Review

Recent advances in research on P-glycoprotein inhibitors

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Summary **The ability of cancer cells to experience intrinsic or acquired resistance to a broad spectrum of structurally and functionally unrelated chemotherapeutic agents, termed multidrug resistance (MDR), is the most common cause of chemotherapy failure. Research has firmly established that most tumors developing MDR are often associated with the over-expression of permeability-glycoprotein (P-gp), the most extensively characterized of the drug efflux pumps. The development of P-gp inhibitors is acknowledged as a viable means of reversing this MDR phenotype and has received considerable attention throughout the past two decades. However, most P-gp inhibitors identified to date have demonstrated limited clinical success due to limitations in potency and specificity. This paper reviews the most recent discoveries relating to the medicinal chemistry of P-gp inhibitors that are presently in development. In light of this information, this paper seeks to suggest new treatment options for the MDR phenotype.**

Keywords: P-glycoprotein, Inhibitor, MDR modulator, Substrate

1. Introduction

Multidrug resistance (MDR) is defined as the simultaneous resistance to various structurally and functionally unrelated drugs, which is believed to be one of the major obstacles of successful cancer chemotherapy (*1*). One of the best-understood MDR-involved mechanisms is the over-expression of the *mdr1* gene-encoded product, P-gp, an ATP-dependent xenobiotic exporter that causes an increased efflux of drugs from cancer cells (*2*).

P-gp (EC 3.6.3.44, CD243 antigen), a 170-kDa phosphoglycoprotein, contains two highly homologous halves, called NH_2- and COOH-terminal halves, respectively, that are 43% identical in human P-gp (*3*). Each homologous half contains six transmembrane (TM) helices and one nucleotide-binding domain (NBD) (*4*). P-gp up-regulation is often found in patients with cancer relapse after chemotherapy and in cultured cells that inevitably become drug resistant after stepwise selection as a result of resistance to chemotherapeutic agents (*5*).

The classical approach to interfering with abnormal P-gp activity is the use of small molecules as P-gp inhibitors, also called MDR modulators, to block the aberrant function of the P-gp pump, eventually leading to the effective accumulation of cytotoxic drugs in tumor cells (*6,7*). The development of P-gp inhibitors, including those of natural origin and those chemically synthesized, is acknowledged as a viable means of reversing this MDR phenotype and has received considerable attention throughout the past two decades (*8*).

Till now, three generations of these molecules have been identified as P-gp inhibitors, which can also be classified into competitive and noncompetitive inhibitors according to their corresponding inhibitory mechanism. Among these inhibitors, many agents that modulate the function of P-gp are able to restore the cytotoxicity of chemotherapeutic drugs to MDR cells *in vitro* and in experimental drug-resistant tumors *in vivo*. Unfortunately, only a few compounds have reached

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the stage of clinical trials, and none has thus far been cleared for clinical therapeutic use (*9*).

There are primarily two main reasons for this difficulty: 1) MDR is a complex phenomenon that may arise from several different biochemical or biomolecular mechanisms, with the consequence that inhibition of transporter proteins may, to some degree, be insufficient to completely reverse it; and 2) Despite years of efforts of functionally analyze its structure, the exact physiological role of P-gp has yet to be fully clarified and still appears to be somewhat of a mystery. Moreover, the current failure to achieve effective MDR control in particular requires more potent modulators with proper selectivity and pharmacokinetics in order to avoid unwanted side effects. This is because P-gp not only contributes to antineoplastic resistance by elevating the cellular apoptotic threshold but is also expressed in normal tissues such as the adrenals, gravid uterus, kidney, liver, colon, and capillary endothelial cells in the brain. Consistent with this, the expression of P-gp in these normal tissues serves to prevent the uptake of xenobiotics and prevent exposure of sensitive tissues to xenobiotic agents (*10*).

Therefore, great efforts are still needed to further identify novel compounds that inhibit abnormal P-gp function in order to reverse the MDR phenotype and sensitize cancer cells to conventional anticancer drugs without unwanted toxicological effects (*11*).

2. Competitive and noncompetitive P-gp inhibitors

2.1. *Competitive inhibitors*

Competitive P-gp inhibitors, as substrates for P-gp, compete with cytotoxic agents for transport by the pump, as shown as Figure 1A. If both the drug substrate and the inhibitor have similar affinity, the greater the concentration of inhibitors, the less chance there is for the substrate to enter the active drug-binding sites of P-gp. Conversely, the greater the concentration of substrate, the less efficient is the inhibition of P-gp. However, if the P-gp inhibitor has a relative low affinity for drug-binding sites, a high concentration of the inhibitor is thus required to achieve the anticipated effect, consequently limiting the efflux of xenobiotics and accordingly increasing its intracellular concentration (*12*).

