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Review

The medicinal chemistry of multidrug resistance (MDR) reversing drugs

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Abstract

Multidrug resistance (MDR) is a kind of resistance of cancer cells to multiple classes of chemotherapic drugs that can be structurally and mechanistically unrelated. Classical MDR regards altered membrane transport that results in lower cell concentrations of cytotoxic drug and is related to the over expression of a variety of proteins that act as ATP-dependent extrusion pumps. P-glycoprotein (Pgp) and multidrug resistance protein (MRP1) are the most important and widely studied members of the family that belongs to the ABC superfamily of transporters. It is apparent that, besides their role in cancer cell resistance, these proteins have multiple physiological functions as well, since they are expressed also in many important non-tumoural tissues and are largely present in prokaryotic organisms. A number of drugs have been identified which are able to reverse the effects of Pgp, MRP1 and sister proteins, on multidrug resistance. The first MDR modulators discovered and studied in clinical trials were endowed with definite pharmacological actions so that the doses required to overcome MDR were associated with unacceptably high side effects. As a consequence, much attention has been focused on developing more potent and selective modulators with proper potency, selectivity and pharmacokinetics that can be used at lower doses. Several novel MDR reversing agents (also known as chemosensitisers) are currently undergoing clinical evaluation for the treatment of resistant tumours. This review is concerned with the medicinal chemistry of MDR reversers, with particular attention to the drugs that are presently in development. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Medicinal chemistry; Multidrug resistance; Reversing drugs

1. Introduction

Drug resistance is a phenomenon that frequently impairs proper treatment of infections and cancer with chemotherapics. Intrinsic drug resistance relates to the failure of many micro-organisms and tumours to respond to initial chemotherapy, while acquired drug resistance occurs when a micro-organism or a tumour initially responds to chemotherapy but later relapses and appears to be strongly resistant to the original treatment [1,2].

Multidrug resistance (MDR) is a case of acquired drug resistance, observed in tumour cells (in vivo and in vitro) and then found operative also in micro-organisms, that consists in the simultaneous emergence of cellular resistance to the toxic action of the chemotherapic drug originally used and to other chemicals, having different structure and different mechanism of action [3,4].

MDR can be the result of a variety of mechanisms that are not fully understood [5]. The most important among them are: (a) altered membrane transport either by decreased drug uptake or by increased drug efflux [6]; (b) perturbed expression of target enzymes or altered target enzymes [7]; (c) altered drug activation or degradation [8]; (d) enhanced DNA repair [9]; and (e) failure to undergo apoptosis [10,11]. In this last case, metabolism of sphingolipids like ceramide seems to have a central role [12]. Some of these mechanisms of drug resistance may coexist, rendering the target tumours refractory to treatment with drugs acting on a single target. However, the most widely implicated mechanism is that concerned with altered membrane transport in tumour cells. This mechanism is often referred to as typical or classical MDR.

Cells exhibiting MDR accumulate a lower intracellular concentration of drug [13]. This effect is associated

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with accelerated efflux of antitumour agents by an ATP-dependent process [14]. A membrane glycoprotein termed P-glycoprotein (also known as Pg-170, Pgp, P-170) was isolated [15] and proposed to be the transporter protein that pumps out the antitumour agents [16,17]. Later on, other proteins of the same superfamily such as multidrug resistance protein (MRP1) [18], lung cancer resistance-related protein (LRP) [19] and breast cancer-resistance protein (BCRP) [20] were identified and shown to act in the same way.

At the moment, the best-known and -studied are Pgp and MRP1 [21]. Several important reviews have been published on the structure, functions and molecular biology of Pgp and MRP1 [22–33]. The reader is referred to these works for detailed information. The present review is dedicated to the medicinal chemistry of MDR reversers, with particular attention to the families of inhibitors that are currently being studied and developed. Also on this subject, several excellent reviews are available [34–44].

2. PGP and MRP1 transporter proteins: the structure

Among the several transporter proteins that were found to be involved in MDR, Pgp and MRP1 are the most important and best known subfamilies. They belong to the ATP-binding cassette (ABC) superfamily of transporters that utilise the energy of ATP hydrolysis to translocate a wide range of substrates across a variety of cellular membranes [45]. In most ABC transporters, the binding and subsequent hydrolysis of adenosine triphosphate (ATP) is believed to be coupled to, and provide the energy for substrate transport.

The Pgps are a highly conserved group of ABC transporters found as multigene families in a wide range of species. Pgp isoforms are encoded by a small family of closely-related genes in mice (mdr1, mdr2, mdr3), rats (mdr1 and mdr2), hamsters (pgp1, pgp2, pgp3) and humans (MDR1, MDR2) [46], although only MDR1 is involved in multidrug resistance [43]. In addition, there are many homologues in human and other animals, insects, yeast and bacteria.

Despite the impressive efforts dedicated to this subject, a clear structural model for P-glycoprotein remains largely elusive [46]. Human MDR1 is expressed as a single polypeptide consisting of 1280 amino acid residues that are organised in two tandem repeats or halves of 610 amino acids, joined by a short segment termed the linker region [29,47]. The two halves share a similar structure. The most widely accepted model of Pgp topology is based on hydrophobicity analyses. In this model, each repeat or half consists of an NH_2-terminal hydrophobic domain containing six potential transmembrane sequences, putative α-helices, separated by hydrophilic loops. These six membrane-spanning segments are believed to form the pathway through which solutes cross the membrane and to play a role in determining substrate specificity. Each hydrophobic domain is followed by a hydrophilic domain (NBD: nucleotide binding domain) containing a nucleotide-binding site that is located at the cytoplasmic face of the membrane and couples ATP hydrolysis to the transport process [29].

Insight into the structure of Pgp has recently been revealed by electron microscopy and single particle images and Fourier projection lamps of 2-dimensional crystalline arrays [24]. The data suggest that the Pgp is a cylinder of approximately 10 nm in diameter with one half of the molecule in the lipid bilayer and the remainder above and below the membrane. There is a large toroidal central pore of approximately 5 nm in diameter surrounded by a roughly hexagonal array of the membrane spanning domains (MSD). The pore is aqueous and larger than is required for the passage of known substrates. In addition, an opening to the lipid phase, within the plane of the membrane, is also apparent.

Substrate recognition and ATP binding are independent of each other [48,49]. Drug-binding studies have suggested that ATP is not necessary for drug binding to Pgp [26], but ATP binding and hydrolysis are required for the Pgp mediated drug transport [26]. Recent studies suggest that there is cross talk between the ATP-binding sites and the transmembrane segments [50] and a major conformational change is induced in the drug binding site of Pgp upon ATP hydrolysis [51].

Pgp is a *N*-glycosylated protein and its level of glycosylation depends on the species: human Pgp is more glycosylated (170 kDa). A recent study indicates that glycosylation may be involved in drug transportation in resistant cells [52].

The multidrug-resistance-associated protein (MRP or MRP1) [53] is a 190 kDa protein encoded by the mrp1 gene and is constituted by 1531 amino acids presenting *N*-linked glycosylation sites. While the human genome encodes only two Pgps, it contains many genes related to MRP [32]. The protein is predominantly localised to the plasma membrane in drug-resistant cells, with detectable levels present in intracellular membrane compartments of some cell types [54]. Whereas Pgp transports neutral and positively charged molecules in their unmodified form, MRP1 overexpression is associated with an increased ATP-dependent glutathione *S*-conjugate transport activity. MRP1 is able to transport a range of substrates as such or conjugated to GSH, glucuronide, and sulfate [31,55,56].

After its discovery, many proteins closely related to MRP1 have been identified in a wide variety of eukaryotic organisms. Included among these are five human MRP1-related proteins, designated MRP2, MRP3, MRP4, MRP5 and MRP6 [54]. MRP7, MRP8 and

MRP9 are recent additions to the family and have not yet been characterised [32,33]. On the basis of studies of post-translational modification of the protein by limited proteolysis and site-directed mutagenesis, a topological model has been proposed. According to this model, MRP1 possesses five, six and four transmembrane segments in MSD1, MSD2 and MSD3, respectively. Recently, structural analysis of reconstituted purified MRP1 was performed by infrared spectroscopy [56].

3. PGP and MRP1 transporter proteins: the mechanism of action

Discovery of the molecular mechanisms by which Pgp and MRP1 proteins exert their action has been one of the major tasks of research in the field of multidrug resistance.

Pgp substrates can structurally be very different, however the physical properties shared by many of them include high hydrophobicity, an amphiphilic nature and a net positive charge, although neutral compounds, among them hydrophobic peptides, have also been described as substrates of Pgp [57]. Although it seems evident that MDR of tumour cells is caused by a lowered cytosolic drug concentration due to Pgp expression, it has to be established whether Pgp is directly involved in the extrusion of multiple drugs or whether MDR is an indirect effect of Pgp expression.

A number of models have been proposed to explain the direct involvement of Pgp in MDR [25]. The first hypothesis is that Pgp functions as a drug transporter (efflux pump) which can act on a broad range of structurally unrelated molecules and uses the energy of ATP hydrolysis to mediate drug efflux. A possible mechanism for the binding of cationic lipophilic drugs by multidrug transporters has been proposed on the basis of crystal structures of the multidrug-binding domain of the transcriptor activator BmrR [58]. It implies a conformational change in the transporter that exposes a buried charged residue in the substrate-binding pocket and allows access to this site only by those drugs that are its steric and electrostatic complements. From this point of view, Pgp is like a regular substrate-specific transporter, the only difference being that the recognition site of this multidrug transporter can interact with a large array of molecules. However, from a theoretical point of view the concept that Pgp is an energy-dependent transporter for so many lipophilic compounds has always represented a problem and has been challenged [59]. As a matter of fact, over a hundred structurally unrelated lipophilic compounds are known to be Pgp substrates.

To address the question of the broad substrate selectivity of Pgp, a novel hypothesis has been proposed [60]. A common, recognisable structure moiety could be readily added to structurally unrelated lipophilic molecules while they are inside the cells, then these molecules could all become the substrates for Pgp as this common structural moiety could serve as a common ''handle'' for selective recognition and subsequent cross-membrane transport. Accordingly, Pgp would be an energy-dependent efflux pump only for certain conjugated metabolites (probably sulfates) of the lipophilic anticancer drugs but not for the parent compounds.

Consistent with the hypothesis of a direct involvement of Pgp, several modes of action can be envisaged. The ''aqueous pore model'' implies the initial capture (binding) of substrates from the cytoplasmatic aqueous phase, followed by translocation across the lipid bilayer, release of the substrate into the aqueous phase out of the membrane, and reorientation of the binding site(s). On the basis of the preferential partitioning of lipophilic molecules in the membrane, it has been proposed that MDR transporters might bind and actively remove hydrophobic drugs at the cell membrane level. In this case, Pgp would function as a ''hydrophobic vacuum cleaner'' to transport drugs from either the inner or the outer leaflet of the lipid bilayer into the external medium. Alternatively, MDR proteins might function as a ''flippase'', a variation on the ''hydrophobic vacuum cleaner'' model, by translocating drugs from the inner to the outer membrane leaflet after which the molecules will diffuse into the external medium.

However, indirect roles for Pgp have also been suggested. According to the ''altered partitioning model'' [59,61], MDR proteins would not transport drugs but increase intracellular pH and lower the electrical membrane potential. As a consequence, charged hydrophobic compounds such as anticancer cytostatics or multidrug resistance reverting drugs (lipophilic cations) might be retained differently in Pgp positive and negative cells. Another indirect mechanism of drug transport suggests that Pgp acts as an outwardly directed ATP channel, thus, generating an electrochemical ATP gradient which drives drugs across the plasma membrane [62,63]. On the other hand, electron paramagnetic resonance (EPR) and fluorescence anisotropy studies have shown that the structural order of membrane lipids increases and membrane fluidity decreases in some MDR cell lines, suggesting that this is a possible reason for the reduced level of chemotherapic in MDR cells [64,65]. However the role of Pgp in reducing the membrane fluidity of MDR cells has not been clarified. In this respect, the finding that Pgp functions can be affected by the lipid environment [66] and that drug–membrane interactions might do the same by influencing the Pgp conformation [67] can be of some importance.

