Discovery of a Daunorubicin Analogue That Exhibits Potent Antitumor Activity and Overcomes P-gp-Mediated Drug Resistance

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Anthracyclines are considered to be some of the most effective anticancer drugs for cancer therapy. However, drug resistance and cardiotoxicity of anthracyclines limit their clinical application. We hypothesize that direct modifications of the sugar moiety of anthracyclines avert P-glycoprotein (P-gp) recognition and efflux, increase drug intracellular concentration in cancer cells, and thus overcome P-gp-mediated drug resistance. Daunorubicin (DNR) analogues with sugar modifications were synthesized by directly transforming the amino group of DNR to an azido group or triazole group. Molecular docking showed that the lead compound (3′-azidodaunorubicin, ADNR) averts P-gp binding, while daunorubicin (DNR) extensively interacts with multidrug-resistance (MDR) protein through H-bonds and electrostatic interactions. FACS assay demonstrated that these new compounds abolished P-gp drug efflux and accumulated high intracellular concentration in the drug-resistant leukemia K562/Dox. P-gp inhibition by CsA confirmed that these new analogues are no longer P-gp substrates. ADNR exhibited potent anticancer activity in both drug-sensitive (K562) and drug-resistant leukemia cells (K562/Dox), with a 25-fold lower drug resistance index than DNR. An in vivo xenograft model demonstrated that ADNR showed more than 2.5-fold higher maximum growth inhibition rate against drug-resistant cancers and significant improvement for animal survival rate versus DNR. No significant body weight reduction in mice was observed for ADNR at the maximum tolerable dose, as compared to more than 70% body weight reduction for DNR. These data suggest that sugar modifications of anthracyclines avert P-gp binding, abolish P-gp-mediated drug efflux, increase intracellular drug concentration, and thus overcome P-gp-mediated drug resistance in cancer therapy.

Introduction

Anthracyclines are considered to be some of the most effective anticancer drugs used either as single agents or in combination for cancer therapy. Since the first isolation of doxorubicin (DOX) and daunorubicin (DNR) from *Streptomyces peucetius* in the 1960s,[1] thousands of analogues have been reported. However, only a few of them have earned clinical approval: idarubicin (IDA),[2] epirubicin (EPI),[3] aclarubicin,[4−7] pirarubicin,[8] and valrubicin (Scheme 1).[9] These drugs are natural or semisynthetic products with similar structures and sugar moieties.[10,11] For instance, DOX and DNR share the same carbon skeleton (Scheme 1), which contains a fused tetracyclic ring system and a carbohydrate moiety (daunosamine). The only difference between DOX and DNR is the side chain at C-14, where DNR carries no functional group and DOX bears a hydroxyl group. Although all these anthracyclines contain similar structures, their therapeutic applications are very different. For instance, DNR and IDA are primarily used in leukemia (ALL and AML) and lymphoma, whereas DOX and EPI have broader anticancer activity against leukemia, lymphomas, and various of solid tumors including breast, small cell lung, cervical, head, and neck cancers.

However, several limitations are frequently associated with the clinical use of anthracyclines. These include drug resistance and cardiotoxicity. MDR is a simultaneous development of resistance to a variety of anticancer drugs in cancer therapy.[12] Although this phenomenon has been studied for a long time, the exact mechanism is still under extensive investigation. Many factors are involved in multidrug resistance.[13] One of the mechanisms is through the overexpression of ABC transporters.[14−18] These ABC transporters use ATP hydrolysis as an energy source to recognize and export very diverse anticancer compounds from cancer cells, decrease intracellular drug concentration, and thereby induce drug resistance. These ABC transporters include 49 different family members, such as multidrug resistance genes (MDR1, ABCB1, P-gp), multidrug-resistance-associated proteins (MRP), and breast cancer resistant proteins (BCRP, ABCG2).[17−20] These transporters actively export a wide range of drugs from cells, which include anticancer drugs anthracyclines, vinca alkaloids, epipodophyllotoxins, and taxanes; HIV-1 protease inhibitors; immunosuppressants; antibacterial reagents; antifungals; and cardiac glycosides.[21−24] The strategy of reversing drug resistance has been explored on the basis of these mechanisms of drug resistance; however, limited success has been achieved.

The second limitation for anthracycline clinical use is the peculiar and often irreversible cardiac toxicity. Cardiac toxicity of anthracyclines is represented by the development of cardiomyopathy and ultimately congestive heart failure. The cardiac

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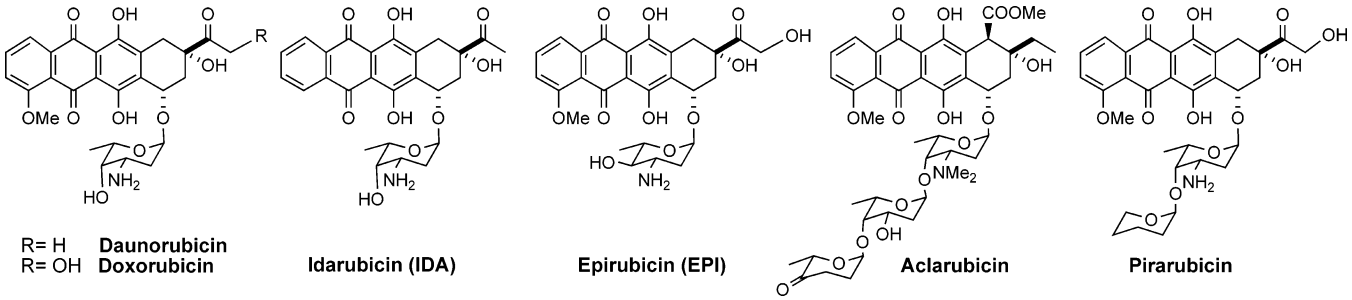
Scheme 1. Clinically Used Anthracyclines



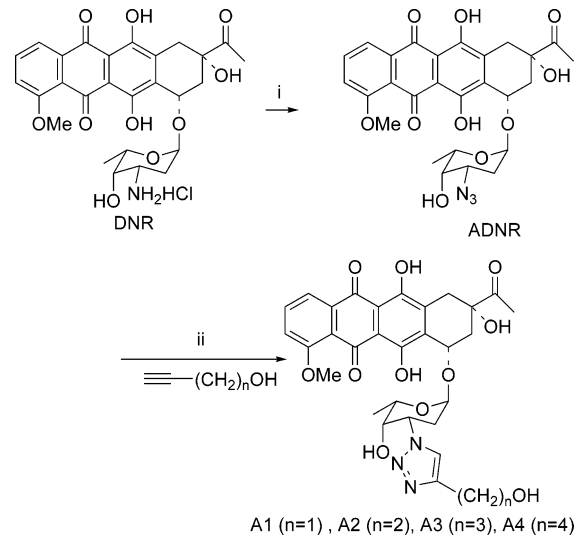
toxicity is often irreversible and related to the total dose accumulation.[25] Therefore, the maximum cumulative doses of DNR and DOX should be less than 500 or 450−600 mg/m^2, respectively.[26] The mechanisms of anthracycline-induced cardiac toxicity are rather complex and multifactorial.[27] These include drug accumulation in heart tissues, free radical generation, apoptosis, C-13 secondary alcohol production, inhibition of nucleic acid and protein synthesis, release of vasoactive amines, changes in adrenergic function, abnormalities in the mitochondria, lysosomal alterations, altered calcium transport, imbalance of myocardial electrolytes, reduction in myocardial antioxidant enzyme activities, increase of lipid peroxidation,[27] and induction of nitric oxide synthase.[27]

Our previous research and literature indicate that modifications on the sugar moiety of anthracyclines markedly alter the anticancer activity, target selectivity, and the sequence specificity of DNA break sites.[28,29] Indeed, modifications on the sugar structures resulted in the development of the second generation of DOX analogues (such as EPI, IDA, valrubicin, and pirarubicin). A third generation of anthracycline analogue [MEN10755, a disaccharide analogue, currently given the generic name of sabarubicin, 3′-deamino-3′-hydroxy-4′-(*O*-L-daunosamyl)-4-demethoxydoxorubicin] shows a different activity spectrum as compared to DOX.[10,30−32] Despite their structure similarity, the second generation of anthracyclines (IDA and EPI) have less cardiac toxicity than DOX and DNR; however, they are still P-gp substrates, and thus, they still exhibit drug resistance.[27,32] Sabarubicin shows partial activity against drug-resistant cancer cells, but it does not avert P-gp recognition.[33] In addition, sabarubicin still shows high cardiac toxicity at higher doses.

