Pergamon

*European Journal of Cancer* Vol. 32A, No. 6, pp. 991–1001, 1996

Printed in Great Britain
0959–8049/96 $15.00+0.00

S0959-8049(96)00047-0

# Experimental Reversal of P-glycoprotein-mediated Multidrug Resistance by Pharmacological Chemosensitisers

J.M. Ford

Departments of Biological Sciences and Internal Medicine (Oncology), Stanford University, Stanford, California 94305-5020, U.S.A.

## INTRODUCTION

CLINICAL DRUG resistance to chemotherapeutic agents is a major obstacle for their curative potential in the treatment of human cancers. Multidrug resistance (MDR) is a common mechanism of cellular resistance to the cytotoxic activity of many chemotherapeutic agents. Tumour cells selected for the MDR phenotype overexpress the *MDR1* gene product, P-glycoprotein (Pgp), a membrane-bound drug efflux pump which confers resistance to a broad range of commonly used chemotherapeutic drugs and other agents. Numerous compounds have been identified which inhibit the efflux activity of Pgp, and reverse cellular resistance to cytotoxic agents in experimental systems [1, 2]. This suggests that clinical drug resistance in human tumours, which often overexpress Pgp, may be potentially circumvented through the concomitant administration to patients of a Pgp inhibitor and chemotherapeutic drugs.

Pgp-mediated MDR is only one of many cellular mechanisms by which tumour cells may evade the cytotoxic effects of anticancer agents, but is one of the best understood and most intensively studied forms of mammalian drug resistance. Remarkable progress has been made in defining the cellular and biochemical pharmacology of MDR since the initial description of the MDR phenotype and its drug efflux characteristics [3, 4]. The membrane protein responsible for drug efflux has been identified and its structure and function characterised, genes encoding for Pgp and other related drug transport proteins have been cloned, and Pgp has been shown to be functionally expressed in many human tumours, as well as in normal tissues.

Of clinical relevance, a large number of drugs have been identified which inhibit the function of Pgp, termed chemosensitisers or MDR modulators, and trials have been designed and carried out in humans to test the potential for pharmacological inhibition of clinical MDR. Many of these chemosensitisers have proven toxic or ineffective *in vivo*, and thus the identification of novel agents for potential clinical use has taken on great importance. This article will briefly summarise our current understanding of the pharmacological mechanism of Pgp inhibition, review in detail those agents most likely to have clinical impact on the circumvention of Pgp-mediated MDR in humans, and discuss novel approaches to the identification of more potent and less toxic chemosensitisers.

## CELLULAR PHARMACOLOGY AND MOLECULAR BIOLOGY OF MDR

The MDR phenotype was first described by Biedler and Riehm who noted that, following selection of mammalian tumour cells for resistance to a single cytotoxic drug, a broad crossresistance developed simultaneously to other structurally and functionally unrelated drugs [3]. Soon after, Dano noted that such drug-resistant cells displayed a decrease in accumulation of the anthracycline chemotherapeutic agent, daunomycin, due to its active outward transport [4]. Chemotherapeutic drugs now known to be affected by MDR include doxorubicin, mitoxantrone, vincristine, vinblastine, VP-16, paclitaxel and topotecan, but not drugs such as bleomycin, methotrexate, cisplatinum or alkylating agents. In addition, naturally occurring carcinogens, such as benzo(*a*)pyrene [5], and physiological substances such as hormonal steroids [6], also serve as substrates for the Pgp pump.

The most consistent alteration found in MDR cell lines is an increased expression of a high molecular weight cell surface glycoprotein (Pgp) not detectable in drug-sensitive cells, associated with an energy-dependent mechanism for the decreased accumulation and retention of cytotoxic drugs [4, 7, 8]. The presence of Pgp was found to correlate with both the degree of resistance and the relative decrease in drug accumulation [9]. Monoclonal antibodies to Pgp and nucleic acid probes for the *MDR1* gene demonstrated that most MDR cell lines from rodent or human origin overexpress this gene [9, 10].

Introduction of expression vectors containing cDNAs coding for Pgp confers the full MDR phenotype when transfected into drug-sensitive cells [11, 12], and these stable transfectants overexpress Pgp and display enhanced efflux of cytotoxic drugs [13]. Furthermore, heterologous expression of mammalian *MDR* genes in bacteria [14] and yeast [15, 16] confers drug resistance and ATP-dependent drug efflux of known Pgp substrates against a strong drug concentration gradient, independent of membrane potential or transmembrane proton gradient [17], and which can be inhibited by chemosensitisers. Extensive studies of the biochemistry and molecular biology of the *MDR1* gene product further support its role as an efflux pump of broad specificity which directly interacts with drug molecules to reduce their intracellular concentration. Structural analyses of Pgp, accomplished by

sequencing cDNA clones from Pgp encoding genes, revealed it consists of two homologous domains containing 12 predicted transmembrane segments, and two nucleotide binding domains [18, 19], and belongs to a superfamily of ATPase proteins that behave as ion channels and transporters [20, 21].

Rigorous proof of the drug efflux pump model for multidrug transport by functional reconstitution of Pgp in a highly defined system has long eluded investigators due to difficulties in achieving efficient biochemical purification of the protein. However, several groups have now reported the purification and reconstitution of Pgp into phospholipid bilayers, which display constitutive ATP-dependent drug transport against a concentration gradient and is inhibited by chemosensitisers, and ATPase activity that is highly stimulated by chemosensitisers and MDR substrates [22–24].

Most models of Pgp suggest it transports drugs across cell membranes in a manner analogous to that defined for active transport proteins (Figure 1). This model predicts that substrates (cytotoxic drugs) bind to specific domains of the protein, which subsequently undergoes an energy-dependent conformational change, allowing the substrate to be released on the exterior side of the membrane. Complementary models have been proposed suggesting that Pgp interacts directly with substrates in the plasma membrane (the 'hydrophobic vacuum cleaner' model [25]), or that Pgp may be involved in the transport of drugs from the inner to the outer leaflet of the plasma membrane, from where they diffuse (the 'flippase' model [26]). Identification and characterisation of Pgp segments responsible for drug recognition and binding suggest that Pgp interacts directly with drug molecules. Efforts to map the drug-binding domains of Pgp by photoaffinity drug analogues and site-directed mutagenesis suggest that Pgp contains multiple non-overlapping or partially overlapping drug binding sites, each having different affinities for different drugs or classes of drugs [20, 27–30].

In summary, Pgp functions as an energy-dependent multidrug transporter and its expression forms the genetic basis for MDR. Furthermore, Pgp appears to function in a manner similar to that of active transport carrier proteins, although evidence remains insufficient to determine how closely it conforms to this model.

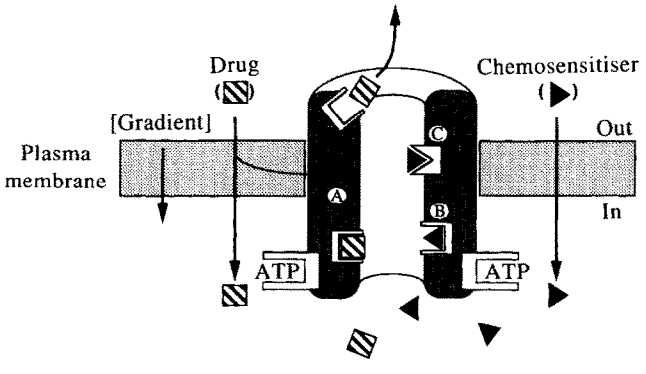


**Figure 1. Functional representation of P-glycoprotein. The model depicts a translocating carrier protein, which utilises ATP energy to actively transport drug substrate across the plasma membrane (A). A chemosensitiser may serve as a competitive inhibitor by occupying drug-binding sites (B) or a non-competitive inhibitor at chemosensitiser binding sites (C).**

## PHARMACOLOGICAL MODULATION OF MDR

A primary goal in the investigation of Pgp-mediated MDR is to discover specific means by which to reverse or circumvent it. Through the understanding of structural and functional features important for the inhibition of the MDR transporter, it is hoped that new agents for potential use in clinical trials will be discovered.

Tsuruo and coworkers first reported the pharmacological reversal of MDR in 1981 by showing that the clinically used drugs, verapamil and trifluoperazine, potentiated the antiproliferative activity of vincristine and produced an increased cellular accumulation of vincristine in an MDR murine leukaemia cell line [31]. Since this original observation, numerous compounds have been shown to antagonise MDR in a variety of tissue culture assays and animal tumour models when co-administered with chemotherapeutic agents to which the cells are resistant [1, 2]. In general, agents used to antagonise MDR alter the drug accumulation defect present in MDR cells, but exhibit little or no effect on drug-sensitive cells.

The primary mechanism by which most chemosensitisers are believed to antagonise MDR is through direct inhibition of drug efflux mediated by Pgp, resulting in restoration of cytotoxic drug accumulation in MDR cells. A simplified model of a potential mechanism of action for the ability of chemosensitisers to inhibit the MDR efflux pump is shown in Figure 1. In this scenario, Pgp functions as an active transport protein, which utilises energy to transport cytotoxic substrates across the plasma membrane. Chemosensitisers may block cytotoxic drug efflux by acting as competitive or non-competitive inhibitors, perhaps by binding to similar drug substrate binding sites, or to other chemosensitiser binding sites which cause allosteric changes resulting in inhibition of cytotoxic drug binding or transport. In support of this model, many studies have now demonstrated that certain chemosensitisers may bind directly to cellular membranes enriched for Pgp, in a specific and saturable manner, and this binding may be inhibited by other chemosensitisers and by chemotherapeutic drugs [27, 32]. In addition, radiolabelled, photoactivated chemosensitiser analogues irreversibly bind to Pgp, and this may be effectively inhibited by many other chemosensitisers [33–37]. However, conflicting evidence exists with regard to whether chemosensitisers of different classes function similarly as inhibitors of drug efflux, where and how many binding sites exist for chemosensitisers on Pgp, and whether chemosensitisers share binding sites with cytotoxic substrates.