Since indirect models do not require direct binding of drugs to transport proteins, they can explain the un-

usual ability of Pgp to act on a vast number of structurally unrelated substrates, which at least share a high degree of hydrophobicity and are positively charged at neutral pH. However, experimental data favour the direct involvement of Pgp, and its functional analogues, in the binding and transport of multiple unrelated drugs. Although the indirect mechanism may in some instances contribute to the drug-resistant phenotype, apparently it plays no critical role in the majority of MDR cells [25].

A fundamental question about Pgp is the number of drug binding sites. There is evidence for the existence of multiple binding sites (at least two) as some substrates bind to Pgp in a mutually non-competitive manner [68]. The presence of two drug-binding sites on Pgp with different specificity is another way in which Pgp may expand the range of substrates it can transport. Other data suggest that the two sites show positive co-operativity for drug binding and transport [69].

Photoaffinity analogues of substrates of Pgp have been used for specific labelling of the binding sites. These photoaffinity probes label two sites, one in the NH_2 terminal half and another in the COOH terminal half of the protein [30]. The binding site in the NH_2 terminal half has been mapped within or close to the putative transmembrane domain TM 4–6, and the other in the COOH terminal half within or close to TM 11–12.

Based on the comparison of some hundred substrates of Pgp, Seelig [27,70] found that a set of well-defined structural elements is required for the interaction: two or three electron donor groups with a fixed spatial separation form the recognition elements. The binding to Pgp increases with the strength and the number of electron donors or hydrogen bonding acceptor groups. Correspondingly, a high percentage of amino acids with hydrogen bonding donor side-chains are found in the transmembrane sequences (TM 4–6 and TM 11–12) of Pgp and are relevant for substrate interaction.

The two NBDs are a critical feature of Pgp. Reconstitution studies with purified Pgp have shown that transport of hydrophobic substrates against a concentration gradient is coupled to ATP hydrolysis [71]. However, the mechanism by which Pgp couples ATP energy to translocation and efflux of a diverse range of substrates is largely an unresolved debate [46]. Both NBDs can hydrolyse nucleotides, and their ATPase activity, that can be blocked by vanadate, is necessary for drug transport [30]. ATP hydrolysis in the catalytic NBD induces a high chemical potential state and its relaxation is coupled to drug movement from an inside-facing, higher affinity substrate-binding site to an outside-facing, lower affinity one [72].

Finally, Pgp is phosphorylated by protein kinase C (PKC) and PKC blockers reduce Pgp phosphorylation and increase drug accumulation. These observations suggest that phosphorylation of Pgp stimulates drug transport. However, there is evidence that PKC inhibitors directly interact with Pgp and inhibit drug transport by a mechanism independent of Pgp phosphorylation [73,74].

The classes of anticancer drugs that are substrates of MRP1 include anthracyclines such as doxorubicin and daunorubicin, vinca alkaloids and etoposide. By pumping these agents out of the tumour cells, MRP1 causes reduced intracellular accumulation of drugs, leading to resistance. Thus MRP1 does confer a multidrug resistant phenotype to cancer cells similar to Pgp and the transport kinetics appear to be very similar [75]. The substrate specificity of both transporter proteins is partly overlapping but is otherwise very distinct [41]. MRP1 has been described as an ATP-dependent export GS-X pump for the endogenous glutathione conjugate leukotriene C4 and structurally related anionic amphiphilic glutathione conjugates.

Several findings indicate that MRP1 reduces drug accumulation by effluxing drugs by a GSH co-transport mechanism or after their conjugation to GSH [31,76]. In the case of daunorubicin, the stoichiometry of the co-transport with GSH has been shown to be 1:1 [77]. The disruption of the gene encoding MRP1 abrogates the co-transport of xenobiotics and GSH [55].

As demonstrated for Pgp and many other ABC superfamily transporters, ATP hydrolysis provides the energy required for transport by MRP1. ATP binding and hydrolysis are essential for the proper functioning of MRP1, but the mechanism by which the energy derived from ATP hydrolysis is transduced into drug transport is not known. In analogy to Pgp, where the mechanism of coupling ATP hydrolysis to drug transport is likely to involve substantial conformational changes, recent studies have investigated tertiary structure changes in MRP1. The results indicate that during the MRP1 catalytic cycle, its secondary structure is not modified. This study also indicates that the secondary structure of MRP1 is not affected upon binding of different nucleotides. In contrast, the tertiary structure of MRP1 changes and adopts different topologies during its catalytic cycle [56].

The mechanism by which GSH facilitates transport of some compounds by MRP1 is still a matter of debate. The proposal that drug efflux by MRP1 might occur after conjugation with GSH has been questioned [18] in favour of a co-transport mechanism involving GSH but also other anions. There are several reasons why the conjugation hypothesis does not provide a convincing explanation of MRP1 ability to confer resistance to such a wide range of xenobiotics. First, there is no evidence that phase II conjugation plays a significant role in the metabolism of natural product drugs. Second, phase II biotransformation reactions occur primarily in the liver and it is unlikely that cell types

which overexpress MRP are competent to carry out such conjugations with the efficiency required to cause resistance. However, recent results seem to confirm that MRP1 mainly transports anionic phase II-conjugates [78].

4. PGP and MRP1 transporter proteins: the physiological functions

After their discovery as determinants of MDR, transporter proteins have been found in a variety of non-tumoural tissues, suggesting a physiological role for Pgp (and possibly MRP1). Several reviews have treated this topic [79–83] and the interested reader should refer to them for a more complete coverage of the topic. Furthermore, drug efflux proteins have been detected and found to have a critical role also in microorganisms [84,85].

In mice, and probably also in rats, the tissue distributions of mdr1a and mdr1b differ from each other, but together they cover the same tissues as the single MDR1 in humans, suggesting that the related protein Pgp performs the same physiological role(s) in the different organisms [86]. In man, Pgp is found in the apical surface of superficial columnar epithelial cells of small and large intestine, in the biliary canicular membranes of hepatocytes, in the apical surface of epithelial cells of small ductile in the pancreas, along endothelial cells in small blood capillaries of the brain and of the testis, and in several other cells and tissues [80]. Localisation suggests that Pgp is primarily involved in the extrusion of some substrates, for instance xenotoxins ingested with food, from the epithelial cell layers into the adjacent luminal space. This would occur either by exclusion of the toxins, as in the intestine and blood–tissue barriers, or by active excretion in the liver, intestine and kidney.

Pgp is present in the mucosal epithelium of the intestine where it makes an important contribution to the direct excretion of transported compounds into the intestinal lumen [81] and is also a major determinant for the reduced uptake of orally administered compounds [87]. At least six ABC-transporter proteins, all homologues of either the Pgp or of the MRP-subfamily, have been characterised in human hepatocytes: by analogy with their intestinal functions they may protect the hepatocyte by returning hydrophobic toxic bile components to the bile [88]. Pgp and MRP1 are also found in the epithelial cells of the proximal tubules of the kidneys, where a function similar to that in the intestine can be envisaged: both a direct excretory role for drugs, and a role in limiting the back diffusion of amphipathic compounds which have entered the preurine by ultrafiltration [86,89].

One of the most important physiological actions of Pgp, and possibly MRP1, is at the level of brain penetration by drugs. The blood–brain barrier (BBB) makes a nearly continuous physical barrier separating the brain compartment from the blood stream. Hydrophilic compounds, which are not small enough to pass the tight junction by passive diffusion, will be excluded from the brain, unless translocated by specific carriers. On the other hand, hydrophobic compounds could cross lipid membranes by passive diffusion and would be able to diffuse through the BBB, entering the brain compartment. However, there are drugs that exhibit poor brain penetration despite their high lipophilicity (doxorubicin and vincristine). The poor distribution of these compounds in the brain led to the proposal that efflux transport systems such as Pgp and MRP1 (whose presence is, at the moment, controversial) can exist at these barriers and actively eliminate drugs from the brain [82]. Pgp in the BBB would be, therefore, the major determinant of the penetration of many amphipathic compounds, including drugs and toxins into the brain [83]. Pgp also seems active in releasing functionally active neurotransmitters/neuromodulator (e.g. opioids, β-endorphin, glutamate) directly from the brain into the blood. This ability of the Pgp transport system to pump functionally active compounds from the brain to periphery defines a potentially important mechanism for the central nervous system to modulate the peripheral system [90,91]. The placental barrier was also shown to express Pgp on the brush-border membrane (maternal side) of trophoblast cells. Pgp is considered to regulate the transfer of several substances from mother to foetus and to protect the foetus from toxic compounds [92].

These properties of extrusion proteins can have profound consequences on the absorption, distribution, clearance, and more in general pharmacokinetics, of many drugs including the chemotherapics used to treat cancer, as will be discussed in more detail in one of the following sections.

5. Pharmacological control of MDR

Since MDR is one of the main obstacles to successful chemotherapy of cancer, a number of biochemical, pharmacological and clinical strategies have been devised to overcome it.

One approach can be that of using high concentrations of cytotoxic drug to overwhelm the effects of cell extrusion. This approach, however, has obvious drawbacks related to the side effects of high doses of chemotherapic.

Another approach is that of using drugs which are not good substrates for Pgp, MRP1 or other members of the family. There are drugs that are not transported

by the carrier protein, such as cyclophosphamide and *cis*-platin [93], and it is conceivable that chemical modification of MDR-producing drugs would result in active, but not transported, analogues [94]. In this field, interesting results have been obtained for instance in the anthracycline class, where small changes in the molecule, namely in position 2′ of the sugar moiety, lead to compounds such as annamycin, showing in vitro activity against MDR cells [94]. Following a similar and very interesting approach, Mazel et al. [95] used doxorubicin–peptide conjugates to skip Pgp extrusion of the drug. In this respect it must be mentioned that even for drugs that are well recognised by the transporters, if the rate of uptake is very high, it is possible to achieve the needed toxicity against MDR cells [96]. However, these aspects of tumour chemotherapy will not be addressed in this review.

As soon as Pgp and sister proteins were recognised as the main responsible of MDR, blocking the efflux of drugs by inhibition of the functions of these transporters has become a realistic way to circumvent MDR [97]. Several chemicals, already known or used as drugs for other purposes, have been tested in vitro and in vivo on resistant tumour cells. Verapamil, a calcium channel antagonist, was the first compound found active in reversing MDR [98] and after it many other compounds, reviewed by Gualtieri [39], have been found effective in the ''resensitisation'' of resistant malignant cells.

