Drugs of the Future 2003, 28(2): 125-136

CCC: 0377-8282/2003

Zosuquidar Trihydrochloride

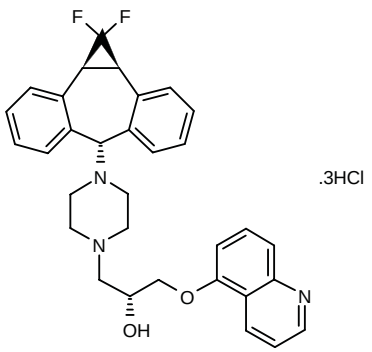
Prop INNM; USAN

Multidrug Resistance Modulator
P-Glycoprotein (MDR-1) Inhibitor

LY-335979
RS-33295-198

(1a*R*,6*S*,10b*S*)-1,1-Difluoro-6-[4-[2(*R*)-hydroxy-3-(quinolin-5-yloxy)propyl]piperazin-1-yl]-1,1a,6,10b-tetrahydrodibenzo[*a*,*e*]cyclopropa[c]cycloheptene trihydrochloride

1-[4-(1,1-Difluoro-1,1a*R*,6*S*,10b*S*-tetrahydrodibenzo[*a*,*e*]cyclopropa[*c*]cyclohepten-6-yl)piperazin-1-yl]-3-(quinolin-5-yloxy)-2(*R*)-propanol trihydrochloride



$C_{32}H_{31}F_2N_3O_2.3HCl$

Mol wt: 636.9946

CAS: 167465-36-3

CAS: 167354-41-8 (as free base)

CAS: 474276-97-6 (as hydrochloride)

CAS: 312905-17-2 (as monohydrate)

EN: 222494

Abstract

The plasma membrane glycoprotein, P-glycoprotein (Pgp), is thought to be the major cause of clinical resistance to certain structurally unrelated oncolytics observed in several human tumor types. Class I Pgps are overexpressed in several cancers and appear to be responsible for the energy-dependent efflux of several oncolytics.The search for small-molecule inhibitors of Pgp drug efflux activity led to first-generation agents which have shown little clinical efficacy due to dose-limiting toxicities and pharmacokinetic interactions with other drugs. Second- and third-generation Pgp modulators have a higher affinity for Pgp but lack other pharmacological activity. Of these novel compounds, zosuquidar trihydrochloride was identified as a potent inhibitor of Pgp function in multidrug resistant (MDR) cells and was selected for further development.

Synthesis

Zosuquidar can be prepared by three related ways:

1) Condensation of dibenzosuberenone (I) with sodium 2-chloro-2,2-difluoroacetate (II) in diglyme at 165 °C gives 10,11-(difluoromethano)benzosuberone (III), which is reduced with $NaBH_4$ in THF/methanol to yield the corresponding *syn*-alcohol (IV). Reaction of alcohol (IV) with hot $SOCl_2$ affords a mixture of the *syn*- and *anti*-chloro derivatives (V). This mixture (V) is treated with 1-formylpiperazine (VI) in refluxing acetonitrile to provide a mixture of *syn*- and *anti*-4-[10,11-(difluoromethano)dibenzosuber-5-yl]piperazine-1-carbaldehyde (VI), which is separated by chromatography. The desired *anti*-isomer (VII) is then treated with KOH in refluxing ethanol/water to give the piperazine derivative (VIII), which is finally condensed with 5-[2(*R*),3-epoxypropoxy]quinoline (IX) in refluxing isopranol (1, 2). Scheme 1.

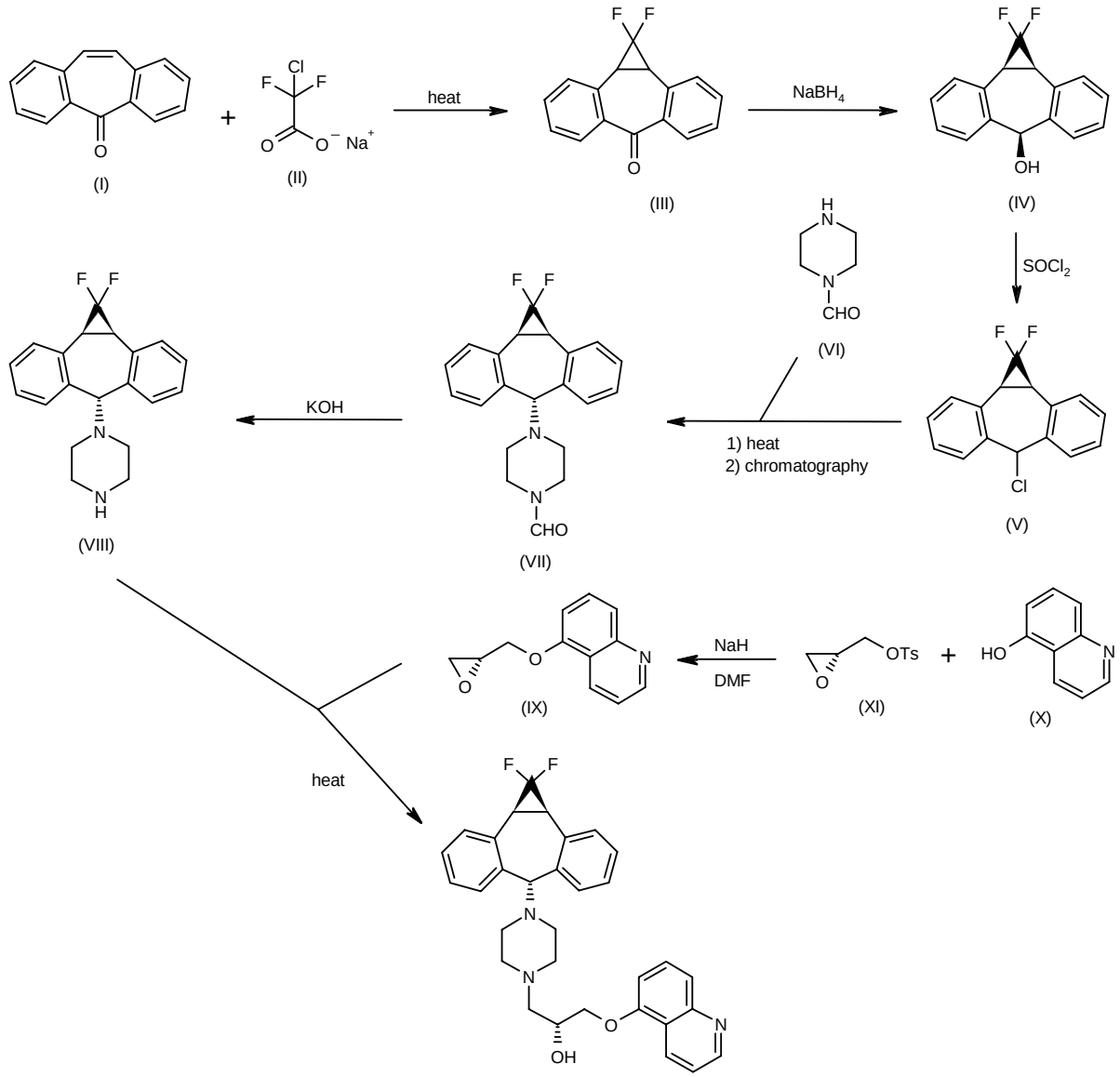
5-[2(*R*),3-Epoxypropoxy]quinoline (IX) is obtained by reaction of 5-hydroxyquinoline (X) with (*R*)-glycidyl tosylate (XI) by means of NaH in DMF (3). Scheme 1.

2) Reaction of alcohol (IV) with 48% HBr or with PBr_3 affords the *anti*-bromo derivative (XII), which is condensed with pyrazine (XIII) in refluxing acetonitrile to give *anti*-1-[10,11-(difluoromethano)dibenzosuber-5-yl]-pyrazinium bromide (XIV). Reduction of compound (XIV) with $NaBH_4$ in ethyl acetate provides the piperazine derivative (VIII), which is finally condensed with 5-[2(*R*),3-epoxypropoxy]quinoline (IX) – obtained by reaction of 5-hydroxyquinoline (X) with (*R*)-glycidyl 4-nitrobenzenesulfonate (XV) by means of K_2CO_3 in DMF – in hot ethanol (4). Scheme 2.

3) Condensation of the *anti*-bromo derivative (XII) with piperazine (XVI) in refluxing acetonitrile provides 1-[10,11-(difluoromethano)dibenzosuber-5-yl]piperazine as a mixture of *syn*- and *anti*-isomers (XVII), which is separated by crystallization. Finally, the desired *anti*-isomer (VIII) is condensed with 5-[2(*R*),3-epoxypropoxy]-quinoline (IX) in hot ethanol (5). Scheme 3.