Recent studies utilising Pgp molecules containing various mutations support the notion that more than one site of interaction for substrates and inhibitors exists. For example, substitution of a serine residue within the eleventh predicted transmembrane domain of murine Pgp by any of six other amino acids resulted in both positive and negative effects on the modulatory abilities of different chemosensitisers [38]. Similarly, diverse effects were seen for the capacity of different chemosensitisers to inhibit drug efflux mediated by Pgp isoforms differing at a codon within the third transmembrane domain [39]. Also, photoactivated binding of the chemosensitiser azidopine to Pgp has identified two distinct sites within the Pgp molecule, one between residues 198 and 440 of the amino half, and the other within the carboxy portion of the protein [40].

Chemosensitisers may themselves serve as substrates for the Pgp multidrug transporter, in support of their possible role as

competitive ligands for drug-binding sites on Pgp. For example, verapamil, *trans*-flupenthixol and cyclosporin A (CsA) are accumulated less in certain MDR cell lines [1, 41, 42]. Alternatively, certain chemosensitisers, such as the phenothiazines, might inhibit Pgp activity by interacting with physically separate sites on Pgp, such as the ATPase site or phosphorylation domains, or may act distantly, such as by inhibiting protein kinase C and altering the phosphorylation pattern of Pgp, thereby altering its activity. The final delineation of Pgp drug binding sites will be necessary to elucidate fully these mechanisms. Hopefully, a more sophisticated understanding of these processes will also lead to more powerful methods to manipulate Pgp function.

## CLASSES OF CHEMOSENSITISERS

The majority of chemosensitisers described to date may be grouped into six broad categories, based on their primary pharmacological activity: (a) calcium channel blockers, (b) calmodulin antagonists, (c) cyclic peptides, (d) steroids and hormonal analogues, (e) dipyridamole, and (f) miscellaneous other compounds. Table 1 displays a partial list of the wide range of agents which have demonstrated ability to reverse MDR in preclinical models. Although these compounds share only broad structural similarities, all are lipophilic, and many are heterocyclic, positively charged substances.

### *Calcium channel blockers*

The first identified compound with the ability to reverse MDR *in vitro* was verapamil [31, 43, 44], and it is an important standard agent for the comparison of potency and mechanism for all subsequently discovered chemosensitisers. This calcium channel blocker inhibits Pgp-associated, energy-dependent drug efflux in MDR cells due to an increase in intracellular accumulation of chemotherapeutic agents, and is an effective antagonist of resistance to a number of drugs in most MDR cell lines *in vitro* [1]. Photoactivated verapamil analogues bind irreversibly to Pgp, and verapamil inhibits the binding of many chemotherapeutic drugs as well as other chemosensitisers to Pgp [27, 34, 45], suggesting that the mechanism of action of verapamil is through competitively blocking the binding of drugs to Pgp. The sensitivity to cytotoxic drugs of most intrinsically sensitive wild-type cells from which these MDR lines were derived is not significantly affected by verapamil. Studies of many other MDR chemosensitisers demonstrate that most appear to function in a manner similar to that of verapamil, though with differing potencies, and perhaps at different sites on the Pgp molecule. Therefore, the initial studies of the effects of verapamil on the cellular pharmacology of MDR cell lines serve as a critical paradigm for the function of most chemosensitisers.

*Table 1. Selected pharmacological agents with ability to reverse multidrug resistance (MDR)*

| | |
|---|---|
| **Calcium channel blockers** | **Cyclic peptides** |
| R-Verapamil (5–10 μM) | Cyclosporin A (0.8–2 μM) |
| Dexniguldipine (0.1–1 μM) | SDZ PSC 833 (0.1–1 μM) |
| Gallopamil (5 μM) | SDZ 280-446 (0.1–1 μM) |
| Ro11-2933 (2–6 μM) | FK506 (3 μM) |
| PAK-200 (5 μM) | Rapamycin (3 μM) |
| **Calmodulin antagonists** | **Vinca alkaloid analogues** |
| Trifluoperazine (3–5 μM) | Vindoline (20–50 μM) |
| Fluphenazine (3 μM) | Thaliblastine (2 μM) |
| *Trans*-Flupenthixol (3 μM) | **Miscellaneous compounds** |
| **Protein kinase C inhibitors** | S 9788 (1–3 μM) |
| Calphostin C (250 nM) | GF120918 (0.02–0.1 μM) |
| Staurosporine (200 nM) | Tolyporphin (0.1–0.5 μM) |
| CGP 41251 (150 nM) | Dipyridamole (5–10 μM) |
| NPC 15437 (60 μM) | BIBW 22 (1 μM) |
| Safingol (20–50 μM) | Quinidine (10 μM) |
| **Steroidal agents** | Terfenadine (3–6 μM) |
| Progesterone (2 μM) | Reserpine (5 μM) |
| Tamoxifen (2–10 μM) | Amiodarone (4 μM) |
| Toremifene (5–10 μM) | Methadone (75 μM) |
| Megestrol acetate (5 μM) | |

Concentrations in parentheses are those shown to have effect in reversing MDR *in vitro*.

In terms of clinical potential for MDR modulation, verapamil is limited by its cardiovascular effects in humans at plasma concentrations needed for antagonism of MDR *in vitro* [46]. Therefore, the chemosensitising activity of many structural analogues of verapamil have now been investigated, in the hope of identifying potent MDR antagonists lacking in negative inotropic and chronotropic effects. The first such identified structural analogue of verapamil, the dithiane tiapamil derivative Ro11-2933, was found to be up to 10-fold more potent than verapamil for increasing the cellular accumulation and sensitising MDR cells to doxorubicin [47, 48]. Subsequent studies have identified several other verapamil and tiapamil analogues which possess similar or greater MDR chemosensitising activity than verapamil, but which lack significant calcium antagonist activity [49–51].

A number of calcium channel blockers structurally dissimilar to verapamil have also been found to possess chemosensitising activity. While nifedipine is known to be a potent calcium channel blocker, it is a poor antagonist of MDR [52, 53]. However, the dihydropyridine analogues, niludipine, nimodipine and nicardapine, have been found to be potent antagonists of MDR [52–54]. Recently, the chemosensitising activity of 200 newly synthesised dihydropyridine analogues in human MDR cells has been reported [55]. The lead compound, PAK-200, possessed the lowest calcium channel blocking activity, yet it fully reversed resistance to vincristine in a human MDR cell line. These studies have confirmed the lack of correlation between pharmacological calcium channel antagonism and anti-MDR activity.

Though most studies of verapamil have used a racemic mixture of the drug, only the *S*-enantiomer selectively binds to calcium channels [56], whereas the *S*- and *R*-enantiomers of verapamil are equally active chemosensitisers [57, 58]. Similarly, stereoisomers of many other calcium channel blockers differ markedly in their calcium channel blocking activity, but are equally effective as MDR chemosensitisers [59]. In particular, the (–)isomer of niguldipine (dexniguldipine) has displayed a dramatically lower affinity for calcium channel binding sites than its (+) isomer, but retained potent anti-MDR activity [60]. In fact, the use of less cardiotoxic enantiomers of verapamil and its analogues may provide a means for reaching clinically effective anti-MDR levels in patients. Already this strategy has been incorporated into ongoing clinical trials, and initial reports of the pharmacokinetics of *R*-verapamil are promising [61–64].

### *Calmodulin antagonists*

The second class of MDR chemosensitisers identified included drugs previously known for their ability to inhibit

calmodulin-mediated processes, and is represented by the phenothiazine calmodulin antagonist, trifluoperazine [43, 44, 65–67]. The examination of structure–activity relationships for a series of 22 phenothiazine derivatives for potentiation of doxorubicin activity in MDR human breast cancer cells revealed structural features important for chemosensitising activity, and it led to the identification of the thioxanthene class of calmodulin antagonist chemosensitisers that possess significantly greater activity against MDR [67] (Figure 2). The thioxanthenes exist as stereoisomers, and the *trans*-isomer of each in a series of 16 thioxanthenes showed greater activity than the *cis*-isomer for reversing MDR [37]. The lead compound, *trans*-flupenthixol, reversed MDR in a number of human and murine MDR cell lines, and in sensitive cells transfected with the *MDR1* gene, increased doxorubicin accumulation to a greater extent than either its stereoisomer *cis*-flupenthixol or verapamil (Figure 2), and inhibited photoactive azidopine binding to Pgp [37].

The clinical pharmacology and toxicology of *trans*-flupenthixol suggests it may be uniquely suited for *in vivo* use. Clinical trials of the antipsychotic effects of thioxanthenes in humans showed that *cis*-flupenthixol was more effective and toxic than *trans*-flupenthixol [68]. This observation is explained by biochemical and crystallographic evidence that *cis*-flupenthixol is a potent antagonist of dopamine receptors [69, 70], whereas *trans*-flupenthixol has virtually no activity as a dopamine antagonist, resulting in its apparent lack of extrapyramidal side-effects [71].

*Cyclic peptides*

Several hydrophobic cyclic peptides with distinctly different pharmacological and structural properties than other known Pgp inhibitors have been found to possess unique and potent activities for modulating MDR. Representative of this class of chemosensitisers is the clinically used drug CsA, an immunosuppressive agent widely used in human organ transplantation. CsA has been found to reverse resistance in MDR cells at concentrations lower than those necessary for most previously identified chemosensitisers (0.5–3 μM) [72–74]. Unlike most other chemosensitisers, CsA has also been observed to potentiate chemotherapeutic drug cytotoxicity in certain sensitive cell lines [75–79].