Many first- and second-generation P-gp inhibitors are just such competitive inhibitors; in other words, they compete as a substrate for P-gp with xenobiotics. In addition, the high degree of similarity in structure, partition coefficient (log *P*), and membrane interaction between substrates and inhibitors has caused great difficulty in defining their molecular properties. Thus, these similarities should be avoided when designing a potent P-gp inhibitor.

2.2. *Noncompetitive inhibitors*

In contrast to their competitive counterparts, noncompetitive inhibitors of the P-gp transporter do not compete with the substrate for active binding sites in the pocket, as shown in Figure 1B. Such inhibitors usually bind to a different region of the protein in comparison to the substrates, and in doing so they can induce a conformational change in the protein via a cross-linking pattern that is affected by the structure of the drug substrate (or what is generally termed an induced-fit mechanism) so that the active site is no longer recognizable to the hydrophobic substrates. This thus prevents ATP hydrolysis and transportation of the anticancer drugs out of the cell, resulting in an increased intracellular concentration. In simple terms, increasing the amount of substrates in cell will have no effect on

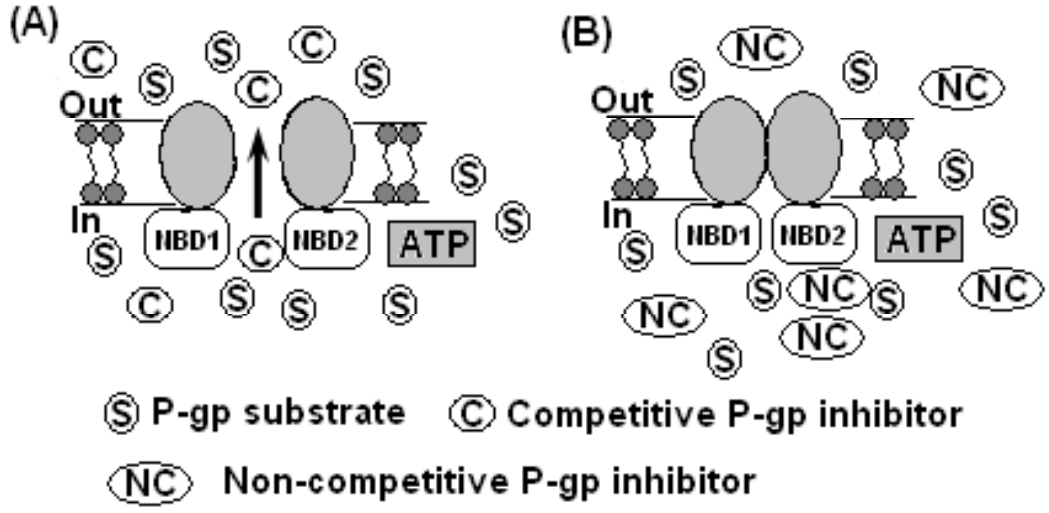

Figure 1. Simple competitive (A) and noncompetitive (B) inhibition modes of P-gp-mediated transport. The picture is referenced from *ref. 10*.

the level of inhibitor (*1*). Third-generation inhibitors of P-gp can bind with high affinity to the transporter pump but are not themselves substrates, so they thus fall under the category of noncompetitive inhibitors.

3. The active drug-binding sites of P-gp

Exploration of the active drug-binding sites of P-gp has always been a major and interesting aspect of the P-gp issue. It is generally accepted that there are least three drug/substrate binding sites and one allosteric site of P-gp; these are independent but interact with each other to perform the transporter function of P-gp (*13*). Many reported P-gp inhibitors primarily interact reversibly with one or more of the three presumed drug/substrate-binding sites and thus are competitive inhibitors (*14*).

Studies with thiol-reactive substrate analogs of P-gp and cysteine mutants have shown the drug-binding site is a "funnel-shaped pocket" surrounded by 12 TM segments; the site is narrow on the cytoplasmic side, at least 9~15Å wide in the middle, and wider at the extracellular side; it allows only stipiamide dimers greater than 11Å but less than 35Å in length (*15*). This drug-binding pocket might be large enough to accommodate more than one substrate at the same time by using an organic combination of amino acid residues from different TM regions to form a specific drug-binding site for a particular drug. Thus, different substrates might occupy different binding sites (*16*).

4. Three generations of P-gp inhibitors

Over the past two decades, P-gp inhibitors have gone through three generations of development, as will be discussed in the following sections.

4.1. *First-generation P-gp inhibitors*

The term "first generation" implies those drugs that already in clinical use for other pharmacological activities but not specifically developed in order to reverse P-gp-mediated MDR. Such drugs, however, were occasionally found to posses such ability in clinical trials. Typical examples are the calcium ionic channel blockers verapamil (VRP) and diltiazem (herbesser), the immunosuppressive agent cyclosporin (cyclosporin A), the estrogen receptor antagonist tamoxifen (TAM), and several calmodulin antagonists, which are themselves substrates of the P-gp pump and inhibit P-gp-mediated drug transport in a competitive manner.