L.A. Sorbera, J. Castañer, J.S. Silvestre, M. Bayés. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of Zosuquidar

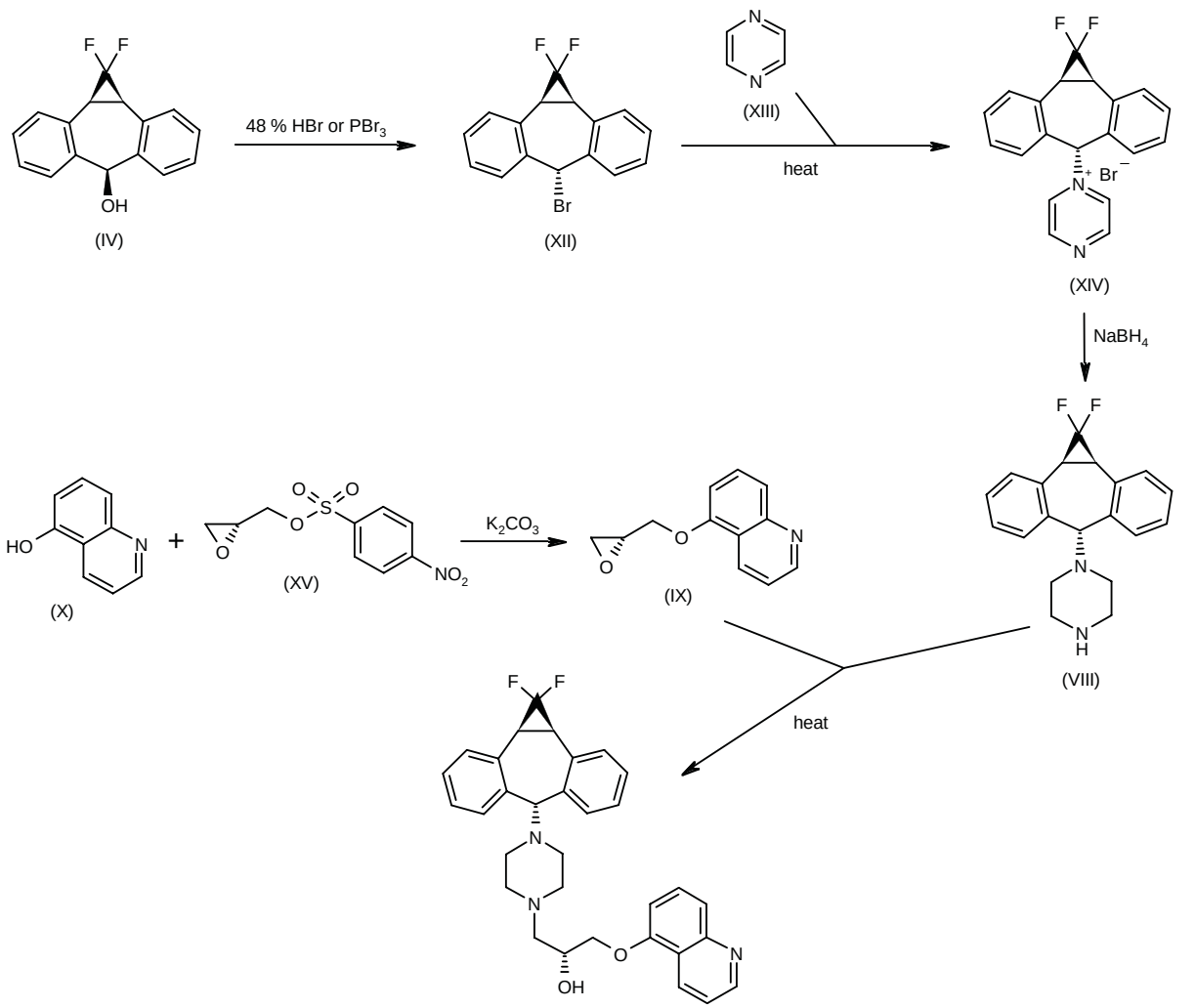


Introduction

Functionally, two different types of P-glycoprotein (Pgp) have been described, but only class I Pgps have been related to drug transport, and their overexpression confers the multidrug resistance (MDR) phenotype in tumoral cells. Class I and II Pgps are a family of plasma membrane glycoproteins (140-180 kDa; class I and II). Pgps were identified when researchers observed that overexpression of certain class I Pgps conferred MDR in several tumor cell types. In humans, class I and II Pgps are encoded by MDR1 and MDR2 genes, respectively, while in mice, the mdr1a and mdr1b genes encode class I Pgps and the mdr2 gene encodes class II Pgps (6-8).

Class I Pgps are normally expressed in several tissue types, although their physiological function and mechanism of action remain to be elucidated. It has been suggested that one function of Pgp is to exclude xenobiotics since Pgp has been shown to be expressed in intestinal, renal and hepatic epithelial cells and in the endothelial cells of the blood-brain barrier. This cannot be the sole function of Pgp due to the fact that high expression is also detected in the adrenal glands and on the apical surface of the choroid plexus epithelial cells (9-12). Class I Pgps have also been shown to be able to translocate short

Scheme 2: Synthesis of Zosuquidar

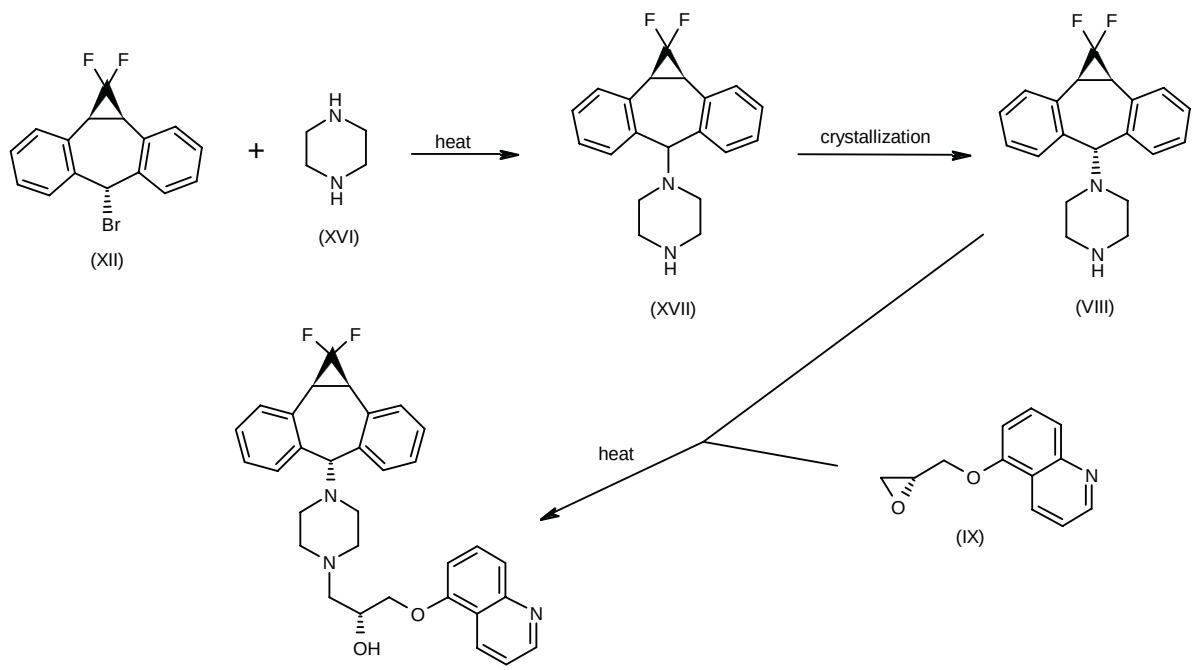


chain analogs of phospholipids from the inner to the outer leaflet of the plasma membrane prompting the hypothesis that Pgp acts as a flippase for phospholipids, particularly for endogenous phosphatidylcholine and sphingomyelin (13-15). An additional function attributed to class I Pgps involves trafficking of sterols within cells and esterification of plasma membrane cholesterol (16, 17).

The function and mechanism of action of Pgp continue to be investigated. It is clear however that the resistance of several tumor types to certain oncolytics is associated with overexpression of class I Pgps. Overexpression of the MDR1 gene has been detected at the time of diagnosis of kidney, colon and adrenocortical cancer, for example, or after relapse and/or treatment failure of breast cancer, lymphoma and leukemia. Class I Pgps have been shown to be responsible for the energy-dependent efflux of several structurally unrelated natural oncolytics such as vinblastine, doxorubicin, etoposide and taxol. Pgp acts as a transporter of the drugs, effluxing them, reducing intracellular drug accumulation thus allowing tumor cells to survive cytotoxic concentrations of drugs (18). However, in 1981, Tsuro *et al.* observed that verapamil, a noncytotoxic calcium channel blocker, could overcome the MDR phenotype to vincristine and vinblastine (19). The search for noncytotoxic modulators that can sensitize MDR cells to oncolytics became a research priority.