There are several in vitro methods to evaluate modulation of MDR. Most of them are based on the study of the effect of a cytotoxic agent, in the presence or in the absence of the modulator, on the growth of resistant cell lines. A resistant cell line shows an IC_{50} of the cytotoxic agent that is higher than that of the original strain. The MDR modulator would take back the IC_{50} of the cytotoxic agent toward the value measured for the parental cell line. Accordingly, the ratio between the IC_{50} of the cytotoxic agent in the absence and presence of the MDR reverter is a measure of its activity. This ratio is called MDR ratio or fold reversal and is widely used to assess the activity of chemosensitisers. Of course this method does not give information on the mechanism of action. A direct involvement of carrier proteins like Pgp may be assessed by binding studies, using labelled compounds such as [^{3}H]azidopine. Drug accumulation or efflux, in the presence and in the absence of the chemosensitiser, can also be used as indicators of Pgp activity. The antitumour drug doxorubicin, the dye Rhodamine 123 and calcein-acetoxymethyl (Calcein-AM) [99] are widely used reporters for this method. In a related approach [75,100], the concentration of the reverter (indicated as $[i]_{0.5}$) that restores the nuclear concentration of the antitumour drug to half that of parental cell line is taken as a measure of MDR activity. Inhibition of ATP-ase activity of the carrier protein has also been used to evaluate the reverting activity of MDR modulators [101]. In recent reviews the most used methods to evaluate the efficiency of drugs to reverse MDR have been critically discussed, also from a quantitative point of view [44,102]. The problem of the choice of model substance for screening drug–Pgp interactions has recently been discussed by Litman [33].

It should be emphasised that, as far as structure–activity relationships are concerned, the variety of methods and parameters used to assess MDR reversion in vitro complicates a simple and sound comparison of the active molecules. For these reasons, in the following section, the activity will be quantified only by the active concentration (AC), when known, without any mention of the assay used. For details on this aspect of the problem the reader is referred to the corresponding references.

6. Modulators of multidrug resistance proteins

The molecules that have shown some action on MDR are innumerable and it is an impossible task to report them all. Table 1 presents a selection of compounds that have shown some kind of activity. Reports are very frequently anecdotal and the activities reported are, most of the time, quite low. Therefore, in the following section we will not attempt to review every compound or class of compounds that has shown some activity, but we will select the molecules and the classes that have been more significant for the design of new MDR reverters and that have produced therapeutically interesting compounds.

The first compounds tested and found active on MDR were easily available and known drugs, usually endowed with some kind of pharmacological activity that represented one of the problems that complicated their clinical use. As a consequence, clinical application was hindered by their side effects when they were used at the concentrations required to overcome MDR. Indeed, none of them has survived preclinical and clinical trials. The most representative, namely those that have given origin to more efficient and safer molecules, are reported in Fig. 1.

Independent of when they were tested, we will call this class of MDR-reverting agents the *first generation chemosensitisers*, intending as the *second generation* all the compounds obtained by aimed manipulations of leads that showed MDR reversing activity.

6.1. Verapamil-related compounds (Fig. 2)

Verapamil (Fig. 1) was the first compound found to reverse MDR in vitro [98] and to reach clinical trials [151]. An initial improvement in this family of

Table 1
Selection of compounds that have shown MDR modulation properties [a]

Comp.	Class	Target protein	Reference
Amphotericin B	antibiotic	Pgp	[93]
Astemizole	antihistaminic	Pgp	[103]
Atemoyacin B	acetogenin	Pgp	[104]
Benzoperimidine	anticancer	Pgp, MRP	[105]
Benzquinamide	antiemetic	Pgp	[40]
Bepridil	calcium blocker	Pgp	[35,106,107]
Bromocriptine	antiparkinsonian	Pgp	[108]
Cefoperazone	antibiotic	Pgp	[35,106]
Cepharantine	alkaloid	Pgp	[109]
Cholesterol	steroid	Pgp	[110]
Cinchonine	alkaloid	Pgp	[111,112]
Colchicine	alkaloid	Pgp	[4]
Diltiazem	calcium blocker	Pgp	[35]
Dmt-Tic	opioid	Pgp	[113]
Erythromycin	antibiotic	Pgp, MRP	[4,35,106,114]
Fangchinoline	alkaloid	Pgp	[115]
Ficus citrifolia extract		Pgp	[116]
Flunarizine	calcium blocker	Pgp	[35]
Forskolin	diterpene	Pgp	[35]
Genistein	isoflavonoid	Pgp, MRP	[117–119]
Glibenclamide	sulfonylurea	Pgp, MRP	[120,121]
Gramicidin D	antibiotic	Pgp	[4]
Grapefruit juice		Pgp	[122]
Haloperidol	antipsychotic	Pgp	[107,123,124]
Hapalosin	depsipeptide	Pgp	[125,126]
Hexanoylglucosylceramide	sphingolipide	Pgp	[127]
Isotrilobine	alkaloid	Pgp	[128]
Kempferol	flavonoid	Pgp, MRP	[117]
Ketoconazole	antimicotic	Pgp	[4,107]
Lamellarins	alkaloid	Pgp	[129]
Lomerizine	calcium blocker	Pgp	[130]
Lovastatin	hypocholesterolemic	Pgp	[131]
Lycaconitine	alkaloid	Pgp	[132]
Mefloquine	antimalarial	Pgp	[133]
Megestrol acetate	steroid	Pgp	[134]
Methadone	opioid	Pgp	[38]
Methylene blue	dye	Pgp	[135]
Ofloxacin	quinolone	MRP	[114]
Oxatomide	antiallergic	Pgp	[136]
Primaquine	antimalarial	Pgp, MRP	[137]
Progesterone	steroid	Pgp	[138,139]
Quercetin	flavonoid	Pgp, MRP	[117,140]
Quinacrine	antimalarial	Pgp	[40]
Quinidine	alkaloid	Pgp, MRP	[4,111,112,137]
Quinine	alkaloid	Pgp, MRP	[111,112,137]
Reserpine	antihypertensive	Pgp	[4,35,141]
Rifampicin	antibiotic	MRP	[142]
Roemerine	alkaloid	Pgp	[143]
Rosemary extract		Pgp	[144]
Secoverine	calcium blocker	Pgp	[107]
Silybin	flavolignan	Pgp	[145]
Staurosporine	bacterial metabolite	Pgp	[146]
Tamolarizine	calcium blocker	Pgp	[147]
Terfenadine	antihistaminic	Pgp	[4,106,141]
Tetracycline	antibiotic	Pgp	[34]
Tetrandine	alkaloid	Pgp	[115]
Thaliblastine	alkaloid	Pgp	[148]
Thioperidone	antidepressant	Pgp	[107,141]
Thioridazine	neuroleptic	Pgp	[139]
Tiamulin	antibiotic	Pgp	[149]
Trazodone	antidepressant	Pgp	[35]
Valinomycin	antibiotic	Pgp	[4,150]
Vindoline	alkaloid	Pgp	[34,35]
Yohimbine	alkaloid	Pgp	[34,35]

[a] In this table the compounds discussed in detail in the section are not mentioned.

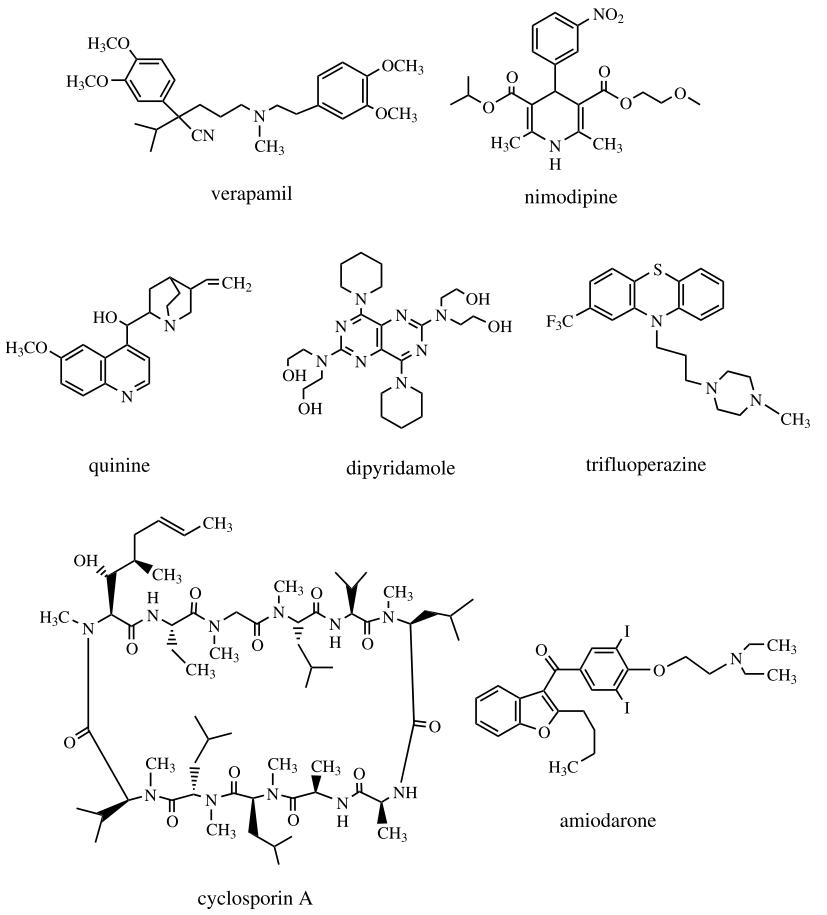


Fig. 1. First generation MDR reverters.

compounds has been the finding that (*R*)-verapamil, which has lower cardiovascular activity, is equipotent with the (*S*) enantiomer as MDR reverter [152]. Thus, the more selective (*R*) enantiomer has been tested in clinical trials [93,153] with encouraging results. The cardiovascular action of verapamil derivatives has always represented a problem in the development of MDR modulators possessing this structure and many efforts have been devoted to identifying more selective compounds.

In an attempt to identify more specific molecules for MDR reversal, a series of verapamil analogues obtained from Knoll [154] was studied. Among them compound LU48895 was two-fold more potent as MDR reverter and four-fold less potent as calcium antagonist than verapamil (Fig. 1).

Within a series of tiapamil-related drugs [155–158], compounds Ro10-6852, Ro11-5160 and Ro11-2933 have been reported to be significantly more potent than verapamil in reverting MDR on a few cell lines, while their cardiovascular activity appeared lower. Compound Ro44-5912 was shown to be slightly more potent than its enantiomer and was selected for further studies.

In a large set of verapamil derivatives with reduced molecular flexibility, originally synthesised as calcium antagonists, remarkable MDR reverting activity in vitro (erithroleukemia K 562 cells) was found. Compound EDP42 showed an AC = 0.5 μM as MDR reverter, while being inactive as cardiovascular agent [159]. In a following investigation, verapamil analogues carrying large aromatic moieties on the amine nitrogen were studied. The rationale of the synthesis was the introduction into the verapamil moiety of other aromatic rings that could favour interaction with the Pgp protein. Although neither the lipophilicity nor the aromaticity of the compounds could be correlated with activity, the research led to the identification of a fairly potent modulator of MDR (MM36; AC = 0.05 μM on K 562 cells) that at the same time, possessed much lower cardiovascular activity [160].

Another series of verapamil derivatives obtained by substitution of the isopropyl chain with thioether groups and by manipulation of the amine residue has been described [161]. Within the series, compound CL 329,753 and CL 347,099 have shown the best pharmacological profile, being some ten times more potent than verapamil in reverting MDR. At the same time, the compounds were found to be some 70-fold less potent than verapamil as calcium antagonists.

6.2. Nimodipine-related compounds (Fig. 3)

The reverting activity of dihydropyridines such as nimodipine (Fig. 3) has stimulated the search for MDR modulators in the series. The (*R*)-enantiomer of niguldipine, dexniguldipine which is fairly potent as MDR reverter (AC = 0.1–1.0 μM [38], depending on the cell line) and presents an affinity for the calcium channel some 40 times lower than that of its enantiomer, has reached phase II clinical trials [162]. The pyridine derivative PAK-104P [163] is active on both Pgp and MRP-dependent MDR [164,165]. Its aromatic structure confirms that MDR modulation is independent from calcium antagonism and the classical structure of DHP calcium antagonists can be significantly changed maintaining good levels of MDR inhibition and greatly reducing cardiovascular activity. Indeed, further reduction of calcium channel antagonism and improvement of MDR modulating properties have been obtained by substitution of the aryl group in 4 position with different groups, as has been done in the series of nicardipine-derived compounds: NIK-250, N276-9 and N276-16 [166,167].

