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Synthesis and Evaluation of (2-(4-Methoxyphenyl)-4-quinolinyl)(2-piperidinyl)methanol (NSC23925) Isomers To Reverse Multidrug Resistance in Cancer

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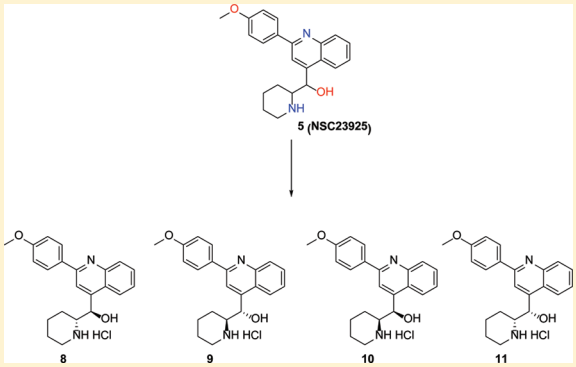
S *Supporting Information*

ABSTRACT: Development of multidrug resistance (MDR) during chemotherapy is a fundamental obstacle associated with cancer care. Prior studies have identified (2-(4-methoxyphenyl)-4-quinolinyl)(2-piperidinyl)methanol (**5**) (NSC23925) to be a small molecule agent that reverses MDR in cancer cells. We synthesized all four isomers of **5** and analyzed them by liquid chromatography−mass spectrometry (LCMS). Structure−activity relationships for reversing MDR were evaluated. Isomer **11** demonstrated the most potent activity. **11** reversed MDR in several drug-resistant cell lines expressing Pgp, including ovarian, breast, colon, uterine, and sarcoma cancer. **11** resensitized these cell lines to paclitaxel, doxorubicin, mitoxantrone, vincristine, and trabectedin with no effect on cell sensitivity to cisplatin, topotecan, and methotrexate. **11** significantly enhanced in vivo antitumor activity of paclitaxel in MDR xenograft models, without increasing the level of paclitaxel toxicity. In conclusion, **11** and derivatives of this compound may hold therapeutic value in the treatment of MDR-dependent cancers.



INTRODUCTION

A major obstacle to cancer therapy is multidrug resistance (MDR), which can arise from either intrinsic or acquired mechanisms of resistance.[1,2] One of the molecular mechanisms for MDR is overexpression of plasma membrane glycoprotein (Pgp).[1−3] Pgp is the gene product of MDR1(ABCB1), a member of the ABC (ATP binding cassette) superfamily of a 170 kDa transporter protein, and it acts as an energy-dependent (ATP) cancer drug efflux by pumping drugs out of the cells.[4] Overexpression of Pgp in tumor cells prevents adequate intracellular accumulation of a large number of cytotoxic drugs including anthracyclines (doxorubicin, daunorubicin), anthracenedione (mitoxantrone), vinca alkaloids (vinblastine, vincristine), taxanes (paclitaxel, docetaxel), and many others.[3−5] A series of homologous ABC proteins such as multidrug-resistance proteins (MRPs) and breast cancer resistance protein (BCRP) have also been discovered.[6,7]

Pgp is a major protein associated with MDR and has a low level expression in normal tissues. A series of compounds that interact with Pgp and block drug efflux have been reported to reverse the MDR phenotype.[1,8] Previous studies have focused on MDR-reversing agents such as verapamil, cyclosporine A, valspodar (PSC833, Amdray),[8] and biricodar (VX710, INCEL).[9] Unfortunately, these compounds, initially developed for pharmacological uses other than MDR reversal, are relatively nonspecific and weak in potency.[8,9] For most of these compounds, clinical toxicities associated with their use at concentrations required to inhibit Pgp have precluded their widespread use. For example, the serum concentration of the calcium channel blocker verapamil that is required to produce in vitro reversal of MDR cannot be achieved in patients because of significant cardiotoxicities. Valspodar and biricodar have shown significant pharmacokinetic interaction with paclitaxel.[1,3,10,11] The addition of biricodar (biricodar blood steady-state concentrations of 10 μM were sustained at a dosage of 120 mg/m^2 per hour) to doxorubicin and vincristine therapy did not significantly increase antitumor activity or survival.[12]

Previously, in an attempt to identify novel small molecules that are more potent and selective as MDR inhibitors, we

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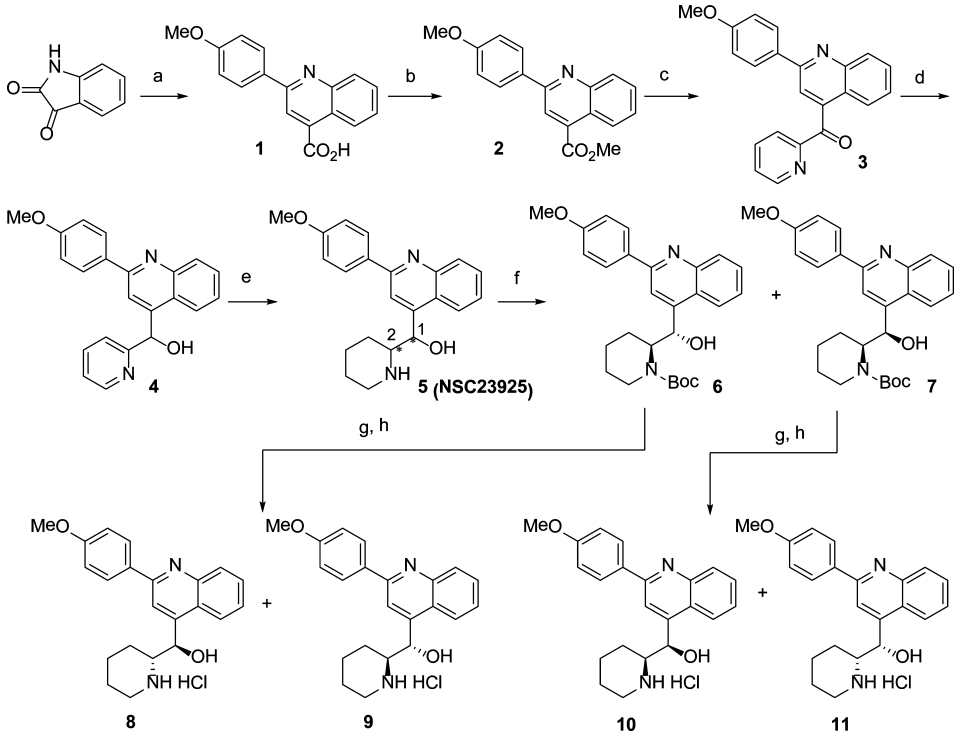
Scheme 1. Synthesis of 5 (NSC23925) Isomers[a]