CsA appears to possess complex pharmacological properties for modulating drug sensitivity, in accordance with its known activity as an inhibitor of many important cellular enzymes. Numerous studies suggest that CsA may serve as a Pgp substrate and antagonise MDR, at least in part, through competitive inhibition of Pgp-mediated outward transport of cytotoxic drugs, but also imply that CsA modulates cytotoxicity by other mechanisms.

There has been great interest in exploring the anti-MDR activity of other, less immunosuppressive or nephrotoxic cyclosporin analogues. Initial studies of several non-immunosuppressive analogues have demonstrated modulation of MDR [72, 73]. Numerous non-immunosuppressive cyclosporin analogues have now been studied for anti-MDR activity, and the cyclosporin D analogue, PSC 833, has been found to

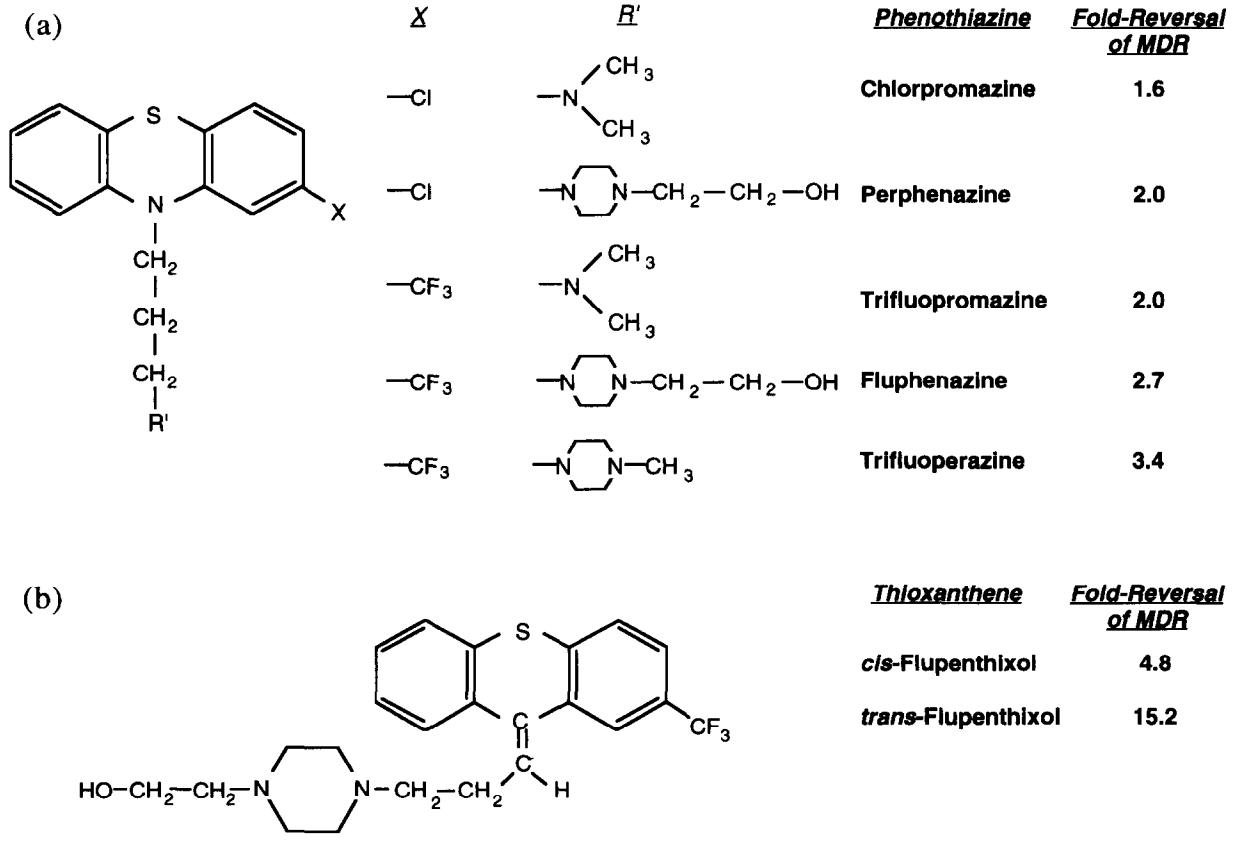


**Figure 2. Structures and anti-MDR activity of calmodulin antagonists with chemosensitising activity. The fold-reversal of MDR was determined by measuring the relative ability of similar concentrations (3–5 μM) of phenothiazines (a), or thioxanthenes (b), to sensitise MCF-7/DOX human MDR breast cancer cells to doxorubicin. Data from [67].**

be 10- to 30-fold more potent than CsA, effective *in vitro* at concentrations as low as 0.1 μM [80]. PSC 833 has emerged to be among the most potent chemosensitisers known [81, 82], and to possess clinical and pharmacological characteristics making it very attractive for clinical use [83]. A second non-immunosuppressive hydrophobic peptide being studied is SDZ 280-446, which appears similarly potent to PSC 833 for antagonism of MDR [84].

In summary, the cyclosporins sensitise MDR cells to a variety of chemotherapeutic drugs, either through a direct effect on Pgp, through alternative mechanisms of potentiation of chemotherapeutic drug toxicity, or a combination of these. This leads to the important possibility that CsA, in combination with other more Pgp-specific chemosensitising agents, may act synergistically to antagonise MDR. The newly developed non-immunosuppressive cyclosporin analogues, particularly PSC 833, appear to be excellent candidates for the clinical reversal of drug resistance, due to their increased potency and specificity for Pgp-mediated MDR.

*Steroids and hormonal analogues*

The expression of high levels of Pgp in human adrenal cortex and placenta [85] suggests a possible role for the pump in physiological transport of steroid hormones. In fact, cortisol and aldosterone are substrates for Pgp transport, although progesterone is not [6]. However, progesterone inhibits cytotoxic drug transport and binding to membranes from MDR cells, and functions as a potent chemosensitiser [6, 86, 87].

An orally active congener of progesterone, megesterol acetate, functions as a chemosensitiser in human MDR cells, and increases vincristine accumulation with 2- to 3-fold greater potency than progesterone [88]. Megestrol acetate may be a potentially useful clinical chemosensitiser, since high oral doses producing plasma levels of up to 2 μM have been safely administered in trials of its anticachexia properties [89].

Synthetic steroid analogues have demonstrated particular promise for clinical use. Tamoxifen and the related anti-oestrogen toremifene are active chemosensitisers in a number of human Pgp-expressing MDR cell lines, independent of their effect on oestrogen receptors [90–94]. For example, isobologram analyses demonstrate that tamoxifen synergistically potentiates vinblastine and mitoxantrone cytotoxicity and cellular accumulation in MDR human breast cancer cells [95, 96]. Tamoxifen appears to act as a chemosensitiser by directly interacting with and inhibiting Pgp, as it has been shown to bind in a specific and saturable manner to Pgp-enriched plasma membranes, and a photoactivated analogue irreversibly labels Pgp [97]. However, like progesterone, transport of tamoxifen by Pgp has not been detected.

The clinical pharmacology of anti-oestrogens makes them attractive for clinical use as chemosensitisers. Because of their relative lack of side-effects, high serum concentrations have been achieved [92, 98]. The major metabolite of toremifene, desmethyl-toremifene, possesses significant chemosensitising activity. Thus, the *in vitro* and clinical pharmacokinetics of these anti-oestrogens suggest they may be well-tolerated, effective chemosensitisers for use in combination with other chemotherapeutic agents in clinical drug resistance.

*Dipyridamole*

The antithrombotic drug dipyridamole has been shown to be a unique biochemical modulator of a variety of cytotoxic drugs, of both MDR and non-MDR classes. Dipyridamole is a potent inhibitor of the salvage pathway for repletion of cellular nucleotide pools by nucleoside transport across cell membranes, and thus potentiates the activity of antimetabolites such as methotrexate [99] and 5-fluorouracil [100]. Dipyridamole also appears to modulate the cytotoxicity of cisplatin in various cell lines, although the mechanism remains obscure [101]. Recently, dipyridamole has been found to enhance the effects of several MDR-related drugs against both sensitive and resistant cell lines, potentially through multiple mechanisms including inhibition of Pgp function. For example, non-toxic concentrations of dipyridamole interacted in a synergistic manner with VP-16, doxorubicin and vinblastine in drug-resistant and drug-sensitive human ovarian carcinoma cells [102, 103].

Recently, Cheng and coworkers described an analogue of dipyridamole, BIBW 22, which displays markedly increased potency for the reversal of MDR and inhibition of nucleoside transport [104]. BIBW 22 is 10-fold more potent than dipyridamole in reversing vinblastine resistance in human MDR cells and completely inhibits photoactive azidopine binding to Pgp at concentrations of 1 μM. Furthermore, BIBW 22 is a 7-fold more potent inhibitor of nucleoside transport than dipyridamole, and results in a 20-fold enhancement of 5-fluorouracil cytotoxicity at 1 μM concentrations. Thus, this compound may have unique clinical properties as a bifunctional modulator in trials of combination chemotherapy employing both antimetabolites and vinca alkaloids.

*Miscellaneous compounds*

The search for agents to circumvent MDR has led to the identification of numerous compounds not belonging to any of the previously discussed classes of chemosensitisers and not otherwise pharmacologically related. Most of these compounds are lipophilic in nature, and share a broad structural similarity that includes a heterocyclic ring nucleus separated at a distance from a cationic amino group. This diverse group of chemosensitising agents includes: anti-arrhythmics such as amiodarone [105] and quinidine [106]; the quinoline amines, chloroquine and quinacrine [107]; the indole alkaloids, reserpine and yohimbine [108, 109]; and the antihistamines, terfenadine [110], azelastine and flezelastine [111]. Most have been reported to overcome partially resistance to cytotoxic drugs, and to increase drug accumulation and retention in various MDR cell lines.