6.3. Quinine-related compounds (Fig. 4)

The MDR reversal activity, evaluated also in clinical trials [168,169], of quinine and related compounds (see Fig. 1 and Table 1) has apparently stimulated the synthesis and study of compounds containing quinoline and/or related heterocycles. Indeed, some of the most interesting and potent MDR modulators so far synthesised such as MK-571 [3], NLCPQ-1 [170,171] and MS-209 [172] belong to this class. The latter compound is active on both Pgp and MRP proteins [173] and has entered clinical trials in Japan [174]. Extended modifications of MS 209 structure showed that a piperazinyldibenzosuberane fragment, such as in MS-073 [175], enhanced MDR reversing activity. Further manipulation of the new lead gave LY335979 (previously known as RS-33295-198) [176], one of the most potent and selective Pgp modulators to date. In fact, this

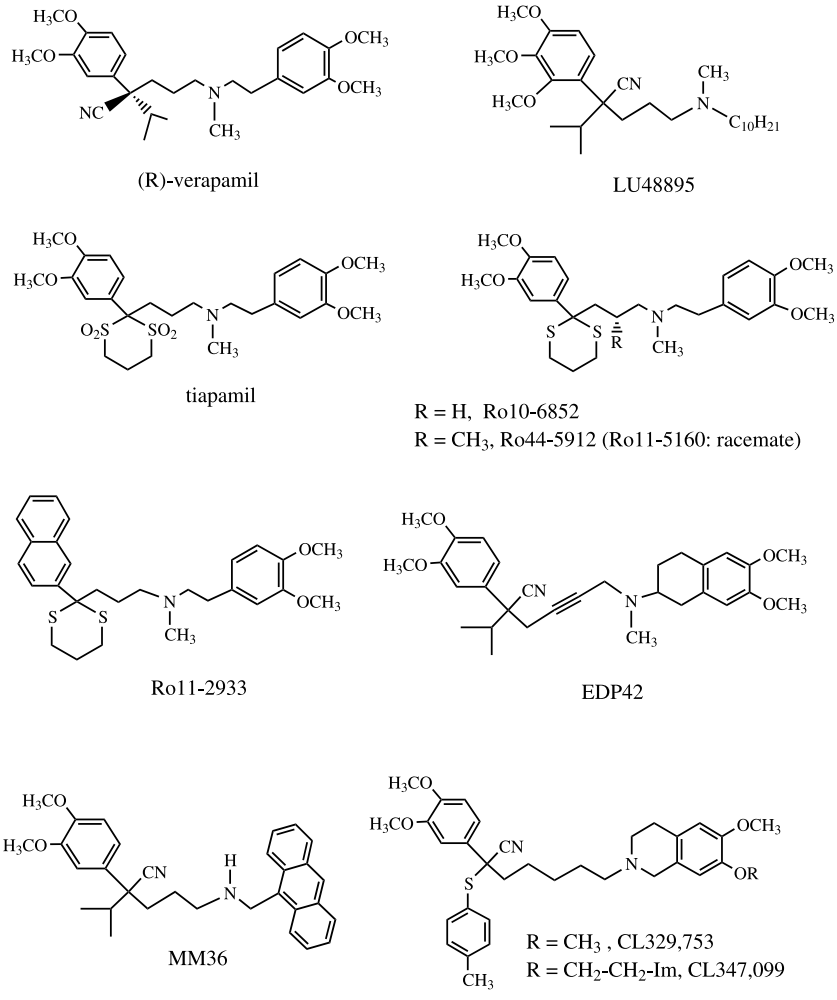


Fig. 2. Verapamil-related compounds.

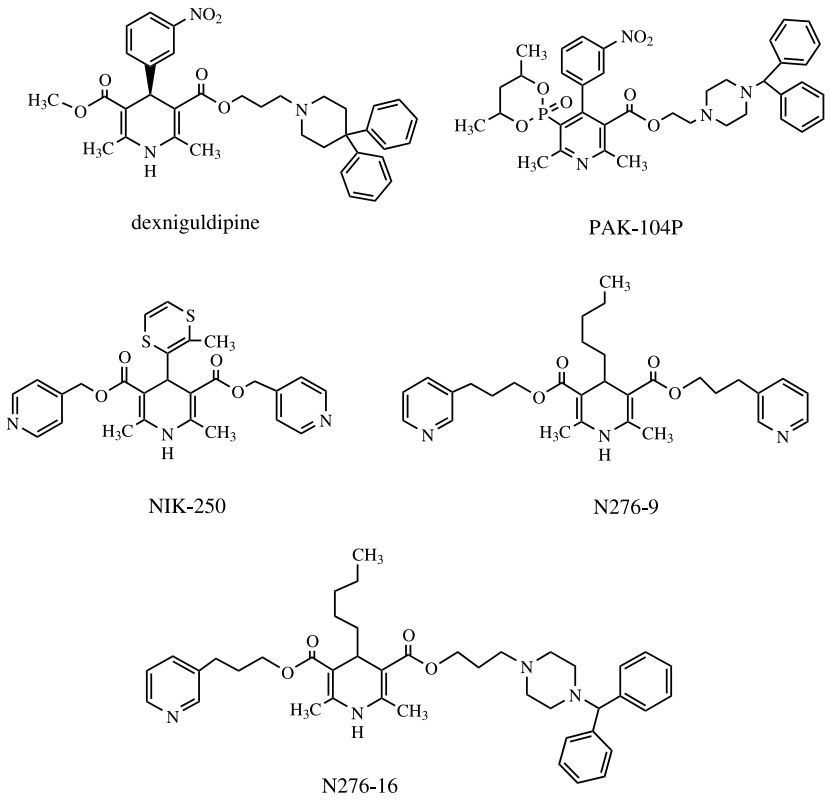


Fig. 3. Nimodipine-related compounds.

substance is highly effective on Pgp-mediated MDR (AC = 0.1–2 μM [177]) and shows a very strong affinity for Pgp (K_i = 59 nM [177]). The compound is specific for Pgp-mediated MDR since it does not modulate MRP-mediated resistance. LY335979 is currently under investigation in phase II clinical trials [177]. The acridone derivative GF120918 [178] (also known as GG918) which is one of the most potent and selective MDR modulators disclosed thus far [179–181] (AC = 20–100 nM [38], depending on the cell line) can also be included in this class. Even this drug, which is now undergoing clinical trials [182], seems selective for Pgp-mediated MDR, as it has shown to be ineffective in MRP-mediated multidrug resistance [183]. A related drug that has been recently disclosed, R101933 [184], is claimed to be a potent orally active MDR inhibitor that does not influence the pharmacokinetics of the chemotherapic used to treat cancer (see the next section) [185]. Finally, Chibale et al. [186] have reported that manipulation of the quinoline antimalarial drugs afforded potent MDR inhibitors exemplified by compound **1**.

6.4. Dipyridamole-related compounds (Fig. 5)

The vasodilator dipyridamole (Fig. 1) has been one of the first compounds to be found effective in controlling MDR [187]. Apparently, its structure has inspired the synthesis of several interesting compounds. A close analogue of dipyridamole is BIBW 22 BS [188] which has been shown to be a potent reverser of MDR (AC = 1 μM [38]). The triazine derivative S-9788, formally derived from almitrine [189], shows high MDR reversing properties (AC = 1–3 μM [38], depending on the cell line) and induced a strong accumulation of adriamycin. Its unexpected cardiovascular activity, leading to ventricular arrhythmia and torsade de pointe, has precluded further clinical development [190,191]. From a medicinal chemistry point of view, the compounds of the XR series seem very interesting. The first useful compound of the series, XR9051, was derived from the chemical modification of a natural product lead carrying a diketopiperazine nucleus, isolated from streptomyces species. Apparently the compound is the result of a hybridisation of the diketopiperazine lead with GF120918 (Fig. 4). It is a potent modulator (AC = 0.3–0.5 μM), shows a fairly good affinity for Pgp (EC_{50} = 1.4 nM) and is active in vivo per os [192,193]. The next compound of the series, XR9576 [194], no longer resembles the original lead while sharing many of the structural features of GF120918. XR9576 is reported to be an extremely potent, selective and an effective modulator in vitro (AC = 0.5 μM on CH(r)B30 cells) and in vivo (AC = 6–12 mg/kg p.o.) with a long duration of action. It holds great promise for the treatment of Pgp-mediated

MDR cancers [195,196]. If viewed as a poly-nitrogen compound, even the newly disclosed OC-144-093 can be described in this class [197,198]. The compound is the result of the optimisation of a lead identified in a library of imidazole derivatives, constructed around structural characteristics of MDR reverting drugs. It is reported to be nontoxic and fairly potent (AC = 0.01–0.5 μM on CEM/VLB1000 cells). A 3D-QSAR analysis of the series has provided a significant and useful CoMFA model [199]. Somehow belonging to this class is a series of quinoxalinones exemplified by compound **2**, which is the most promising of the family. The 2-oxoquinoxaline scaffolding was identified by screening several thousands of commercially available compounds. The research was aimed at identifying MDR modulators selectively active on Pgp protein, on the basis that interaction with MRP1 protein would be responsible of unwanted side effects. Compound **2** is indeed fairly active as MDR modulator but does not interact with MRP1 protein [200,201].

6.5. Cyclosporin A-related compounds (Fig. 6)

Cyclosporin A, the complex hydrophobic cyclic undecapeptide commonly used for organ transplantation (Fig. 1), as well as other immunosuppressants of the same family (FK506 [202] and rapamicin [203]) was identified as one of the most effective MDR reversing agents [204]. In this class of chemosensitisers, the original immunosuppressant activity was a severe obstacle for clinical use and the main goal of the researchers working in this field has been that of getting rid of this dangerous aspect.

Among the many compounds synthesised and studied by the Sandoz group [205–207], the oxidation product of cyclosporin D, SDZ-PSC-833 [208] has

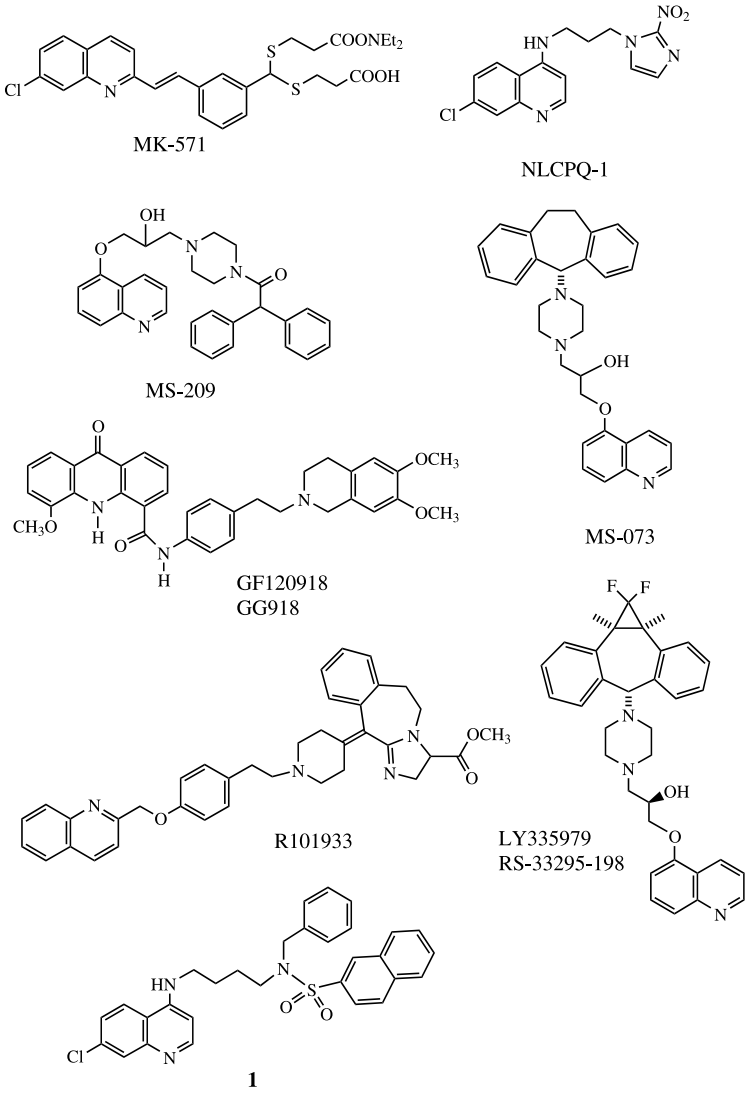


Fig. 4. Quinine-related compounds.