Most first-generation inhibitors proved to be effective *in vitro*, and some were even extremely efficient at MDR reversal. Unfortunately, they were minimally effective, non-specific, and toxic. Due to their mechanism of competitive action and the resulting low binding affinities with transporters, high *in vivo* serum concentrations of inhibitors were thereby required to obtain sufficient intracellular concentrations of the cytotoxic drugs as well as effective inhibition of P-gp, resulting in, to a large extent, severely unacceptable toxicities for cancer patients. Therefore, these drugs often produced disappointing results *in vivo* and have limited clinical usefulness mainly due to their severe side effects and toxicity. What is worse, some, *e.g.* quinine, even proved to specifically beneficial at reversing the overexpression of P-gp when used in the treatment of MDR (*17*).

In addition, numerous studies have found that many of these inhibitors are substrates both for transporter proteins and other physiological drug transporting enzyme systems, *e.g.* drug-metabolizing cytochrome P450 isoenzyme 3A4 (CYP3A). Because both CYP3A4 and P-gp are expressed in metabolic-related tissues, such as the intestine and the liver, they may work together to eliminate xenobiotics. Thus, the inhibition of P-gp will interfere with normal metabolism and possibly retard the biotransformation of drugs, leading to unpredictable drug-drug interactions and unexpected pharmacokinetic effects, as well as increased toxicity (*18*).

4.2. *Second-generation P-gp inhibitors*

To overcome the limitations of first-generation drugs, MDR modulators with improved potency, specificity, and P-gp-binding affinity were exhaustively examined. Second-generation inhibitors were thus developed and can be divided into two categories. The first, which accounts for the majority of the drugs, is limited to analogues of first-generation drugs, including dexverapamil, dexniguldipine, *trans*-flupentixol, cyclosporine A analog valspodar (PSC833), and quinidine analog MS-209. The second category mostly consists of those investigational agents with novel chemical structures, such as S-9788, GF-12918, and VX-710 (biricodar).

These drugs proved to have a better pharmacologic profile with relatively high P-gp inhibitory capacity and fewer toxic side effects. Some, such as PSC833, VX-710, and S9788, have undergone phase I, I/II, or even III clinical trials, but results have been unsatisfactory (*19*,*20*).

Given that these inhibitors still detain intracellular xenobiotics by a competitive approach, together with the fact that many are still substrates for CYP3A4, they present similar issues to those with first-generation P-gp inhibitors. That is, the unpredictable pharmacokinetic interactions between the inhibiting agent and anticancer drugs remained unresolved.

Furthermore, the relatively lower specificity and P-gp affinity of these inhibitors led to many, *e.g.* valspodar and verapamil, affecting several other ABC transporter family members besides the P-gp transporter, such as MRP1 (multidrug resistance protein 1), another

important ABC transporter protein involved in the cancer cell MDR phenotype (*21*). Studies by Lawrence *et al.* revealed that although P-gp and MRP1, two of the most often-studied MDR-related transporters, have pharmacological properties that only partially overlap, they do possess transporter selectivity. Inhibitors that alternatively antagonize P-gp over MRP1 are an optimal choice for effective MDR modulators (*22*).

Finally, in spite of the acknowledged high correlation between overexpression of P-gp and the MDR phenotype, the localized expression of P-gp has also been detected in normal cells or tissues with a secretory function, including the pancreatic gland, adrenal cortex (AdC), kidney, hepatocytes, epithelial tissue (*e.g.* intestinal epithelium), and cells that constitute the barrier and metabolic functions in the intestine, brain microvascular endothelia, and blood brain barrier (BBB) (*10*). The abundant expression of P-gp in these normal tissues or organs suggests that it may play a protective role in absorption, transportation, or secretion of proteins or hormones, distribution or detoxification of xenobiotics, and similar processes (*23*). Consistent with these facts, inhibition of the overexpression of P-gp might also lessen the ability of normal cells or tissues to endure cytotoxic agents. In other words, this inhibition of nonselective transporters may lead to greater adverse effects of anticancer drugs.

4.3. *Third-generation P-gp inhibitors*

Given the drawbacks of traditional first- and second P-gp inhibitors, such as targeting interrelated proteins, undesirable intrinsic toxicity, and pharmacokinetic interactions, MDR modulator studies have tended to emphasize trying to overcome these disadvantages. The compounds studied are expected to have specific and potent interaction with the P-gp transporter without inhibiting other ABC family transporters.