[a]Reagents and conditions: (a) 1-(4-methoxyphenyl)ethanone, KOH, EtOH, 85 °C, 24 h, 50%; (b) concd H_2SO_4, MeOH, reflux overnight, 70%; (c) 2-bromopyridine, n-BuLi, Et_2O−THF, −78 °C, 2 h, 91%; (d) $NaBH_4$, EtOH, 0 °C, 1 h, 60%; (e) H_2, PtO_2, concd HCl, MeOH, 20 °C, 2 h; (f) $(Boc)_2O$, Et_3N, THF, 0 to rt,overnight; (g) chiral HPLC; (h) HCl/EtOH, 20 °C.

screened 2000 small molecule compounds from the NCI Diversity Set library to look for compounds that can reverse MDR in drug-resistant cell lines. We identified 2 out 2000 compounds, tetrandrine (NSC77037)[13] and (2-(4-methoxyphenyl)-4-quinolinyl)(2-piperidinyl)methanol (**5**) (NSC23925), as compounds with the highest activities as MDR reversal agents.[5,13]

5 is the most potent MDR reversing compound that we observed in the NCI compound set.[5,14] In comparison with tetrandrine, **5** is a unique compound with essentially very little chemical or biologic characterization since its synthesis as an antimalarial agent in the 1940s.[14] Analysis of the structure of **5** indicates that it has two chiral centers which lead to four different stereoisomers **8**, **9**, **10**, and **11**. In theory, each of the compound **5** isomers may have distinct bioactivities.

In this study, we synthesized and separated all four isomers of **5** and biologically characterized each of them in several multidrug-resistant cell lines. The most potent **5** isomer, **11**, was selected for further evaluation in vivo in a multidrug-resistant ovarian cancer xenograft mouse model.

CHEMISTRY

By using the route as indicated in Scheme 1, the synthesis of all four isomers of **5** was accomplished with Pfitzinger's method.[15] 2-(4-Methoxyphenyl)quinoline-4-carboxylic acid (**1**) was prepared in 50% yield from indoline-2,3-dione and 1-(4-methoxyphenyl)ethanone, and then it was esterified to afford methyl 2-(4-methoxyphenyl)quinoline-4-carboxylate (**2**) in 70% yield. Using n-BuLi as a base, 2-bromopyridine was reacted with **2** to give (2-(4-methoxyphenyl)quinolin-4-yl)(pyridin-2-yl)-methanone (**3**) in 91% yield. Subsequent reduction of **3** by $NaBH_4$ afforded (2-(4-methoxyphenyl)quinolin-4-yl)(pyridin-2-yl)methanol (**4**) in 60% yield. Hydrogenation of **4** with 30 mol % PtO_2 in a mixture of methanol and concd HCl (40:1) afforded (2-(4-methoxyphenyl)quinolin-4-yl)(piperidin-2-yl)-methanol (**5**).[16] Because attempts to separate isomers of **5** by chiral HPLC failed to get optically pure products, derivation of **5** was carried out, and Boc-protected derivatives **6** and **7** were prepared and separated away from each other on silica gel chromatography. The structure of **6** and **7** was confirmed to be threo isomer and erythro isomer, respectively, according to the common trend in which the coupling constant of CH(NH)-CH(OH) of the erythro isomer (^3J-erythro) is smaller than that of threo isomer (^3J-threo).[17] Then threo isomer **6** was separated by chiral HPLC to give optically pure Boc-**8** and Boc-**9**, and erythro isomer **7** gave optically pure Boc-**10** and Boc-**11**. Finally deprotection of these Boc-derivatives of **8** to **11** by HCl−EtOH solution afforded four isomers **8**, **9**, **10**, and **11**. We cultivated the single crystal from these synthesized compounds and determined the absolute configuration for all four isomers (Supporting Information Figure 1) which confirmed the judgment on the erythro/threo isomers. Liquid chromatography−mass spectrometry (LCMS, chiral HPLC) analysis demonstrated that the four isomers **8**, **9**, **10**, and **11** have the desired mass with significant purity (Supporting Information Figure 2).

BIOLOGICAL EVALUATION AND DISCUSSION

Identification of 11 as the Most Potent Isomer That Reverses MDR in Human Cancer Cell Lines. Structure−activity relationships of four **5** isomers for reversing MDR were evaluated in human ovarian cancer MDR cell line SKOV-3_{TR} by MTT assay. We observed that among the **5** isomers, **11** is the most potent one in reversing drug resistance (Figure 1). **8** and **9** showed modest but lesser activity, while **10** showed the least potency.

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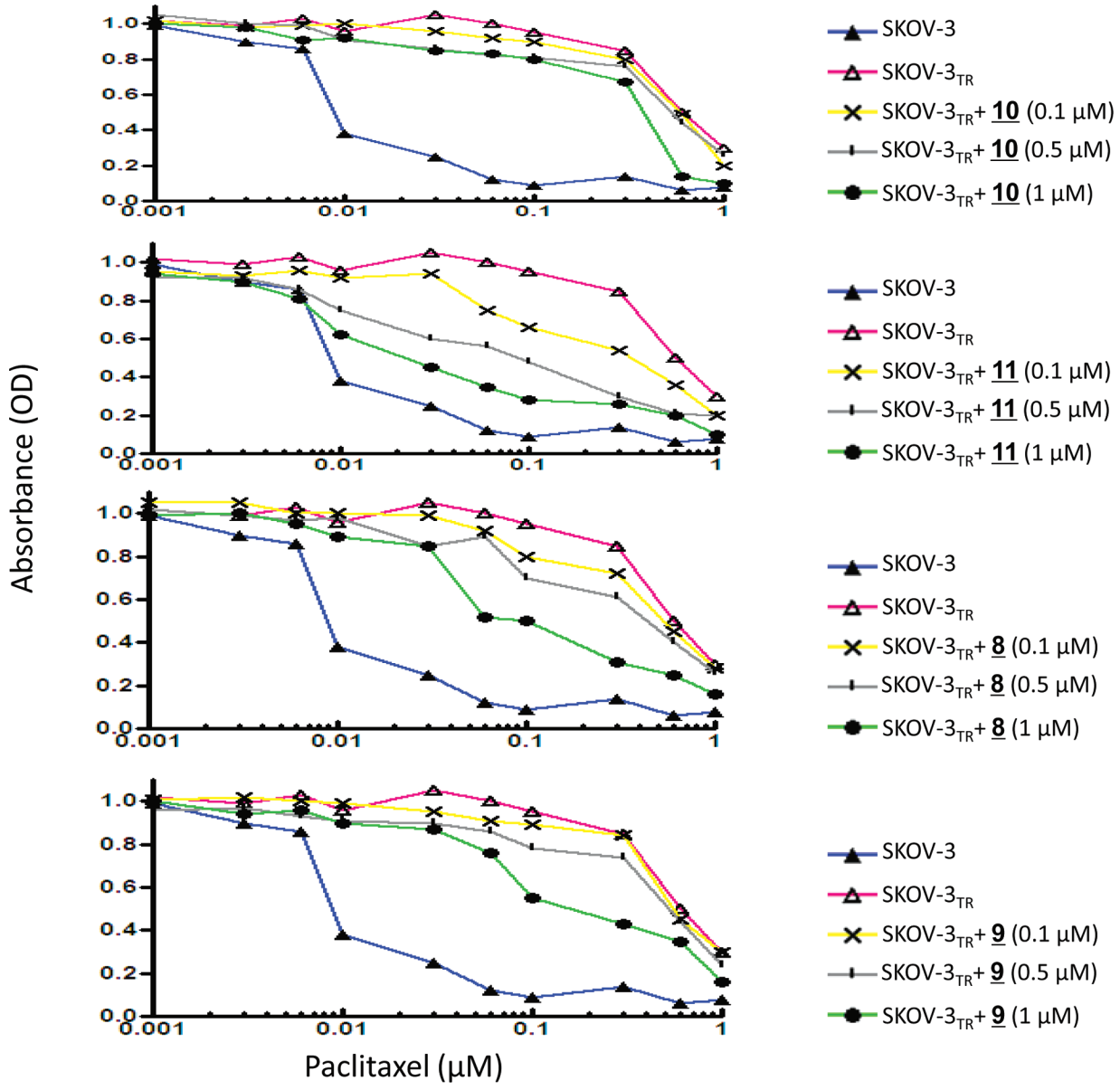