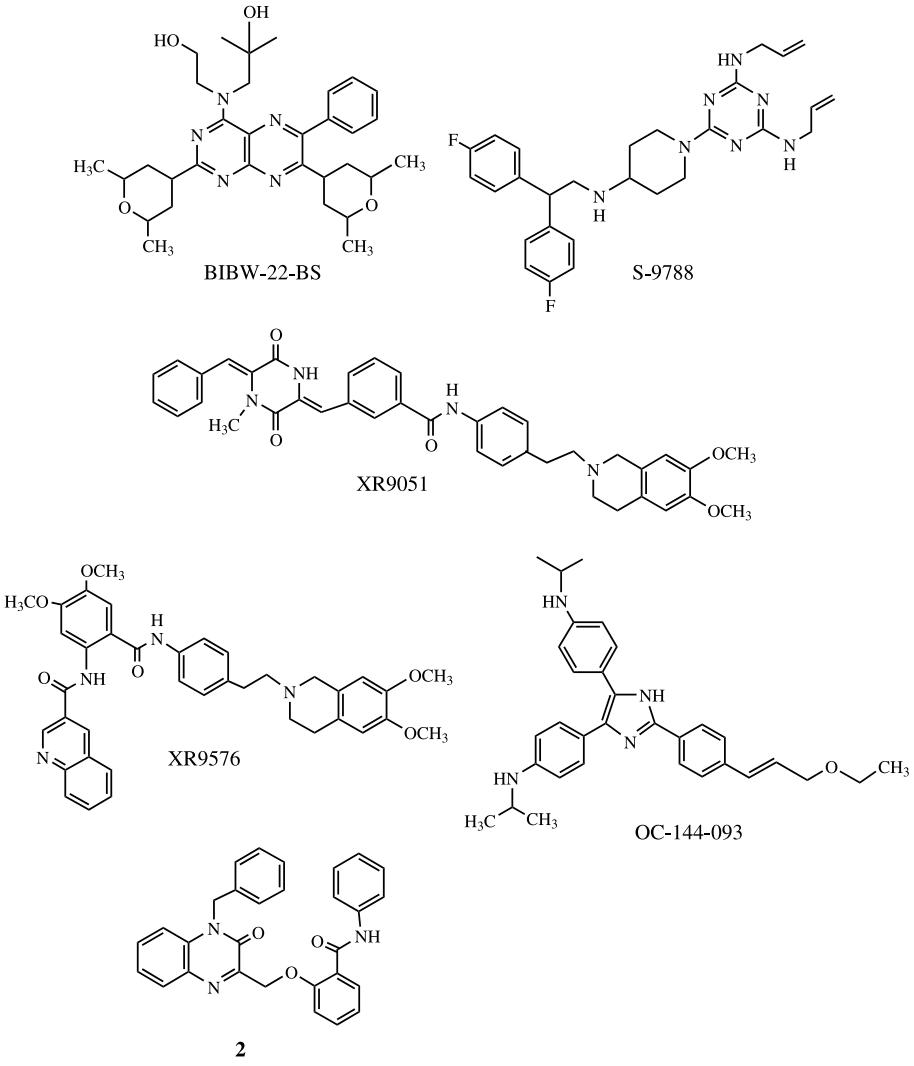


Fig. 5. Dipyridamole-related compounds.

emerged as the most promising. It is ten times more potent than cyclosporin A in reversing MDR (AC = 0.1–1 μM [38], depending on the cell line) and, most important, has little or no immunosuppressive action [209]. Moreover, it is selective for Pgp protein (being practically inactive on MRP1) and shows acceptable systemic bioavailability. SDZ-PSC-833, designated as valspodar, is being developed and is currently in phase III clinical trials [209]. Besides its use in cancer chemotherapy, the drug has the potential to increase exposure or to modulate the biodistribution of other chemotherapeutics, such as HIV protease inhibitors, to the brain. It is likely that valspodar will be the first MDR reversing drug to reach the market. A cyclosporin A derivative, FR 901459, which differs from the parent compound for the 2nd, 5th and 10th aminoacid, has been reported to be a potent Pgp inhibitor [210]. A family of hydrophobic peptides called reversins has been described as potent and specific MDR reverters [211]. Two members of the family, reversin 121 and reversin 205, have recently become commercially available from Sigma/RBI. Very recently, dendroamide A, a cyclopeptide isolated from blue–green alga reported to be a potent MDR reverser, has been made available by total synthesis [212]. Aureobasidin A and analogues are other cyclopeptides that have shown promising MDR reversing activity [213,214]. It is interesting that a peptidomimetic like ritonavir, a potent HIV protease inhibitor, has been reported to be more potent than SDZ-PSC-833 in inhibiting Pgp [215].

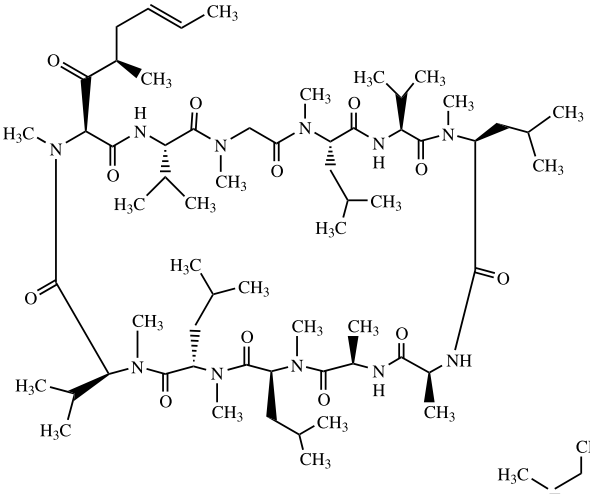
6.6. Taxanes (Fig. 7)

Several noncytotoxic natural taxanes from the Japanese yew tree *Taxus cuspidata* like taxuspine C (**3**) [216] [217], its benzoyl analogue **4** [218] and taxinine A (**5**) [219], have been reported to increase the cellular accumulation of vincristine in MDR tumour cells. This has paved the way to the synthesis and study of new potent chemosensitisers of the taxane family [217,219].

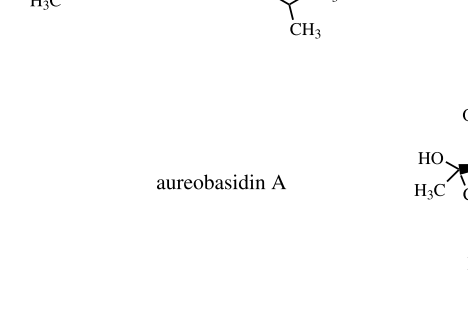
In a series of new taxanes synthesised through the modification of 10-deacetylbaccatin III (DAB) and 14-hydroxy-10-deacetylbaccatin III (14-OH DAB), several compounds exhibited >99% MDR reversal activity against breast cancer cell lines, at 1–3 μM level. Among them, compound SB-RA-31012 was still active at 0.1 μM [220,221]. Morihira et al. [222,223] reported the synthesis and activity of a series of C-aromatic taxoids and derivatives thereof, among which compound **6** was as potent as verapamil.

6.7. Trifluoperazine-related compounds (Fig. 8)

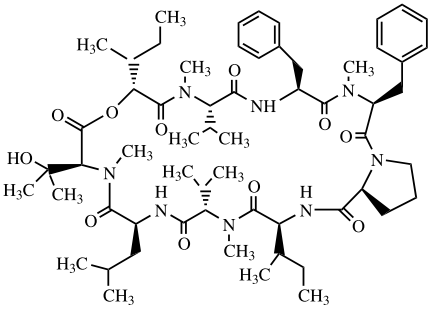
Tricyclic molecules like trifluoperazine (Fig. 8) [224], as well as some related CNS drugs (Table 1), show MDR reverting activity. Pajieva et al. have extensively studied phenothiazines and thioxanthenes like fluphenazine and flupentixol to gain information on the molecular requirements for MDR modulation. It was found that this class of compounds interact specifically with the membrane phospholipids [225]. Molecular modelling studies suggested that the small difference in MDR reversion between *cis* and *trans* flupentixol could be due to a different orientation of the two isomers in the membrane [67,226]. By using a CoMFA approach, the same authors found support for the membrane interaction mechanism of this kind of compound, suggesting that the molecular profile of hydrophobicity is a specific structural determinant of their MDR reverting activity [227].



valspodar
SDZ-PSC-833
PSC-833
amdray



aureobasidin A



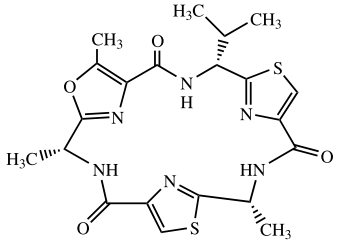
BOC-Asp(OBzl)-Lys(Z)-OtBu

reversin 121

[BOC-Glu(OBzl)]$_2$-Lys-OMe

reversin 205

BOC =t-butyloxycarbonyl; OBzl = O-benzyl; Z = benzyloxycarbonyl; OtBu = O-t-butyl
OMe = O-methyl



dendroamide A

Fig. 6. Cyclosporin A-related compounds.

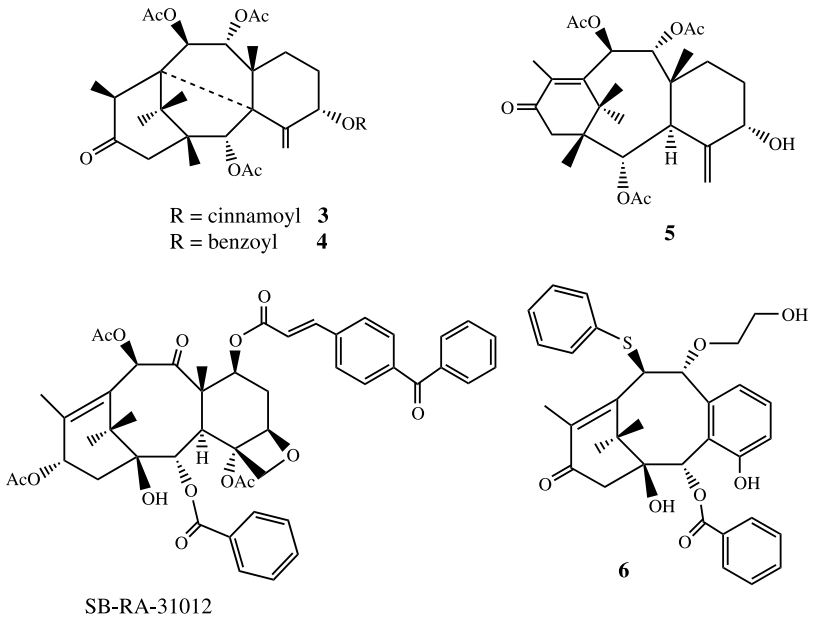


Fig. 7. Taxanes.