We hypothesize that direct modifications of the sugar moieties of anthracyclines avert P-gp recognition and efflux, increase drug intracellular concentration in cancer cells, and thus overcome P-gp-mediated drug resistance. Indeed, this hypothesis has been validated by other previous reports.[34−40] For instance, replacement of the 3′-NH_2 of DOX with 3′-OH or 3′-*N*-methylation was shown to reverse the drug resistance.[34−43] In addition, modification of disaccharides in antibiotics (such as vancomycin) has been successfully applied in overcoming bacterial drug resistance.[44,45]

In this research, we report the syntheses and biological activities of a novel class of DNR analogues, 3′-azido-2,3,6-trideoxy-α-L-lyxo-hexopyranosyldaunorubicinone (ADNR), where the 3′-amino group is replaced with an azido group and its triazole derivatives (Scheme 2). These compounds were modeled by molecular docking with a MDR protein. The drug efflux by P-gp was tested in drug-resistant leukemia K562/dox with high expression levels of P-gp. The anticancer activity and toxicity were tested in K562/Dox in vitro and xenograft models in vivo. New anthracycline analogues avert P-gp binding, abolish P-gp mediated drug efflux, increase intracellular drug concentration, and thus overcome P-gp-mediated drug resistance. The lead

Scheme 2[a]



[a] Reagents and conditions: (i) K_2CO_3, $CuSO_4$, TfN_3 solution, overnight; (ii) $(EtO)_3PCuI$, DIPEA.

compound ADNR shows significant anticancer activity against drug-resistant cancers in cell culture in vitro and in a xenograft model in vivo, increases animal survival rate, and decreases general toxicity in animal model.

Results and Discussion

Chemistry. Synthesis of Daunorubicin Analogues. ADNR was readily prepared by treatment of DNR with a TfN_3 solution[46] in 70% yield. To prepare triazole analogues **A1**−**A4** through Huisgen 1,3-dipolar cycloaddition reaction, three catalytic systems were tested, including CuI/DIPEA (diisopropylethylamine),[47] $CuSO_4 \cdot 5H_2O$/sodium ascorbate,[48] and $(EtO)_3PCuI$/DIPEA.[49] The results showed that only $(EtO)_3PCuI$/DIPEA gave a satisfactory yield. Thus, all the triazole analogues were obtained using this catalytic system in THF at room temperature. Reaction of ADNR with the corresponding alkynes in the presence of $(EtO)_3PCuI$ and DIPEA afforded compounds **A1**−**A4** in yields of 53, 67, 60, and 77%, respectively (Scheme 2).

Biology. Molecular Docking of DNR and ADNR to Multidrug Resistance (MDR) Protein. P-gp exports many anticancer drugs (including anthracyclines) to confer drug resistance. After drugs are diffused into cells, they bind to intracellular domains of P-gp and trigger ATP binding to the nucleoside binding domain (NBD) and the hydrolysis of ATP. The energy from the ATP hydrolysis exports the drugs out of cells.[50]

The positive charge from the amine (NH_2) in the sugar structure of anthracyclines is very critical in P-gp recognition.[27] We hypothesize that replacement of the NH_2 with an azido or

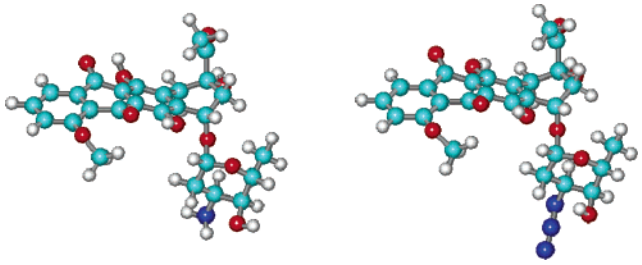


Figure 1. 3D Conformations of DNR (left) and ADNR (right).

triazole group will abolish its positive charge, provide hindrance with the linear molecular arrangement ($-N_3$) or a large substituent (trizole), and create a higher electron density ($-N_3$) to prevent P-gp binding (Figure 1). Since no crystal structure of mammalian P-gp is available, it is impossible to perform the molecular docking to mammalian P-gp. Fortunately, a distant ortholog(MsbA) in bacteria, which has 30% homology to human P-gp, has been crystallized with 3.8 Å resolution.[51−53] More importantly, MsbA is also a multidrug resistance protein in bacteria that effluxes many drugs out of bacteria to confer drug resistance. Also, MsbA and P-gp share many common substrates. Therefore, we utilized the MsbA crystal structure as a target to perform molecular docking to test our compounds.

The molecular docking results showed that DNR binds to the cavity between the intracellular domain (ICD) and NBD (Figure 2). The C4−OH in the sugar structure forms H-bonds with histidine 202 (His202) in the ICD2 of ICD domain. It also interacts with aspartic acid 431 (Asp431) in the α2 of NBD domain. The NH_3^+ in the sugar structure forms a H-bond with Asp431 in the NBD domain, in addition to the electrostatic interaction between the positive and negative charge (Figure 2). However, the four rings in DNR lie in the cavity without any specific interactions with any amino acid residues. In contrast, the azido sugar in ADNR turns away from His202 and Asp431. No specific interaction is found between ADNR and any amino acid residue. The four rings of ADNR are in the same position as in DNR, which lie in the cavity with no specific interactions with any amino acid residue (Figure 2). These data indicate that DNR strongly binds to MsbA while ADNR has no binding or interaction with MsbA. Therefore, ADNR may avert recognition by MDR protein to overcome P-gp-mediated drug resistance.

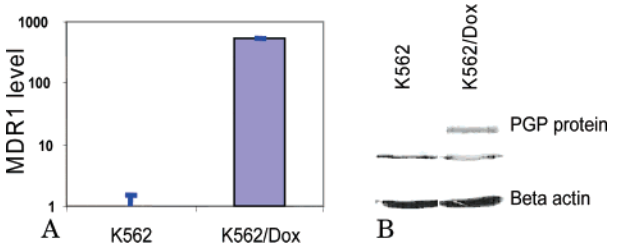


Figure 3. Real-time PCR and Western blotting results of MDR1 mRNA (A) and P-gp protein (B) levels in drug-resistant leukemia K562/Dox compared to drug-sensitive leukemia K562 cells.

New Anthracycline Analogues Avert P-gp Recognition and Efflux in Drug-Resistant Leukemia Cells (K562/Dox) in Vitro. To confirm our molecular modeling findings, we tested the P-gp export of these new anthracycline analogues (ANDR and A1-A4) in K562/Dox cells in the presence or absence of a P-gp inhibitor (cyclosporine, CsA). If a drug is a P-gp substrate, the P-gp inhibitor will inhibit the drug efflux and increase the intracellular concentrationof the drug. Therefore, we performed a flow cytometry (FACS) study to quantify the drug uptake and efflux in K562/Dox cells for all new anthracycline analogues. This cell line was induced to exhibit drug resistance by the treatment of a low concentration of Dox. One of the mechanisms for Dox-induced drug resistance is the overexpression of an ABC transporter (ABCB1, MDR1, gene product is P-glycoprotein, P-gp). To confirm the high expression of P-gp in K562/Dox cells, MDR1 mRNA and P-gp protein expression levels were measured by real-time PCR and Western blotting in both K562 and K562/Dox cells. In drug-resistant K562/Dox cells, MDR1 mRNA was induced by 600-fold higher than drug-sensitive K562 cells as measured by real-time PCR. The P-gp protein was undetectable in drug-sensitive K562 cells, while P-gp was significantly induced in K562/Dox cells as confirmed by Western blot (Figure 3). Since P-gp is significantly overexpressed in K562/Dox cancer cells, these cells provide good models for a P-gp-mediated drug resistance study.

As shown in Figure 4, DNR readily diffused to the K562/Dox cells after 30 min of incubation. P-gp in the cell membrane exported the drug out of the cells. However, when CsA (5 μM) was coincubated with DNR for 30 min, much more drug was accumulated in the cells (uptake phase) as measured by FACS. After the uptake, the cells were washed with PBS, and the drug

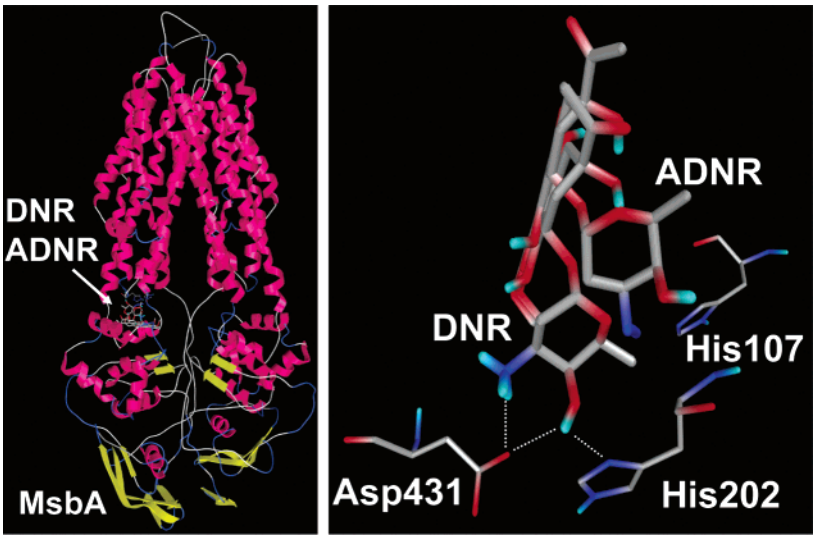


Figure 2. Molecular docking of daunorubicin (DNR) and ADNR to multidrug resistance protein (MsbA). The left panel is the overall docking view of drugs and MsbA. The right panel is the detailed interaction between DNR or ADNR with amino acid residues in MsbA.