Several classes of Pgp modulators have been identified. First-generation agents included compounds developed for other indications, such as the calcium channel blocker verapamil, immunosuppressive agents (*e.g.*, ciclosporin, FK-506), analogues of the antihypertensives reserpine and yohimbine, the neuroleptic trifluoperazine and antiestrogens (tamoxifen, toremifene). Unfortunately, Pgp modulation by these compounds has shown limited efficacy in the clinic due to several reasons, including the

Scheme 3: Synthesis of Zosuquidar



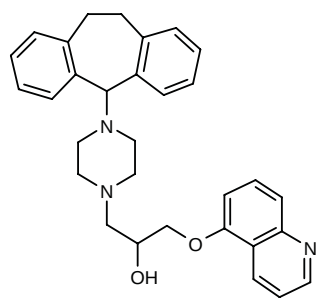
failure to achieve concentrations of the modulators high enough to inhibit Pgp, dose-limiting toxicities and marked interactions with coadministered chemotherapeutic agents (20-22). Second- and third-generation agents were subsequently synthesized displaying a higher affinity for Pgp and lacking the additional pharmacological activity of the original compounds (see Table I). A comprehensive review has recently been published in this regard (23).

However, continued research has identified other Pgp modulators which hold promise for efficacy in the clinic. The most potent modulator to emerge thus far is zosuquidar trihydrochloride (LY-335979; RS-33295-198), a derivative of MS-073 [I] containing a difluorocyclopropyl substitution in the dibenzosuberane moiety resulting in enhanced activity. The agent has no other pharmacological effects and a high affinity for Pgp. Zosuquidar has displayed efficacy *in vitro* and *in vivo* and was selected for further development to circumvent MDR in human tumors (1, 24-26).

Pharmacological Actions

Several *in vitro* studies have demonstrated that zosuquidar selectively and potently modulates Pgp-mediated drug resistance, increasing the sensitivity of MDR cells to anticancer agents.

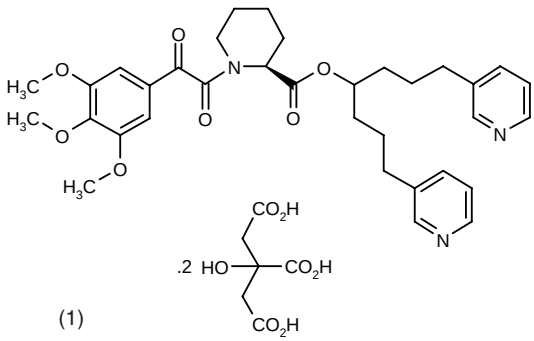
A study using MDR CHO cells (CH^RC5), KB-V1 cells (a HeLa cell derivative displaying vinca alkaloid resistance), MDR human uterine sarcoma cells (MES-SA/Dx5) and MDR murine leukemia cell lines (P388/ADR and P388/VCR), demonstrated in an MTT assay that zosuquidar enhanced the antiproliferative activity of doxorubicin, vincristine, etoposide and paclitaxel. The EC_{50} values for zosuquidar to increase the sensitivity of all cell types to doxorubicin ranged from 10-100 nM. In addition, zosuquidar was shown to dose-dependently increase doxorubicin (2 μg/ml) levels in CH^RC5 cells with a maximal efficacy observed at concentrations between 100-300 nM (24). Similarly, zosuquidar at a concentration of 0.1 μM was shown to restore sensitivity of



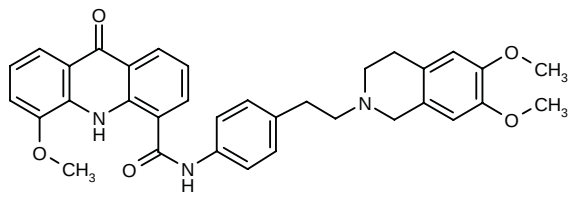
[I]

Table I: P-Glycoprotein (MDR-1) inhibitors under active development (from Prous Science Integrity®).

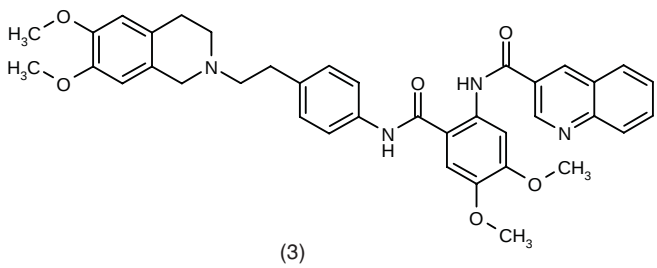
Drug	Source	Status
1. Biricodar Dicitrate	Vertex	Phase II
2. Elacridar	GlaxoSmithKline	Phase I
3. Tariquidar	Xenova/QLT Phototherapeutics	Phase III
4. Zosuquidar HCl	Lilly	Phase III



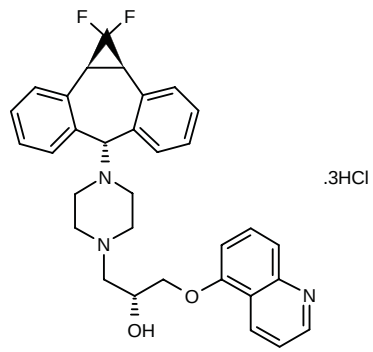
(1)



(2)



(3)



(4)

MDR CEM/VLB100 cells to vinblastine, doxorubicin, etoposide and paclitaxel so that the IC_{50} values for the agent were shiifted 440-, 13-, 19- and 1200-fold, respectively. Zosuquidar was also effective in enhancing the sensitivity of the following resistant cells to antitumor agents: P388/ADR and MCF-7/ADR to vinblastine, doxorubicin and taxol; human ovarian carcinoma (2780AD) to doxorubicin; and human non-small cell lung carcinoma (UCLA-P3.003VLB) to taxol. The drug-sensitive parental cells were unaffected by treatment with zosuquidar. Experiments using CEM/VLB100 plasma membranes showed that zosuquidar binds to Pgp in the presence of ATP, by competitively displacing [^{3}H]-vinblastine (40 nM) with an apparent K_i value of 0.059 μM, and inhibited [^{3}H]-azidopine photoaffinity labeling of the M_r 170,000 Pgp. Further data suggest that zosuquidar does not serve as a substrate for the Pgp pump indicating that the agent may have a long duration of action. Similar results were obtained in another study (27, 28).

The selectivity of zosuquidar to modulate the Pgp-expressing cells was demonstrated in an *in vitro* study in which the agent (1 μM) was shown to have no effect on drug cytotoxicity in HL60/ADR cells (MDR due to overexpression of multidrug resistance-associated protein [MRP1] and not Pgp) and HeLa-T5 cells transfected with MRP1 (resistant to doxorubicin and vincristine). Leukotriene C_4 (an MRP1 and MRP2 substrate) uptake into MRP1-transfected HeLa-T5 and MRP2-transfected MDCK cell membranes was unaffected by the agent. However, the agent (0.01 μM) enhanced the cytotoxicity of doxorubicin to a Pgp-overexpressing HL60/Vinc cell line (MDR due to overexpression of Pgp and not MRP1). Results indicate that zosuquidar selectively modulates Pgp-mediated resistance with no effects on MRP1 or MRP2 (28).

The ability of zosuquidar to modulate Pgp-mediated MDR was also demonstrated in a study using MDR human acute myeloid leukemia cells with Pgp^+/MRP^- (KG1a/200, K562/150) and Pgp^-/MRP^+ (HL60/130) phenotypes and shown to be significantly resistant to daunorubicin. The agent was found to significantly sensitize Pgp^+/MRP^- cells to daunorubicin-induced growth inhibition and increase daunorubicin accumulation. In contrast, the agent had no effect on daunorubicin accumulation or cytotoxicity in Pgp^-/MRP^+ cells (29, 30).

Zosuquidar was not only not ineffective against MRP1-mediated resistance but also had no influence on breast cancer resistant protein (BCRP)-mediated drug resistance. Although zosuquidar (0.5 μM) sensitized Pgp-expressing HL60/Vinc cells to mitoxantrone and vinorelbine, it did not influence drug resistance of MRP1-expressing HL60/ADR or BCRP-transfected MCF-7 cells even at concentrations of 5 μM. In addition, while [^{125}I]-iodomycin photolabeling of Pgp in CEM/VLB100

Table II: In vitro *effects of zosuquidar and other P-glycoprotein (MDR-1) inhibitors under development (data from Prous Science Integrity®).*

Drug	Pgp affinity[a] K_i [IC_{50}] (μM)	Pgp inhibition[d] IC_{50} (μM)
Biricodar	[0.55][b] (48)	2.50[e] (48)
Ciclosporin*	[1.70]-[3.50][b] (48, 49)	0.44[f] (49)
Elacridar	0.0014-0.005[c] (50, 51)	0.048[f] (49)
Tariquidar	0.0026[c] (50)	0.033-0.038[e,f] (49, 52)
Zosuquidar	0.060[c] (27)	0.0067-0.020[e,f] (32)

*Included for comparison. [a]P-glycoprotein affinity evaluated in different mutlidrug-resistant (MDR) cells by displacement of [b][^{3}H]-azidopine or [c][^{3}H]-vinblastine. [d]P-glycoprotein inhibition evaluated in MDR cells by means of [e]rhodamine or [f]daunorubicin accumulation assay. References in parentheses.

membranes was inhibited by approximately 70% by zosuquidar (5 μM), photolabeling of MRP1 and BCRP in membrane preparations of MRP1-overexpressing H69AR and BCRP-transfected MCF-7 cells, respectively, was unaffected by treatment (31).