Figure 1. Effect of **5** isomers on reverse drug resistance in SKOV-3_{TR} cell line. Cells were treated with the paclitaxel and isomers (**8**, **9**, **10**, and **11**) in RPMI1640 complete media at the indicated concentrations. The relative sensitivity of each line to paclitaxel was determined by MTT analysis 6 days posttreatment.

Further studies showed that **11** is able to reverse paclitaxel, doxorubicin, and mitoxantrone resistance in human breast cancer MDR cell line MCF-7adr and human colon cancer MDR cell line $SW480_{TR}$ (Figure 2). In addition, **11** is much more potent (10 to 60 fold) than that of known drug resistance reversing agents verapamil or CsA (data not shown). In summary, **11** is able to reverse MDR in several MDR cell lines, including ovarian, breast, uterine, colon, and sarcoma MDR cancer cell lines to paclitaxel, docetaxel, doxorubicin, vincristine, daunorubicin, gemcitabine, trabectedin (ET-743, Yondelis),[5] and mitoxantrone. Table 1 shows representative findings of **11** on reversing drug resistance in ovarian, breast, and colon MDR cell lines while similar results have been found in uterine sarcoma, osteosarcoma, and Ewing sarcoma cell lines (data not shown). In the profile of reverse drug resistance to chemotherapy drugs, the results showed that **11** could reverse drug resistance to paclitaxel, doxorubicin, mitoxantrone, vincristine, and ET-743 but has no significant effects on drug sensitivities of cisplatin, carboplatin, topotecan, and methotrexate. Overexpression of MDR1 is a well-known mechanism of drug resistance for paclitaxel, doxorubicin, mitoxantrone, and vincristine. In contrast, the mechanism of drug resistance for cisplatin, carboplatin, and topotecan are thought to be due to increased DNA repair mechanisms. Interestingly, our previous study showed reversal of MDR by **5** was through selective and potent inhibition of MDR1 (Pgp) function. Furthermore, we also evaluated the effects of **11** on MDR1 (Pgp)-independent MRP1 or BCRP-mediated drug resistance in H-69AR and MCF-7/MX cell lines. The results showed that **11** was unable to reverse drug resistance in these non-Pgp-expressing drug-resistant cell lines, suggesting that **11** activity is specific for Pgp.

11 Modulates Pgp-Mediated Uptake and Efflux of Calcein AM. Reversal of MDR is usually displayed as an increased intracellular accumulation of chemotherapeutic drugs.

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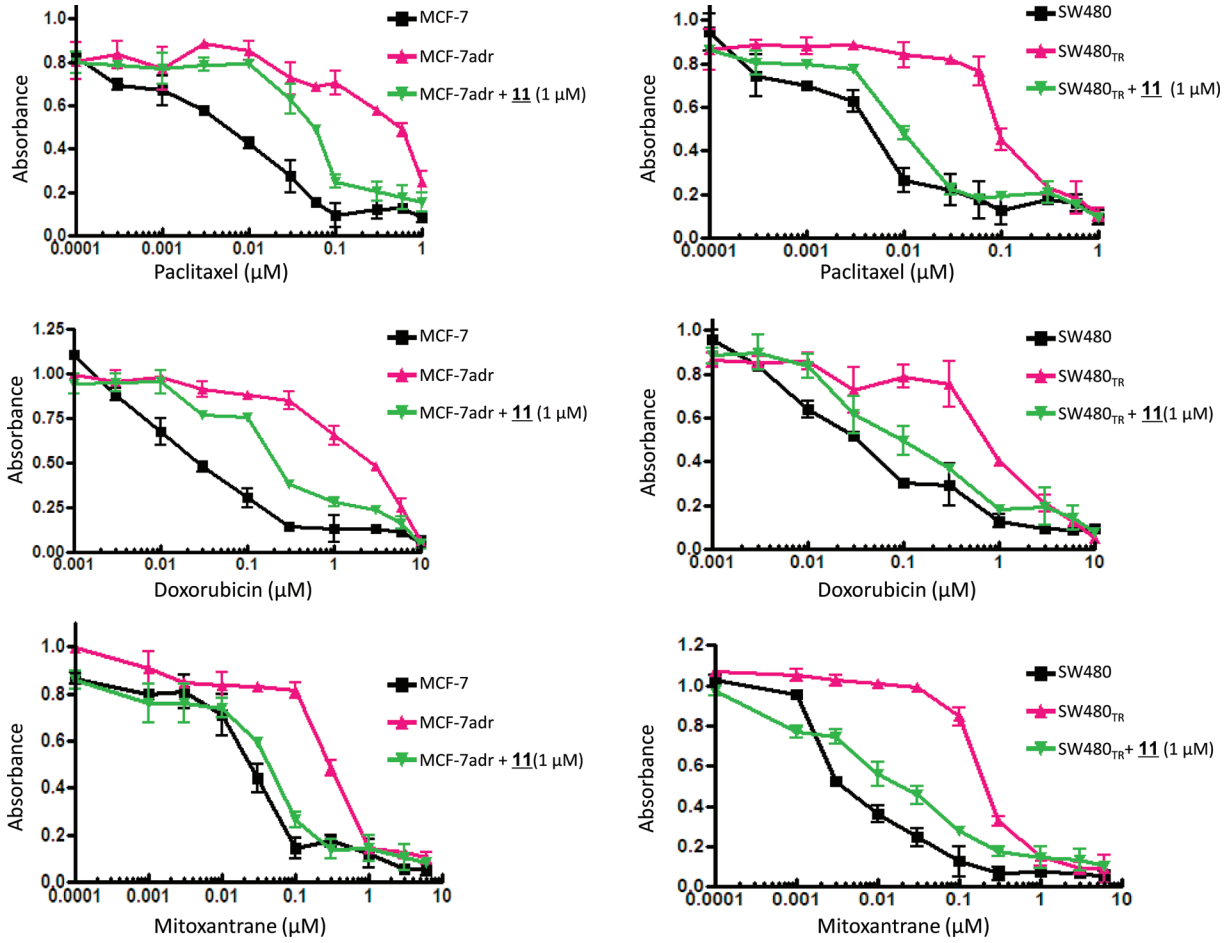

Figure 2. Reverse multidrug resistance isomer **11** in MCF-7adr and $SW480_{TR}$ cell lines. Cells were treated with paclitaxel, doxorubicin, or mitoxantrone and isomer **11** in RPMI1640 complete media at the indicated concentrations. The relative sensitivity of each line to the indicated drugs was determined by MTT analysis 6 days posttreatment.