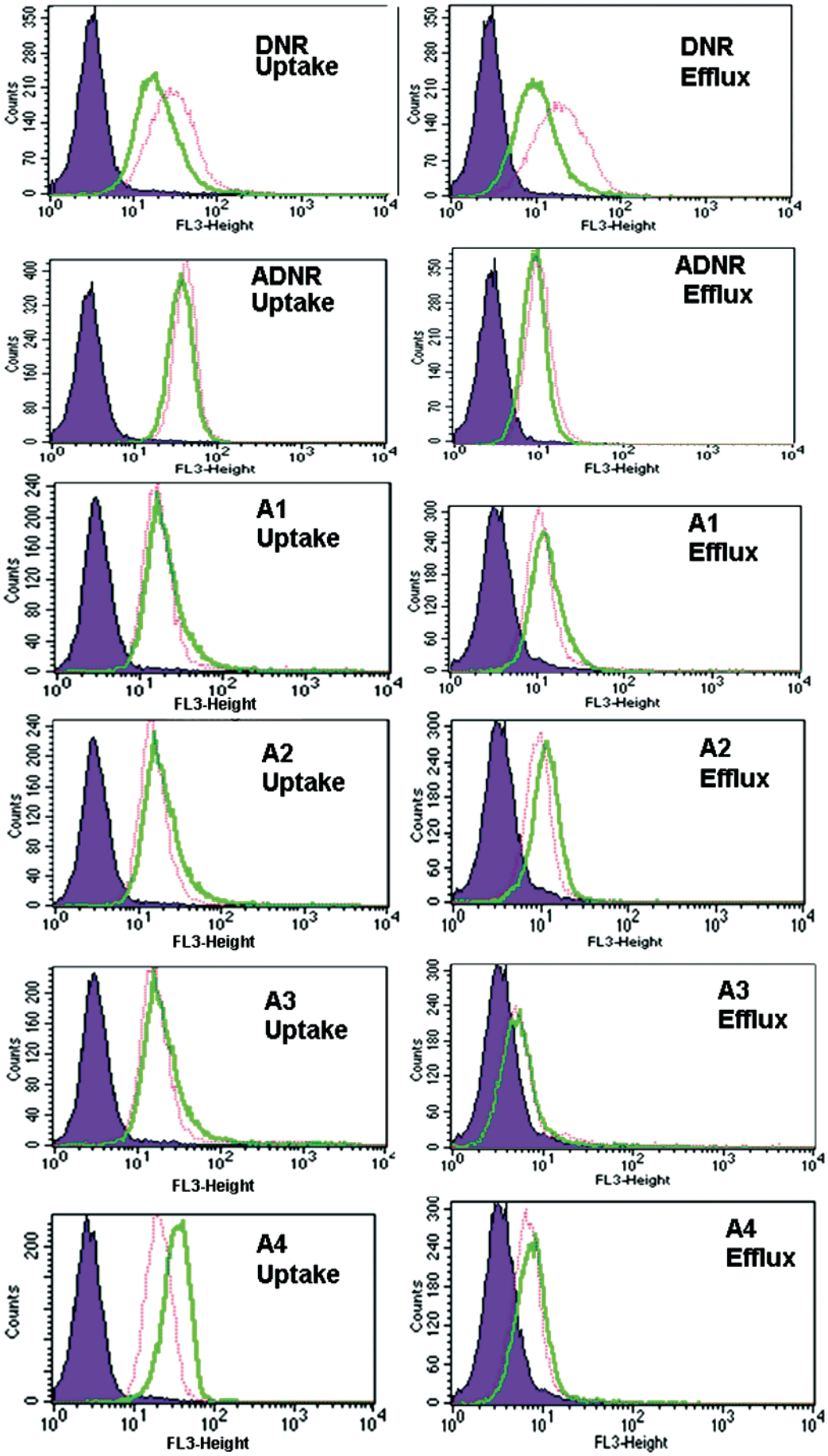


Figure 4. Drug uptake and efflux of DNR and its analogues (ADNR, **A1**−**A4**, 2 μM) in drug-resistant leukemia cells (K562/Dox) in the presence of P-gp inhibitor (5 μM CsA, red line) or the absence of CsA (green line) by flow cytometry.

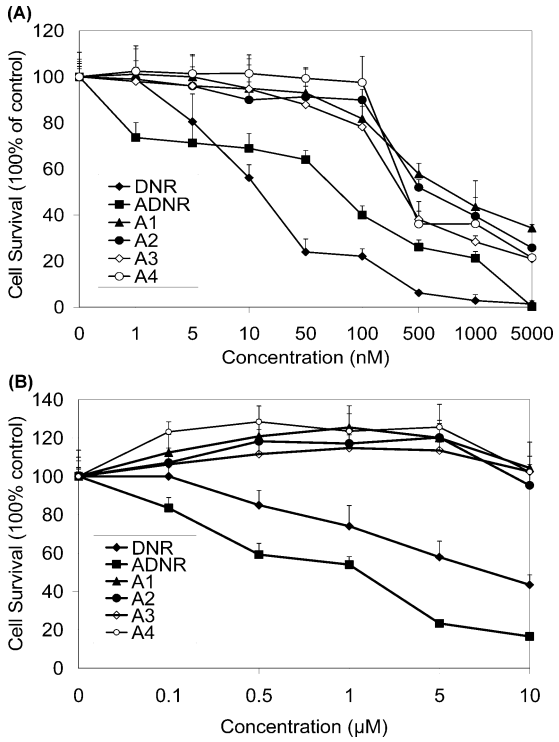


Figure 5. Cytotoxicity of daunorubicin (DNR) and its analogues in drug-sensitive leukemia K562 cells (Figure 5A) and drug resistant K562/Dox cells (Figure 5B).

efflux from the cancer cells was monitored again for another 30 min. Similarly, P-gp inhibitor (CsA) significantly inhibited the drug efflux and increased drug accumulation in the cells. These data confirmed that DNR is a substrate of P-gp, and inhibition of P-gp increased intracellular DNR accumulation. In contrast, the new anthracycline analogues (ADNR and A1-A4) also readily diffused into K562/Dox cells. However, the P-gp inhibitor (CsA) did not show any effect on the retention of these new compounds in both uptake and efflux phases in the cancer cells. These results strongly suggest that modifications of the sugar structures of DNR avert P-gp binding, diminish the drug efflux, and may well overcome P-gp-mediated drug resistance.

Cytotoxicity of New Anthracycline Analogues against Drug-Resistant Leukemia Cells K562/Dox in Vitro. To test if the new anthracycline analogues will overcome drug resistance, we measured their cytotoxicity in K562/Dox cells by MTS assay in comparison with DNR. As shown in Figure 5, DNR showed much higher IC_{50} (>5 μM) against K562/Dox cells than against K562 (15 nM). The drug resistance index (DRI, ratio of IC_{50} in drug-resistant cells over IC_{50} in drug-sensitive cells) is a good indicator of a drug's ability to overcome resistance. The DRI for DNR is more than 333. These data strongly suggests that K562/Dox cells are resistant to DNR, which is due to the drug efflux by P-gp as measured by FACS. In contrast, the lead compound ADNR exhibited potent anticancer activity with IC_{50} of 0.075 μM in K562 and of 1.0 μM in drug-resistant cells. Compared to DNR, ADNR was less active in K562; however, ADNR was at least 5-fold more active than DNR against drug-resistant K562 cells. Its DRI is only 13, which is 25-fold lower than that of DNR. These data indicate that ADNR overcomes P-gp-mediated drug resistance.