Recently, the advancement of computational chemistry, chemoinformatics, and molecular pharmacology, together with technologies for combinatorial chemistry, have led to MDR modulator discovery shifting from blind screening to rational drug design. As a consequence, third-generation P-gp inhibitors with novel chemical structures have quickly been developed. These inhibitors were designed with the guidance and elucidation provided by quantitative structure-activity relationship (QSAR) studies, as well as use of noncompetitive mechanisms. Many of the inhibitors thus developed are in the clinical stage and hold promise for future treatment, including VX-710 (biricodar), tetrandrine, FG020326, S-9788, GF-120918 (elacridar), LY-335979 (zosuquidar), tariquidar (XR9576, Xenova), laniquidar (R101933), and ONT-093 (OC144-093) (*24*). These investigational agents have minimal effect on other members of the ABC transporter family and dramatically reduced the interaction with CYP 3A4 at relevant concentrations (*25*).

More importantly, the vast majority of the agents tested thus far have caused minimal clinically relevant alterations in the pharmacokinetics of co-administered anticancer drugs *in vitro*. Such specificity for the P-gp pump minimizes the possibility that the blockade of more than one pump might result in altered bioavailability or excretion of the chemotherapy agents. The advantages of these noncompetitive P-gp inhibitors make them potential MDR reversing agents and offer significant improvements in chemotherapy without the need for chemotherapy dose reductions (*5*).

5. Newly identified P-gp inhibitors

Despite the strong potency of third-generation P-gp inhibitors *in vitro*, there is no commercial drug for use in MDR therapy. This is due to disappointing results from either *in vivo* or preclinical assays.

Given the lessons learned from failed trails, novel P-gp inhibitors with high specificity and potency, little effect on metabolic enzymes, and reduced pharmacokinetic effects are in great demand to increase survival for cancer patients. Recently, a number of natural and synthetic inhibitors have thus appeared, expanding MDR research.

5.1. *Several predicted pharmacophore models concerning P-gp inhibitors*

Identifying molecules that specifically interact with P-gp is pivotal for drug discovery; useful pharmacophore models provide direct and tangible information for rational drug design. Current studies are concentrating on two alternative approaches, pharmacophore models using computational methods and database screening from reported molecules in the literature to predict their affinities as inhibitors *in vitro*.

Over the last several years, several pharmacophore models concerning P-gp inhibitors have been built through three-dimensional QSAR analysis of various reported P-gp substrates and inhibitors. As a consequence, P-gp inhibitors with potential MDR reversal activity might generally consist of the following physico-chemical characteristics: (a) Liposolubility with hydrophobic centers (*e.g.* aromatic rings or hydrophobic substituents); (b) Cationic species or a basic center in the physiological pH range; (c) At least two coplanar aromatic rings, and hydrophobic substituted aromatic rings to help activity; (d) Appropriate hydrogen bond acceptors (O or N atom), and/or hydrogen bond donors (OH or NH group) with certain spatial separation (*26-29*) .

In 1997, supplementary information was provided by QSAR studies by Klopman *et al.* (*30*). They found that the introduction of a carboxyl group, quaternary

ammonium salt, and substituent groups like aniline, phenol, and N=CH-CH= were detrimental to activity, so such substituents should thus be avoided.

In 2002, Garrigues *et al.* (*31*) derived two predictive MDR reversal pharmacophore models by means of a molecular modeling approach, suggesting that the azimuthal distribution of hydrophobic and polar elements (rather than chemical motifs) as well as the size of ligands of various MDR modulators affect the patterns of interaction with P-gp. They characterized two different but partially overlapping models and indicated that ligand size affected their ability to bind with P-gp.

At nearly the same time, Ekins *et al.* (*32*) used *in vitro* data associated with inhibition of P-gp function to build catalyst 3D-QSAR models that could qualitatively rank, order, and predict IC_{50} values for P-gp inhibitors that may modulate one or more P-gp binding sites. As an extension of their work, they further deduced a new pharmacophore to validate their previous results, suggesting that digoxin and vinblastine are likely to bind similar or overlapping P-gp binding sites summarized from inhibition of verapamil binding (*33*).

In 2006, Crivori *et al.* (*34*) used two different computational models with calculated molecular descriptors and multivariate analysis to identify potential P-gp substrates and inhibitors. The highly predictive models were capable of predicting correctly the behavior of 72% of an external set of 272 proprietary P-gp-associated compounds. Their results should prove highly useful to medicinal chemists in the search for prospective drug candidates with high success rates and in structure-based virtual screening.

To investigate plausible explanations for the pharmacokinetic profiles of published inhibitors, Chang *et al.* (*35*) used both pharmacophore models and database screening to rapidly and accurately confirm the affinity properties (inhibitor or substrate) of tested molecules when binding with P-gp. At the same time, they used biopharmaceutics and drug disposition classification to also provide insight into anticipated drug-drug interactions, which is especially useful in efficiently facilitating screening *in vitro* in order to avoid compounds with a potential and specific P-gp interaction.