6.8. Propafenone-related compounds (Fig. 9)

Propafenone is an antiarrythmic drug that shows fairly good MDR reverting activity (AC = 0.3 μM on CEM vcr1000 human T-lymphoblast cell line). Chiba et al. [228–232] have thoroughly investigated structure–activity relationships in this family of MDR reversers. Structures A and B represent the main variations introduced into the propafenone molecule to give a large set of derivatives that have been subjected to QSAR studies. In this series of compounds, the authors were able to correlate lipophilicity with Pgp-dependent MDR. The same results were obtained measuring ATPase activity of Pgp [101]. A series of structurally related pyrazole derivatives (general structure C) was then synthesised to gain further insights into the structural requirements necessary for interaction with Pgp [233]. QSAR studies showed a good correlation of MDR modulating activity with lipophilicity. However, inclusion of hydrogen bond acceptor strength and steric parameters as descriptors led to remarkably increased predictivity of the QSAR equation [233]. Particularly interesting is the study [234] of the four optical isomers of a related compound where the carbonyl function of propafenone has been reduced: GP-88. It was found that there is a very low enantioselectivity which parallels that of the propafenone enantiomers (AC = 0.26 μM for the (*R*)- and AC = 0.37 μM for the (*S*)-form on CEM vcr1000 human T-lymphoblast cell line). This result seems to confirm that chirality does not play a major role in MDR reversers [235].

6.9. Amiodarone-related compounds (Fig. 10)

The potassium channel blocker amiodarone (Fig. 1) has found use as an anti-arrhythmic and is known as a potent MDR inhibitor [236]. Its severe side effects prevent its use as MDR modulator but, apparently, its structure has guided the design and synthesis of compounds with interesting chemosensitising activity. Even if it has been reported that they were derived from raloxifene, compounds LY117018 and LY329146 bear close relationships with amiodarone. Of them, LY329146 is reported to be a potent and selective MRP1 modulator [237]. A similar structure presents a series of indole derivatives exemplified by N-1, reported to be effective MDR inhibitors characterised by low cytotoxicity and hydrophobicity [238].

6.10. Flavonoid-related compounds (Fig. 11)

Some flavonoids and isoflavonoids have been shown to modulate both Pgp- and MRP-dependent MDR

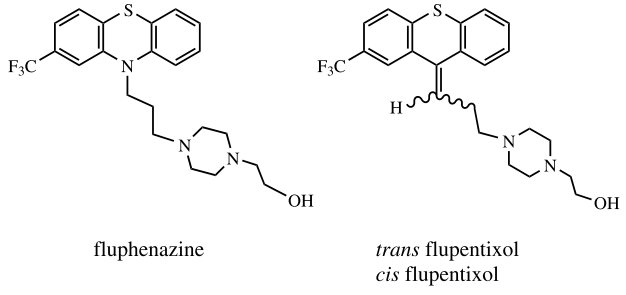


Fig. 8. Trifluoperazine-related compounds.

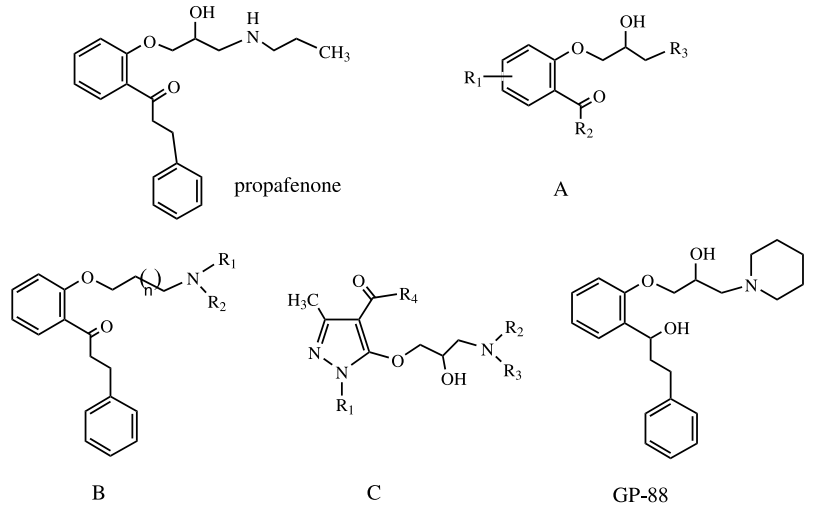


Fig. 9. Propafenone-related compounds.

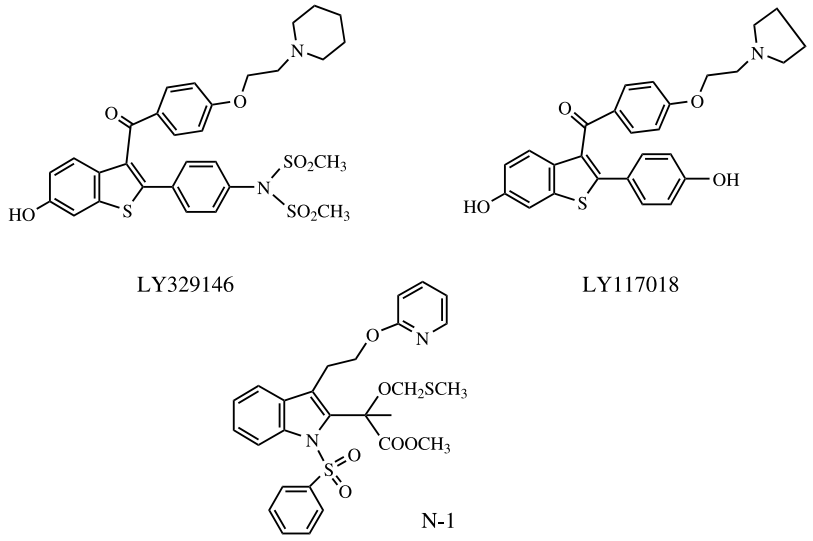


Fig. 10. Amiodarone-related compounds.

(Table 1) by acting through bifunctional interactions at vicinal ATP-binding site and steroid-interacting region within a cytosolic domain of Pgp [140]. This finding has prompted several modifications of their structure to improve the pharmacological profile. A series of halogenated and alkoxy chalcones (general structure A), open analogues of flavones, have been synthesised and found to possess high affinity for Pgp [239,240]. The introduction of an amino group, usually present in MDR reverters, has been performed by Ferté et al. [117] who introduced an *N*-benzylpiperazine side-chain to give a series of compounds such as **7** that were found to be more potent than verapamil. C-isoprenylation of flavonoids gives compounds such as 8-dimethylallylchrysin (**8**) which is claimed to be more potent that cyclosporin A [241]. Accordingly, simple substitution of the 4′ hydroxy group of galangin seems to produce compounds (**9** and **10**) with higher affinity with the cytosolic nucleotide-binding domain of Pgp [242]. Somehow related to this series is the leukotriene D4 receptor antagonist ONO-1078 that has been found to inhibit MRP transporting activity [243]. Finally, it has been reported that flavonolignans and some simple alkylated flavones are quite active on bacterial MDR pumps [244].

6.11. Alkaloids (Fig. 12)

In addition to the quinine alkaloids, several other compounds of this class (Table 1) have shown MDR reversing activity. In particular, ardeemins [245] have been the object of synthetic studies and have been taken as leads for the design of new MDR reverters [246–248]. Very recently, fumitremorgin C and some

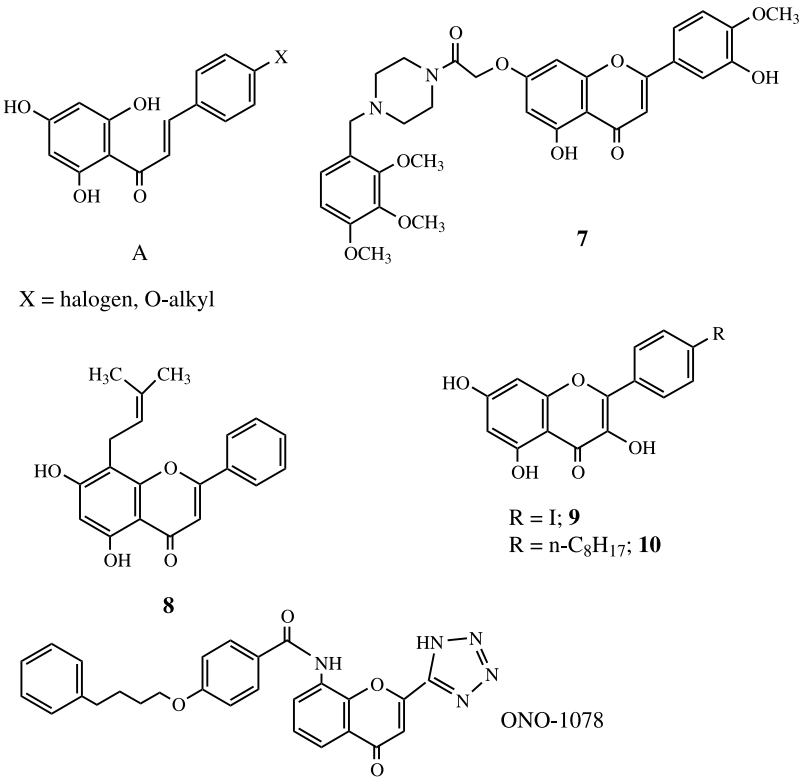


Fig. 11. Flavonoid-related compounds.

synthetic analogues have been shown to be effective on breast cancer resistance protein (BCRP) [249].

6.12. Terpenes (Fig. 13)

Although they generally lack one of the features considered critical for MDR-reversing molecules (i.e. basic nitrogen; a property shared with taxanes and flavonoids), several members of this family possess MDR modulating activity. Euphosalicin, a new diterpene polyester, was reported to be more active than verapamil in reversing multidrug resistance in mouse lymphoma cells [250]. The sesquiterpene torilin potentiated the cytotoxicity of adriamicin and other chemotherapics against multidrug resistant KB-V1 and MCF7/ADR cells [251]. New natural sesquiterpenes from celastraceae, exemplified by **11**, have been reported to inhibit Pgp functions [252,253]. Other natural compounds that are MDR active and lack a basic nitrogen, are of interest since their structure could suggest new directions to the design of MDR reversing agent. Among them are the oligoacylated sucroses atractysucrose I, II, and III, that have been reported to be as potent as verapamil in modulating MDR [254], the irciniasulfonic acid, recently isolated from marine sponge [255], and the natural polyene (−)-stipiamide and its synthetic and semisynthetic derivatives like DHS [256,257].

6.13. Steroid-related compounds (Fig. 14)

Progesterone, megestrol acetate and related hormones show chemosensitising properties (Table 1). Megestrol acetate has reached phase III in clinical trials [258]. The oestrogen antagonists toremifene [259] and tamoxifen [260], as well as some other analogues among which the steroid compound ICI164,384 [261], have been studied in more detail and have been found to completely revert MDR in some culture cells. The antiprogestatin drug RU486 [262] has been reported to reverse MRP-mediated drug resistance in human lung cancer cells. A series of naturally occurring sterols was shown to modulate MDR [263]. Among them, agosterol A and 4-deacetoxyagosterol [264] are the most promising, being active on Pgp and MRP pumps.