However, although compounds **A1**−**A4** with larger substituents (triazole derivatives) also averted P-gp recognition and drug efflux, they showed much less activity in both K562 and K562/Dox cells. These data indicates that modification of the sugar structure at the 3′ position with large substituents averts P-gp binding, but it also results in decreased anticancer activity. Therefore, the following animal studies only focused on the lead compound ADNR that averts P-gp recognition and maintains anticancer activity in drug-resistant cancer cells.

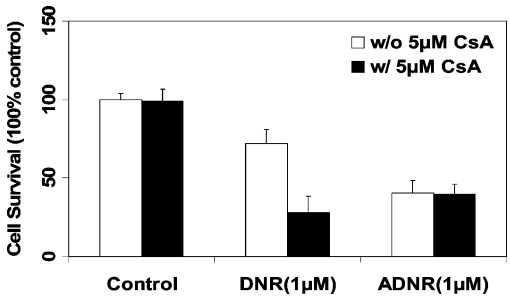


Figure 6. Cytotoxicity of daunorubicin (DNR) and ADNR in drug-resistant leukemia K562/Dox cells in the presence or absence of 5 μM cyclosporine A (CsA).

To further confirm that drug resistance to DNR is partially due to P-gp efflux and that ADNR can avert P-gp recognition, we tested the cytotoxicity of DNR and ADNR against K562/Dox in the presence of a P-gp inhibitor (CsA). As shown in Figure 6, CsA alone (5 μM) did not show any cytotoxicity to K562/Dox, while DNR (1 μM) alone showed 30% cell killing effect; however, the combination of CsA and DNR showed more than 70% cell killing effects under the same condition. These data indicates that CsA inhibits P-gp for drug efflux and increases DNR intracellular concentration for better cytotoxicity. However, ADNR at 1 μM concentration kills more than 50% of the cancer cells, and CsA did not change the cell killing effects of ADNR. This further confirmed that ADNR is no longer a P-gp substrate, thus overcoming P-gp-mediated drug resistance.

However, one question still remains to be answered: why does ADNR show a different IC_{50} value in K562 and K562/Dox cells when it is not a P-gp substrate? This may be due to the complexity of multidrug resistance in this drug-resistant leukemia cell line (K562/Dox). P-gp may not be the only determining factor contributing to anthracycline resistance in K562/Dox cells. The oncogene alterations (such as Bcl-2, Bax, and P53) may also contribute to the drug resistance. For instance, our data showed that BCL-2 is also overexpressed by 14-fold higher in K562/Dox than K562 (data not shown). It was also reported that other transporter molecules, such as MRP, LRP, and ABCG, may also be induced by doxorubicin.[54] Therefore, although ADNR fully averts P-gp-mediated resistance, it may not overcome another factor-mediated drug resistance. To confirm our speculation, we conducted cytotoxicity studies of these compounds on human epidermoid carcinoma cell line (KB-3-1with low expression of P-gp) and its corresponding drug-resistant cell line with high P-gp expression (KB-V). As expected, the IC_{50} values of ADNR in these two cell lines are identical (0.3 μM), while DNR shows a 5.3-fold higher IC_{50} in drug-resistant KB-V cells than in KB-3-1. The results indicate that ADNR completely overcomes P-gp-mediated drug resistance.

Anticancer Activity of ADNR against Drug-Resistant Cancers in Xenograft Model in Vivo. On the basis of the in vitro data, we selected the lead compound ADNR to be evaluated for its anticancer activity against drug-resistant cancers in the xenograft mouse model. In this animal model, 10^7 drug-resistant leukemia K562/Dox cells were injected subcutaneously into nude mice. After 14 days, the tumor reached 100 mm^3. From day 15, ADNR (5 or 10 mg/kg) and DNR (5 or 10 mg/

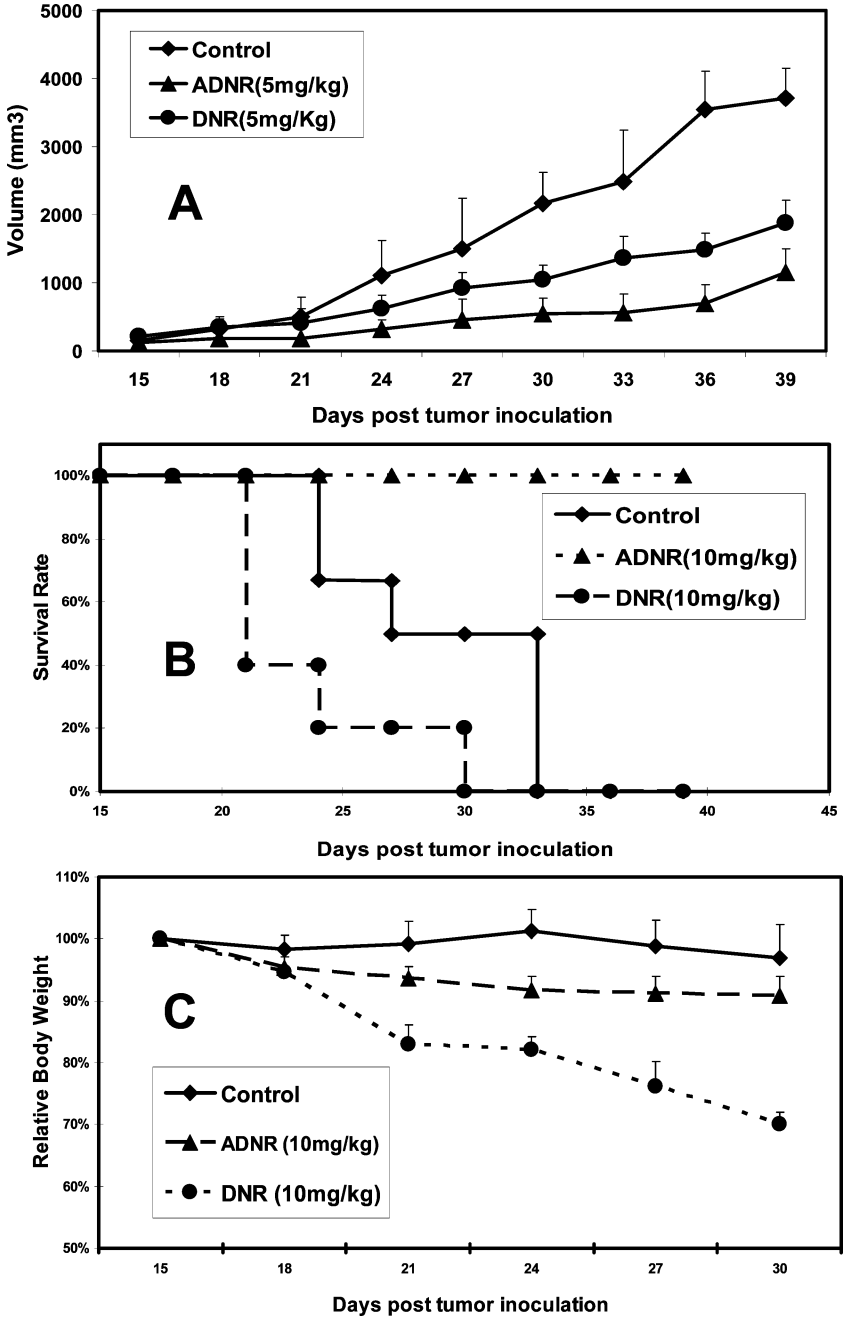


Figure 7. Anticancer activity (A), animal survival rate (B), and relative body weight change (C) of lead compound (ADNR) and daunorubicin (DNR) in xenograft mice model of drug-resistant leukemia cells (K562/Dox). $N = 5$, 8, and 6 for control, ADNR and DNR groups, respectively. Each point represents the mean ± SE of each group.

kg) were injected in to the mice intraperitoneally twice per week for 3 weeks. The tumor volume was measured every 3 days. As shown in Figure 7, tumor growth was very rapid in the control group (without drug treatment). However, ADNR and daunorubicin (DNR) significantly inhibited tumor growth 1 week after dose. As expected, ADNR (5 mg/kg) showed 2.5-fold higher maximum growth inhibition rate against drug-resistant cancers than DNR (Figure 7A). This result indicates that ADNR is more effective than DNR against drug-resistant cancers.

When ADNR and DNR were given to the xenograft model at the maximum tolerable dose of 10 mg/kg twice a week for 3 weeks, the body weight of DNR-treated mice decreased more than 70% and all of them died after 2 weeks of DNR treatment, while the ADNR treatment group and control group did not show any significant body weight change (Figure 7C). All animals (8/8) in the ADNR group survived (100%) after 50 days, while the mice in the DNR group (6/6) died before 30 days (due to both tumor growth and drug toxicity; 50% of the mice died before 20 days). The mice in the control group (5/5) all died in 33 days (50% of the mice died in 25 days) (Figure 7B). These data indicate that ADNR overcomes P-gp-mediated drug resistance and is effective in treatment of drug-resistant cancers with a better safety profile in the K562/Dox leukemia xenograft model.