Table 1. Effect of Isomer 11 on Reverse Drug Resistance in MDR Cell Lines[a]

	$SKOV\text{-}3_{TR}$ IC_{50} (μM)	MCF-7adr IC_{50} (μM)	$SW480_{TR}$ IC_{50}(μM)
pactlitaxel	0.57 ± 0.03	0.62 ± 0.12	0.12 ± 0.08
with **11** 0.1 μM	0.33 ± 0.04 (2)	0.46 ± 0.1 (1.3)	0.09 ± 0.01 (1.3)
with **11** 0.5 μM	0.08 ± 0.06 (7)	0.16 ± 0.03 (4)	0.016 ± 0.006 (8)
with **11** 1 μM	0.02 ± 0.03 (28)	0.05 ± 0.02 (12)	0.009 ± 0.002 (13)
doxorubicin	38.5 ± 2.6	3.8 ± 0.12	0.84 ± 0.16
with **11** 0.1 μM	24.6 ± 1.2 (2)	2.8 ± 0.4 (1.4)	0.5 ± 0.1 (1.7)
with **11** 0.5 μM	4.2 ± 0.5 (9)	0.96 ± 0.22 (4)	0.28 ± 0.08 (3)
with **11** 1 μM	1.2 ± 0.3 (32)	0.25 ± 0.06 (15)	0.11 ± 0.04 (8)
mitoxantrone	0.46 ± 0.06	0.32 ± 0.06	0.27 ± 0.03
with **11** 0.1 μM	0.37 ± 0.05 (1.2)	0.3 ± 0.001 (1.1)	0.23 ± 0.03 (1.2)
with **11** 0.5 μM	0.18 ± 0.02 (2.6)	0.12 ± 0.02 (3)	0.09 ± 0.03 (3)
with **11** 1 μM	0.06 ± 0.01 (8)	0.05 ± 0.01(6)	0.02 ± 0.01 (14)

[a]Cell survival was determined by MTT assay as described in Materials and Methods. IC_{50} is the concentration of drug (μM) that produced 50% inhibition of cell growth. Results were calculated from one experiment with triplicate wells. The numbers in the parentheses represent fold-reversal of drug resistance (IC_{50} of drugs divided by IC_{50} of drugs with **11**).

We examined the effects of **11** on the uptake and efflux of calcein AM in MCF-7adr and $SW480_{TR}$. Fluorescence microscopic analysis showed **11**'s ability to increase intracellular accumulation of calcein AM in these cell lines in a dose-dependent manner (Figure 3). **11** has a prominent effect on the accumulation of calcein AM in these cells starting at a concentration as low as 10 nM. Half-maximal reversal of accumulation deficit was observed at 100 nM and near maximal at 500 nM.

Combination of 11 and Doxorubicin Induces Apoptosis in MDR Cancer Cells. The effect of **11** on the doxorubicin induction of apoptosis was assessed in MCF-7adr or $SW480_{TR}$ cell lines. The cells were treated with either doxorubicin (0.5 μM) alone, **11** (1 μM) alone, or with a combination of doxorubicin and **11** for 72 h. Apoptosis was scored using the M30-Apoptosense ELISA assay. The combination of doxorubicin and **11** resulted in significantly

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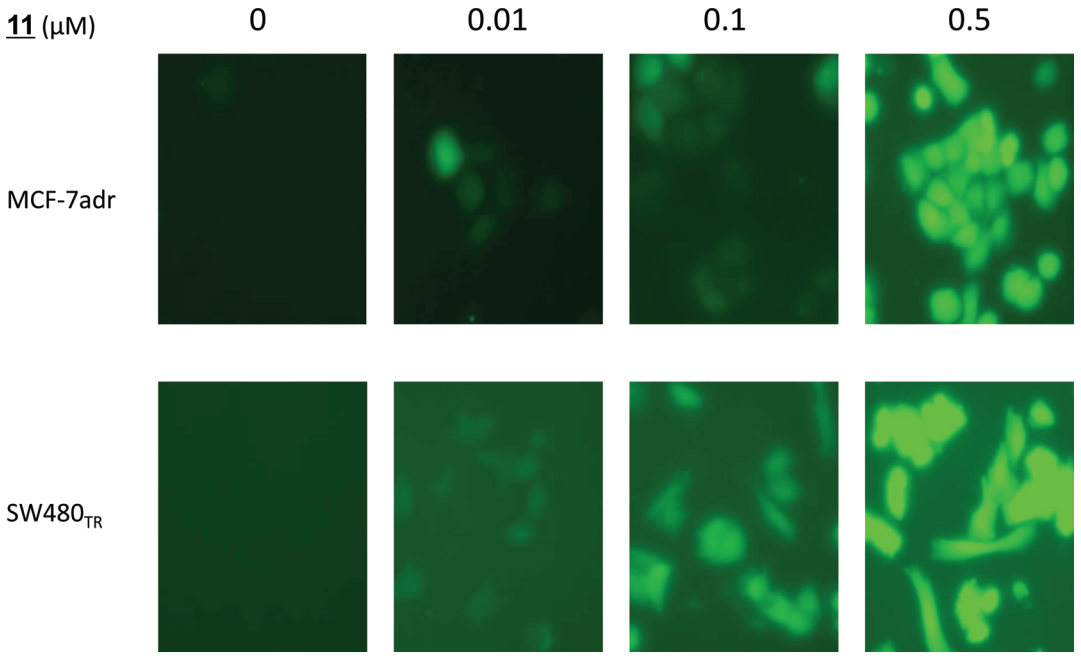

Figure 3. Calcein AM efflux from isomer **11**-treated MCF-7adr and $SW480_{TR}$ cell line cells. The calcein AM assay was optimized and performed using the Vybrant Multidrug Resistance kit. Cells were seeded at 50 000 cells/well (100 μL of culture medium) in a 96-well plate and incubated for 24 h. MCF-7adr or $SW480_{TR}$ cells in triplicate were treated with **11** for 1 h and then incubated in calcein AM for 30 min. The cell fluorescence images were acquired by a fluorescence microscope.