Several newly identified classes of chemosensitisers are of particular interest. The acridone carboxamide derivative, GF120918, possesses remarkable potency for reversing MDR, with concentrations as low as 0.02 μM displaying similar *in vitro* activity as 5 μM verapamil [112]. The triazinoaminopiperadine derivative, S 9788, has displayed potent *in vitro* activity [48, 113], favourable *in vivo* characteristics [114], and increased intracellular daunorubicin accumulation in Pgp-positive clinical samples from patients with haematological malignancies more effectively than verapamil or CsA, as assessed by flow cytometry [115].

A novel class of compounds active against MDR has been identified by screening natural products extracted from strains of cyanobacteria (blue-green algae). Two such compounds, tolytoxin and cryptophycin, displayed significant cytotoxicity alone to both sensitive and MDR cells [116, 117]. They did not effect vinblastine accumulation and were poor substrates for Pgp, and their activity for modulating vinca alkaloid cytotoxicity appeared to be through microfilament depolymeris-

ation, and thus independent of Pgp. However, another cyanobacterial isolate, termed tolyporphyin, functioned as a potent chemosensitiser [118]. This agent sensitised MDR cells to daunamycin, vinblastine, VP-16 and paclitaxel at doses of 0.1 μM, and inhibited labelled vinblastine and azidopine binding to Pgp.

*Combination chemosensitisers*

The major limiting factors for achieving what are thought to be adequate human serum concentrations of chemosensitisers to reverse MDR are their intrinsic side-effects. Therefore, a similar strategy to that originally proposed for combination chemotherapy may prove effective for the pharmacological use of chemosensitisers; that is, by combining several chemosensitising agents with non-overlapping toxicities to achieve an overall anti-MDR effect greater than that possible with individual agents at higher doses. Studies of the pharmacological effects of chemosensitisers have revealed that two agents, when used in combination, often result in additive [1], and occasionally supra-additive [119, 120] activity for sensitising MDR cells to cytotoxic drugs. Although determining the precise effects of combination chemosensitisers together with chemotherapeutic drugs on clinical pharmacokinetic parameters will prove daunting, the ability of this strategy to influence tumour growth and survival, deserves *in vivo* testing.

## STRUCTURE–ACTIVITY RELATIONSHIPS AMONG CHEMOSENSITISERS

Although the broad specificity of Pgp for substrates and inhibitors has enabled the successful identification of many agents with chemosensitising activity through *in vitro* screening, very few of these hold significant potential for clinical use due to undesirable side-effects. Therefore, rational approaches to drug development may provide more potent and effective agents. Unfortunately, the lack of detailed crystallographic structural information of membrane bound Pgp, or even detailed knowledge of the important protein binding sites of inhibitors, impedes a purely molecular approach to drug design for this target. However, most data suggest that, while different chemosensitisers may have multiple cellular targets, they probably share common targets for reversal of MDR. Therefore, studies have been designed to identify and define those structural features that enhance the interaction of chemosensitisers with Pgp.

For example, critical structure–activity relationships were identified for a series of phenothiazines with individual molecular alterations for their ability to sensitise a human MDR breast cancer cell line to doxorubicin, and included a hydrophobic tricyclic ring, a positively charged tertiary amine, and incorporation of the amino moiety into a cyclic structure [67] (Figure 2). The structural principles derived from this study allowed the identification of the thioxanthenes, a class of chemosensitisers structurally similar to the phenothiazines, but which contain an exocyclic double bond to the side chain, and thus exist as stereoisomers. For a group of 16 thioxanthenes, the *trans* isomer of each thioxanthene pair was a more effective chemosensitiser than the *cis*-isomer, providing additional information regarding the spatial relationships between the amino side chain and hydrophobic tricyclic ring structure important for resultant chemosensitising activity [37]. Several other large studies of phenothiazines, as well as compounds of different chemical classes, reached similar conclusions regarding the structural features important for anti-MDR activity [107, 121, 122].

Recently, several groups of investigators have discovered novel chemosensitising drugs by rationally synthesising compounds to contain the particular structural features and spatial relationships found to be important for phenothiazine and thioxanthene anti-MDR activity. For example, Dodic and associates synthesised a series of 85 tricyclic carboxamides and tested their ability to reverse Pgp-mediated MDR in rodent cells [123]. The structure of these agents consisted of a cationic side chain of various length, linked through an amide bond to a lipophilic, tricyclic nuclei. Similar to the structure–activity relationships originally observed for thioxanthene molecules, the carboxamide compound displaying the greatest chemosensitising activity within the series tested contained a cationic basic nitrogen located at a fixed distance and orientation from a lipophilic tricyclic nucleus. Using a similar approach, a series of propafenone-like propanolamines was synthesised and evaluated for MDR modulating activity [124]. Again consistent with the earlier thioxanthene results, compounds possessing the greatest anti-MDR activity possessed side chains containing tertiary amino groups and, in particular, a nitrogen incorporated into a cyclic non-aromatic ring structure.

## POST-TRANSLATIONAL MODIFICATION OF P-GLYCOPROTEIN FUNCTION

An alternative strategy of reversing MDR pharmacologically is to target mechanisms that appear to modify the function of Pgp. Post-translational modification of Pgp through phosphorylation has been appreciated for many years and a functional role has been suggested [125–131]. Exposure of cells to substrates for Pgp including chemotherapeutic drugs and chemosensitisers results in phosphorylation of the molecule above basal levels [127, 132], suggesting that interfering with this reaction could sensitise cells to chemotherapy.

Pgp can be phosphorylated by a variety of serine/threonine kinases, and contains serine residues that resemble the consensus phosphorylation sites of protein kinase C (PKC), cAMP-dependent protein kinase and calmodulin-dependent protein kinase II [130, 131, 133, 134]. PKC has been shown to phosphorylate Pgp in cell membranes [135] and in immunoprecipitates [136, 137], and is translocated to cell membranes in response to phorbol 12-myristate 13-acetate in a temporally consistent manner with changes in drug accumulation [138]. Treatment of sensitive cells with activators of PKC, such as phorbol esters, decreases the accumulation of chemotherapeutic drugs [139, 140] and mimics the MDR phenotype [128, 140], and treatment of MDR cells with PKC activators further increases drug resistance [127, 128]. The role of individual isozymes of PKC in MDR cell lines has been recently investigated. PKCα and -β have been shown to be overexpressed in P388/ADR cells [141], and a 10-fold increase in enzymatic activity in MCF-7/AdrR cells occurs due to a selective increase in the expression of PKCα [142]. These results suggest that a selective alteration of PKC activity in tumour MDR cells may be possible.

Therefore, it is plausible that inhibition of PKC activity may reduce Pgp activity [143, 144]. In fact, many commonly used chemosensitisers, such as the phenothiazines, thioxanthrenes, tamoxifen and CsA, are weak inhibitors of PKC [37, 145–149]. However, most studies of the effect of PKC inhibitors on MDR are complicated by the fact that many of these drugs interact directly with Pgp as well [37, 148, 150–153]. However, evidence is now emerging that specific inhibitors

of PKC may modulate Pgp function through alteration of phosphorylation. For example, staurosporine at concentrations well below those required to inhibit azidopine binding to Pgp, increase the phosphorylation of Pgp and result in the decreased accumulation of vinblastine in MCF-7 MDR cells [137]. In addition, it appears that increasing cellular levels of sphingosine leads to inhibition of Pgp due to antagonism of PKC. For example, the calcium channel blocker SR33557 has been shown to enhance cellular sphingosine levels 5-fold in MDR, but not drug-sensitive cells, and to inhibit PKC and Pgp function without significantly altering MDR drug substrate binding to Pgp [154]. Similarly, the lysosphingolipid PKC inhibitor safingol is a saturated analogue of sphingosine, and inhibits phosphorylation of PKC substrates including Pgp in both MCF-7-sensitive and MDR cells, but selectively enhances vinblastine accumulation and cytotoxicity in the MDR line [155]. In support of a role for PKC inhibition in this effect, safingol did not alter Pgp expression, Pgp ATPase activity, or MDR substrate binding to Pgp.

Despite the intense interest in PKC activity in MDR, relatively little is known about phospholipase C (PLC), an enzyme which is critical to the activity of this kinase. There is reason to believe that the activation of PLC in MDR cells may be responsible for the cascade of events leading to the activation of PKC and phosphorylation of Pgp. Heat shock, a cellular stress mechanism known to activate PLC [156, 157], markedly increases the phosphorylation of Pgp in MCF-7 MDR cells to a degree similar to that seen when PKC is directly activated by phorbol esters [158]. The PKC inhibitor staurosporine blocks the phosphorylation of Pgp induced by heat shock. These experiments provide evidence that the phosphorylation of Pgp can be regulated by cellular stress responses that activate PLC, perhaps including chemotherapeutic drugs, and suggest additional targets for potential pharmacological manipulation of Pgp function.

Pgp is also modified post-translationally by N-glycosylation [159], suggesting additional methods of affecting the biological function of Pgp. Initial studies using the specific N-glycosylation inhibitor tunicamycin in MDR CCRF-CEM human leukaemic lymphoblasts found that while Pgp glycosylation is indeed inhibited by the drug, drug resistance is unaltered [160]. However, a more recent investigation observed that tunicamycin treatment of MDR cells reduces membrane associated Pgp levels and enhances anthracycline cellular accumulation [161]. Thus, further studies of the effects of glycosylation on Pgp function and its interactions with cytotoxic drugs appear warranted.