Conclusion

In summary, a novel class of anthracyclines with sugar modifications was synthesized by transforming the amino group of DNR to an azido group or triazole group. These new anthracycline analogues were shown to avert P-gp binding by molecular docking. While DNR extensively interacts with the

MDR proteins, the new anthracycline analogues do not show any specific interactions with the MDR protein. Therefore, these new analogues with sugar modifications abolish the drug efflux by P-gp and accumulate at high intracellular concentration in the drug-resistant leukemia K562/Dox by FACS assay. P-gp inhibition by CsA confirmed that these new analogues are no longer P-gp substrates. One lead compound (ADNR) exhibited potent anticancer activity in both drug-sensitive and drug-resistant leukemia cells, with a 25-fold lower DRI value than DNR. Its cytotoxicity in drug-resistant cancer cells is independent of P-gp inhibition. In vivo xenograft results further demonstrated that ADNR showed 2.5-fold higher maximum growth rate inhibition against drug-resistant cancers and a significant increase for animal survival rate. No significant body weight reduction in mice was observed for ADNR at the maximum tolerable dose as compared to more than 70% body weight reduction for DNR. These data suggest that sugar modifications of anthracyclines can avert P-gp recognition and drug efflux, increase drug intracellular concentration, and overcome P-gp-mediated drug resistance in cancer therapy.

Experimental Section

Chemistry. All solvents were dried with a solvent-purification system (Innovative Technology, Inc). Daunorubicin hydrochloride (Greenfield Chemicals Inc.) was directly used without further purification. Analytical TLC was carried out on E. Merck silica gel 60 F254 aluminum-backed plates. The preparative TLC was carried out on silica gel 60 F254 plates (20 × 20 cm, 1 mm) from EMD Chemicals, Inc. The 230−400 mesh size silica gel (EMD Chemicals Inc.) was utilized for all chromatographic purifications. ^{1}H and ^{13}C NMR spectra and the high-resolution mass spectra were collected at The Ohio State University Campus Chemical Instrumentation Center.

7-(3-Azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)daunorubicinone (ADNR). Daunorubicin hydrochloride (5.24 g, 9.3 mmol) was dissolved in water (30 mL) and treated with potassium carbonate (1.92 g, 13.9 mmol) and $CuSO_4 \cdot 5H_2O$ (14 mg, 88 μmol). MeOH (60 mL) was added to the reaction mixture, and a TfN_3 solution (made using 2 equiv of Tf_2O[46]) was added. Then, adequate MeOH was added to homogeneity. The reaction was stirred overnight. The mixture was diluted with H_2O (100 mL) and extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified through a silica gel column using MeOH/CH_2Cl_2 (1:100−50) to afford ADNR as a red solid (70%): HRMS $(M + Na)^+$ (ESI) calcd for $C_{27}H_{27}N_3O_{10}Na^+$ 576.1589, found 576.1612; ^{1}H NMR (500 MHz, $CDCl_3$) 13.95 (1H, s, HO-6), 13.19 (1H, s, HO-11), 7.98 (1H, d, J = 7.4 Hz, H-1), 7.74 (1H, t, J = 8.2 Hz, H-2), 7.36 (1H, d, J = 8.4 Hz, H-3), 5.54 (1H, d, J = 3.6 Hz, H-1′), 5.23 (1H, d, J = 1.9 Hz, H-7), 4.37 (1H, s, HO-9), 4.10 (1H, m, H-5′), 4.05 (3H, s, MeO-4), 3.69 (1H, br, H-4′), 3.60 (1H, m, H-3′), 3.15 (1H, dd, J = 1.6 Hz, J = 18.8 Hz, Ha-10), 2.87 (1H, d, J = 18.8 Hz, Hb-10), 2.38 (3H, s, H-14), 2.28 (1H, m, Ha-8), 2.09 (2H, m, Hb-8, Ha-2′), 1.91 (1H, m, Hb-2′), 1.30 (3H, d, J = 6.6 Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$) 211.5, 186.9, 186.7, 161.1, 156.3, 155.7, 135.7, 135.5, 134.2, 133.9, 120.8, 119.8, 118.5, 111.5, 111.3, 100.6, 76.7, 70.1, 69.5, 67.1, 56.8, 56.7, 34.9, 33.3, 28.5, 24.7, 16.8.

General Procedures A for the Preparation of the Daunorubicin Derivatives Containing Triazole Ring (A1−A4). To a solution of ADNR (52 mg, 0.094 mmol) and terminal alkynes (1.2 equiv) in THF (1.5 mL) were added $(EtO)_3PCuI$ (0.2 equiv) and diisopropylethylamine (0.3 equiv). The reaction was stirred at room temperature for 48 h. Water (2 mL) and $(NH_4)_2S$ solution (22%) were added. The resulting mixture was stirred for an additional 2 h. The product was extracted with CH_2Cl_2. The combined organic layers were washed with saturated $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. After concentration and purification through Prep-TLC (CH_2Cl_2/MeOH, 18:1), the products were provided.

7-[3-(4-Hydroxymethyl-[1,2,3]triazol-1-yl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (A1). Compound **A1** was obtained as a red solid (53%) according to the procedure A: HRMS $(M + Na)^+$ (ESI) calcd for $C_{30}H_{31}N_3O_{11}Na^+$ 632.1851, found 632.1860; ^{1}H NMR (500 MHz, DMSO) 13.97 (1H, s, HO-6), 13.19 (1H, s, HO-11), 7.88 (1H, s, H-triazole), 7.82 (2H, m, H-1, H-2), 7.57 (1H, d, J = 7.9 Hz, H-3), 5.51 (1H, s, OH), 5.39 (1H, d, J = 2.9 Hz, H-1′), 5.14 (1H, d, J = 7.0 Hz, H-7), 5.10 (1H, t, J = 2.6 Hz, HO), 4.95 (2H, m, OH, H-3′), 4.48 (2H, d, J = 5.5 Hz, CH_2), 4.39 (H, q, J = 6.7 Hz, H-5′), 3.93 (3H, s, MeO-4), 3.69 (1H, m, H-4′), 2.90 (2H, m, H-10), 2.49 (1H, m, Ha-8), 2.29 (3H, s, H-14), 2.21(1H, m, Hb-8), 2.06 (1H, m, Ha-2′), 1.85 (1H, m, Hb-2′), 1.17-(1H, d, J = 6.5 Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$) 212.4, 186.9, 186.7, 161.2, 156.6, 154.9, 147.8, 136.6, 135.9, 135.0, 134.9, 121.9, 120.4, 120.1, 119.4, 111.1, 111.0, 100.0, 75.5, 70.3, 69.2, 67.3, 57.0, 56.6, 55.6, 36.5, 32.0, 29.8, 24.6, 17.3.

7-[3-[4-(2-Hydroxyethyl)-[1,2,3]triazol-1-yl]-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (A2). Compound **A2** was obtained as a red solid (67%) according to the procedure A: HRMS $(M + Na)^+$ (ESI) calcd for $C_{31}H_{33}N_3O_{11}Na^+$ 646.2007, found 646.1981; ^{1}H NMR (500 MHz, DMSO) 13.87 (1H, s, HO-6), 13.07 (1H, s, HO-11), 7.79 (1H, s, H-triazole), 7.71 (2H, m, H-1, H-2), 7.45 (1H, d, J = 7.9 Hz, H-3), 5.45 (1H, s, OH), 5.36 (1H, br, H-1′), 4.88 (2H, m, H-7, H-3′), 4.36 (H, q, J = 6.5 Hz, H-5′), 3.86 (3H, s, MeO-4), 3.68 (1H, br, H-4′), 3.58 (2H, t, J = 6.9 Hz, CH_2), 2.78 (2H, m, H-10), 2.71 (2H, t, J = 6.9 Hz, CH_2), 2.47 (1H, m, Ha-8), 2.28 (3H, s, H-14), 2.21(1H, m, Hb-8), 2.07 (1H, m, Ha-2′), 1.81 (1H, m, Hb-2′), 1.16(1H, d, J = 6.5 Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$) 212.4, 186.6, 186.5, 161.1, 156.5, 154.9, 144.1, 136.4, 135.7, 134.8, 134.7, 121.6, 120.1, 119.9, 119.2, 110.8, 110.7, 100.0, 75.4, 70.3, 69.2, 67.2, 60.9, 56.9, 56.6, 36.3, 31.9, 29.8, 29.7, 24.6, 17.3.