5.2. *Natural inhibitors of P-gp*

Piperine (Figure 2), a major component of black pepper (*Piper nigrum* Linn) and long pepper (*Piper longum* Linn) used as a spice and nutritional supplement, is a common dietary constituent and phytochemical. Research has verified that piperine can inhibit the P-gp-mediated transport of digoxin and cyclosporine in the Caco-2 cell monolayers (*36*). More recently, Han *et al.* (*37*) performed *in vitro* and *in vivo* assays at dietary levels. They indicated that piperine can affect P-gp function and expression in a manner that is time- and concentration-dependent. Nevertheless, the mechanisms for the piperine-mediated modulation of P-gp expression have yet to be verified, In addition, dietary piperine could affect plasma concentrations of both P-gp and metabolizing enzyme CYP3A4 substrates in humans, so its pharmacokinetic effects are still unclear (*36*).

Fumagillin (Figure 3) isolated from aspergillus fumigatus is an antibiotic effective against sporidiae to treat nosema disease in bees and fish. Dupuy *et al.* (*38*) proved that fumagillin can interfere with P-gp function, revealing that proper optimization of the fumagillin scaffold or derivatization might generate optimal novel fumagillin-derived P-gp inhibitors.

5.3. *Synthetic inhibitors of P-gp*

Recently developed WK series compounds target tetrahydroisoquinolin-ethyl-phenylamine based WK-X-34, WK-X-50, and WK-X-84, as shown in Figure 4. These compounds were found to be very potent, specific, and non-toxic inhibitors of P-gp- and breast cancer resistance protein (BCRP)-mediated MDR in different cell lines using specific *in vitro*- and *in vivo*-imaging techniques. They may thus be potential candidates for therapy to treat MDR-resistant tumors.

Within the WK series, WK-X-34 has proven to be the most promising P-gp inhibitor with IC_{50} values on the order of the nM (*39*). It has also displayed reduced cellular toxicity and increased potency of both P-gp (IC_{50} = 82.1 ± 6 nM) and BCRP inhibition (IC_{50} = 26.5 ± 4.6 μM) in comparison to cyclosporine A, a first-generation P-gp inhibitor with broad-spectrum MDR modulating activity in clinical trials. WK-X-34 does so by inhibiting not only P-gp but also other MDR-associated proteins such as BCRP, MRP1, and lung-resistance protein (LRP) (*5,40*).

Two synthetic taxane-based compounds (Figure 5), Ortataxel (formerly called BAY 59-8862 or IDN-5109)

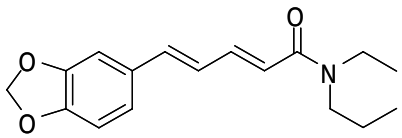

Figure 2. Chemical structures of piperine.

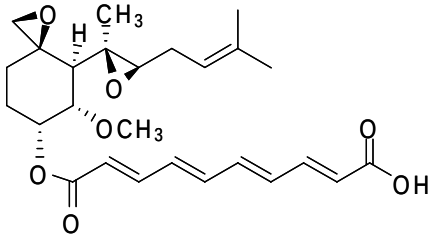

Figure 3. Chemical structures of fumagillin.

and tRA 96023 (or SB-RA-31012), have recently emerged as interesting clinical candidates. These candidates could overcome resistance to paclitaxel with broad-spectrum ABC protein (P-gp, MRP1, BCRP) modulating activity and allow oral administration as well. Ortataxel is a cytotoxic taxane that can effectively block its own efflux from P-gp-overexpressing cells by virtue of modulation of P-gp-mediated transport. In contrast, tRA 96023, also can modulate P-gp function but is noncytotoxic, which may be attributed to the removal of the tubulin-binding side chain at the C-13 position of the taxane scaffold. Such a compound is in preclinical development (*4,12*).

Pleban *et al. (42)* demonstrated that the combination of autocorrelation vectors and self-organizing artificial neural networks is an extremely valuable method of identifying P-gp inhibitors with a structurally new backbone. Another approach involving pharmacophore-driven photoaffinity as well as protein homology modeling was used to glean more useful information about P-gp inhibitors. Results indicated that two of the propafenone-type inhibitors of P-gp, AG-690/11972772 together with AN-989/14669159 (Figure 6), were highly active with IC_{50} values below 1 μM (*42*).