## CONCLUSION

Over the 15 years since the initial discovery that experimental MDR could be reversed through pharmacological inhibition of the Pgp drug pump [31], a vast number of compounds have been identified which possess such chemosensitising activity [1, 2]. However, only a small proportion of these drugs possess clinical pharmacological characteristics appropriate and safe for their use as potential modulators of clinical MDR. Furthermore, although the most promising chemosensitisers to emerge from *in vitro* studies have not yet been adequately tested in clinical trials, it remains unclear if clinical tumour drug resistance may be modified by Pgp inhibitors.

However, even if Pgp-associated MDR proves to be a relevant and reversible cause of clinical drug resistance, many complex issues have to be addressed before effective clinical chemosensitisation can be achieved. Factors such as chemosensitiser absorption, distribution and metabolism, the effect of chemosensitisers on chemotherapeutic drug clearance, toxicity to normal tissues expressing Pgp, and the most efficacious modulator regimens and appropriate tumours to treat all need to be defined *in vivo*. Clearly, the identification of more specific, potent and less clinically toxic chemosensitisers for clinical use remains critical to the possible success of this approach.

Exploration of novel approaches for the modification of MDR may provide powerful and more selective approaches to use instead of, or in addition to, pharmacological Pgp inhibitors. For example, experimental approaches to reversing MDR have been reported using monoclonal antibodies against Pgp, ribozyme constructs targeting MDR1 mRNA, and factors regulating the expression of the *MDR1* gene [2], and are discussed in detail elsewhere in this issue. However, the finding that a number of pharmacological agents can antagonise a well-characterised form of experimental drug resistance provides promise for potential clinical applications. Further study of chemosensitisers in humans and the rational design of novel chemosensitisers with improved activity should define the importance of MDR to clinically resistant cancer.


1. Ford JM, Hait WN. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* 1990, **42**, 156–199.
2. Ford JM. Modulators of multidrug resistance: preclinical studies. *Hematol Oncol Clin North Am* 1995, **9**, 337–355.
3. Biedler JL, Riehm H. Cellular resistance to actinomycin D in Chinese hamster cells *in vitro*: cross-resistance, radioautographic and cytogenetic studies. *Cancer Res* 1970, **30**, 1174–1184.
4. Dano K. Active outward transport of daunomycin in resistant Ehrlich ascites tumour cells. *Biochim Biophys Acta* 1973, **323**, 466–483.
5. Yeh GC, Lopaczynska J, Poore CM, Phang JM. A new functional role for P-glycoprotein: efflux pump for benzo(*a*)pyrene in human breast cancer MCF-7 cells. *Cancer Res* 1992, **52**, 6692–6695.
6. Ueda K, Okamura N, Hirai M, *et al.* Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J Biol Chem* 1992, **267**.
7. Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 1976, **455**, 152–162.
8. Skovsgaard T. Mechanisms of resistance to daunorubicin in Ehrlich ascites tumour cells. *Cancer Res* 1978, **38**, 1785–1791.
9. Kartner N, Riordan JR, Ling V. Cell surface P-glycoprotein is associated with multidrug resistance in mammalian cell lines. *Science* 1983, **221**, 1285–1288.
10. Gerlach JH, Kartner N, Bell DR, Ling V. Multidrug resistance. *Cancer Surv* 1986, **5**, 25–46.
11. Gros P, Neriah BY, Croop JM, Housman DE. Isolation and expression of a complementary DNA that confers multidrug resistance. *Nature* 1986, **323**, 728–731.
12. Ueda K, Cardarelli C, Gottesman MM, Pastan I. Expression of a full-length cDNA for the human *mdr1* gene confers resistance to colchicine, doxorubicin, and vinblastine. *Proc Natl Acad Sci USA* 1987, **84**, 3004–3008.
13. Hammond JR, Johnstone RM, Gros P. Enhanced efflux of [$^3$H]vinblastine from Chinese hamster ovary cells transfected with a full-length complementary DNA clone for the *mdr1* gene. *Cancer Res* 1989, **49**, 3867–3871.
14. Bibi E, Gros P, Kaback HR. Functional expression of mouse mdr1 in *Escherichia coli*. *Proc Natl Acad Sci USA* 1993, **90**, 9209–9213.
15. Ruetz S, Raymond M, Gros P. Functional expression of p-glycoprotein encoded by the mouse *mdr3* gene in yeast cells. *Proc Natl Acad Sci USA* 1993, **90**, 11588–11592.
16. Raymond M, Ruetz S, Thomas DY, Gros P. Functional

expression of P-glycoprotein in *Saccharomyces cerevisiae* confers cellular resistance to the immunosuppressive and antifungal agent FK520. *Mol Cell Biol* 1994, **14**, 277–286.
17. Ruetz S, Gros P. Functional expression of P-glycoproteins in secretory vesicles. *J Biol Chem* 1994, **269**, 12277–12284.
18. Gros P, Croop J, Housman D. Mammalian multidrug resistance gene: complete cDNA sequence indicates strong homology to bacterial transport proteins. *Cell* 1986, **47**, 371–380.
19. Chen CJ, Chin E, Ueda K, *et al.* Internal duplication and homology with bacterial transport proteins in the *mdr*1 (P-glycoprotein) gene from multidrug resistant human cells. *Cell* 1986, **47**, 381–389.
20. Gros P, Shustik C. Multidrug resistance: a novel class of membrane-associated transport proteins is identified. *Cancer Invest* 1991, **9**, 563–569.
21. Higgins CF. The ABC of channel regulation. *Cell* 1995, **82**, 693–696.
22. Sharom FJ, Yu X, Doige CA. Functional reconstitution of drug transport and ATPase activity in proteoliposomes containing partially purified P-glycoprotein. *J Biol Chem* 1993, **268**, 24197–24202.
23. Shapiro AB, Ling V. ATPase activity of purified and reconstituted P-glycoprotein from Chinese hamster ovary cells. *J Biol Chem* 1994, **269**, 3745–3754.
24. Shapiro AB, Ling V. Reconstitution of drug transport by purified P-glycoprotein. *J Biol Chem* 1995, **27**, 16167–16175.
25. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* 1993, **62**, 385–427.
26. Higgins CF. To flip or not to flip? *Curr Biol* 1994, **4**, 259–260.
27. Cornwell MM, Pastan I, Gottesman MM. Certain calcium channel blockers bind specifically to multidrug resistant human KB carcinoma membrane vesicles and inhibit drug binding to P-glycoprotein. *J Biol Chem* 1987, **262**, 2166–2170.
28. Loo TW, Clarke DM. Functional consequences of glycine mutations in the predicted cytoplasmic loops of P-glycoprotein. *J Biol Chem* 1994, **269**, 7243–7248.
29. Loo TW, Clarke DM. Functional consequences of phenylalanine mutations in the predicted transmembrane domains of P-glycoprotein. *J Biol Chem* 1994, **268**, 19965–19972.
30. Morris DI, Greenberger LM, Bruggemann EP, *et al.* Localization of the forskolin labeling sites to both halves of P-glycoprotein: similarity of the sites labeled by forskolin and prazosin. *Mol Pharmacol* 1994, **46**, 329–337.
31. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y. Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res* 1981, **41**, 1967–1972.
32. Naito M, Tsuruo T. Competitive inhibition by verapamil of ATP-dependent high affinity vincristine binding to the plasma membrane of multidrug-resistant K562 cells without calcium ion involvement. *Cancer Res* 1989, **49**, 1452–1455.
33. Safa AR, Glover CJ, Sewell JL, Meyers MB, Bielder JL, Felsted RL. Identification of the multidrug resistance-related membrane glycoprotein as an acceptor for calcium channel blocks. *J Biol Chem* 1987, **262**, 7884–7888.
34. Safa AR. Photoaffinity labeling of the multidrug-resistance-related P-glycoprotein with photoactive analogs of verapamil. *Proc Natl Acad Sci USA* 1988, **85**, 7187–7191.
35. Safa AR. Inhibition of azidopine binding to the multidrug resistance related gp 150-180 (P-glycoprotein) by modulators of multidrug resistance. *Proc Am Assoc Cancer Res* 1988, **29**, 1160.
36. Yang CH, Mellado W, Horwitz SB. Azidopine photoaffinity labeling of multidrug resistance-associated glycoproteins. *Biochem Pharmacol* 1988, **37**, 1417–1421.
37. Ford JM, Bruggeman E, Pastan I, Gottesman MM, Hait WN. Cellular and biochemical characterization of thioxanthenes for reversal of multidrug resistance in human and murine cell lines. *Cancer Res* 1990, **50**, 1748–1756.
38. Kajiji S, Dreslin JA, Grizzuti K, Gros P. Structurally distinct MDR modulators show specific patterns of reversal against P-glycoproteins bearing unique mutations at serine$^{939/941}$. *Biochemistry* 1994, **33**, 5041–5048.
39. Cardarelli CO, Aksentijevich I, Pastan I, Gottesman MM. Differential effects of P-glycoprotein inhibitors on NIH3T3 cells transfected with wild-type (G185) or mutant (V185) multidrug transporters. *Cancer Res* 1995, **55**, 1086–1091.
40. Bruggemann EP, Currier SJ, Gottesman MM, Pastan I. Characterization of the azidopine and vinblastine binding site of P-glycoprotein. *J Biol Chem* 1992, **267**, 21020–21026.
41. Yusa K, Tsuruo T. Reversal mechanism of multidrug resistance by verapamil: direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM cells. *Cancer Res* 1989, **49**, 5002–5006.
42. Tamai I, Safa A. Azidopine noncompetitively interacts with vinblastine and cyclosporin A binding to P-glycoprotein in multidrug resistant cells. *J Biol Chem* 1991, **266**, 16796–16800.
43. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y. Increased accumulation of vincristine and adriamycin in drug-resistant P388 tumor cells following incubation with calcium antagonists and calmodulin inhibitors. *Cancer Res* 1982, **42**, 4730–4733.
44. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y. Potentiation of vincristine and adriamycin in human hematopoietic tumor cell lines by calcium antagonists and calmodulin inhibitors. *Cancer Res* 1983, **43**, 2267–2272.
45. Akiyama S, Cornwell MM, Kuwano M, Pastan I, Gottesman MM. Most drugs that reverse multidrug resistance inhibit photoaffinity labelling of P-glycoprotein by a vinblastine analog. *Mol Pharmacol* 1988, **33**, 144–147.
46. Ozols RF, Cunnion RE, Klecker RW, *et al.* Verapamil and adriamycin in the treatment of drug-resistant ovarian cancer patients. *J Clin Oncol* 1987, **5**, 641–647.
47. Kessel D, Wilberding C. Promotion of daunorubicin uptake and toxicity by the calcium antagonist tiapamil and its analogs. *Cancer Treat Rep* 1985, **69**, 673–676.
48. Plumb JA, Wishart GC, Setanoians A, *et al.* Identification of a multidrug resistance modulator with clinical potential by analysis of synergistic activity *in vitro*, toxicity *in vivo* and growth delay in a solid human tumor xenograft. *Biochem Pharmacol* 1994, **47**, 257–266.
49. Pereira E, Teodori E, Dei S, Gualtieri F, Guarnier-Suillerot A. Reversal of multidrug resistance by verapamil analogues. *Biochem Pharmacol* 1995, **50**, 451–457.
50. Toffoli G, Simone F, Corona G, *et al.* Structure–activity relationship of verapamil analogs and reversal of multidrug resistance. *Biochem Pharmacol* 1995, **50**, 1245–1255.
51. Eliason JF, Ramuz H, Yoshikubo T, Ishikawa T, Yamamoto T, Tsuruo T. Novel dithiane analogues of tiapamil with high activity to overcome multidrug resistance *in vitro*. *Biochem Pharmacol* 1995, **50**, 187–196.
52. Tsuruo T, Iida H, Nojiri M, Tsukagoshi S, Sakurai Y. Circumvention of vincristine and adriamycin resistance *in vitro* and *in vivo* by calcium influx blockers. *Cancer Res* 1983, **43**, 2905–2910.
53. Ramu A, Spanier R, Rahamimoff H, Fuks Z. Restoration of doxorubicin responsiveness in doxorubicin-resistant P388 murine leukaemia cells. *Br J Cancer* 1984, **50**, 501–507.
54. Tsuruo T, Kawabata H, Nagumo N, *et al.* Potentiation of antitumor agents by calcium channel blockers with special reference to cross-resistance patterns. *Cancer Chemother Pharmacol* 1985, **15**, 16–19.
55. Niwa K, Yamada K, Furukawa T, *et al.* Effect of a dihydropyridine analogue, 2-[benzyl(phenyl)amino]ethyl-1,4-dihydro-2,6-dimethyl-5-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-1-(2-morpholino-ethyl)-4-(3-nitrophenyl)-3-pyridinecarboxylate on reversing *in vivo* resistance of tumor cells to adriamycin. *Cancer Res* 1992, **52**, 3655–3660.
56. Weir MR, Peppler R, Gomolka D, Handwerger BS. Evidence that the antiproliferative effect of verapamil on afferent and efferent immune responses is independent of calcium channel inhibition. *Transplantation* 1992, **54**, 681–685.
57. Gruber A, Peterson C, Reizenstein P. D-verapamil and L-verapamil are equally effective in increasing vincristine accumulation in leukemic cells *in vitro*. *Int J Cancer* 1988, **41**, 224–226.
58. Plumb JA, Milroy R, Kaye SB. The activity of verapamil as a resistance modifier *in vitro* in drug resistant human tumor cell lines is not stereospecific. *Biochem Pharmacol* 1990, **39**, 787–792.
59. Hollt V, Kouba M, Dietel M, Vogt G. Stereoisomers of calcium antagonists which differ markedly in their potencies as calcium blockers are equally effective in modulating drug transport by P-glycoprotein. *Biochem Pharmacol* 1992, **43**, 2601–2608.
60. Boer R, Haas S, Schodl A. Influence of dexniguldipine-HCl on rhodamine-123 accumulation in a multidrug-resistant leu-