7-[3-[4-(3-Hydroxypropanyl)-[1,2,3]triazol-1-yl]-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (A3). Compound **A3** was obtained as a red solid (60%) according to the procedure A: HRMS $(M + Na)^+$ (ESI) calcd for $C_{32}H_{35}N_3O_{11}Na^+$ 660.2164, found 660.2177; ^{1}H NMR (500 MHz, DMSO) 13.90 (1H, s, HO-6), 13.10 (1H, s, HO-11), 7.76 (1H, s, H-triazole), 7.74 (2H, m, H-1, H-2), 7.49 (1H, d, J = 7.8 Hz, H-3), 5.46 (1H, s, OH), 5.37 (1H, br, H-1′), 5.11 (1H, d, J = 6.8 Hz, OH), 4.89 (2H, m, H-7, H-3′), 4.43 (1H, t, J = 5.2 Hz, OH), 4.39 (1H, q, J = 6.7 Hz, H-5′), 3.88 (3H, s, MeO-4), 3.69 (1H, d, J = 6.1 Hz, H-4′), 3.41 (1H, q, J = 5.2 Hz, CH_2), 2.81 (2H, m, H-10), 2.60 (2H, t, J = 7.6 Hz, CH_2), 2.48 (1H, m, Ha-8), 2.29 (3H, s, H-14), 2.22 (1H, m, Hb-8), 2.08 (1H, m, Ha-2′), 1.70 (2H, quint, J = 7.8 Hz, CH_2), 1.17 (1H, d, J = 6.5 Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$) 212.4, 186.6, 186.4, 161.1, 156.5, 154.9, 146.5, 136.5, 135.8, 134.8, 134.7, 121.1, 120.2, 119.9, 119.3, 110.9, 110.8, 100.0, 75.4, 70.3, 69.2, 67.2, 60.5, 56.9, 56.6, 36.3, 32.7, 31.9, 29.7, 24.6, 22.2, 17.3.

7-[3-[4-(4-Hydroxybutyl)-[1,2,3]triazol-1-yl]-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (A4). Compound **A4** was obtained as a red solid (77%) according to the procedure A: HRMS $(M + Na)^+$ (ESI) calcd for $C_{33}H_{37}N_3O_{11}Na^+$ 674.2320, found 674.2363; ^{1}H NMR (500 MHz, DMSO) 13.84 (1H, s, HO-6), 13.05 (1H, s, HO-11), 7.75 (1H, s, H-triazole), 7.69 (2H, m, H-1, H-2), 7.43 (1H, d, J = 8.4 Hz, H-3), 5.44 (1H, s, OH), 5.36 (1H, br, H-1′), 5.09 (1H, br, OH), 4.88 (2H, m, H-7, H-3′), 4.39 (1H, q, J = 6.2 Hz, H-5′), 3.84 (3H, s, MeO-4), 3.69 (1H, br, H-4′), 3.37 (1H, m, CH_2), 2.72 (2H, m, H-10), 2.56 (2H, t, J = 7.4 Hz, CH_2), 2.48 (1H, m, Ha-8), 2.29 (3H, s, H-14), 2.22(1H, m, Hb-8), 2.05 (1H, m, Ha-2′), 1.58 (2H, m CH_2), 1.43 (2H, m, CH_2), 1.16 (1H, d, J = 6.5 Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$) 212.4, 186.5, 186.3, 161.0, 156.5, 154.9, 146.7, 136.4, 135.7, 134.8, 134.7, 121.0, 120.1, 119.8, 119.2, 110.9, 110.8, 100.1, 75.4, 70.3, 69.3, 67.2, 60.9, 56.8, 56.6, 36.3, 32.5, 31.9, 29.7, 25.9, 25.4, 24.6, 17.3.

Biology. Molecular Docking. The structure of MsbA from *Vibrio cholera* (PDB ID 1PF4) was chosen for the docking template. The functional dimer from chains A and B was selected, polar hydrogens were added, and Kollman charges[55] were assigned. Three-dimensional affinity grids (120 × 120 × 100) covering the central cleft bordering NBDs with 0.375 Å spacing were calculated

for each of the following atom types: C, A (aromatic C), N, O, S, H, and e (electrostatic) using Autogrid3.[56] For the two ligands, all hydrogens were added and Gasteiger charges were assigned.[57] The rotatable bonds were assigned via AutoTors.[56] AutoDock version 3.0.7[56] was used for the docking simulation. For each compound, the docking parameters were as follows: trials of 100 dockings, population size of 150, random starting position and conformation, translation step ranges of 2.0 Å, rotation step ranges of 50°, elitism of 1, mutation rate of 0.02, crossover rate of 0.8, local search rate of 0.06, and 10 million energy evaluations. Final docked conformations were clustered using a tolerance of 2.0 Å root-mean-square deviation (rmsd).

Cell Culture. Drug-sensitive leukemia cells K562 and drug-resistant leukemia cells K562/Dox were a gift from Dr. J. P. Marie (Institut National de la Sante' et de la Recherche Me'dicale, E9912, University of Paris 6, Paris, France). Human epidermoid carcinoma cell line (KB-3-1 with low expression of P-gp) and its corresponding drug-resistant cell line with high P-gp expression (KB-V) were generously provided by Dr. M. M. Gottesman (Laboratory of Cell Biology, National Cancer Institute, National Institute of Health, Bethesda, MD 20892). The cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acid, and penicillin (100 units/mL)/streptomycin (100 μg/mL) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. The culture mediums were changed every 2−3 days. Before each experiment, K562/Dox cells were stimulated with 0.1 μM doxorubicin at least for 1 week and then cultured for 10 days without doxorubicin stimulation. It was assured that P-gp expression level was similar in every experiment.

RNA Extraction and Real-Time PCR Analysis. RNA was extracted from cells using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacture's instructions. Real-time PCR was performed using SYBR Green as the dye. The MDR1 mRNA expression level in each cell line is calculated against β-actin as a control. The MDR1 level in K562/Dox was calculated using K562 as control.

Western Blot Analysis. The cell membrane fractions (20 μg) were subjected to electrophoresis and transferred to nitrocellulose membranes. Western blotting follows standard procedure with the monoclonal anti-P-gp antibody.[58−60]

Flow Cytometry (FACS). The assay was performed on a Becton-Dickinson FACS calibur (San Jose, CA) equipped with an ultraviolet argon laser (excitation at 488 nm, emission at 530/30 and 570/30 nm band-pass filters). Analysis was stopped on acquisition of 30 000 cells. Log fluorescence was collected and displayed as single parameter histograms. Since DNR analogues have fluorescence at similar wavelength, a direct intracellular retention of each compound in K562/Dox cells was performed with the flow cytometer. When a fluorescent substrate, is diffused into the cell, P-gp actively pumps out the fluorochrome. If a P-gp inhibitor is coincubated along with the fluorescence marker, the fluorescent marker accumulates in the cell, resulting in a higher intensity of fluorescence.

On the day of the experiment, the cell media was replaced with fresh 10% FBS RMPI 1640 containing no P-gp inhibitor (CsA) 60 min before experiment. The cells were centrifuged (1000 rpm for 3 min) at room temperature and resuspended in 10% FBS RMPI1640. One million cells (in 50 μL) were transferred to plastic tubes containing 1.95 mL of incubation media with DNR, ADNR, or A1-A4. In the uptake phase, cells were incubated with 2 μM DNR (ADNR, or A1-A4) in 10% FBS RMPI1640 in the presence or absence of 5 μM CsA for 30 min at 37 °C. After centrifugation (1000 rpm for 3 min at 4 °C), the cells were separated into two tubes equally. One tube was washed once with ice cold RMPI (no FBS) and transferred to the FACS tube in the staining buffer on ice. These cells represent the drug uptake phase. The other tubes were reincubated in 10% FBS RMPI1640 in the presence or absence of 5 μM CsA for an additional 30 min at 37 °C. This represents the drug efflux phase. After final centrifugation (1000 rpm for 3 min at 4 °C), the supernatant was removed. The cells were washed once with cold RMPI 1640, and the cell suspension in staining buffer was transferred to FACS tubes. The drug accumulation in the cells was analyzed by FACS.

Cytotoxicity of Daunorubicin Analogues by MTS Assay. Drug-sensitive K562 and drug-resistant K562/Dox cells (2000−10 000) were seeded in 96-well plates in RPMI-1640 and incubated for 24 h, while KB-3-1 and KB-V cells were seeded in 96-well plates in complete DMEM medium for 24 h. The exponentially growing cancer cells were incubated with various concentrations of compounds for 72 h at 37 °C (5% CO_2, 95% humidity). After 72 h of incubation, tetrazolium[3-(4,5-dimethythiazol-2-yl)]-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS, 2 mg/mL), and phenazine methosulfate (PMS, 25 μM) were mixed and added directly to the cells. After incubated for 3 h at 37 °C, the absorbance of formazan (the metabolite of MTS by viable cells) was measured at 490 nm. The IC_{50} values of the compounds for cytotoxicity were calculated by WinNonlin software from the dose−response curves.