An *in vitro* study using both MDR cell lines and human natural killer (NK) cells further characterized the effects of zosuquidar on Pgp function. Results suggest that the agent is the most potent Pgp inhibitor described to date. Zosuquidar was 500-1500 times more potent than ciclosporin or verapamil in restoring Pgp substrate (rhodamine 123 [Rh123] and daunorubicin) accumulation in vincristine-resistant HL60/VCR cells with IC_{50} values of 6.7 and 20 nM, respectively. Moreover, the agent suppressed Pgp function (indicated by Rh123 accumulation) on isolated $CD56^+$ lymphocytes (IC_{50} = 1.18 ± 0.2 nM) and on $CD56^+$ lymphocytes present in human whole blood (IC_{50} = 174 ± 1.61 nM) (32). Table II shows the *in vitro* Pgp affinity and Pgp inhibition of zosuquidar compared with other selected Pgp multidrug-resistance modulators.

The efficacy of zosuquidar to enhance MDR tumor cells was also demonstrated in several studies using murine xenograft models *in vivo*.

Treatment of nude mice implanted with P388/ADR murine leukemia cells with zosuquidar (1, 3, 10, 20 or 30 mg/kg i.v. once daily for 5 days starting on implantation day) and coadministered doxorubicin (1, 2 or 4 mg/kg i.p. 30 min later), etoposide (20 mg/kg i.p. 30 min later) or vincristine (0.1 mg/kg i.p.), resulted in a significant increase in survival rate as compared to controls. Zosuquidar did not significantly alter the pharmacokinetics of doxorubicin or etoposide in this model (24, 27, 33).

Zosuquidar (30 mg/kg i.p. starting 5 days after implantation) also potentiated the antitumor activity of paclitaxel (20 mg/kg i.v. 1 h after zosuquidar) in experiments using nude mice bearing MDR Pgp-expressing human non-small cell lung carcinoma (UCLA-P3.003VLB) xenografts with significant suppression of tumor growth observed on days 12 and 19. The pharmacokinetics of paclitaxel were not significantly altered by zosuquidar coadministration (27, 33).

Significant delays in tumor growth were observed in nude mice bearing established s.c. MDR human sarcoma (MES-SA/Dx5) tumors and coadministered zosuquidar (30 mg/kg i.p. on days 4 and 8 after implantation) and doxorubicin (7.5 mg/kg i.v. on days 4 and 8 after implantation) (24).

Metabolism and Pharmacokinetics

Cytochrome P-450 (CYP) isozymes are involved in the oxidative metabolism and therefore elimination of many oncolytics. Many Pgp substrates interact with CYP3A and it has been reported that there is an overlap between the substrate specificities of Pgp and CYP3A (34). Thus, it is possible that a Pgp modulator may affect the activity of CYPs which could lead to drug-drug interactions. In this regard a study using human microsome preparations examined the ability of zosuquidar to inhibit the catalytic activities of the following CYP enzymes known to be involved in the metabolism of natural product oncolytics: CYP3A (midazolam 1'-hydroxylation), CYP2C9 (diclofenac 4'-hydroxylation), CYP2D6 (bufuralol 1'-hydroxylation) and CYP1A2 (phenacetin O-deethylation). Zosuquidar was found to be a competitive inhibitor of CYP3A (apparent K_i = 3.8 ± 0.8 μM). Inhibition of the other 3 CYPs with the agent was even less. Competitive and noncompetitive inhibition of CYP2C9 and CYP2D6, respectively, were observed with apparent K_i values of 12.3 ± 3.0 and 25.3 ± 2.7 μM obtained, respectively; inhibition of CYP1A2 by zosuquidar was only slight, even at concentrations of 50 μM. Thus, zosuquidar modulates Pgp (K_i = 0.059 μM) more potently (60-fold or more) than any of the CYPs examined. These results suggest that zosuquidar (at doses of 1 μM or less) would have little effect on the pharmacokinetics of coadministered oncolytics *in vivo* (28).

A study using bile from [^{3}H]-zosuquidar (20 mg/kg i.v.)-treated rats in addition to rat, dog, monkey and human liver microsome preparations, examined the metabolism of zosuquidar and identified several novel metabolites. Use of LC/NMR revealed the presence of an N-oxide metabolite (LY-389551) resulting from oxidation of the quinoline nitrogen in microsomes and 3 glucuronide metabolites in rat bile which were conjugates of products from oxidation of the quinoline ring (35).

No drug-related toxicities were observed in a 1-month study involving beagle dogs administered zosuquidar (2, 10 and 100 mg/kg/day p.o.). In animals administered the highest dose, plasma levels of the agent surpassed the K_i value for Pgp inhibition by more than 80 times. When dogs were coadministered zosuquidar (10 or 100 mg/kg/day days 4-16) with vinorelbine (10 mg/m^2 once weekly), no effects were observed on vinorelbine pharmacokinetics or on survival, clinical observations, body weights or food consumption as compared to either treatment alone. Zosuquidar alone was not associated with any hematological changes. One animal receiving 100 mg/kg zosuquidar experienced emesis and fine tremors.

Both zosuquidar and vinorelbine were associated with abnormal stools. Zosuquidar was found to worsen vinorelbine-induced myelosuppression, indicating that suppression of Pgp in normal tissue can potentiate the effects of oncolytics. It was concluded, however, that the myelosuppression observed with coadministration was clinically manageable (36).

Similarly, zosuquidar (10 or 90/45 mg/kg/day continuous 120-h i.v. infusion) had no effect on the pharmacokinetics of doxorubicin (1 mg/kg 20-min i.v. infusion starting after 72 h of zosuquidar) in dogs coadministered the agents. The C_{max} values obtained were 351, 458 and 322 ng/ml and $AUC_{0-\infty}$ values were 115, 142 and 121 ng·h/ml in animals given doxorubicin alone or in combination with 10 or 90/45 mg/kg zosuquidar, respectively. Once again, zosuquidar enhanced doxorubicin-induced myelosuppression (37).

Clinical Studies

The safety and tolerability of zosuquidar (20, 40, 80, 10, 320, 300 or 400 mg/m^2 p.o. every 12 h for 7 doses or every 8 h for 10 doses on days 1-4 of a 21 [cycle 1 only]- or 35-day cycle) in combination with doxorubicin (45, 60 or 75 mg/m^2 30-min i.v. infusion starting 2 h after the AM dose of zosuquidar on day 15 of cycle 1 or day 3 of subsequent cycles) were reported from results of a phase I, dose-escalation study involving 38 patients with advanced nonhematological malignancies. The dose-limiting toxicity (DLT) for zosuquidar was neurotoxicity manifested as cerebellar dysfunction, hallucinations and palinopsia, and the maximum tolerated dose (MTD) was 300 mg/m^2 every 12 h for 4 days. Coadministration of zosuquidar did not affect the pharmacokinetics of doxorubicin or doxorubicin-induced myelosuppression. *Ex vivo* analysis of patient peripheral blood CD56$^+$ NK cells at 72, 73 and 96 h postdosing with zosuquidar revealed that greater Pgp inhibition in NK cells was associated with higher plasma zosuquidar concentrations (38). The results of this study and those that follow are summarized in Table III.

A phase I study involving 7 patients with advanced solid malignancies (sarcoma, melanoma, head and neck and lung) examined the safety and tolerability of zosuquidar (100, 200 or 300 mg/m^2 p.o. b.i.d. on cycle days 1-3) combined with docetaxel (75 mg/m^2 i.v. on days 1-3 alone in cycle 1 and in combination in subsequent cycles). A total of 17 cycles were completed. Reversible grade 3/4 toxicities observed were neutropenia in 11 cycles and myalgia in 2 cycles. Analysis of cycles 1 and 2 suggests that none of the zosuquidar doses enhanced docetaxel-induced neutropenia. To date, 3 patients have stable disease but no responses have been observed. The pharmacokinetics of the agent alone and in combination are being examined and accrual to 300 mg/m^2 zosuquidar is ongoing (39).

The safety, tolerability and efficacy of zosuquidar (100, 200 or 300 mg/m^2 p.o. every 8 or 12 h for 3 days every 28 days starting 1 day before vinorelbine) in combination with vinorelbine (22.5 or 30 mg/m^2 weekly for 3 weeks every 28 days) were investigated in a phase I trial involving patients with advanced solid tumors. Of the 16 patients entered, 14 were evaluable for toxicity and 9 for response; 14 patients have discontinued due to death (1 patient), progressive disease (5 patients), toxicity (4 patients) or symptomatic progression (4 patients). The grade 3/4 toxicities reported were neutropenia, arthralgia, myalgia, fever, constipation, fatigue and stomatitis. Two of the 3 patients receiving 30 mg/m^2 vinorelbine and 200 mg/m^2 zosuquidar (every 8 h) experienced the DLT of grade 4 neutropenia for more than 1 week. Although no responses were reported, 6 patients had stable disease for 2.1-5.3 months. Accrual is ongoing (40).