kaemia cell line: comparison with other chemosensitisers. *Eur J Cancer* 1994, **30A**, 1117–1123.
61. Berg SL, Tolcher A, O'Shaughnessy JA, *et al.* Effect of R-verapamil on the pharmacokinetics of paclitaxel in women with breast cancer. *J Clin Oncol* 1995, **13**, 2039–2042.
62. Motzer RJ, Lyn P, Fischer P, *et al.* Phase I/II trial of dexverapamil plus vinblastine for patients with advanced renal cell carcinoma. *J Clin Oncol* 1995, **13**, 1958–1965.
63. Wilson WH, Jamis-Dow C, Bryant G, *et al.* Phase I and pharmacokinetic study of the multidrug resistance modulator dexverapamil with EPOCH chemotherapy. *J Clin Oncol* 1995, **13**, 1985–1994.
64. Wilson WH, Bates SE, Fojo A, *et al.* Controlled trial of dexverapamil, a modulator of multidrug resistance, in lymphomas refractory to EPOCH chemotherapy. *J Clin Oncol* 1995, **13**, 1995–2004.
65. Ganapathi R, Grabowski D, Rouse W, Riegler F. Differential effect of the calmodulin inhibitor trifluoperazine on cellular accumulation, retention and cytotoxicty of anthracyclines in doxorubicin (adriamycin)-resistant P388 mouse leukemia cells. *Cancer Res* 1984, **44**, 5056–5061.
66. Ganapathi R, Grabowski D, Schmidt H. Factors governing the modulation of vinca-alkaloid resistance in doxorubicin-resistant cells by the calmodulin inhibitor trifluoperazine. *Biochem Pharmacol* 1986, **35**, 673–678.
67. Ford JM, Prozialeck WC, Hait WN. Structural features determining activity of phenothiazines and related drugs for inhibition of cell growth and reversal of multidrug resistance. *Mol Pharmacol* 1989, **35**, 105–115.
68. Johnstone EC, Crow TJ, Frith CD, Carney MWD, Price JS. Mechanism of the antipsychotic effect in the treatment of acute schizophrenia. *Lancet* 1978, **1**, 848–851.
69. Post ML, Kennard U, Horn AS. Stereoselective blockade of the dopamine receptor and the X-ray structures of alpha and beta-flupenthixol. *Nature* 1975, **256**, 342–343.
70. Huff RM, Molinoff B. Assay of dopamine receptors with [α-3H] flupenthixol. *J Pharmacol Exp Ther* 1984, **232**, 57–61.
71. Nielsen IM, Pedersen V, Nymark M, *et al.* Comparative pharmacology of flupenthixol and some reference neuroleptics. *Acta Pharmacol Toxicol* 1973, **33**, 353–362.
72. Twentyman PR, Fox NE, White DJG. Cyclosporin A and its analogues as modifiers of adriamycin and vincristine resistance in a multi-drug resistant human lung cancer cell line. *Br J Cancer* 1987, **56**, 55–57.
73. Hait WN, Stein JM, Koletsky AJ, Harding MW, Handschumacher RE. Activity of cyclosporin A and a non-immunosuppressive cyclosporin on multidrug resistant leukemic cell lines. *Cancer Commun* 1989, **1**, 35–43.
74. Silbermann MH, Boersma AWM, Jansen ALW, Scheper RJ, Herweijer H, Nooter K. Effects of cyclosporin A and verapamil on the intracellular daunorubicin accumulation in Chinese hamster ovary cells with increasing levels of drug-resistance. *Int J Cancer* 1989, **44**, 722–726.
75. Slater LM, Sweet P, Stupecky M, Wetzel MW, Gupta S. Cyclosporin A corrects daunorubicin resistance in Ehrlich ascites carcinoma. *Br J Cancer* 1986, **54**, 235–238.
76. Osieka R, Seeber S, Pannenbacker R, Soll D, Glatte P, Schmidt CG. Enhancement of etoposide-induced cytotoxicity by cyclosporin A. *Cancer Chemother Pharmacol* 1986, **18**, 198–202.
77. Chambers SK, Hait WN, Kacinski BM, Keyes SR, Handschumacher RE. Enhancement of anthracycline growth inhibition in parent and multidrug-resistant Chinese hamster ovary cells by cyclosporin A and its analogues. *Cancer Res* 1989, **46**, 6275–6279.
78. Gaveriaux C, Boesch D, Boilsterli JJ, *et al.* Overcoming multidrug resistance in Chinese hamster ovary cells *in vitro* by cyclosporin A (Sandimmune) and non-immunosuppressive derivatives. *Br J Cancer* 1989, **60**, 867–871.
79. Slater L, Sweet P, Wetzel M, Stupecky M, Osann K. Comparison of cyclosporin A, verapamil, PSC-833 and cremophor EL as enhancing agents of VP-16 in murine lymphoid leukemias. *Leuk Res* 1995, **19**, 543–548.
80. Boesch D, Muller K, Pourtier-Manzanedo A, Loor F. Restoration of daunomycin retention in multidrug-resistant P388 cells by submicromolar concentrations of SDZ PSC 833, a nonimmunosuppressive cyclosporin derivative. *Exp Cell Res* 1991, **196**, 26–32.
81. Jachez B, Nordmann R, Loor F. Restoration of taxol sensitivity of multidrug-resistant cells by the cyclosporine SDZ PSC 833 and the cyclopeptolide SDZ 280-446. *J Natl Cancer Inst* 1993, **85**, 478–483.
82. Jiang XR, Kelsey SM, Wu YL, Newland AC. Circumvention of P-glycoprotein-mediated drug resistance in human leukaemic cells by non-immunosuppressive cyclosporin D analogue, SDZ PSC 833. *Br J Haematol* 1995, **90**, 375–383.
83. Fisher GA, Sikic BI. Clinical studies with modulators of multidrug resistance. *Hematol Oncol Clin North Am* 1995, **9**, 363–382.
84. Loor F, Boesch D, Gaveriaux C, Jachez B, Dourtier-Manzanedo A, Emmer G. SDZ 280-446, a novel semisynthetic cyclopeptide: *in vitro* and *in vivo* circumvention of P-glycoprotein-mediated tumor cell multidrug resistance. *Br J Cancer* 1992, **65**, 11–18.
85. Arceci RJ, Croop JM, Horwitz SB, Housman D. The gene encoding multidrug resistance is induced and expressed at high levels during pregnancy in the secretory epithelium of the uterus. *Proc Natl Acad Sci USA* 1988, **85**, 4350–4354.
86. Yang C-PH, DePinho SH, Greenberger LM, Arceci RJ, Horwitz SB. Progesterone interacts with P-glycoprotein in multidrug-resistant cells and in the endometrium of gravid uterus. *J Biol Chem* 1989, **264**, 782–788.
87. Yang C-PH, Cohen D, Greenberger LM, Hsu SH, Horwitz SB. Differential transport properties of two mdr gene products are distinguished by progesterone. *J Biol Chem* 1990, **265**, 10282–10288.
88. Fleming GF, Amato JM, Agresti M, Safa AR. Megestrol acetate reverses multidrug resistance and interacts with P-glycoprotein. *Cancer Chemother Pharmacol* 1992, **29**, 445–449.
89. Aisner J, Tchekmedyian NS, Tait N, Parnes H, Novak M. Studies of high-dose megestrol acetate: potential applications in cachexia. *Semin Oncol* 1988, **15S**, 68–75.
90. Ramu A, Glaubiger D, Fuks Z. Reversal of acquired resistance to doxorubicin in P388 murine leukemia cells by tamoxifen and other triparanol analogues. *Cancer Res* 1984, **44**, 4392–4395.
91. Foster BJ, Grotzinger KR, McKoy WM, Rubinstein LV, Hamilton TC. Modulation of induced resistance to adriamycin in two human breast cancer cell lines with tamoxifen or perhexiline maleate. *Cancer Chemother Pharmacol* 1988, **22**, 147–152.
92. DeGregorio MW, Ford JM, Benz CC, Wiebe VJ. Toremifene: pharmacologic and pharmacokinetic basis of reversing multidrug resistance. *J Clin Oncol* 1989, **7**, 1359–1364.
93. Chatterjee M, Harris A. Enhancement of adriamycin cytotoxicity in a multidrug resistant Chinese hamster ovary (CHO) subline, CHO-Adr$^r$, by toremifene and its modulation by alpha 1 acid glycoprotein. *Eur J Cancer* 1990, **26**, 432–436.
94. Kirk J, Houlbrook S, Stuart NSA, Stratford IJ, Harris AL, Carmichael J. Differential modulation of doxorubicin toxicity to multidrug and intrinsically drug resistant cell lines by antioestrogens and their major metabolites. *Br J Cancer* 1993, **67**, 1189–1195.
95. Leonessa F, Jacobson M, Boyle B, Lippman J, McGarvey M, Clarke R. Effect of tamoxifen on the multidrug-resistant phenotype in human breast cancer cells: isobologram, drug accumulation and $M_r$ 170,000 glycoprotein binding studies. *Cancer Res* 1994, **54**, 441–447.
96. Desai PB, Bhardwaj R, Damle B. Effect of tamoxifen on mitoxantrone cytotoxicity in drug-sensitive and multidrug-resistant MCF-7 cells. *Cancer Chemother Pharmacol* 1995, **36**, 368–372.
97. Callaghan R, Higgins CF. Interaction of tamoxifen with the multidrug resistance P-glycoprotein. *Br J Cancer* 1995, **71**, 294–299.
98. Jordan VC, Bain RR, Brown RR, Gosden B, Santos MA. Determination and pharmacology of a new hydroxylated metabolite of tamoxifen observed in patient sera during therapy for advanced breast cancer. *Cancer Res* 1983, **43**, 1446–1450.
99. Nelson JA, Drake S. Potentiation of methotrexate toxicity by dipyridamole. *Cancer Res* 1984, **44**, 2493–2496.
100. Grem JL, Fischer PH. Augmentation of 5-flourouracil cytotoxicity in human colon cancer cells by dipyridamole. *Cancer Res* 1985, **45**, 2967–2972.
101. Jekunen A, Vick J, Sanga R, Chan TCK, Howell SB. Synergism between dipyridamole and cisplatin in human ovarian carcinoma cells *in vitro*. *Cancer Res* 1992, **52**, 3566–3571.
102. Howell SB, Hom D, Sanga R, Vick JS, Abramson IS. Comparison of the synergistic potentiation of etoposide, doxorubicin and