Drug Resistance Assay of Daunorubicin Analogues. K562/Dox leukemia cells (2000−10 000) were seeded in 96-well plates in RPMI-1640 and incubated overnight. The cells were pretreated with 5 μM cyclosporine A (CsA) for 10 min. Then the compounds (1 μM) were added. After 72 h, tetrazolium[3-(4,5-dimethythiazol-2-yl)]-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS, 2 mg/mL), and phenazine methosulfate (PMS, 25 μM) were mixed and added directly to the cells. After incubation for 3 h at 37 °C, the absorbance of formazan (the metabolite of MTS by viable cells) was measured at 490 nm. The drug resistance index (DRI), which is the ratio of the IC_{50} in K562/DOX to the IC_{50} in K562, was calculated.

In Vivo Antitumor Activity in K562/Dox Xenograft Model. Female, 5−7-week-old athymic nu/nu mice were purchased from the Charles River laboratories. Cells (1×10^7) were injected subcutaneously into the right flanks of the mice. Mice bearing tumors of 100−300 mm^3 in volume (usually 10−15 days after tumor inoculation) were randomized into five to eight mice per group. Mice were treated with: (1) vehicle alone; (2) DNR at 5 or 10 mg/kg, ip, twice per week for six injections; or (3) ADNR at 5 or 10 mg/kg, ip, twice per week for six injections. Tumor volume (V) was recorded every 3 days as $V = {}^1/_2 lw^2$, where l and w are the longest and shortest diameters of the tumor mass (in mm), respectively. Body weight was also recorded every 3 days to monitor the toxicity of the treatment. The statistical significance was analyzed using Student's t test, and the differences were considered significant at $p < 0.05$.

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Supporting Information Available: NMR, HRMS, and HPLC data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Weiss, R. B. The anthracyclines: Will we ever find a better doxorubicin? *Semin. Oncol.* **1992**, *19*, 670−86.
(2) Arcamone, F.; Bernardi, L.; Giardino, P.; Patelli, B.; Di Marco, A.; Casazza, A. M.; Pratesi, G.; Reggiani, P. Synthesis and antitumor activity of 4-demethoxydaunorubicin, 4-demethoxy-7,9-diepidaunorubicin, and their b anomers. *Cancer Treat. Rep.* **1976**, *60*, 829−34.
(3) Coukell, A. J.; Faulds, D. Epirubicin. An updated review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the management of breast cancer. *Drugs* **1997**, *53*, 453−82.
(4) Oki, T. New anthracycline antibiotics. *Jpn. J. Antibiot.* **1977**, *30 Suppl*, 70−84.