The safety and tolerability of oral zosuquidar (100, 200, 250 or 300 mg/m^2 t.i.d. for 10 doses) in combination with paclitaxel (175 mg/m^2 single 3-h i.v. infusion on day 2 or 3) were examined in a phase I, dose-escalation, pharmacokinetic study involving 29 patients with solid tumors (ovary, breast, gastric, non-small cell lung and esophagus). The DLT of neurotoxicity was reached at 250 mg/m^2 zosuquidar requiring a reduction in the number of doses from 10 to 7, 5 and 3 for doses of 200, 250 and 300 mg/m^2, respectively. The toxicities observed related to zosuquidar were hallucinations (grade 3 observed at the highest dose in 3 patients) and tremor (grade 2 appeared to be related to duration of zosuquidar treatment); moderate paclitaxel-related toxicities were also observed. It was concluded that combination treatment was well tolerated. Two patients with ovarian cancer and 1 patient with breast cancer had partial remissions. The AUC, C_{max} or $t_{1/2}$ values for paclitaxel were not altered by zosuquidar coadministration in most patients with the exception of the high paclitaxel AUC values seen in 3 of 8 patients receiving 3 doses of 300 mg/m^2 zosuquidar (38.4 ± 17.6 *vs.* 25.4 ± 7.2 μM·h with paclitaxel alone). The paclitaxel dose has been increased to 225 mg/m^2 (41).

The toxicity and pharmacokinetics of oral zosuquidar (200, 400 or 500 mg every 12 h on days 1 and 2 for 3 doses) in combination with first-line CHOP (starting on day 2 in cycles 1, 3 and subsequent cycles every 3 weeks: 750 mg/m^2 cyclophosphamide, 50 mg/m^2 doxorubicin, 1.4 mg/m^2 vincristine and 100 mg p.o. prednisolone [for 5 days]) were evaluated in a phase I trial involving 11 *de novo* patients with non-Hodgkin's lymphoma. Reported toxicities related to zosuquidar were moderate tremor (4 patients), moderate ataxia (1 patient) and mild dysphagia (1 patient); minor to moderate CHOP-related toxicities were also seen. Grade 3 constipation was reported in 1 patient receiving 400 mg/m^2 zosuquidar in cycle 1 requiring a 50% reduction in vincristine dose in subsequent cycles. No significant pharmacokinetic interactions were detected with vincristine and doxorubicin combined with zosuquidar (42).

A phase I, dose-ranging trial involving 12 patients with acute myeloid leukemia (AML; median WBC = 3.5 x 10^9/l) is examining the efficacy and pharmacodynamics of

Table III: Cinical studies of zosuquidar (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Cancer	Open	Zosuquidar, 20 mg/m^2 po bid x 7 doses + Doxorubicin, 45 mg/m iv over 30 min on d 15 1x/21 d (n=3) Zosuquidar, 40 mg/m^2 po bid x 7 doses + Doxorubicin, 45 mg/m^2 iv over 30 min on d 15 1x/21 d (n=4) Zosuquidar, 80 mg/m^2 po bid x 7 doses + Doxorubicin, 45 mg/m^2 iv over 30 min on d 15 1x/21 d (n=3) Zosuquidar, 160 mg/m^2 po bid x 7 doses + Doxorubicin, 45 mg/m^2 iv over 30 min on d 15 1x/21 d (n=4) Zosuquidar, 320 mg/m^2 po bid x 7 doses + Doxorubicin, 45 mg/m^2 iv over 30 min on d 15 1x/21 d (n=3) Zosuquidar, 400 mg/m^2 po tid x 10 doses + Doxorubicin, 45 mg/m^2 iv over 30 min on d 15 1x/21 d (n=3) Zosuquidar, 300 mg/m^2 po tid x 10 doses + Doxorubicin, 45 mg/m^2 iv over 30 min on d 15 1x/21 d (n=3) Zosuquidar, 300 mg/m^2 po tid x 10 doses + Doxorubicin, 60mg/m2 iv over 30 min on d 15 1x/21 d (n=3) Zosuquidar, 300 mg/m^2 po tid x 10 doses + Doxorubicin, 75 mg/m^2 i.v. over 30 min on d 15 1x/21 d (n=2) Zosuquidar, 300 mg/m^2 po bid x 7 doses + Doxorubicin, 75 mg/m^2 iv over 30 min on d 15 1x/21 d (n=10)	38	Zosuquidar inhibited P-glycoprotein and enhanced the antitumor activity of doxorubicin with no pharmacokinetic interaction or increase in toxicity. The maximum tolerated dose for zosuquidar was 300 mg/m^2	38
Cancer	Open	Zosuquidar, 100 mg/m^2 po bid on d 1-3 + Docetaxel, 75 mg/m^2 iv Zosuquidar, 200 mg/m^2 po bid on d 1-3 + Docetaxel, 75 mg/m2 iv Zosuquidar, 300 mg/m^2 po bid on d 1-3 + Docetaxel, 75 mg/m^2 iv	7	Zosuquidar plus docetaxel was well tolerated in patients with solid tumors	39
Cancer	Open	Zosuquidar, 100 mg/m^2 po tid on d 2-4 + Vinorelbine, 30 mg/m^2 1/wk 1x/28 d (n=3) Zosuquidar, 200 mg/m^2 po bid on d 2-4 + Vinorelbine, 22.5 mg/m^2 1/wk 1x/28 d (n=7) Zosuquidar, 200 mg/m^2 po tid on d 2-4 + Vinorelbine, 30 mg/m^2 1/wk 1x/28 d (n=3) Zosuquidar, 300 mg/m^2 po bid on d 2-4 + Vinorelbine, 22.5 mg/m^2 1/wk 1x/28 d (n=3)	16	Zosuquidar plus vinorelbine had manageable toxicity, with grade 4 neutropenia being the dose-limiting toxicity at doses of 200 mg/m^2 b.i.d. combined with vinorelbine 30 mg/m^2 once weekly in patients with advanced solid tumors	40
Cancer	Open	Zosuquidar, 100 mg/m^2 po tid x 10 doses + Paclitaxel, 175 mg/m^2 iv over 3 h on d 3 Zosuquidar, 200 mg/m^2 po tid x 7 doses + Paclitaxel, 175 mg/m^2 iv over 3 h on d 3 Zosuquidar, 300 mg/m^2 po tid x 7 doses + Paclitaxel, 175 mg/m^2 iv over 3 h on d 3 Zosuquidar, 300 mg/m^2 po tid x 3 doses + Paclitaxel, 175 mg/m^2 iv over 3 h on d 2 Zosuquidar, 250 mg/m^2 po tid x 3 doses + Paclitaxel, 175 mg/m^2 iv over 3 h on d 2	29	Aside from reversible neurotoxicity, zosuquidar was well tolerated in patients with solid tumors. Combination of zosuquidar with paclitaxel did not show drug interactions	41
Non-Hodgkin's lymphoma	Open	Zosuquidar, 200 mg po bid x 3 doses + Cyclophosphamide, 750 mg/m^2 + Doxorubicin, 50 mg/m^2 + Vincristine, 1.4 mg/m^2 + Prednisolone, 100 mg po 1x/21 d (n=3) Zosuquidar, 400 mg po bid x 3 doses + Cyclophosphamide, 750 mg/m^2 + Doxorubicin, 50 mg/m^2 + Vincristine, 1.4 mg/m^2 + Prednisolone, 100 mg po 1x/21 d (n=3) Zosuquidar, 500 mg po bid x 3 doses + Cyclophosphamide, 750 mg/m^2 + Doxorubicin, 50 mg/m^2 + Vincristine, 1.4 mg/m2 + Prednisolone, 100 mg po 1x/21 d (n=5)	11	Combination of oral zosuquidar with full doses of cyclophosphamide, doxorubicin, vincristine and prednisolone was effective in producing maximal inhibition of P-glycoprotein, with no drug interactions being observed 42	42