vinblastine cytotoxicity by dipyridamole. *Cancer Res* 1989, **49**, 3178–3183.
103. Howell SB, Hom DK, Sanga R, Vick JS, Chan TCK. Dipyridamole enhancement of etoposide sensitivity. *Cancer Res* 1989, **49**, 4147–4153.
104. Chen HX, Bamberger U, Heckel A, Guo X, Cheng YC. BIBW 22, a dipyridamole analogue, acts as a bifunctional modulator on tumor cells by influencing both P-glycoprotein and nucleoside transport. *Cancer Res* 1993, **53**, 1974–1977.
105. Chauffert B, Martin M, Hammann A, Michel MF, Martin F. Amiodarone-induced enhancement of doxorubicin and 4'-deoxydoxorubicin cytotoxicity to rat colon cancer cells *in vitro* and *in vivo*. *Cancer Res* 1986, **46**, 825–830.
106. Tsuruo T, Iida H, Kitatani Y, Yokota K, Tsukagoshi S, Yakurai Y. Effects of quinidine and related compounds on cytotoxicity and cellular accumulation of vincristine and adriamycin in drug-resistant tumor cells. *Cancer Res* 1984, **44**, 4303–4307.
107. Zamora JM, Pearce HL, Beck WT. Physical-chemical properties shared by compounds that modulate multidrug resistance in human leukemic cells. *Mol Pharmacol* 1988, **33**, 454–462.
108. Beck WT, Cirtain MC, Glover CJ, Felsted RL, Safa AR. Effects of indole alkaloids on multidrug resistance and labeling of P-glycoprotein by a photoaffinity analog of vinblastine. *Biochem Biophys Res Commun* 1988, **153**, 959–966.
109. Pearce HL, Safa AR, Bach NJ, Winter MA, Cirtain MC, Beck WT. Essential features of the P-glycoprotein pharmacophore as defined by a series of reserpine analogs that modulate multidrug resistance. *Proc Natl Acad Sci USA* 1989, **86**, 5128–5132.
110. Hait WN, Gesmonde JF, Murren JR, Yang JM, Chen HX, Reiss M. Terfenadine (Seldane): a new drug for restoring sensitivity to multidrug resistant cancer cells. *Biochem Pharmacol* 1993, **45**, 401–406.
111. Hu YP, Robert J. Azelastine and flezelastine as reversing agents of multidrug resistance: pharmacological and molecular studies. *Biochem Pharmacol* 1995, **50**, 169–175.
112. Hyafil F, Vergely C, Du Vignaud P, Grand-Perret T. *In vitro* and *in vivo* reversal of multidrug resistance by GF120918, an acridonecarboxamide derivative. *Cancer Res* 1993, **53**, 4595–4602.
113. Pierre A, Dunn TA, Kraus-Berthier L, *et al. In vitro* and *in vivo* circumvention of multidrug resistance by Servier 9788, a novel triazinoaminopiperidine derivative. *Invest New Drugs* 1992, **10**, 137–148.
114. Soudon J, Berlion M, Lucas C, Haddad P, Bizzari JP, Calvo F. *In vitro* activity of S 9788 on a multidrug-resistant leukemic cell line and on normal hematopoietic cells—reversal of multidrug resistance by sera from phase I-treated patients. *Cancer Chemother Pharmacol* 1995, **36**, 195–203.
115. Merlin JL, Guerci A, Marchal S, *et al.* Comparative evaluation of S9788, verapamil and cyclosporine A in K562 human leukemia cell lines and in P-glycoprotein-expressing samples from patients with hematologic malignancies. *Blood* 1994, **84**, 262–269.
116. Smith CD, Carmeli S, Moore RE, Patterson GML. Scytophycins, novel microfilament-depolymerizing agents which circumvent P-glycoprotein-mediated multidrug resistance. *Cancer Res* 1993, **53**, 1343–1347.
117. Smith CD, Zhang X, Mooberry SL, Patterson GML, Moore RE. Cryptophycin: a new antimicrotubule agent active against drug-resistant cells. *Cancer Res* 1994, **54**, 3779–3784.
118. Smith CD, Prinsep MR, Caplan FR, Moore RE, Patterson GM. Reversal of multiple drug resistance by tolyporphin, a novel cyanobacterial natural product. *Oncol Res* 1994, **6**, 211–218.
119. Hu XF, Martin TJ, Bell DR, Luise M, Zalcberg JR. Combined use of cyclosporin A and verapamil in modulating multidrug resistance in human leukemia cell lines. *Cancer Res* 1990, **50**, 2953–2957.
120. Osann K, Sweet P, Slater LM. Synergistic interaction of cyclosporin A and verapamil on vincristine and daunorubicin resistance in multidrug-resistant human leukemia cells *in vitro*. *Cancer Chemother Pharmacol* 1992, **30**, 152–154.
121. Ramu A, Ramu N. Reversal of multidrug resistance by phenothiazines and structurally related compounds. *Cancer Chemother Pharmacol* 1992, **30**, 165–173.
122. Ramu A, Ramu N. Reversal of multidrug resistance by bis(phenylalkyl)amines and structurally related compounds. *Cancer Chemother Pharmacol* 1994, **34**, 423–430.
123. Dodic N, Dumaitre B, Daugan A, Pianetti P. Synthesis and activity against multidrug resistance in Chinese hamster cells of new acridone-4-carboxamides. *J Med Chem* 1995, **38**, 2418–2426.
124. Chiba P, Burghofer S, Richter E, Tell B, Moser A, Ecker G. Synthesis, pharmacologic activity, and structure–activity relationships of a series of propafenone-related modulators of multidrug resistance. *J Med Chem* 1995, **38**, 2789–2793.
125. Carlsen SA, Till JE, Ling V. Modulation of drug permeability in Chinese hamster ovary cells—possible role for phosphorylation of surface glycoproteins. *Biochim Biophys Acta* 1977, **467**, 238–250.
126. Center MS. Evidence that adriamycin resistance in Chinese hamster lung cells is regulated by phosphorylation of a plasma membrane glycoprotein. *Biochem Biophys Res Commun* 1983, **115**, 159–166.
127. Hamada H, Hagiwara K-I, Nakajima T, Tsuruo T. Phosphorylation of the $M_r$ 170,000 to 180,000 glycoprotein specific to multidrug-resistant tumor cells: effects of verapamil, trifluoperazine, and phorbol esters. *Cancer Res* 1987, **47**, 2860–2865.
128. Fine RL, Patel Ja, Chabner BA. Phorbol esters induce multidrug resistance in human breast cancer cells. *Proc Natl Acad Sci USA* 1988, **85**, 582–586.
129. Ma L, Marquardt D, Takemoto L, Center MS. Analysis of P-glycoprotein phosphorylation in HL-60 cells isolated for resistance to vincristine. *J Biol Chem* 1991, **266**, 5593–5599.
130. Meyers MB. Protein phosphorylation in multidrug resistant Chinese hamster cells. *Cancer Commun* 1989, **1**, 233–241.
131. Mellado W, Horwitz SB. Phosphorylation of the multidrug resistance associated glycoprotein. *Biochemistry* 1987, **26**, 6900–6904.
132. Center MC. Mechanisms regulating cell resistance to adriamcyin—evidence that drug accumulation in resistant cells is modulated by phosphorylation of a plasma membrane glycoprotein. *Biochem Pharmacol* 1985, **34**, 1471–1476.
133. Kemp BE, Pearson RB. Protein kinase recognition sequence motifs. *Trends Biochem Sci* 1990, **15**, 342–346.
134. Kennelly PJ, Krebs EG. Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases. *J Biol Chem* 1991, **266**, 15555–15558.
135. Chambers TC, McAvoy EM, Jacobs JW, Eilon G. Protein kinase C phosphorylates P-glycoprotein in multidrug resistant human KB carcinoma cells. *J Biol Chem* 1990, **265**, 7679–7686.
136. Aftab DT, Hait WN. Effects of phorbol 12-myristate 13-acetate on drug accumulation and P-glycoprotein phosphorylation in sensitive and multidrug resistant MCF-7 cells. *Proc Am Assoc Cancer Res* 1992, **33**, 2821.
137. Aftab DT, Yang JM, Hait WN. Functional role of phosphorylation of the multidrug transporter (P-glycoprotein) by protein kinase C in multidrug-resistant MCF-7 cells. *Oncol Res* 1994, **6**, 59–70.
138. Chambers TC, Chalikonda I, Eilon G. Correlation of protein kinase C translocation, P-glycoprotein phosphorylation and reduced drug accumulation in multidrug resistant human KB cells. *Biochem Biophys Res Commun* 1990, **169**, 253–259.
139. Ido M, Asao T, Sakurai M, Inagaki M, Saito M, Hidaka H. An inhibitor of protein kinase C, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7) inhibits TPA-induced reduction of vincristine uptake from P388 murine leukemic cell. *Leuk Res* 1986, **10**, 1063–1069.
140. Ferguson PF, Chen Y. Transient protection of cultured human cells against antitumor agents by 12-*O*-tetradecanoylphorbol-13-acetate. *Cancer Res* 1987, **47**, 433–441.
141. Gollapudi S, Patel K, Jain V, Gupta S. Protein kinase C isoforms in multidrug resistant P388/ADR cells: a possible role in daunorubicin transport. *Cancer Lett* 1992, **62**, 69–75.
142. Blobe GC, Sachs CW, Khan WA, *et al.* Selective regulation of expression of protein kinase C isoenzymes in multidrug-resistant MCF-7 cells: functional significance of enhanced expression of PKCα. *J Biol Chem* 1993, **268**, 658–664.
143. Dong Z, Ward NE, Fan D, Gupta KP, O'Brian CA. *In vitro* model for intrinsic drug resistance: effects of protein kinase C activators on the chemosensitivity of cultured human colon cancer cells. *Mol Pharmacol* 1991, **39**, 563–569.
144. O'Brian CA, Fan C, Ward NE, *et al.* Transient enhancement of multidrug resistance by the bile acid deoxycholate in murine