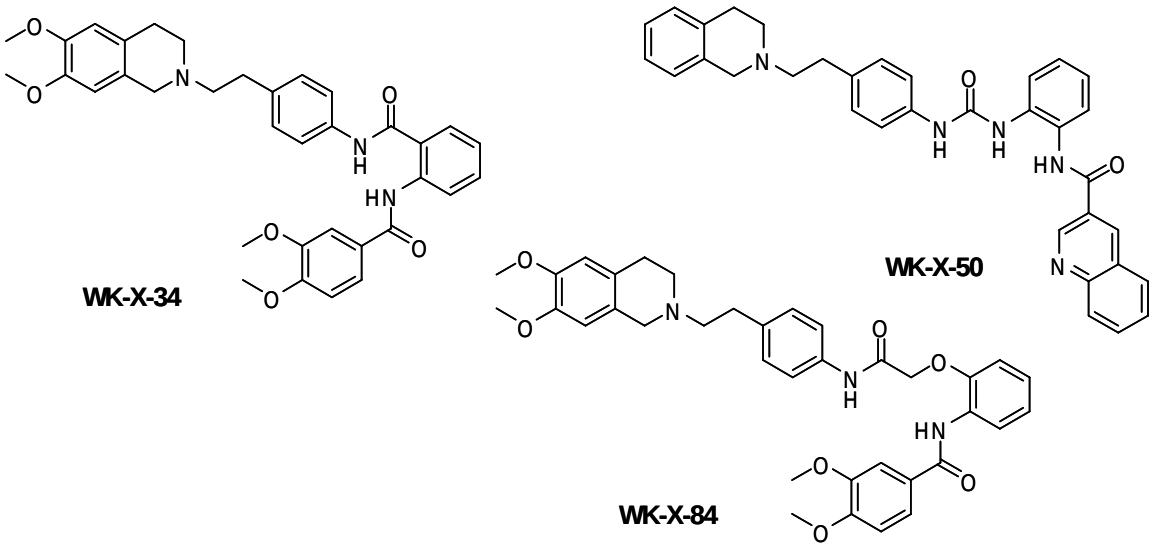

Figure 4. Chemical structures of WK series compounds.

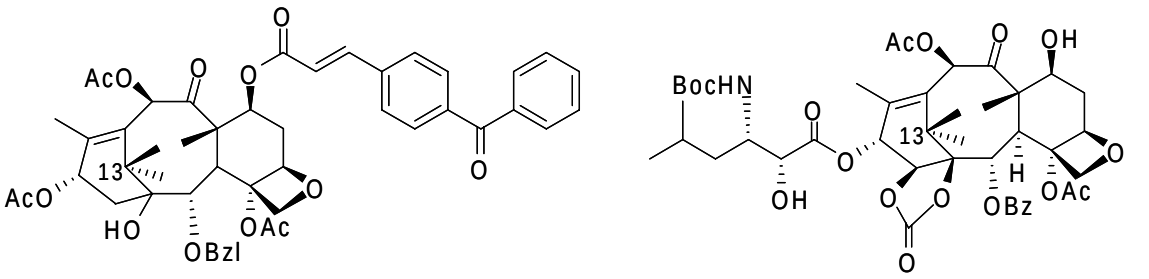

Figure 5. Chemical structures of synthetic taxane-based compounds Ortataxel and tRA 96023.

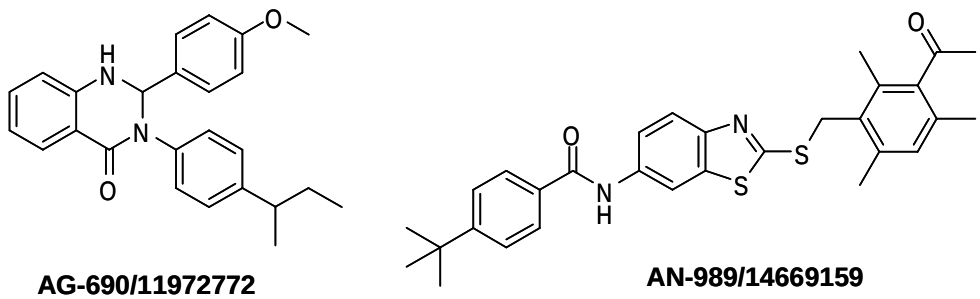

Figure 6. Chemical structures of propafenone-type inhibitors of P-gp.

Given the fact that the paclitaxel-based taxoid IDN5109 and docetaxel-based IDN5390 are potential clinical candidates to eliminate resistance to paclitaxel, Barboni *et al.* (*43*) prepared their corresponding methoxylated analogs **1** and **2**, as depicted in Figure 7. They did so in order to improve cytotoxic potency and substantially retain bioavailability. They found that a modification in the form of inserting of a *meta*-methoxy group in the C-2 benzoate caused a general increase in cytotoxicity and had minimal effect on water solubility. However, the assumption that introduction of a methoxyl group might dramatically effect drug metabolism must still be confirmed.