Continuation

Table III Cont.: Cinical studies of zosuquidar (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Acute myeloid leukemia	Open	Zosuquidar, 200 mg/m^2 iv over 6 h on d 3-5 + Daunorubicin, 50 mg/m^2 on d 1, 3 and 5+ Cytarabine, 200 mg/m^2 d 1-7 (n=4) Zosuquidar, 300 mg/m^2 iv over 6 h on d 3-5 + Daunorubicin, 50 mg/m^2 on d 1, 3 and 5+ Cytarabine, 200 mg/m^2 d 1-7 (n=3) Zosuquidar, 400 mg/m^2 iv over 3 h on d 3-5 + Daunorubicin, 50 mg/m^2 on d 1, 3 and 5+ Cytarabine, 200 mg/m^2 d 1-7 (n=5)	12	Zosuquidar was effective in inhibiting P-glycoprotein in patients with acute myeloid leukemia	43
Acute myeloid leukemia	Open	HD: Daunorubicin, 45 mg/m^2/d iv over 10 min on d 2-4 + Cytarabine, 1.5 mg/m^2 iv over 2 h bid on d 5-8 + Zosuquidar, 480 mg/m^2/d iv over 96 h on d 1-4 (n=3) LD: Daunorubicin, 45 mg/m^2/d iv over 10 min on d 2-4 + Cytarabine, 1.5 mg/m^2 iv over 2 h bid on d 5-8 + Zosuquidar, 320 mg/m^2/d iv over 72 h on d 1-4 (n=49)	52	Zosuquidar plus daunorubicin and cytarabine combination was effective in inhibiting P-glycoprotein and showed antitumor activity with similar response rates irrespective of the A or T genotypes at positions 2677 and 3435 in patients with high risk acute myeloid leukemia. Nevertheless, neurologic toxicity should be monitored	44-46

zosuquidar (200, 300 or 400 mg/m^2 as a 3-h or 6-h i.v. infusion on days 3 and 5) in combination with daunorubicin (50 mg/m^2 on days 1, 3 and 5) and cytarabine (cytosine arabinoside; 200 mg/m^2 on days 1-7). Eight patients had a complete response and 1 patient had a partial response; 2 patients with refractory leukemia and 1 with an infection died. *Ex vivo* analysis of CD56$^+$ NK cells showed that zosuquidar treatment was related to rapid and complete inhibition of Rh123 efflux (*i.e.*, Pgp modulation) from cells (95% inhibition at 1 h of infusion, 90.5% at 2-3 h postinfusion); similar results were obtained for CD33$^+$ gated blast cells from 4 patients. Four patients had overexpression of Pgp while no Pgp overexpression was observed in 8 others according to quantitation using an MRK16 monoclonal antibody (43).

A phase II trial involving 52 patients with newly diagnosed high risk AML, refractory anemia with excess blasts in transformation (RAEB-t) or relapsed/refractory AML/RAEB-t examined the efficacy and tolerability of zosuquidar (480 mg/m^2 96-h continuous i.v. infusion on days 1-4 or 320 mg/m^2 72-h i.v. infusion on days 1-4; 4 patients received a second cycle on day 10) in combination with daunorubicin (45 mg/m^2/day i.v. over 10 min on days 2-4) and high-dose cytarabine (1.5 g/m^2 i.v. over 2 h every 12 h for 8 doses starting on day 5). Of 3 patients treated with the highest dose of zosuquidar, 2 developed reversible grade 3 ataxia. Of the 49 patients treated with the lower zosuquidar dose, 3 patients developed reversible grade 3 ataxia and 8 patients experienced confusion and agitation. Of the 39 patients evaluable for response, 15 had a complete response, 9 had a partial complete response (< 5% marrow blasts but a platelet count of > 100,000), 5 had a partial response and 10 patients were refractory; 8 patients died during induction therapy. *Ex vivo* analysis of Pgp function from bone marrow mononuclear cells (CD34$^+$ and CD34$^-$) from 45 patients showed that 28 (90%) of the 31 samples that had positive Rh123 efflux activity before zosuquidar dosing exhibited Pgp inhibition postdosing. In addition, genetic polymorphism of the MDR1 gene was investigated in zosuquidar-treated patients. Data from normal subjects have identified single nucleotide polymorphisms at nucleotide position 2677 within exon 21 or position 3435 within exon 27 as being possibly related to altered expres-sion and/or activity of the MDR1 transporter. In this phase II study, exons 21 and 27 of the MDR1 gene were amplified from isolated genomic DNA of leukemic myeloblasts from 27 and 29 patients, respectively. Analysis of sequence data found no consistent trends between clinical responses to combination treatment including zosuquidar and genotype at position 2677 or 3435 (44-46).

Several randomized, placebo-controlled, double-blind phase III trials involving zosuquidar in combination with oncolytics are currently recruiting patients. One trial will examine whether addition of zosuquidar during conventional induction and postremission standard chemotherapy will increase survival of patients older than 60 years of age with newly diagnosed AML, RAEB-t or high-risk RAEB-t. Another phase III trial is planned also in elderly patients (60 years or older) with newly diagnosed AML, RAEB-t or high-risk RAEB. This study will compare the overall survival and progression-free survival of patients treated with daunorubicin and cytarabine with or without zosuquidar (47).

Zosuquidar continues to undergo phase III trials in patients with acute myeloid leukemia.

Source

Eli Lilly and Company (US); licensed from Roche Bioscience (US).

References

1. Pfister, J.R., Makra, F., Muehldorf, A.V., Wu, H., Nelson, J.T., Cheung, P., Bruno, N.A., Casey, S.M., Zutshi, N., Slate, D.L. *Methanodibenzosuberylpiperazines as potent multidrug resistance reversal agents.* Bioorg Med Chem Lett 1995, 5: 2473-6.

2. Pfister, J.R., Slate, D.L. (Syntex USA). *10,11-Methanodibenzosuberane derivs. used as chemosensitizing agents.* EP 0695293, EP 0866063, JP 1996509223, WO 9424107.

3. Vo, D., Wolowyk, M.W., Knaus, E.E. *Synthesis and cardioselective β-adrenergic antagonist activity of quinolyloxypropanolamines.* Drug Des Discov 1992, 9: 69-78.

4. Huff, B.E., Le Tourneau, M.E., Wilson, T.M., Bush, J.K., Reutzel-Edens, S.M. (Eli Lilly and Company). *Process for preparing 10,11-methanobenzosuberane derivs.* EP 1189887, JP 2003501421, WO 0075121.

5. Astleford, B.A., Barnett, C.J., Kobierski, M.E., Wilson, T.M. (Eli Lilly and Company). *Process for preparing a 10,11-methanodibenzosuberane deriv.* EP 1198462, WO 0075132.

6. Gottesman, M.M., Pastan, I. *Biochemistry of multidrug resistance mediated by the multidrug transporter.* Annu Rev Biochem 1993, 62: 385-427.

7. Ruetz, S., Gros, P. *Functional expression of P-glycoproteins in secretory vesicles.* J Biol Chem 1994, 269: 12277-84.

8. Bosch, I., Croop, J. *P-glycoprotein multidrug resistance and cancer.* Biochim Biophys Acta 1996, 1288: F37-54.

9. Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M.M., Pastan, I., Willingham, M.C. *Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues.* Proc Natl Acad Sci USA 1987, 84: 7735-8.

10. Cordon-Cardo, C., O'Brien, J.P., Boccia, J., Casals, D., Bertino, J.R., Melamed, M.R. *Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues.* J Histochem Cytochem 1990, 38: 1277-87.

11. Schinkel, A.H., Mayer, U., Wagenaar, E. et al. *Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins.* Proc Natl Acad Sci USA 1997, 94: 4028-33.

12. Rao, V., Dahlheimer, J.L., Bardgett, M.E., Snyder, A.Z., Piwnica-Worms, D. *MDR1 Pgp mediates the blood-cerebrospinal fluid permeability barrier in choroid plexus epithelium.* Proc Am Assoc Cancer Res 1998, 39: Abst 517.

13. van Helvoort, A., Smith, A.J., Sprong, H., Fritzsche, I., Schinkel, A.H., Borst, P., van Meer, G. *MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine.* Cell 1996, 87: 507-17.

14. Bosch, I., Dunussi-Joannopoulos, K., Wu, R.L., Furlong, S.T., Croop, J. *Phosphatidylcholine and phosphatidylethanolamine behave as substrates of the human MDR1 P-glycoprotein.* Biochemistry 1997, 36: 5685-94.

15. Bezombes, C., Maestre, N., Laurent, G., Levade, T., Bettaieb, A., Jaffrezou, J.P. *Restoration of TNF-α-induced ceramide generation and apoptosis in resistant human leukemia KG1a cells by the P-glycoprotein blocker PSC833.* FASEB J 1998, 12: 101-9.

16. Lange, Y., Steck, T.L. *Cholesterol homeostasis. Modulation by amphiphiles.* J Biol Chem 1994, 269: 29371-4.

17. Luker, G.D., Nilsson, K.R., Covey, D.F., Piwnica-Worms, D. *Multidrug resistance (MDR1) P-glycoprotein enhances esterification of plasma membrane cholesterol.* J Biol Chem 1999, 274: 6979-91.

18. Sandor, V., Foto, T., Bates, S.E. *Future perspectives for the development of P-glycoprotein modulators.* Drug Resist Updates 1998, 1: 190-200.

19. 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: 1967-72.

20. Lum, B.L., Fisher, G.A., Brophy, N.A., Yahanda, A.M., Adler, K.M., Kaubisch, S., Halsey, J., Sikic, B.I. *Clinical trials of modulation of multidrug resistance. Pharmacokinetic and pharmacodynamic considerations.* Cancer 1993, 72: 3502-14.