(5) Oki, T.; Shibamoto, N.; Matsuzawa, Y.; Ogasawara, T.; Yoshimoto, A.; Kitamura, I.; Inui, T.; Naganawa, H.; Takeuchi, T.; Umezawa, H. Production of nineteen anthracyclic compounds by *Streptomyces galilaeus* MA144-M1. *J. antibiot.* **1977**, *30*, 683−7.
(6) Hori, S.; Shirai, M.; Hirano, S.; Oki, T.; Inui, T.; Tsukagoshi, S.; Ishizuka, M.; Takeuchi, T.; Umezawa, H. Antitumor activity of new anthracycline antibiotics, aclacinomycin-A and its analogs, and their toxicity. *Gann = Gan.* **1977**, *68*, 685−90.
(7) Oki, T.; Matsuzawa, Y.; Yoshimoto, A.; Numata, K.; Kitamura, I. New antitumor antibiotics aclacinomycins A and B. *J. Antibiot.* **1975**, *28*, 830−4.
(8) Umezawa, H.; Takahashi, Y.; Kinoshita, M.; Naganawa, H.; Masuda, T.; Ishizuka, M.; Tatsuta, K.; Takeuchi, T. Tetrahydropyranyl derivatives of daunomycin and adriamycin. *J. Antibiot.* **1979**, *32*, 1082−4.
(9) Israel, M.; Modest, E. J.; Frei, E., 3rd *N*-Trifluoroacetyladriamycin-14-valerate, an analog with greater experimental antitumor activity and less toxicity than adriamycin. *Cancer Res.* **1975**, *35*, 1365−8.
(10) Arcamone, F. *Doxorubicin, Anticancer Antibiotics;* Medicinal Chemistry Series; Academic Press: New York, 1981; Vol. 17, pp 1−369.
(11) Weiss, R. B.; Sarosy, G.; Clagett-Carr, K.; Russo, M.; Leyland-Jones, B. Anthracycline analogs: The past, present, and future. *Cancer Chemother. Pharmacol.* **1986**, *18*, 185−97.
(12) Kimchi-Sarfaty, C.; Gribar, J. J.; Gottesman, M. M. Functional characterization of coding polymorphisms in the human MDR1 gene using a vaccinia virus expression system. *Mol. Pharmacol.* **2002**, *62*, 1−6.
(13) Gottesman, M. M.; Fojo, T.; Bates, S. E. Multidrug resistance in cancer: Role of ATP-dependent transporters. *Nat. Rev. Cancer* **2002**, *2*, 48−58.
(14) Gottesman, M. M.; Ambudkar, S. V. Overview: ABC transporters and human disease. *J. Bioenerg. Biomembr.* **2001**, *33*, 453−8.
(15) Ambudkar, S. V.; Kimchi-Sarfaty, C.; Sauna, Z. E.; Gottesman, M. M. P-glycoprotein: From genomics to mechanism. *Oncogene* **2003**, *22*, 7468−85.
(16) Komatani, H.; Kotani, H.; Hara, Y.; Nakagawa, R.; Matsumoto, M.; Arakawa, H.; Nishimura, S. Identification of breast cancer resistant protein/mitoxantrone resistance/placenta-specific, ATP-binding cassette transporter as a transporter of NB-506 and J-107088, topoisomerase I inhibitors with an indolocarbazole structure. *Cancer Res.* **2001**, *61*, 2827−32.
(17) Hirose, M.; Hosoi, E.; Hamano, S.; Jalili, A. Multidrug resistance in hematological malignancy. *J. Med. Invest.* **2003**, *50*, 126−35.
(18) di Bartolomeo, S.; Spinedi, A. Differential chemosensitizing effect of two glucosylceramide synthase inhibitors in hepatoma cells. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 269−74.
(19) Gottesman, M. M. How cancer cells evade chemotherapy: Sixteenth Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res.* **1993**, *53*, 747−54.
(20) Kaye, S. B. The multidrug resistance phenotype. *Br. J. Cancer* **1988**, *58* (6), 691−4.
(21) Yu, D. K. The contribution of P-glycoprotein to pharmacokinetic drug-drug interactions. *J. Clin. Pharmacol.* **1999**, *39*, 1203−11.
(22) Yu, D. S.; Chang, S. Y.; Ma, C. P. The correlation of membranous glycoprotein-gp-170, cytoplasmic glutathione and glucose-6-phosphate dehydrogenase levels with multidrug resistance in transitional cell carcinoma cell lines of the urinary tract. *J. Urol.* **1997**, *157*, 727−31.
(23) Yu, D. S.; Chang, S. Y.; Ma, C. P. The expression of mdr-1-related gp-170 and its correlation with anthracycline resistance in renal cell carcinoma cell lines and multidrug-resistant sublines. *Br. J. Urol.* **1998**, *82*, 544−7.
(24) Chiou, W. L.; Chung, S. M.; Wu, T. C.; Ma, C. A comprehensive account on the role of efflux transporters in the gastrointestinal absorption of 13 commonly used substrate drugs in humans. *Int. J. Clin. Pharmacol. Ther.* **2001**, *39*, 93−101.
(25) Singal, P. K.; Li, T.; Kumar, D.; Danelisen, I.; Iliskovic, N. Adriamycin-induced heart failure: Mechanism and modulation. *Mol. Cell. Biochem.* **2000**, *207*, 77−86.
(26) Monneret, C. Recent developments in the field of antitumor anthracyclines. *Eur. J. Med. Chem.* **2001**, *36*, 483−493.
(27) Minotti, G.; Menna, P.; Salvatorelli, E.; Cairo, G.; Gianni, L. Anthracyclines: Molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol. Rev.* **2004**, *56*, 185−229.
(28) Zhang, G.; Fang, L.; Zhu, L.; Sun, D.; Wang, P. G. Syntheses and biological activity of bisdaunorubicins. *Bioorg. Med. Chem.* **2005**, in press.
(29) Zhang, G.; Shen, J.; Cheng, H.; Zhu, L.; Fang, L.; Luo, S.; Muller, M. T.; Lee, G. E.; Wei, L.; Du, Y.; Sun, D.; Wang, P. G. Syntheses and biological activities of rebeccamycin analogues with uncommon sugar. *J. Med. Chem.* **2005**, *48*, 2600−2611.
(30) Arcamone, F.; Animati, F.; Capranico, G.; Lombardi, P.; Pratesi, G.; Manzini, S.; Supino, R.; Zunino, F. New developments in antitumor anthracyclines. *Pharmacol. Ther.* **1997**, *76*, 117−124.
(31) Arcamone, F. M. From the pigments of the actinomycetes to third generation antitumor anthracyclines. *Biochimie* **1998**, *80*, 201−206.
(32) Binaschi, M.; Bigioni, M.; Cipollone, A.; Rossi, C.; Goso, C.; Maggi, C. A.; Capranico, G.; Animati, F. Anthracyclines: Selected new developments. *Curr. Med. Chem.: Anti-Cancer Agents* **2001**, *1*, 113−130.
(33) Arcamone, F.; Animati, F.; Berettoni, M.; Bigioni, M.; Capranico, G.; Casazza, A. M.; Caserini, C.; Cipollone, A.; De Cesare, M.; Franciotti, M.; Lombardi, P.; Madami, A.; Manzini, S.; Monteagudo, E.; Polizzi, D.; Pratesi, G.; Righetti, S. C.; Salvatore, C.; Supino, R.; Zunino, F. Doxorubicin disaccharide analogue: Apoptosis-related improvement of efficacy in vivo. *J. Natl. Cancer Inst.* **1997**, *89*, 1217−23.
(34) Ishikawa, T.; Akimaru, K.; Kuo, M. T.; Priebe, W.; Suzuki, M. How does the MRP/GS-X pump export doxorubicin? *J. Natl. Cancer Inst.* **1995**, *87*, 1639−40.
(35) Solary, E.; Ling, Y. H.; Perez-Soler, R.; Priebe, W.; Pommier, Y. Hydroxyrubicin, a deaminated derivative of doxorubicin, inhibits mammalian DNA topoisomerase II and partially circumvents multidrug resistance. *Int. J. Cancer* **1994**, *58*, 85−94.
(36) Borrel, M. N.; Fiallo, M.; Priebe, W.; Garnier-Suillerot, A. P-glycoprotein-mediated efflux of hydroxyrubicin, a neutral anthracycline derivative, in resistant K562 cells. *FEBS Lett.* **1994**, *356*, 287−99.
(37) Priebe, W.; Perez-Soler, R. Design and tumor targeting of anthracyclines able to overcome multidrug resistance: A double-advantage approach. *Pharmacol. Ther.* **1993**, *60*, 215−34.
(38) Priebe, W.; Van, N. T.; Burke, T. G.; Perez-Soler, R. Removal of the basic center from doxorubicin partially overcomes multidrug resistance and decreases cardiotoxicity. *Anticancer Drugs* **1993**, *4*, 37−48.
(39) Ling, Y. H.; Priebe, W.; Yang, L. Y.; Burke, T. G.; Pommier, Y.; Perez-Soler, R. In vitro cytotoxicity, cellular pharmacology, and DNA lesions induced by annamycin, an anthracycline derivative with high affinity for lipid membranes. *Cancer Res* **1993**, *53*, 1583−9.
(40) Consoli, U.; Priebe, W.; Ling, Y. H.; Mahadevia, R.; Griffin, M.; Zhao, S.; Perez-Soler, R.; Andreeff, M. The novel anthracycline annamycin is not affected by P-glycoprotein-related multidrug resistance: Comparison with idarubicin and doxorubicin in HL-60 leukemia cell lines. *Blood* **1996**, *88*, 633−44.
(41) Priebe, W.; Van, N. T.; Burke, T. G.; Perez-Soler, R. Removal of the basic center from doxorubicin partially overcomes multidrug resistance and decreases cardiotoxicity. *Anti-Cancer Drugs* **1993**, *4*, 37−48.
(42) Gate, L.; Couvreur, P.; Nguyen-Ba, G.; Tapiero, H. *N*-methylation of anthracyclines modulates their cytotoxicity and pharmacokinetic in wild type and multidrug resistant cells. *Biomed. Pharmacother.* **2003**, *57*, 301−308.
(43) Priebe, W. A.; Perez-Soler, R. Sugar-modified anthracyclines: A search for drugs able to overcome multidrug resistance. *Carbohydr. Drug Des.* **1997**, 551−578.
(44) Ge, M.; Chen, Z.; Onishi, H. R.; Kohler, J.; Silver, L. L.; Kerns, R.; Fukuzawa, S.; Thompson, C.; Kahne, D. Vancomycin derivatives that inhibit peptidoglycan biosynthesis without binding D-Ala-D-Ala. *Science* **1999**, *284*, 507−511.
(45) Ge, M.; Thompson, C.; Kahne, D. Reconstruction of vancomycin by chemical glycosylation of the pseudoaglycon. *J. Am. Chem. Soc.* **1998**, *120*, 11014−11015.
(46) Alper, P. B.; Hung, S.-C.; Wong, C.-H. Metal catalyzed diazo transfer for the synthesis of azides from amines. *Tetrahedron Lett.* **1996**, *37*, 6029−6032.
(47) Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C.-H. Synthesis of sugar arrays in microtiter plate. *J. Am. Chem. Soc.* **2002**, *124*, 14397−14402.
(48) Lee, J. K.; Chi, Y. S.; Choi, I. S. Reactivity of acetylenyl-terminated self-assembled monolayers on gold: Triazole formation. *Langmuir* **2004**, *20*, 3844−3847.
(49) Perez-Balderas, F.; Ortega-Munoz, M.; Morales-Sanfrutos, J.; Hernandez-Mateo, F.; Calvo-Flores, F. G.; Calvo-Asin, J. A.; Isac-Garcia, J.; Santoyo-Gonzalez, F. Multivalent neoglycoconjugates by regiospecific cycloaddition of alkynes and azides using organic-soluble copper catalysts. *Org. Lett.* **2003**, *5*, 1951−1954.
(50) Gottesman, M. M.; Fojo, T.; Bates, S. E. Multidrug resistance in cancer: Role of ATP-dependent transporters. *Nat. Rev. Cancer* **2002**, *2*, 48−58.
(51) Reyes, C. L.; Chang, G. Structure of the ABC transporter MsbA in complex with ADP. Vanadate and lipopolysaccharide. *Science* **2005**, *308*, 1028−1031.

(52) Chang, G. Structure of MsbA from *Vibrio cholera*: A multidrug resistance ABC transporter homolog in a closed conformation. *J. Mol. Biol.* **2003**, *330*, 419−430.
(53) Chang, G.; Roth, C. B. Structure of MsbA from E. coli: A homolog of the multidrug resistance ATP binding cassette (ABC) transporters. *Science* **2001**, *293*, 1793−1800.
(54) Grandjean, F.; Bremaud, L.; Verdier, M.; Robert, J.; Ratinaud, M.-H. Sequential gene expression of P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP) and lung resistance protein: Functional activity of P-gp and MRP present in the doxorubicin-resistant human K562 cell lines. *Anti-Cancer Drugs* **2001**, *12*, 247−258.
(55) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S., Jr.; Weiner, P. A new force field for molecular mechanical simulation of nucleic acids and proteins. *J. Am. Chem. Soc.* **1984**, *106* (3), 765−84.
(56) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* **1998**, *19*, 1639−1662.
(57) Gasteiger, J.; Marsili, M. Iterative partial equalization of orbital electronegativity: A rapid access to atomic charges. *Tetrahedron* **1980**, *36*, 3219−22.
(58) Arora, A.; Seth, K.; Kalra, N.; Shukla, Y. Modulation of P-glycoprotein-mediated multidrug resistance in K562 leukemic cells by indole-3-carbinol. *Toxicol. Appl. Pharmacol.* **2005**, *202*, 237−43.
(59) Wang, J.; Chen, Z.; Xia, X.; Lu, D.; Xue, J.; Ruan, C. Improvement of combination chemotherapy tolerance by introduction of polycistronic retroviral vector drug resistance genes MGMT and MDR1 into human umbilical cord blood CD34+ cells. *Leuk. Res.* **2002**, *26*, 281−8.
(60) Cullen, K.; Davey, R.; Davey, M. The drug resistance proteins, multidrug resistance-associated protein and P-glycoprotein, do not confer resistance to Fas-induced cell death. *Cytometry* **2001**, *43*, 189−94.

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