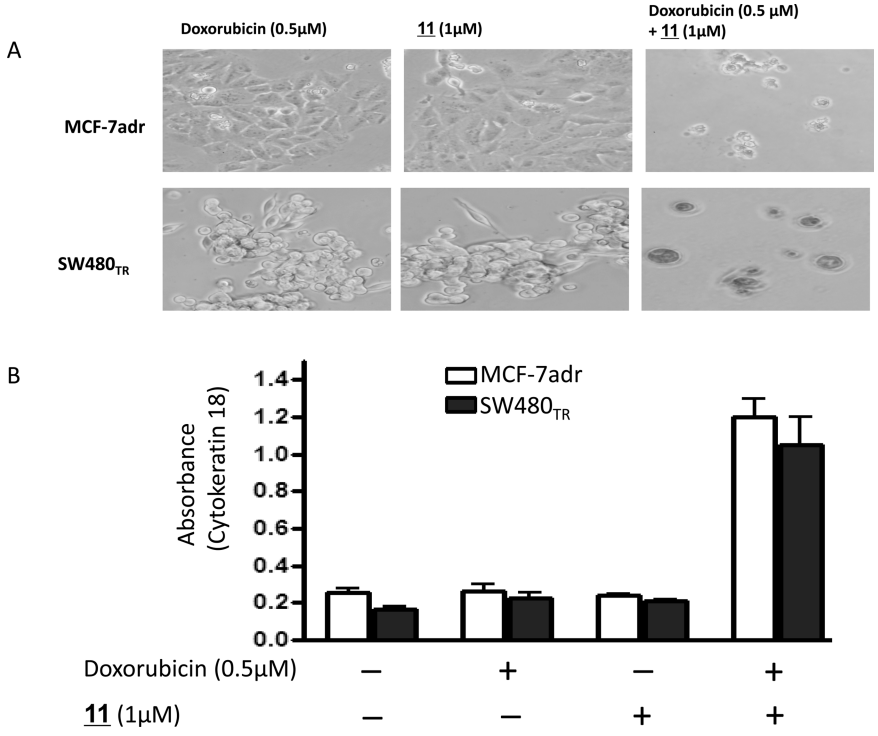

Figure 4. Isomer **11** in combination with doxorubicin significantly induces cell death and apoptosis. A: Cell death induced by isomer **11** in combination with doxorubicin on MCF-7adr and $SW480_{TR}$ cells. B: Cell apoptosis induced by **11** in combination with doxorubicin on MCF-7adr and $SW480_{TR}$ cells. Cells were seeded at a density of 8000 cells per well in a 96-well plate for 24 h. The cells were then treated with **11**, doxorubicin, or a combination of the two drugs for an additional 48 h. The cells were lysed with 10% NP-40, and the M30-Apoptosense ELISA assay was performed as described in Materials and Methods.

greater cell death as compared with doxorubicin or **11** alone (Figure 4). Additionally, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) cytotoxicity assay also showed that **11** has a synergistic effect on doxorubicin-induced cell death.

Effect of Combination Therapy with 11 on Tumor Growth in Vivo. To further evaluate the effects of **11** and paclitaxel combination therapy on the tumor growth of resistant cells, the growth of xenograft tumors was examined. A well-characterized ovarian cancer MDR cell line, $SKOV\text{-}3_{TR}$, was

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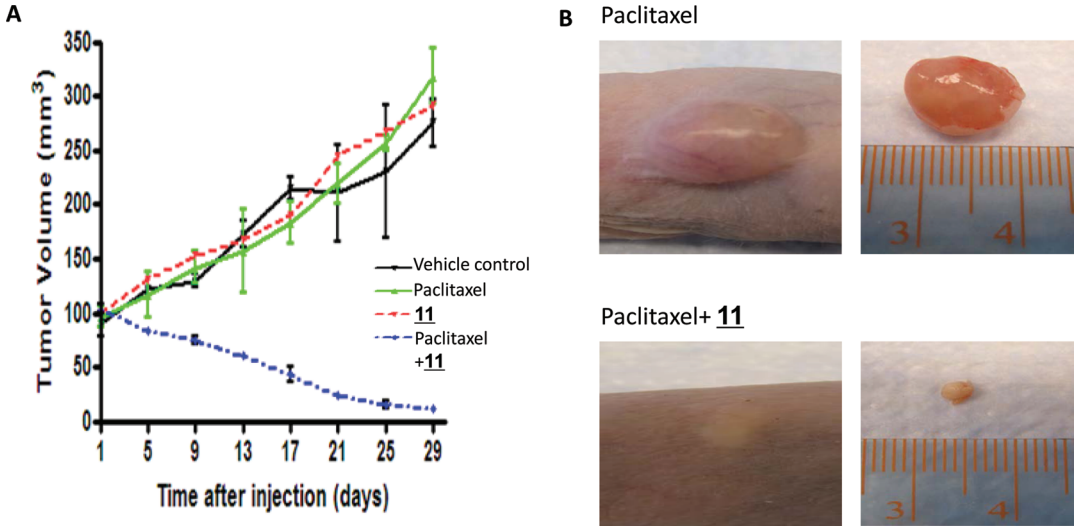

Figure 5. Effect of combination treatment with **11** and paclitaxel on tumor growth in vivo. A: Tumors were measured twice a week with a digital caliper. Tumor volume (mm^3) was calculated as $(W^2 \times L)/2$, where W is width and L is length. B: Representative images of dissected tumor tissues from treated nude mice.

chosen for in vivo studies. The results showed that **11** or paclitaxel alone had no significant effects on the paclitaxel-resistant tumor cell growth in xenograft model. In contrast, the combination of paclitaxel and **11** produced an inhibitory effect on the resistant tumor growth as compared with animals treated with paclitaxel alone (20 mg/kg, Figure 5). Intraperitoneal (ip) injection of **11** (50 mg/kg) was found to almost completely reverse resistance to paclitaxel in this model. In addition, on the basis of animal weight and mortality, no significant toxicity was observed and the animals appeared to have tolerated it well when **11** was administered alone.

CONCLUSIONS

5 is a newly identified small molecular compound found to efficiently reverse MDR in a wide variety of MDR cell lines.[5] The present studies were undertaken to synthesize **5** isomers **8**, **9**, **10**, and **11** based on its molecular chirality and to characterize these novel isomers in vitro and in vivo. We observe that **11** is a selective and very potent inhibitor of Pgp-mediated MDR, and the data provide valuable clues for the further optimization of this structure. The in vitro potency was evaluated by several assays (including cytotoxic drug activity by MTT assay, enhancement of drug uptake by calcein AM assay, and increased drug-induced apoptosis by M-13 apoptosense assay) using a panel of human MDR cell lines with different expressions of Pgp, MRP1, and BCRP. Not surprisingly, we observe that **11** recapitulates the activity of the diasteromeric mixture of **5** isomers; **11** reverses MDR in (Pgp) overexpressing cell lines for several chemotherapy drugs, is a highly potent, specific, nontoxic Pgp inhibitor, and does not inhibit MRP1 or BCRP1 at therapeutically relevant doses. **11** appears to possess all of the desired properties for treating Pgp-mediated MDR.