21. Goldstein, L.J. *Clinical reversal of drug resistance.* Curr Probl Cancer 1995, 19: 65-124.

22. Shustik, C., Dalton, W., Gros, P. *P-glycoprotein-mediated multidrug resistance in tumor cells: Biochemistry, clinical relevance and modulation.* Mol Aspects Med 1995, 16: 1-78.

23. Johnson, W.W. *P-glycoprotein-mediated efflux as a major factor in the variance of absorption and distribution of drugs: Modulation of chemotherapy resistance.* Methods Find Exp Clin Pharmacol 2002, 24: 501-14.

24. Slate, D.L., Bruno, N.A., Casey, S.M., Zutshi, N., Garvin, L.J., Wu, H., Pfister, J.R. *RS-33295-198: A novel, potent modulator of P-glycoprotein-mediated multidrug resistance.* Anticancer Res 1995, 15: 811-4.

25. Dantzig, A.H., Law, K.L., Cao, J., Starling, J.J. *Reversal of multidrug resistance by the P-glycoprotein modulator, LY335979, from the bench to the clinic.* Curr Med Chem 2001, 8: 39-50.

26. Starling, J.J., Shepard, R.L., Cao, J. et al. *Pharmacological characterization of LY335979: A potent cyclopropyldibenzosuberane modulator of P-glycoprotein.* Adv Enzyme Regul 1997, 37: 335-47.

27. Dantzig, A.H., Shepard, R.L., Cao, J., Law, K.L., Ehlhardt, W.J., Baughman, T.M., Bumol, T.F., Starling, J.J. *Reversal of P-glycoprotein-mediated multidrug resistance by a potent cyclopropyldibenzosuberane modulator, LY335979.* Cancer Res 1996, 56: 4171-9.

28. Dantzig, A.H., Shepard, R.L., Law, K.L., Tabas, L., Pratt, S., Gillespie, J.S., Binkley, S.N., Kuhfeld, M.T., Starling, J.J., Wrighton, S.A. *Selectivity of the multidrug resistance modulator, LY335979, for P-glycoprotein and effect on cytochrome P-450 activities.* J Pharmacol Exp Ther 1999, 290: 854-62.

29. Lehne, G., Moerkrid, L., den Boer, M., Rugstad, H.E. *Diverse effects of P-glycoprotein inhibitory agents on human leukemia cells expressing the multidrug resistance protein (MRP).* Int J Clin Pharmacol Ther 2000, 38: 187-95.

30. Lehne, G., De Angelis, P., den Boer, M., Rugstad, H.E. *Growth inhibition, cytokinesis failure and apoptosis of multidrug-resistant leukemia cells after treatment with P-glycoprotein inhibitory agents.* Leukemia 1999, 13: 768-78.

31. Shepard, R.L., Cao, J., Starling, J.J., Dantzig, A.H. *Modulation of P-glycoprotein but not MRP1- or BCRP-mediated drug resistance by LY335979.* Int J Cancer 2003, 103: 121-5.

32. Green, L.J., Marder, P., Slapak, C.A. *Modulation by LY335979 of P-glycoprotein function in multidrug-resistant cell lines and human natural killer cells.* Biochem Pharmacol 2001, 61: 1393-9.

33. Baughman, T.M., Ehlhardt, W.J., Cao, J., Law, K.L., Starling, J.J. *The effect of LY335979 on plasma levels of doxorubicin, etoposide and Taxol in mouse and rat.* 7th North Am Issx Meet (Oct. 20-24, San Diego) 1996, Abst 269.

34. Wacher, V.J., Wu, C.Y., Benet, L.Z. *Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: Implications for drug delivery and activity in cancer chemotherapy.* Mol Carcinog 1995, 13: 129-34.

35. Ehlhardt, W.J., Woodland, J.M., Baughman, T.M., Vandenbranden, M., Wrighton, S.A., Kroin, J.S., Norman, B.H., Maple, S.R. *Liquid chromatography/nuclear magnetic resonance spectroscopy and liquid chromatography/mass spectrometry identification of novel metabolites of the multidrug resistance modulator LY335979 in rat bile and human liver microsomal incubations.* Drug Metab Dispos 1998, 26: 42-51.

36. Truex, L.L., Zimmermann, J., Luffer-Atlas, D., Reddy, V. *Effects of co-administered LY335979 on vinorelbine pharmacokinetics and pharmacodynamics in beagle dogs.* Proc Am Assoc Cancer Res 2001, 42: Abst 5105.

37. Reddy, V.R., Buben, J.A., Truex, L.L., Brown, D.E., Fletcher, D.J., Zimmerman, J.L., Vahle, J.L. *The toxicity and pharmacokinetic interaction of LY335979, an inhibitor of P-glycoprotein, with doxorubicin in beagle dogs.* Proc Am Assoc Cancer Res 1998, 39: Abst 511.

38. Rubin, E.H., de Alwis, D.P., Pouliquen, I. et al. *A phase I trial of a potent P-glycoprotein inhibitor, zosuquidar.3HCl trihydrochloride (LY335979), administered orally in combination with doxorubicin in patients with advanced malignancies.* Clin Cancer Res 2002, 8: 3710-7.

39. Fracasso, P.M., Tan, B.R., Arquette, M.A. et al. *A phase I and pharmacokinetic study of docetaxel and LY335979 in patients with advanced maligancies.* Proc Am Soc Clin Oncol 2000, 19: Abst 701.

40. Moore, M.J., Siu, L.L., Oza, A., MacLean, M., Fisher, B., Jordan, C., Slapak, C., Seymour, L. *A phase I study of vinorelbine plus multidrug resistance (MDR) inhibitor LY 335979 in patients with advanced solid tumors.* Clin Cancer Res 2000, 6(Suppl.): Abst 190.

41. Schellens, J.H.N., Kruytzer, M.F., Vasey, P.A., Harris, A.L., Deplanque, G., Burgess, M., deAlwis, D., O'Callahan, M., Kaye, S.B., Beijnen, J.H. *Phase I and pharmacologic study of the P-glycoprotein (P-gp) inhibitor L-335979 (LY) and paclitaxel (P) in patients with solid tumors.* Proc Am Assoc Cancer Res 2001, 42: Abst 2882.

42. Tura, S., Morschhauser, F., Zinzani, P., Sloots, L., Burgess, M., Dumontet, C. *A phase I trial of the P-glycoprotein (P-gp) inhibitor zosuquidar (LY335979) and CHOP chemotherapy in patients with non-Hodgkin's lymphoma.* Blood 2001, 98(11, Part 2): Abst 4734.

43. Gerrard, G., Ganeshaguru, K., Baker, R., Potter, M., Prentice, H.G., Burgess, M., Herbert, L., McCullough, H., Mehta, A.B. *Preliminary pharmacodynamic analysis on a phase 1 study of the P-gp inhibitor zosuquidar (LY335979) given by short intravenous infusion in combination with daunorubicin and cytarabine in AML patients.* 7th Congr Eur Hematol Assoc (June 6-9, Florence) 2002, Abst 0566.

44. Cripe, L.D., Tallman, M., Karanes, C., List, A.F., Kinney, P.A., Burgess, M.F., Slapak, C.A. *A phase II trial of zosuquidar (LY335979), a modulator of P-glycoprotein (PgP9 activity, plus daunorubicin and high-dose cytarabine in patients with newly-diagnosed secondary acute myelod leukemia (AML), refractory anemia with excess blasts in transformation (RAEB-t) or relapsed/refractory AML.* Blood 2001, 98(11, Part 1): Abst 2492.

45. Green, L., Cripe, L.D., Tallman, M.S., Karanes, C., List, A.F., Slapak, C.A., Marder, P. *Blockade of P-gp function in acute myeloid leukemia bone marrow cells after zosuquidar (LY335979) administration.* Blood 2001, 98(11, Part 2): Abst 4399.

46. Ma, L., Cripe, L.D., Tallman, M.S., Karanes, C., List, A.F., Jordan, C.L., Slapak, C.A. *Genetic polymorphism of the MDR1 gene in patients with acute myeloid leukemia (AML) treated with zosuquidar (LY335979), daunorubicin, and cytarabine.* Blood 2001, 98(11, Part 2): Abst 4411.

47. www.clinicaltrials.gov.

48. Germann, U.A., Shlyakhter, D., Mason, V.S., Zelle, R.E., Duffy, J.P., Galullo, V., Armistead, D.M., Saunders, J.O., Boger, J., Harding, M.W. *Cellular and biochemical characterization of VX-710 as a chemosensitizer: Reversal of P-glycoprotein-mediated multidrug resistance in vitro.* Anticancer Drugs 1997, 8: 125-40.

49. Mistry, P., Stewart, A.J., Dangerfield, W., Okiji, S., Liddle, C., Bootle, D., Plumb, J.A., Templeton, D., Charlton, P. *In vitro and in vivo reversal of P-glycoprotein-mediated multidrug resistance by a novel potent modulator, XR9576.* Cancer Res 2001, 61: 749-58.