fibrosarcoma cells *in vitro*. *Biochem Pharmacol* 1991, **41**, 797–806.
145. Mori T, Takai Y, Minakuchi R, Yu B, Nishizuka Y. Inhibitory action of chlorpromazine, dibucaine and other phospholipid-interacting drugs on calcium-activated, phospholipid-dependent protein kinase. *J Biol Chem* 1980, **255**, 8378–8380.
146. Schatzman RC, Wise BC, Kuo JF. Phospholipid-sensitive calcium-dependent protein kinase: inhibition by anti-psychotic drugs. *Biochem Biophys Res Commun* 1981, **98**, 669–676.
147. O'Brian CA, Liskamp RM, Solomon DH, Weinstein IB. Inhibition of protein kinase C by tamoxifen. *Cancer Res* 1985, **45**, 2462–2465.
148. Walker RJ, Lazzaro VA, Duggin GG, Horvath JS, Tiller DJ. Cyclosporin A inhibits protein kinase C activity: a contributing mechanism in the development of nephrotoxicity? *Biochem Biophys Res Commun* 1989, **160**, 409–415.
149. Aftab DT, Ballas LM, Loomis CR, Hait WN. Structure–activity relationships of phenothiazines and related drugs for inhibition of protein kinase C. *Mol Pharmacol* 1991, **40**, 798–805.
150. Foxwell BMJ, Mackie A, Ling V, Ryffel B. Identification of the multidrug resistance-related P-glycoprotein as a cyclosporin binding protein. *Mol Pharmacol* 1989, **36**, 543–546.
151. Posada JA, McKeegan EM, Worthington KF, Morin MJ, Jaken S, Tritton TR. Human multidrug resistant KB cells overexpress protein kinase C: involvement in drug resistance. *Cancer Commun* 1989, **1**, 285–292.
152. Galski H, Sullivan M, Willingham MC, *et al.* Expression of a human multidrug resistance cDNA (MDR-1) in the bone marrow of transgenic mice: resistance to daunomycin induced leukopenia. *Mol Cell Biol* 1989, **9**, 4357–4363.
153. Smith CD, Zilfou JT. Circumvention of P-glycoprotein-mediated multiple drug resistance by phosphorylation modulators is independent of protein kinases. *J Biol Chem* 1995, **270**, 28145–28152.
154. Jaffrezou JP, Herbert JM, Levade T, Gau MN, Chatelain P, Laurent G. Reversal of multidrug resistance by calcium channel blocker SR33557 without photoaffinity labeling of P-glycoprotein. *J Biol Chem* 1991, **266**, 19858–19864.
155. Sachs CW, Safa AR, Harrison SD, Fine RL. Partial inhibition of multidrug resistance by safingol is independent of modulation of P-glycoprotein substrate activities and correlated with inhibition of protein kinase C. *J Biol Chem* 1995, **270**, 26639–26648.
156. Stevenson MA, Calderwood SK, Hahn GM. Rapid increases in inositol trisphosphate and intracellular $Ca^{++}$ after heat shock. *Biochem Biophys Res Commun* 1986, **137**, 826–833.
157. Calderwood SK, Stevenson MA. Inducers of the heat shock response stimulate phospholipase C and phospholipase A2 activity in mammalian cells. *J Cell Physiol* 1993, **155**, 248–256.
158. Yang JM, Chin KV, Hait WN. Involvement of phospholipase C in heat-shock-induced phosphorylation of P-glycoprotein in multidrug resistant human breast cancer cells. *Biochem Biophys Res Commun* 1995, **210**, 21–30.
159. Ichikawa M, Yoshimura A, Furukawa T, Sumizawa T, Nakazima Y, Akiyama S. Glycosylation of P-glycoprotein in a multidrug-resistant KB cell line, and in the human tissues. *Biochem Biophys Acta* 1991, **1073**, 309–315.
160. Beck WT, Cirtain MC. Continued expression of Vinca alkaloid resistance by CCRF-CEM cells after treatment with tunicamycin or pronase. *Cancer Res* 1982, **42**, 184–189.
161. Kramer R, Weber TK, Arceci R, *et al.* Inhibition of N-linked glycosylation of P-glycoprotein by tunicamycin results in a reduced multidrug resistance phenotype. *Br J Cancer* 1995, **71**, 670–675.