Direct comparison with **8**, **9**, **10**, and **11** shows that **11** is the most potent modulator (Figure 1). Previous and current studies have clearly indicated that reversal of MDR by **5** and its isomer **11** was through selective and potent inhibition of Pgp function.[5] For example, in contrast to reversing MDR in Pgp-overexpressed cell lines, **11** had no effect on drug sensitivities in MRP1- or BCRP-overexpressed MDR cell lines, nor did it affect the sensitivities to cisplatin or carboplatin (data not shown).

In this study, we show new in vivo data confirming in mouse xenografts the expected activities seen in vitro. **11** exhibited potent activity after ip administration in mice bearing MDR human xenografts, restoring antitumor activity of paclitaxel without an apparent increase in toxicity. All of the efficacious combination schedules with **11** appeared to be well tolerated, as indicated by the lack of significant changes in body weights of various treatment groups. These results demonstrate that the **5** isomer **11** is an extremely potent, selective, and effective inhibitor of Pgp with a long duration of action. It exhibits potent activity without apparently enhancing the toxicity of coadministrated drugs.

In conclusion, **11** is a potent in vitro and in vivo modulator of MDR1 (Pgp)-mediated MDR. The properties of **11** indicate that it will be useful in the treatment of Pgp-mediated chemoresistant cancers.

EXPERIMENTAL SECTION

General Procedures for the Synthesis of 5 Isomers. All evaporations were carried out in vacuo with a rotary evaporator. Analytical samples were dried in vacuo (1−5 mmHg) at room temperature. Thin layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator. Spots were visualized by UV light (214 and 254 nm). Purification by column and flash chromatography was carried out using silica gel (300−400 mesh). Solvent systems are reported as volume percent mixture. All NMR spectra were recorded on a Bruker WH-300 (300 MHz) spectrometer. ^1H chemical shifts are reported in δ values in ppm with the deuterated solvent as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constant (Hz), integration. LCMS spectra were obtained on an Agilent 1200 series 6110 or 6120 mass spectrometer with electrospray ionization. LCMS: Method 1 (5-100a and 5-100b) = (Waters X Bridge C18, 50 mm × 4.6 mm × 3.5 μm, 2.3 min gradient, 5% [0.05% TFA/CH_3CN]: 95% [0.05% TFA/H_2O] to 100% [0.05% TFA/CH_3CN] for 1.6 min and hold 100% [0.05% TFA/CH_3CN] for 0.7 min; method 2 (5-100a (test) and 5-100b (test)) = (Waters X Bridge C18, 50 mm × 4.6 mm × 3.5 μm, 3.5 min gradient, 5% [0.05% TFA/CH_3CN]: 95% [0.05% TFA/H_2O] to 100% [0.05% TFA/CH_3CN] for 1.6 min and hold 100% [0.05% TFA/CH_3CN] for 1.9 min; method 3 (5−100A 5) = (Waters X Bridge C18, 50 mm × 4.6 mm × 3.5 μm, 5.8 min gradient, 5% [0.05% TFA/CH_3CN]: 95% [0.05% TFA/H_2O] to 100% [0.05% TFA/CH_3CN] for 5.0 min and hold 100% [0.05% TFA/CH_3CN] for 0.8 min. Preparative chiral HPLC purification was performed on a Shimadzu

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LC 20 with UV detector SPD-20A at 35 °C using CHIRALPAK IA (0.46 cm I.D. × 25 cm length) and using hexane/ispropanol/DEA = 70/30/0.1 (V/V/V) as the mobile phase. Flow rate was 1.0 mL/min (all solvents were HPLC grade). The HPLC system was monitored at 254 nm. Analytical chiral HPLC spectra were run on a Shimadzu LC 20 with UV detector SPD-20A at 35 °C using Chiralpak AD-H (0.46 × 25 cm) and using hexane/ispropanol/DEA = 70/30/0.1 (V/V/V) during 20 min as the mobile phase. Flow rate was 1.0 mL/min (all solvents were HPLC grade). The HPLC system was monitored at 254 nm. Optical rotation was run on a Polarimeter Perkin-Elmer Model 341 at 20 °C.

2-(4-Methoxyphenyl)quinoline-4-carboxylic Acid (1). Indoline-2,3-dione (30.6 g, 203.9 mmol), 1-(4-methoxyphenyl)ethanone (25.0 g, 169.9 mmol), and KOH (28.6 g, 509.7 mmol) were dissolved in EtOH (200 mL). The mixture was stirred at 80 °C for 24 h. Evaporation of the solvent afforded a residue which was diluted in water, and then the solution was extracted by ether. The aqueous phase was acidified at 0 °C to pH = 1 with concd HCl, and then the precipitate was collected by suction filtration, washed with water and CH_2Cl_2, and dried in vacuo to afford 28.4 g (50% yield) of analytically pure **1** (yellow solid) confirmed by LCMS. Analytical LCMS (method 1): single peak (214, 254 nm), t_R = 1.65 min, MS (ESI$^+$) m/z 279.09 (M + H)$^+$.

Methyl 2-(4-Methoxyphenyl)quinoline-4-carboxylate (2). To a solution of **1** (15 g, 53.8 mmol) in MeOH (50 mL) was added concd H_2SO_4 (13 mL). The mixture was stirred under reflux overnight, diluted by water, and extracted by AcOEt. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated under reduced pressure to afford 11.1 g (70%) of analytically pure **2** (yellow solid) confirmed by LCMS. Analytical LCMS (method 2): single peak (214, 254 nm), t_R = 2.05 min, MS (ESI$^+$) m/z 293.11 (M + H)$^+$.

(2-(4-Methoxyphenyl)quinolin-4-yl)(pyridin-2-yl)methanone (3). To a solution of n-BuLi (2.5 M, 26.4 mL, 66.1 mmol) in Et_2O (80 mL) cooled to −40 °C under N_2 was added a solution of 2-bromopyridine (10.4 g, 66.1 mmol) in Et_2O (20 mL). The reaction was maintained at −78 °C for 20 min, and then a solution of **2** (6.5 g, 22.0 mmol) in THF was added at −70 °C under N_2. The reaction mixture was stirred at −70 °C for 1.5 h, quenched with water, and extracted by AcOEt. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated in vacuo to give a residue. The residue was triturated with Et_2O to afford 7 g (91%) of analytically pure **3** (yellow solid) confirmed by LCMS. Analytical LCMS (method 1): single peak (214, 254 nm), t_R = 1.87 min, MS (ESI$^+$) m/z 341.2 (M + H)$^+$.

(2-(4-Methoxyphenyl)quinolin-4-yl)(pyridin-2-yl)methanol (4). To a solution of **3** (7.7 g, 22.6 mmol) in EtOH (110 mL) cooled to 0 °C was added $NaBH_4$ (2.12 g, 45.3 mmol). After the addition was complete, the mixture was stirred for 0.5 h and then quenched with water. Then AcOEt was added to the solution, and the organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated in vacuo to afford 4.6 g (60% yield) of **4** (white solid) which was used for the next step without further purification. Analytical LCMS (method 2): single peak (214, 254 nm), t_R = 1.53 min, MS (ESI$^+$) 343.2 (M + H)$^+$.