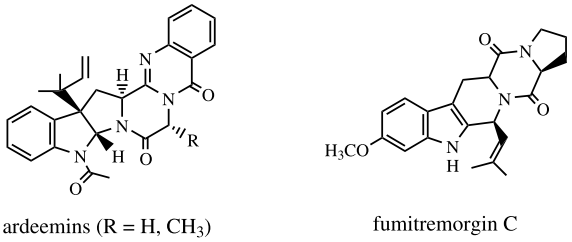


Fig. 12. Alkaloids.

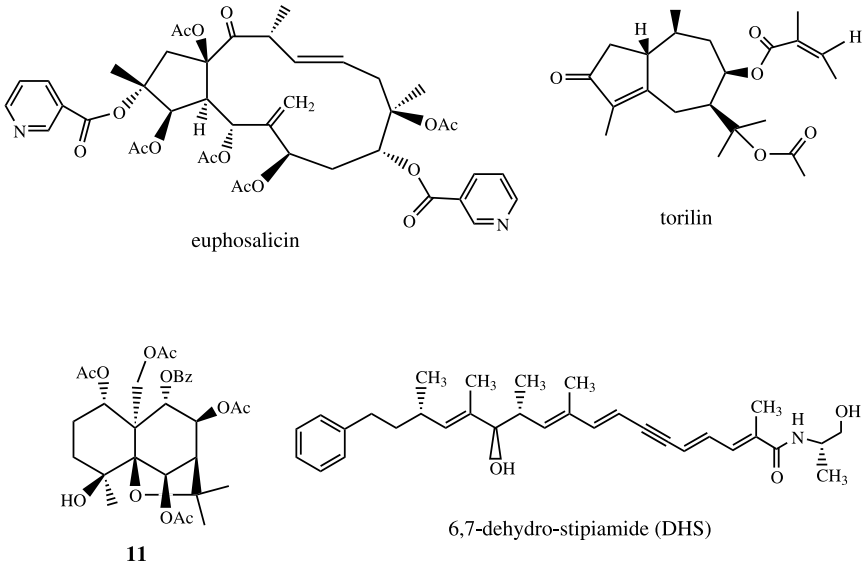


Fig. 13. Terpenes.

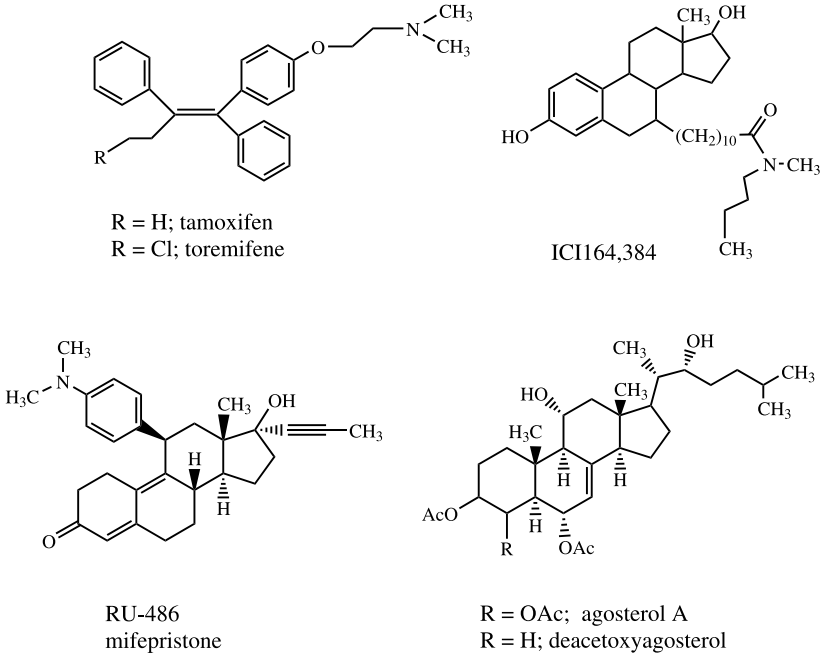


Fig. 14. Steroid-related compounds.

6.14. Oligonucleotides

Antisense oligonucleotides can reduce the level of gene message and inhibit the expression of transporting proteins. As a consequence, the activity of Pgp, as well as that of MRP1 and sister proteins, can be reduced. Phosphorothioate antisense oligonucleotides have proven effective against both Pgp and MRP1 transporters [265–267]. Recently it has been shown that antisense having a phosphorothioate backbone and a methoxyethoxy group at the 2′ position of the ribose ring, had the greatest potency [268].

6.15. Affinity labels (Fig. 15)

Knowledge of the binding site of protein transporters is critical not only for understanding how drugs interact with it, but also for designing better and more specific inhibitors. Therefore, potent and selective ligands that, after labelling, can establish covalent bonds with the transporter protein molecule are particularly useful [269]. The subject has been reviewed [270,271]. Verapamil [272,273] and dihydropyridine [274,275] analogues like **12** and azidopine are the most popular, but representatives of other known chemosensitisers

[276–279] like **13** and IAAQ have also been proposed. Frequently, they are photoactivable molecules carrying an azido group, but also mustard and aziridine derivatives like *trans* flupentixol mustard and ^{3}H TAMA [280,281] have been studied. The vast majority of them are labelled with ^{3}H or an equivalent radioisotope. However, biotinylated compounds [282], like EDP 141 [283] are also useful.

6.16. Others (Fig. 16)

As shown in Table 1, a variety of molecular structures present various degrees of MDR reversing activity. Some of them that have been studied in more detail than most of those reported in the table and that cannot be included in the families described above will be described here. The most studied compound of this heterogeneous class is VX-710 (biricodar, incel) that is more effective than verapamil, is active also on MRP1-expressing cells, produces minimal toxicity and has reached phase I clinical trials [284–286]. A glutathione derivative, GIF-0019, has been reported to restore the cellular sensitivity of MRP1-overexpressing drug-resistant cancer cells [287,288]. In this group some protein kinase C modulators that affect MDR can be examined. Among them, derivatives of staurosporine (Table

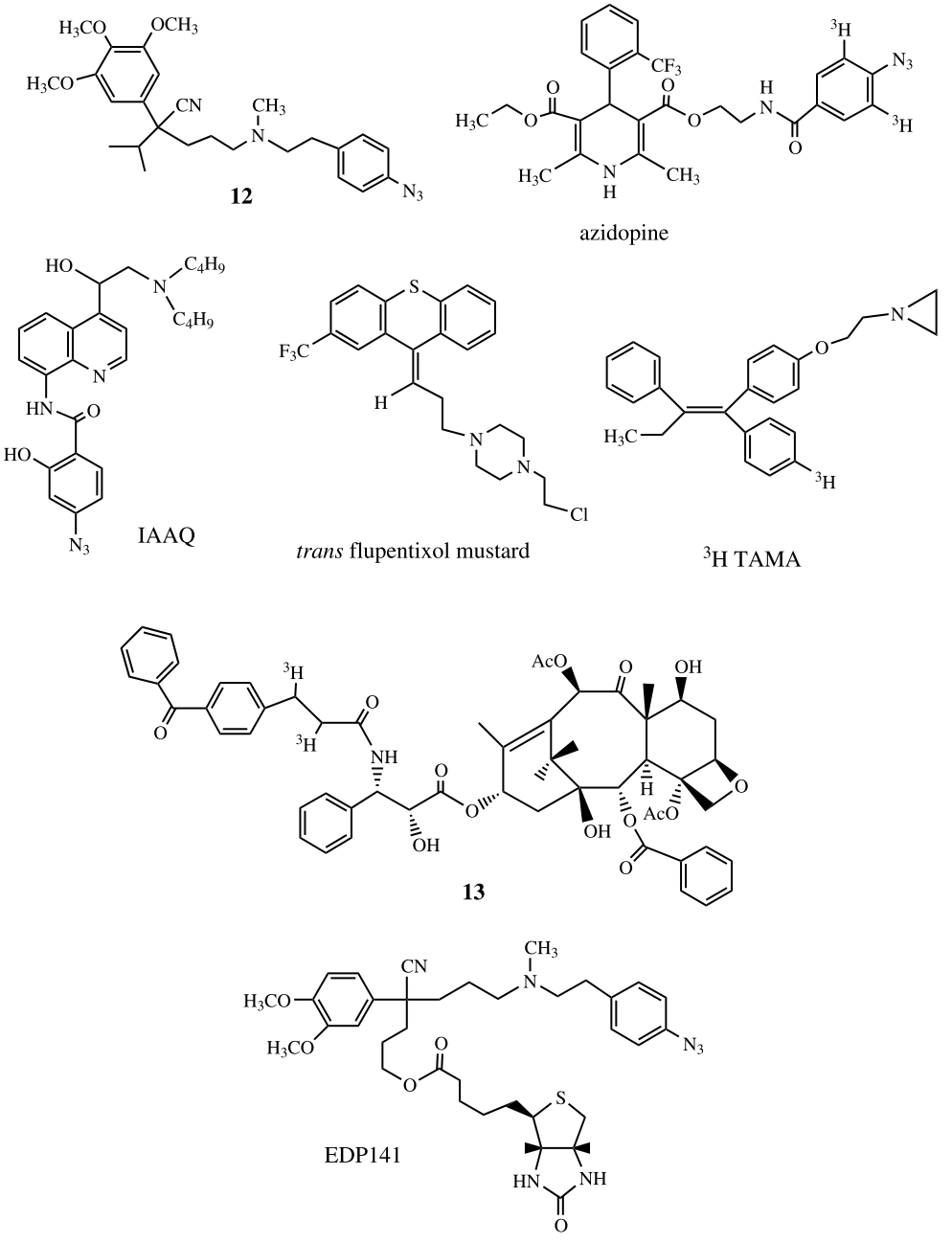


Fig. 15. Affinity labels.

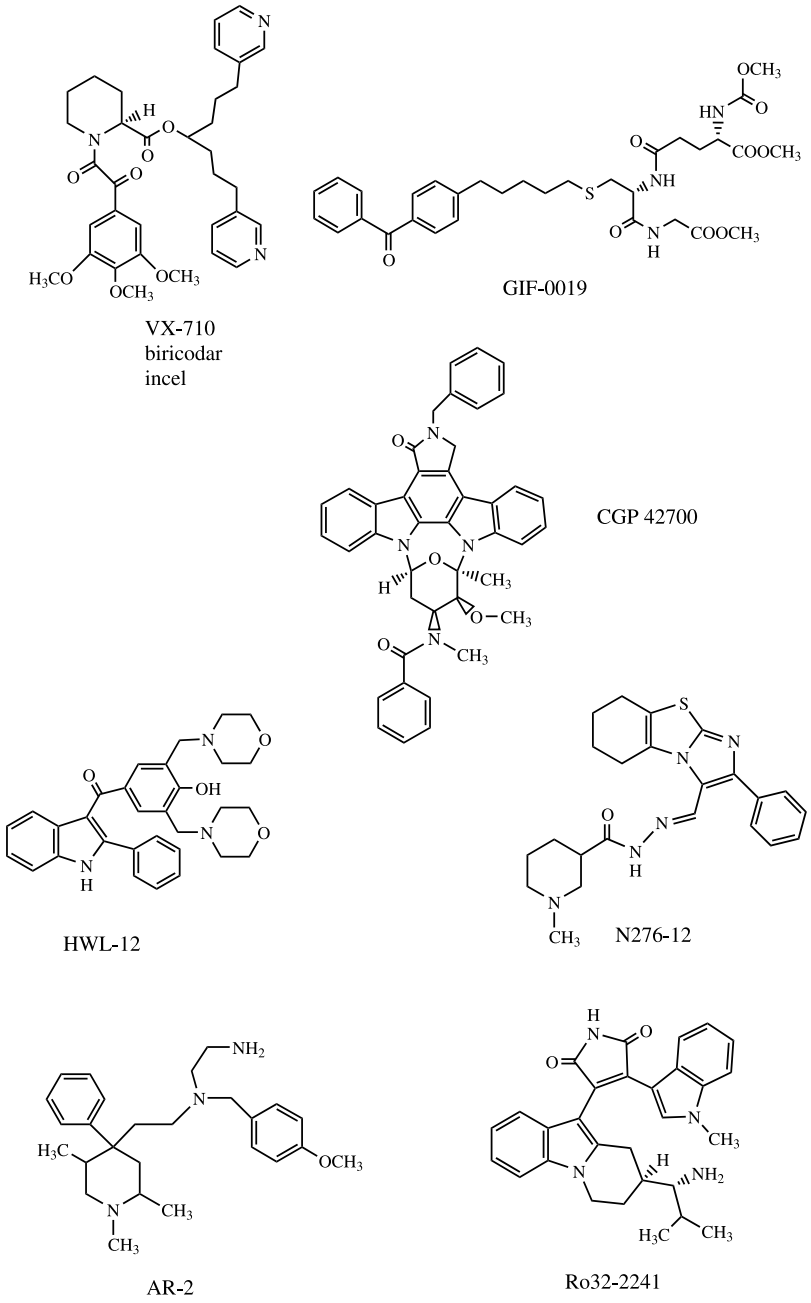


Fig. 16. Others.