Similar modifications have also been made by Fong *et al.* (±)-3′-*O*,4′-*O*-dicinnamoyl-*cis*-khellactone (DCK) is a noncompetitive P-gp inhibitor *via* derivatization of (±)-praeruptorin A (PA), which is a major component of the extract of *Peucedanum praeruptorum* Dunn. Since DCK possesses better P-gp-mediated MDR reversal activity than PA itself or even verapamil, the methoxylation of the cinnamoyl groups on DCK was therefore investigated in order to enhance its bioactivity (*44*). Two novel pyranocoumarins, (±)-3′-*O*,4′-*O*-bis(3,4-dimethoxycinnamoyl)-*cis*-khellactone (DMDCK) and (±)-3′-*O*,4′-*O*-bis(4-methoxycinnamoyl)-*cis*-khellactone (MMDCK), resulted, as shown in Figure 8. They were found to have different P-gp-inhibitory activity. The successful outcome of the modification is the co-existence of 3- and 4-methoxy groups on cinnamoyl (DMDCK), resulting in markedly enhanced activity through a noncompetitive mechanism. To be precise, Fong *et al.* speculated that the additional 3-methoxy group on cinnamoyl allows DMDCK to interact more efficiently with the P-gp substrate site(s). Meanwhile, the lone existence of the 4-methoxy group on cinnamoyl (MMDCK) reduced activity, and removal of the 4-methoxy group on cinnamoyl (DCK) resulted in moderate activity. A further pharmacophore search with a verapamil-based template also provided supplementary proof that four functional groups of DMDCK could be simultaneously involved in interaction with P-gp whereas for DCK or MMDCK only three groups were involved.

Given that the sigma-2 receptor agonist PB28 demonstrates good P-gp inhibitory activity with an EC_{50} value of 0.55 μM, Colabufo *et al.* (*7*) recently developed its analogs as lead compound **3**. They consequently prepared the corresponding series of biphenyl and 2-naphthyl isoquinoline derivatives, as shown in Figure 9.

SAR studies showed that in the biphenyl series (**4**~**6**), **5a** (X=CH_2, R=OH) provided the best results with an EC_{50} value of 0.05 μM, which is superior to the lead compound (EC_{50} = 0.55 μM), while its amide derivative **5b** was found to be less active with an EC_{50} value of 3.5 μM. This suggests the importance of basicity and the apparent unimportance of the presence of –OH or $–OCH_3$ substituents. In the 2-naphthyl series, both basicity and the presence of an H-bond

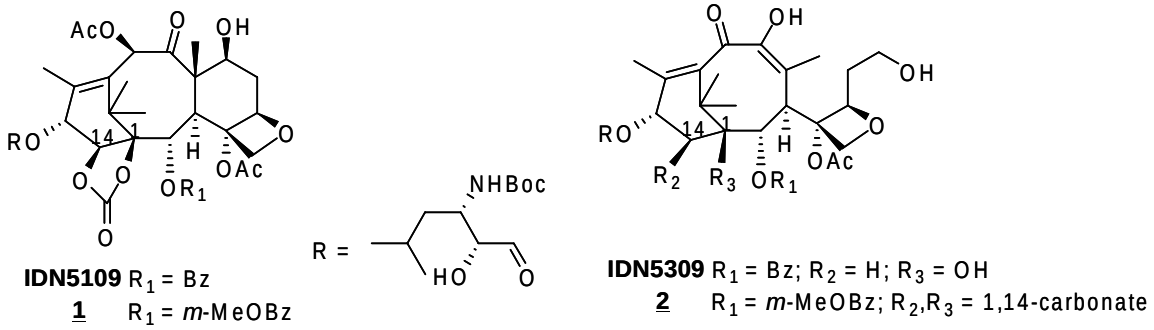

Figure 7. Chemical structures of methoxylated analogs derived from IDN5109 and IDN5390.

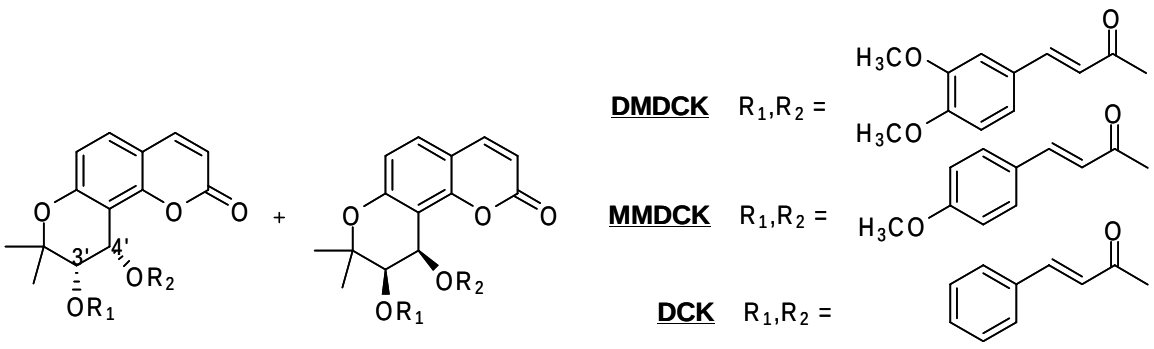

Figure 8. Chemical structures of methoxylated analogs DMDCK and MMDCK derived from DCK.