(2-(4-Methoxyphenyl)quinolin-4-yl)(piperidin-2-yl)methanol (5) (NSC23925). To a solution of **4** (3.72 g, 10.88 mmol) in MeOH (360 mL) were added concd HCl (9 mL) and PtO_2 (741 mg, 3.26 mmol). The mixture was then hydrogenated at 20 °C for about 2 h. The catalyst was filtered off and washed with MeOH (3 × 10 mL), and the filtrate was concentrated under reduced pressure. The residue obtained was a dark green solid, which was used for the next step reaction without further purification. (We tried to directly separate the four isomers by using chiral prep HPLC but failed.) Analytical LCMS (method 2): single peak (214, 254 nm), t_R = 1.28 min, MS (ESI$^+$) 349.3 (M + H)$^+$.

***tert*-Butyl 2-(Hydroxy(2-(4-methoxyphenyl)quinolin-4-yl)-methyl)piperidine-1-carboxylate (6, 7).** To a solution of **5** (the residue above) and Et_3N (3.48 g, 34.48 mmol) in THF (50 mL) cooled at 0 °C was added a solution of $(Boc)_2O$ (4.0 g, 11.49 mmol) in THF (15 mL) dropwise. The mixture was stirred at r.t. overnight, and then water (50 mL) was added. The mixture was extracted with AcOEt (3 × 100 mL), and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated in vacuo, and the crude residue was flash chromatographed on silica gel and eluted with 1:10−1:6 EA/PE to afford 1.0 g of **6** (threo isomer) (analytical LCMS (method 1): single peak (214, 254 nm), t_R = 1.62 min, MS (ESI$^+$) 449.0 (M + H)$^+$) and 1.3 g of **7** (erythro isomer) (analytical LCMS (method 1): single peak (214, 254 nm), t_R = 1.68 min, MS (ESI$^+$) 449.3 (M + H)$^+$).

(2-(4-Methoxyphenyl)quinolin-4-yl)(piperidin-2-yl)methanol Hydrochloride (8−11). After purification of **7** (1.3 g) by chiral HPLC, two Boc-protected chiral compounds were separated from each other. Then each compound was deprotected with a solution of HCl in EtOH to give the corresponding HCl salt. Thus, **10** (0.6 g) and **11** (0.6 g) were obtained, respectively. Using the same methods, **8** (0.37 g) and **9** (0.26 g) were produced, respectively, from 1 g of **6**.

(1*R*,2*R*)-(2-(4-Methoxyphenyl)quinolin-4-yl)(piperidin-2-yl)-methanol Hydrochloride (8). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (m, 2H), 1.72 (m, 4H), 2.81 (m, 1H), 3.24 (m, 1H), 3.49 (m, 1H), 3.89 (s, 3H), 4.5 (brs, 1H), 5.68 (s, 1H), 7.20 (d, J = 8.7 Hz, 2H), 7.74 (m, 1H), 7.93 (m, 1H), 8.26−8.38 (m, 5H), 8.53 (m, 1H), 9.25 (m, 1H); analytical LCMS (method 3): 97% purity (214 nm), t_R = 1.77 min, MS (ESI$^+$) m/z 349.0 (M + H)$^+$; $[\alpha]_D^{20}$ = −15.3 (c = 0.16, MeOH); chiral HPLC purity: 99.9% ee (254 nm) (t_R (major) = 6.52 min, t_R (minor) = 8.69 min).

(1*S*,2*S*)-(2-(4-Methoxyphenyl)quinolin-4-yl)(piperidin-2-yl)-methanol Hydrochloride (9). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (m, 2H), 1.72 (m, 4H), 2.81 (m, 1H), 3.24 (m, 1H), 3.49 (m, 1H), 3.89 (s, 3H), 4.5 (brs, 1H), 5.68 (s, 1H), 7.20 (d, J = 8.7 Hz, 2H), 7.74 (m, 1H), 7.93 (m, 1H), 8.26−8.38 (m, 5H), 8.53 (m, 1H), 9.25 (m, 1H); analytical LCMS (method 3): 99% purity (214 nm), t_R = 1.78 min, MS (ESI$^+$) m/z 349.0 (M + H)$^+$; $[\alpha]_D^{20}$ = +15.0 (c = 0.16, MeOH); chiral HPLC purity: 99.9% ee (254 nm) (t_R (major) = 8.70 min, t_R (minor) = 6.53 min).

(1*R*,2*S*)-(2-(4-Methoxyphenyl)quinolin-4-yl)(piperidin-2-yl)-methanol Hydrochloride (10). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 (m, 2H), 1.68 (m, 4H), 2.97 (m, 1H), 3.25 (m, 1H), 3.45 (m, 1H), 3.90 (s, 3H), 4.6 (brs, 1H), 6.11 (s, 1H), 7.23 (d, J = 8.7 Hz, 2H), 7.78 (m, 1H), 8.01 (m, 1H), 8.26 (m, 3H), 8.50 (m, 2H), 8.69 (t, J = 8.7 Hz, 1H), 10.46 (m, 1H); analytical LCMS (method 3): 95% purity (214 nm), t_R = 1.83 min, MS (ESI$^+$) m/z 349.0 (M + H)$^+$; $[\alpha]_D^{20}$ = −15.8 (c = 0.2, MeOH); chiral HPLC purity: 99.9% ee (254 nm) (t_R (major) = 4.96 min, t_R (minor) = 12.7 min).

(1*S*,2*R*)-(2-(4-Methoxyphenyl)quinolin-4-yl)(piperidin-2-yl)-methanol Hydrochloride (11). ^{1}H NMR (300 MHz, DMSO-d_6) δ1.29 (m, 2H), 1.68 (m, 4H), 2.97 (m, 1H), 3.25 (m, 1H), 3.45 (m, 1H), 3.90 (s, 3H), 4.6 (brs, 1H), 6.11 (s, 1H), 7.23 (d, J = 8.7 Hz, 2H), 7.78 (m, 1H), 8.01 (m, 1H), 8.26 (m, 3H), 8.50 (m, 2H), 8.69 (t, J = 8.7 Hz, 1H), 10.46 (m, 1H); analytical LCMS (method 3): 98% purity (214 nm), t_R = 1.83 min, MS (ESI$^+$) m/z 349.0 (M + H)$^+$; $[\alpha]_D^{20}$ = +15.6 (c = 0.2, MeOH); chiral HPLC purity: 97.2% ee (254 nm) (t_R (major) = 12.7 min, t_R (minor) = 4.7 min).