1) like CGP 42700 seem most promising [74]. As already mentioned, the activity of these compounds does seem independent from their PKC activity as they interact directly with the carrier protein. The other compounds shown in Fig. 16, HWL-12 [289], N276-12 [290], AR-2 [291] and Ro32-2241 [292] are claimed to be endowed with interesting activity, but no further studies have appeared so far. Finally, it has to be mentioned that some well-known compounds frequently used in formulation, like the fatty acid ester surfactant cremophor EL [293,294], salutol HS15 [295], triton-X-160 and thesit [35] have shown unexpected and interesting MDR modulating properties.

7. Structure–activity relationships

The highly heterogeneous chemical structure of the compounds found active in reversing MDR makes it a formidable task to establish sound structure–activity relationships. The problem is complicated for several reasons. First, as stated before, acquired resistance can

be due to several mechanisms of action and MDR itself can be overcome in different ways [35,139,296]. More over, even classical MDR can be due to different carrier proteins (Pgp, MRP, LRP, and BCRP), each with its peculiar mechanism of action and probably different interacting sites. Second, MDR reversers can act by a direct interaction with the carrier proteins at the substrate or at the nucleotide binding site. Alternatively, they may act by indirect mechanisms, such as altering the membrane functionality and the partitioning of the chemotherapic, or inhibiting phosphorylation of the extruding proteins by protein kinase C. Third, MDR reversers might modulate gene expression, as has been reported for verapamil, doxorubicin and mitomycin C for mdr1 [297,298].

As a consequence, MDR reversion appears to be a complex process for which, at the moment, there is no precise molecular-level description. In general, for most MDR reversers the selectivity toward the extruding proteins has not been specifically studied and is largely unknown. There are few compounds that are reported to be selective for Pgp such as LY 335979 [177] and SDZ-PSC 833 [209] or for MRP, such as genistein [119] and MK571 [3]. Some MDR-reversing agents are able to inhibit the activity of both Pgp and MRP1 proteins, but in that case high concentrations are required for MRP1 inhibition. In this respect, it is interesting to note that recently it has been suggested that specific inhibition of Pgp would be preferable from a clinical point of view [200]. Moreover, the broad substrate selectivity of extruding pumps, in particular that of Pgp, suggests that there might be more than a single binding site and it is possible that the same compound has more than one mechanism of action. In fact, it has been observed that Pgp antagonists fall into two groups: those that are transported themselves and those that are not, even if overall, the transport of Pgp antagonists is quite poor [299]. The reasons why inhibitors can be found in both classes are not completely understood at the moment and have been thoroughly discussed by Litman [33].

Therefore it is not surprising that the efforts to establish structure–activity relationships for MDR reversers have been somewhat frustrating. As a matter of fact, the following discussion will refer mainly to Pgp-dependent MDR reversers where, on the other hand, sound SARs have been established only for compounds that are members of closely related families.

Several attempts to establish general SARs [300–307] for the most important classes of compounds showing MDR reversing activity have indeed reached the rather vague conclusion that activity is normally present in lipophilic compounds containing a hydrophilic *N*-alkyl group that is protonated at physiological pH. Such drugs are often characterised by a two-ring structure linked by a single alkyl bridge to a secondary or tertiary amino group. However, recently in a series of propafenone derivatives, the role of the basic nitrogen atom, present in almost all MDR modulators, has been questioned. It was proposed that it does not interact with Pgp in a charged form but functions as an electron donor group, which can be replaced by other hydrogen bond acceptors [308].

A large number of different compounds was taken into account by Ramu [107] and Klopman [309] but the conclusions reached did not differ substantially from those found previously. As a matter of fact, significant relationships of MDR reversing activity with lipophilicity have been obtained for some classes of compounds [101,229,233], while in other cases such a correlation failed to show up [159,160], even if a trend suggesting a role for lipophilicity was detectable. As previously mentioned, Pajeva and Wiese [227], using a CoMFA approach on phenothiazines and related drugs, obtained the best models either with hydrophobic fields alone or in combination with steric and electrostatic fields, pointing to hydrophobicity as a property of primary importance. In this respect hydrophobic fields seem more appropriate descriptors than log P or Log D. On the other hand, other factors besides lipophilicity seem to control inhibitors interaction with Pgp. In fact, adding hydrogen bond acceptor strength and steric parameters to lipophilicity descriptors led to remarkably increased predictivity of the QSAR equation [233]. Moreover, it was advanced that relative inhibitory potency of Pgp antagonist could be predicted based upon the surface area or volume of the compound [310], a conclusion substantially in agreement with the findings of Litman et al. [311]. On the contrary, chirality does not seem a critical issue in MDR modulators [152,235,311,312]. Apparently, the chirality of the molecule does not play a role in the case of pump substrates also, since daunorubicin and its enantiomer WP900 were found to be transported in the same way both by Pgp and MRP1 proteins [313].

Very recently, Wiese and Pajeva have thoroughly reviewed SAR and QSAR studies on MDR inhibitors [44]. Again, while their precious work identified a number of relevant structural parameters for MDR inhibitors, it hardly provided a generally valid indication. Perhaps the derivation of structure–activity relationships for chemosensitisers should be restricted to chemically related compounds, carefully checking that MDR reversion is due to the same mechanism, relinquishing the efforts to establish general rules.

8. Problems related to the therapeutic use of multidrug resistance modulators

Several chemosensitisers found active in culture cells have reached clinical trials but only a few are in devel-

opment. Among the first generation MDR-reversing drugs, verapamil and dexverapamil have been the most studied, but quinidine, trifluoroperazine, tamoxifene, cyclosporin and a few others have also been tested [168]. The severe problems connected with the development of MDR modulators have been reviewed [168,293,314,315].

Unfortunately, the results of most early clinical studies have been inconclusive and rather disappointing so that the debate among clinicians supporting the use of chemosensitisers and those not yet convinced of their utility is still ongoing [293,315,316]. There are a number of possible explanations for the predominantly negative therapeutic outcomes in in vivo studies: the small number of patients involved in some trials; the inclusion of patients with unknown Pgp or MRP expression; the low doses used because of toxic side effects and the poor pharmacokinetics of several drugs. To overcome some of these problems the use of synergic combinations of chemosensitisers, that would permit a reduction of doses, has been proposed [317]. Nevertheless, despite the disappointing results of many early clinical trials, the idea that chemosensitisers with proper pharmacokinetics, high potency and reduced toxicity hold promise for a positive solution of MDR problem has gained consensus.

However, there are some aspects connected with the therapeutic use of chemosensitisers that need to be discussed in more detail [106,293].

One is related to the presence of Pgp and MRP in many non-tumoural human tissues. Tampering with the function of "normal" extrusion proteins present in healthy tissues might lead to disruption of fundamental physiological functions and to high levels of toxicity from naturally occurring substrates, xenobiotics as well as the drugs used in chemotherapy. Accordingly, proper functioning of biological structure such as the blood–brain barrier could be impaired, leading to unacceptable toxicity. At the same time, Pgp impairment could be therapeutically useful to improve drug distribution. For instance, inhibition of Pgp has been proposed to increase HIV protease concentrations in the brain, foetus and in other tissues and cells expressing Pgp [318,319].

A reason for further concern is the influence of Pgp and MRP1 modulation on the pharmacokinetics (and then efficiency and/or toxicity) of other drugs [320], among which is the chemotherapic itself. Many clinical trials with MDR reversers have evidenced a decreased systemic clearance of the anticancer drug, calling for a dose reduction of the chemotherapic to prevent exacerbated toxicity. Fortunately, in recent years, drugs endowed with high potency, such as R101933, LY 335979 and GF 120918 (Fig. 4) which appear to lack significant kinetic interactions, have been disclosed [293]. These chemosensitisers do not necessitate dose reduction of the chemotherapic and appear more promising as medicines. Carefully designed clinical trials are necessary to evaluate the incidence of such problems on the eventual use of MDR modulators.

Another important problem arises from the vast array of compounds that seem able to interact with extrusion pumps. In Table 1 are reported a few representative compounds that have shown MDR reversing properties. Some of them are widely used drugs. It is likely that several food components and currently used drugs would share similar properties [321]. As a consequence, it can be expected that food regimens and medical treatment of patients with several drugs simultaneously may result in modulation of the naturally expressed carrier proteins. This may adversely affect the natural functions of the pumps and might cause side effects by drug–drug or drug–food interactions.

On the other hand, it is conceivable that exalting the physiological action of carrier proteins would prevent, by extrusion of toxic molecules from healthy cells, the insurgence of tumours [322]. Building on this idea, Phang et al. have reported the Pgp stimulatory effect of some flavonols, like quercetin, galangin and the like, which are widely distributed in fruits and vegetables and would be able to increase the efflux of 7,12-dimethylbenz(*a*)antracene in MDR breast cancer cells [323]. However, opposing results indicating these compounds as Pgp antagonists have been reported [117]. Most likely, flavonoids should be considered individually rather than as a class of compounds because their effects on protein pumps, namely MRP, are variable [324]. Very recently, Kondratov et al. [325] have identified several small synthetic molecules able to activate the functions of Pgp and that could be useful to facilitate chemoprotection of cells and tissues from a variety of cytotoxic drugs.

9. Conclusions

Progress in knowledge of the mechanisms of multidrug resistance and in the molecular biology of extruding proteins has clarified several aspects of MDR since its discovery. Nevertheless, although the first drug able to reverse MDR was discovered more than 20 years ago, a potent, specific and safe drug, able to reverse multidrug resistance and to assist in the chemotherapy of cancer is still lacking. However, several drug candidates presently under development look very promising and some of them, such as valspodar (Fig. 6), biricodar (Fig. 16) and LY 335979 (Fig. 4), are in a good position to gain approval in the near future.

The variety of chemical structures that can modulate MDR has been a major difficulty, as have been the generally modest potency and high toxicity of the molecules tested. It is obvious that potent and safer

drugs are badly needed to definitely establish the practical value of inhibiting extruding pumps to fight resistance in cancer. A few compounds that seem to fulfil these characteristics are being evaluated, but a further effort by medicinal chemists appears necessary to shed more light on the problem.

Besides the efforts to control tumours, modulation of extruding pumps seems to offer other interesting opportunities and could represent a new option for the future. The recent disclosure [326] of the involvement of Pgp in the efflux of β-amyloid, a putative cause of Alzheimer's disease, is an example of the exciting perspectives opened by the modulation of the physiological functions of Pgp and similar carrier proteins.

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