50. Martin, C., Berridge, G., Mistry, P., Higgins, C., Charlton, P., Callaghan, R. *The molecular interaction of the high affinity reversal agent XR9576 with P-glycoprotein.* Br J Pharmacol 1999, 128: 403-11.

51. Traunecker, H.C.L., Stevens, M.C., Kerr, D.J., Ferry, D.R. *The acridonecarboxamide GF120918 potently reverses P-glycoprotein-mediated resistance in human sarcoma MES-Dx5 cells.* Br J Cancer 1999, 81: 942-51.

52.Mellows, G., Norris, D., Waterfall, J. *The effect of XR9576 (tariquidar), a specific P-glycoprotein inhibitor, on cytochrome P450 activities in human liver microsomes.* Proc Am Assoc Cancer Res 2002, 43: Abst 4714.

Additional References

Gerrard, G., Ganeshaguru, K., Baker, R., Potter, M., Prentice, H.G., Burgess, M., Herbert, L., McCullough, H., Mehta, A.B. *A phase I study of a P-gp inhibitor zosuquidar (LY 335979), given by short intravenous infusion in combination with daunorubicin and cytarabine in AML/MDS patients.* Blood 2001, 98(11, Part 2): Abst 4398.

Green, L., Marder, P., Skerjanec, A., Rubin, E., Sandler, A., Slapak, C. *P-glycoprotein targeted pharmacological activity detected during clinical evaluations of LY335979.* Proc Am Assoc Cancer Res 1999, 40: Abst 437.

Skerjanec, A., Sandler, A., Green, L., Marder, P., Kuan, S., Chaudhary, A., Slapak, C. *A phase I dose-escalating trial of LY335979, a potent multidrug-resistance modulator.* Farm Vestn 1999, 50: 292-3.

Choo, E.F., Leake, B., Wandel, C., Imamura, H., Wood, A.J., Wilkinson, G.R., Kim, R.B. *Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes.* Drug Metab Dispos 2000, 28: 655-60.

Fontaine, M., Yang, H., Elmquist, W.F. *The effect of LY-335979, a novel P-glycoprotein inhibitor, on the permeability of mannitol across the blood-brain and blood-CSF barriers: An in vivo microdiaslysis study in freely-moving rats.* Pharm Res 1997, 14(11, Suppl.): Abst 2364.

Sun, H., Elmquist, W.F. *The effect of LY-335979, a novel P-glycoprotein inhibitor, on the free fraction of quinidine in rat plasma.* Pharm Res 1997, 14(11, Suppl.): Abst 3293.

Starling, J.J., Law, K.L., Shepard, R.L., Cao, J., Bumol, T.F., Dantzig, A.H. *LY335979, a potent cyclopropyldibenzosuberane inhibitor of P-glycoprotein, significantly reverses taxol resistance in a multidrug resistant human non-small cell lung carcinoma nude mouse xenograft model.* Proc Am Assoc Cancer Res 1996, 37: Abst 2264.

Slapak, C.A., Dahlheimer, J., Piwnica-Worms, D. *Reversal of multidrug resistance with LY335979: Functional analysis of P-glycoprotein-mediated transport activity and its modulation in vivo.* J Clin Pharmacol 2001, 41: 29S-38S.

Kurumboor, S.K., Miller, D.W., Elmquist, W.F. *Effect of LY335979 on the brain distribution of doxorubicin in wild-type and P-glycoprotein deficient mice.* Proc Am Assoc Cancer Res 1999, 40: Abst 4370.

van der Sandt, I.C.J., Gaillard, P.J., Voorwinden, H.H., de Boer, A.G., Breimer, D.D. *P-glycoprotein inhibition leads to enhanced disruptive effects by anti-microtubule cytostatics at in vitro blood-brain barrier.* Pharm Res 2001, 18: 587-92.

Shepard, R.L., Law, K.L., Starling, J.J., Dantzig, A.H. *Modulation of navelbine and mitoxantrone resistance by the P-glycoprotein modulator, LY335979.* Proc Am Assoc Cancer Res 2000, 41: Abst 1703.

Shepard, R.L., Slate, D.L., Pfister, J., Cao, J., Starling, J.J., Dantzig, A.H. *Evaluation of a highly potent modulator of multidrug resistance (MDR), RS33295-198.* Proc Am Assoc Cancer Res 1995, 36: Abst 2051.

Shepard, R.L., Dantzig, A.H. *The affinity of P-glycoprotein (Pgp) differs for stereoisomers of the potent modulator LY335979.* Proc Am Assoc Cancer Res 1996, 37: Abst 2272.

Dantzig, A.H., Shepard, R.L., Law, K.L., Tabas, L., Gillespie, J., Binkley, S., Starling, J.J., Wrighton, S. *Selectivity of the multidrug resistance modulator, LY335979, for P-glycoprotein and effect on cytochrome P450 activities.* 10th NCI-EORTC Symp New Drugs Cancer Ther (June 16-19, Amsterdam) 1998, Abst 555.

Norman, B.H., Kroin, J.S., Dantzig, A.H., Shepard, R.L., Cao, J., Winter, M.A., Bell, M.G., Starling, J.J. *Structure activity relationship (SAR) studies of cyclopropyldibenzosubarane inhibitors of P-glycoprotein and their effects on multidrug resistance in vitro and in vivo.* 9th NCI-EORTC Symp New Drugs Cancer Ther (March 12-15, Amsterdam) 1996, Abst 414.

Ekins, S., Kim, R.B., Leake, B.F. et al. *Three-dimensional quantitative structure-activity relationships of inhibitors of P-glycoprotein.* Mol Pharmacol 2002, 61: 964-73.

Ekins, S., Kim, R.B., Leake, B.F. et al. *Application of three-dimensional quantitative structure-activity relationships of P-glycoprotein inhibitors and substrates*. Mol Pharmacol 2002, 61: 974-81.

Liu, L., Nussbaum, M.A. *Systematic screening approach for chiral separations of basic compounds by capillary electrophoresis with modified cyclodextrins.* J Pharm Biomed Anal 1999, 19: 679-94.

Ekins, S., Bravi, G., Binkley, S., Gillespie, J.S., Ring, B.J., Wikel, J.H., Wrighton, S.A. *Three- and four-dimensional quantitative structure activity relationship analyses of cytochrome P-450 3A4 inhibitors.* J Pharmacol Exp Ther 1999, 290: 429-38.

Ekins, S., Bravi, G., Binkley, S., Gillespie, J.S., Ring, B.J., Wikel, J.H., Wrighton, S.A. *Three- and four-dimensional-quantitative structure activity relationship (3D/4D QSAR) analyses of CYP2C9 inhibitors.* Drug Metab Dispos 2000, 28: 994-1002.

Baron, J.M., Goh, L.B., Yao, D., Wolf, C.R., Friedberg, T. *Modulation of P450 CYP3A4-dependent metabolism by P-glycoprotein: Implications for P450 phenotyping.* J Pharmacol Exp Ther 2001, 296: 351-8.

Sun, H., Bungay, P.M., Elmquist, W.F. *Effect of capillary efflux transport inhibition on the determination of probe recovery during in vivo microdialysis in the brain.* J Pharmacol Exp Ther 2001, 297: 991-1000.

van der Sandt, I.C., Vos, C.M., Nabulsi, L., Blom-Roosemalen, M.C., Voorwinden, H.H., de Boer, A.G., Breimer, D.D. *Assessment of active transport of HIV protease inhibitors in various cell lines and the in vitro blood-brain barrier.* AIDS 2001, 15: 483-91.

Florea, B.I., van der Sandt, I.C., Schrier, S.M., Kooiman, K., Deryckere, K., de Boer A.G., Junginger, H.E., Borchard, G. *Evidence of P-glycoprotein mediated apical to basolateral transport of flunisolide in human broncho-tracheal epithelial cells (Calu-3).* Br J Pharmacol 2001, 134: 1555-63.

Molnár, L., Keseru, G.M. *A neural network based virtual screening of cytochrome P450 3A4 inhibitors.* Bioorg Med Chem Lett 2002, 12: 419-21.

de Alwis, D.P., Pouliquen, I., Burgess, M., Green, L., Chaudhary, A., Jordan, C., Slapak, C., Sandler, A. *A phase I dose escalating study of LY335979, a novel Pgp modulator-administered intravenously in combination with doxorubicin.* Proc Am Soc Clin Oncol 2001, 20(Part 1): Abst 284.

Sweeney, C., Sandler, A., Gordon, M. et al. *Continuous intravenous infusion of LY335979, a P-glycoprotein modulator, administered in combination with doxorubicin: Results of a phase I dose-escalating trial.* Proc Am Soc Clin Oncol 1999, 18: Abst 703.

Rubin, E., Zamek, R., Medina, M. et al. *A phase I trial of LY335979, a potent multidrug-resistance modulator, administered orally in combination with doxorubicin.* Proc Am Soc Clin Oncol 1999, 18: Abst 705.