X-ray Crystallography of 5 Isomers. The absolute configuration of the isomer was determined by X-ray crystallography technique at the Center for Crystallographic Studies at Department of Chemistry and Chemical Biology, Harvard University. A crystal of a **5** isomer was mounted on a diffractometer, and data were collected at 100 K. The intensities of the reflections were collected by means of a Bruker APEX II CCD diffractometer ($Mo_{K\alpha}$ radiation, λ = 0.71073 Å) and equipped with an Oxford Cryosystems nitrogen flow apparatus. The collection method involved 0.5° scans in ω at 28° in 2θ. Data integration down to 0.76 Å resolution was carried out using SAINT V7.46 A with reflection spot size optimization. Absorption corrections were made with the program SADABS. The structure was solved by the direct methods procedure and refined by least-squares methods again F^2 using SHELXS-97 and SHELXL-97. Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were allowed to ride on the respective atoms. Crystal data, hydrogen bond parameters, and geometric parameters of the isomer were collected. The Ortep plots

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produced with the SHELXL-97 program, and the other drawings, were produced with Accelrys DS Visualizer 2.0.

Pharmacology, Cell Lines, Cell Culture, and Drugs. A panel of a parental cancer cell line and their daughter resistant cell lines, displaying a MDR phenotype, were used in this study. Human ovarian cancer cell line SKOV-3, breast cancer cell line MCF-7, colon cancer cell line SW480, osteosarcoma cell line U-2OS, KHOS, uterine sarcoma cell line MESSA and its doxorubicin selected drug-resistant cell line MESSA/Dx5, and nonsmall cell lung cancer cell line H-69 and it is doxorubicin-selected drug-resistant cell line H-69AR (overexpresses MRP1) were obtained from the ATCC (Rockville, MD). The multidrug-resistant SKOV-3_{TR}, MCF-7adr, and $SW480_{TR}$ cell lines were established as previously reported. Dr. Efstathios Gonos (Institute of Biological Research & Biotechnology, Athens, Greece) provided the doxorubicin-resistant U-2OS R2 (referred in the text below as U-$2OS_{DR}$), KHOS R2 cell lines. Dr. Erasmus Schneider (Wadsworth Center, Albany) provided the mitoxantrone-resistant breast cancer MCF-7/MX (overexpresses BCRP) cell line. All the cell lines were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin (Invitrogen). Cells were incubated at 37 °C in 5% CO_2−95% air atmosphere and passaged when near-confluent monolayers were achieved using 2% trypsin−EDTA solution. Drug-resistant cell lines were periodically cultured in the respective drug to confirm their drug resistance characteristics. The chemotherapy drugs were obtained from the pharmacy at the Massachusetts General Hospital and stored at −20 °C.

Cytotoxicity MTT Assay and Reversals of MDR. To evaluate for reversal of Pgp-mediated MDR, SKOV-3_{TR}, MCF-7adr, and $SW480_{TR}$ cell lines were seeded into a 96-well tissue culture plate at 2000 cells per well with varying concentrations of chemotherapy drugs and **11** for 6 days. Cell proliferation IC_{50} (concentration required for 50% inhibition) and MDR reversal IC_{50} were determined from dose−response curves carried out in triplate in a 96-well plate. The fold of reverse resistance for individual drugs were defined as the IC_{50} of MDR cells without **11** treatment divided by the IC_{50} of MDR cells treated with **11**. To test for the effect of reversal of MRP1- or BCRP-mediated MDR, H-69AR or MCF-7/MX cells were placed into 96-well plates and treated with chemotherapy drugs similar to that described above. Drug cytotoxicity was assessed in vitro using the MTT assay as previously described.[18] Drugs at the concentrations utilized in the MTT assay were performed in the absence of cells to verify no change in absorbances. Response curves were fitted with the use of GraphPad PRISM 4 software (GraphPad Software).

Drug Efflux Fluorescence Microscopy Assay. The Vybrant multidrug resistance assay kit (Invitrogen/Molecular Probes) was used to measure drug efflux properties of different resistant cell lines. This assay utilizes the fluorogenic dye calcein acetoxymethyl ester (calcein AM) as a substrate for efflux activity of Pgp or other membrane pump ABC proteins. Calcein AM is taken up by cells and hydrolyzed by cytoplasmic esterases to fluorescent calcein. Calcein AM is well retained in the cytosol. However, multidrug-resistant cells expressing high levels of Pgp rapidly extrude nonfluorescent calcein AM from the plasma membrane, reducing accumulation of fluorescent calcein in the cytosol. Studies were carried out as described. In brief, drug-resistant cells (5000 cells per well) were cultured in a 96-well plate for 24 h. Triplicated cultures of cells were treated with different concentrations of **11** for 1 h and then incubated in calcein AM in 100 μL of total volume. After 30 min, images were acquired by Nikon Eclipse Ti-U fluorescence microscope (Nikon Corp.) equipped with a SPOT RT digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI).

Apoptosis Assays. Apoptosis was evaluated using the M30-Apoptosense ELISA assay kit (Peviva AB, Bromma, Sweden) according to the manufacturer's instructions. For the doxorubicin and **11** treatment, MCF-7adr or $SW480_{TR}$ cells were seeded at 8000 cells/per well in a 96-well plate for 24 h before treatment. The cells were then treated with 0.5 μM doxorubicin, 1 μM **11**, or a combination of the two drugs for an additional 72 h. The cells were then lysed by an additional 10 μL of 10% NP40 per well, following the manufacturer's instructions for apoptosis assay.

Effect of Combination 11 and Paclitaxel on Tumor Growth in Vivo. The Crl:SHO-*Prkdc*^{SCID}*Hr*^{hr} nude female mice at approximately 3−4 weeks of age were purchased from The Charles River Laboratories (Ann Arbor, MI). Animal studies were carried out with protocols approved by the Massachusetts General Hospital Subcommittee on Research Animal Care (SRAC). To determine the effect of **11** on paclitaxel sensitivity in the xenograft model, 5×10^6 SKOV-3_{TR} cells were injected subcutaneously with matrigel (BD Biosciences, San Jose, CA) on day one. Two weeks after injection, the mice were divided into four groups to start the treatments. Group one received intraperitoneal (ip) injection with normal saline, group two with paclitaxel (20 mg/kg), and group three with **11** (50 mg/kg). The final group of mice received paclitaxel (20 mg/kg) and **11** (50 mg/kg). The dose of paclitaxel (20 mg/kg) was based on previous studies.[19,20] All four groups were treated twice a week for two weeks. The health of the mice and evidence of tumor growth were examined daily. Tumors were measured twice a week with a digital caliper. Tumor volume (mm^3) was calculated as $(W^2 \times L)/2$, where W is width and L is length. The curve of tumor growth was drawn according to tumor volume by using of GraphPad PRISM 4 software.

■ ASSOCIATED CONTENT

Supporting Information

Perspective views showing 50% probability displacement ellipsoids of absolute configuration for all four isomers (Figure 1). Chiral HPLC spectra of four compounds **8**, **9**, **10** and **11** (Figure 2). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

MDR, multidrug resistance; Pgp, P-glycoprotein; MRP1, multidrug-resistant protein 1; BCRP, breast cancer-resistant protein

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