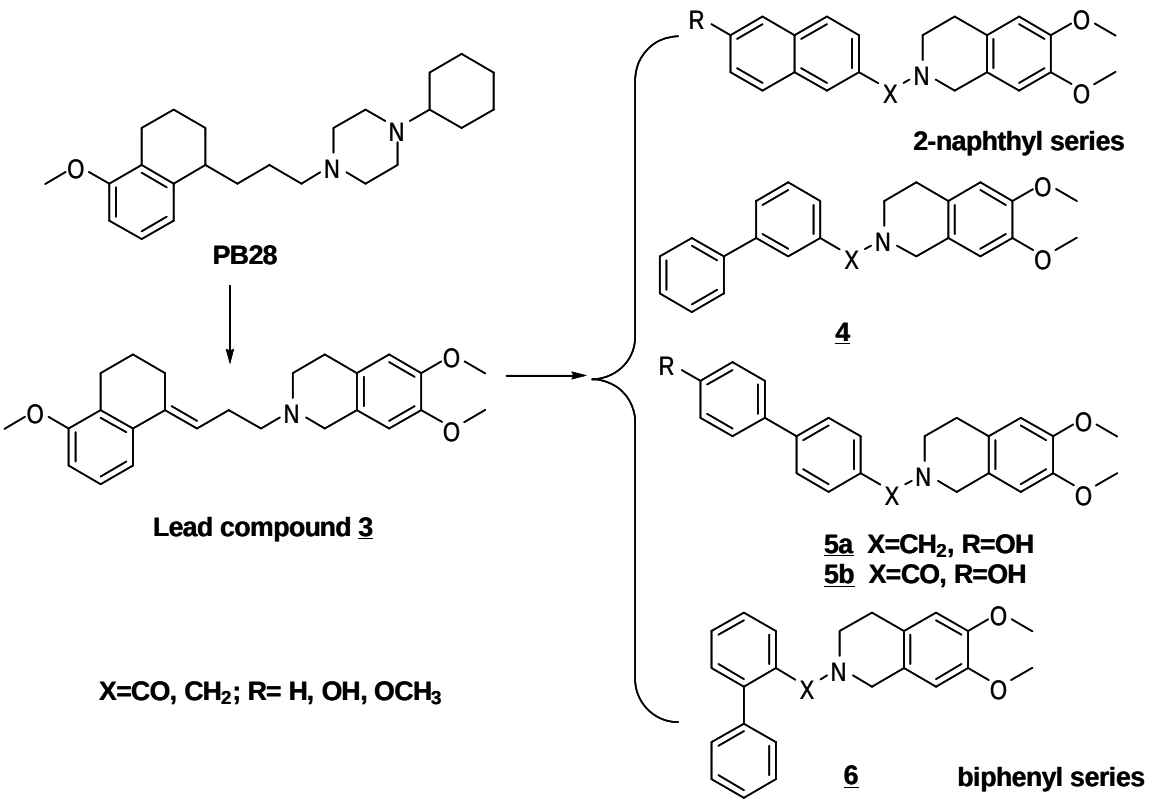

Figure 9. Chemical structures of isoquinoline derivatives.

donor or acceptor seem to be of negligible importance. In addition, Colabufo *et al*. (*7*) also proposed that lipophilicity did not affect the P-gp inhibitory activity among the compounds.

6. Conclusions and perspectives

During the past two decades, significant progress has been made in understanding the pharmacological and physiological role of P-gp. Concomitant use of P-gp inhibitors is hopefully an effective and safe way to perform further preclinical and clinical investigations with the hope of providing new treatment options to overcome the MDR phenotype.

However, the complex mechanisms of tumor MDR in the body makes the effective use of P-gp-targeted MDR reversing agents/modulators a difficult task. This is largely attributable to, in spite of years of efforts, uncertainty about MDR-associated mechanisms and interaction of inhibitors/substrates with P-gp as well as lack of agreement in those areas (*45,46*). The formation of the MDR phenotype is a complex and multi-factor process, so focusing attention on certain aspects would prove unfruitful. In addition, this is why, to a large extent, many P-gp inhibitors have been identified but failed to lead to MDR modulating drugs (*47*).

For instance, one fascinating mystery of P-gp is how its complex protein transport system can recognize and transport a wide variety of structurally unrelated compounds and perform different functions. Requirements for potential clinical candidates are basically that they are novel MDR modulators with broad-spectrum modulation, they lack significant toxicity, and they lack significant pharmacokinetic interactions with cytotoxic drugs. The potency of inhibition of metabolizing enzymes (*e.g.* CYP3A4) does not necessarily predict a drug's potency of inhibition for P-gp and vice versa, despite the fact that many molecules interact with CYP3A4 and P-gp to a similar extent (*48*).

Although P-gp inhibition seems to be a complex and difficult task, a large amount of work is still needed to optimize this strategy. The continued development of detection technology, *e.g.* computational virtue screening techniques, 3-D QSAR studies, molecular pharmacology, and chemoinformatics, together with the technologies of chemogenomics, will deepen the understanding of P-gp's structure and efflux mechanisms. Accordingly, this should offer the opportunity for novel therapeutically effective P-gp inhibitor candidates.

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