

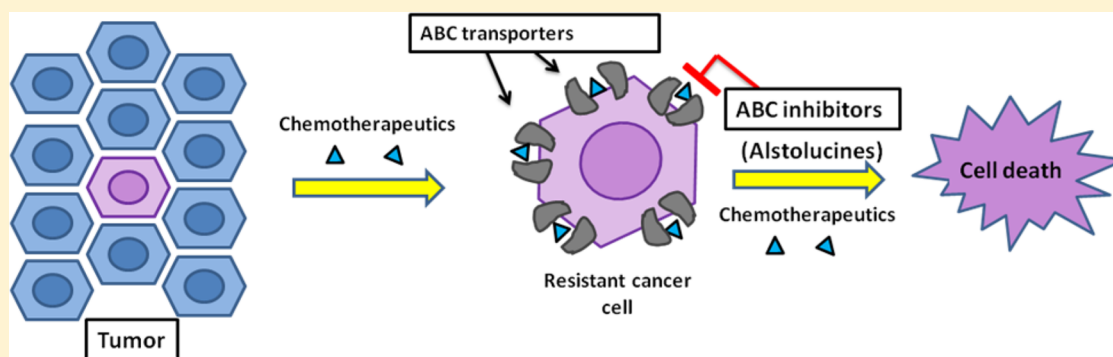
Synthesis and Biological Evaluation of Pentacyclic *Strychnos* Alkaloids as Selective Modulators of the ABCC10 (MRP7) Efflux Pump

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S Supporting Information



ABSTRACT: The selective modulation of ATP-binding cassette (ABC) efflux pumps overexpressed in multidrug resistant cancers (MDR) and attendant resensitization to chemotherapeutic agents represent a promising strategy for treating cancer. We have synthesized four novel pentacyclic *Strychnos* alkaloids alstolucines B (2), F (3), and A (5) and *N*-demethylalstogucine (4), in addition to known *Strychnos* alkaloid echitamidine (16), and we evaluated compounds 1–5 in biochemical assays with ABCC10 and P-glycoprotein (P-gp). Alstolucines B (2) and F (3) inhibited ABCC10 ATPase activity at 12.5 μM without affecting P-gp function; moreover, they resensitized ABCC10-transfected cell lines to paclitaxel at 10 μM . Altogether, the alstolucines represent promising lead candidates in the development of modulators of ABCC10 for MDR cancers overexpressing this pump.

INTRODUCTION

Cellular resistance to anticancer agents constitutes a major obstacle in the successful chemotherapeutic treatment of cancers with multidrug resistance (MDR) being responsible for failure in >90% of metastatic cancer patients.¹ Over the last few decades the notions of tumor heterogeneity and cancer stem cells have transformed our understanding of cancer and chemoresistance. Previously, tumors were depicted using the clonal evolution model as a collection of homogeneous cancer cells, with little variation, that had equal potential to initiate and propagate tumorigenesis. However, current research depicts tumors as hierarchically organized with intratumor heterogeneity giving rise to a subclass of cells with increased ability to initiate tumor formation.² This subclass of cells, referred to as tumor-initiating cells or cancer stem cells (CSCs), was initially reported by Bonnet and Dick in 1997³ and since then has been isolated in various types of cancers including breast cancer, ovarian cancer, acute myeloid leukemia (AML), glioblastoma, and pancreatic cancer, among others.⁴ Furthermore, it has been shown that CSCs play a major role in resistance through a variety of acquired and intrinsic mechanisms that include overexpression of ATP-binding cassette (ABC) efflux trans-

porters and detoxification enzymes, increased ability to repair DNA, down-regulation of apoptotic pathways, and changes in the cell cycle kinetics and microenvironment.^{5–8} One of many reported contributing factors to CSC resistance and MDR is the overexpression of a class of efflux pumps belonging to the ABC superfamily of proteins. Resistance is conferred from ABC proteins by their ability to specifically efflux chemotherapeutic agents out of cells and may contribute to resistance in CSCs. Initially, ABC transporters, particularly P-glycoprotein (P-gp, ABCB1),⁹ emerged as a promising strategy for directly addressing the mechanism of MDR and has been actively pursued for the past 30 years.¹⁰ While several reasons for the clinical failure of this approach have been put forward, an overarching problem of targeting P-gp is the concomitant toxicity. P-gp is expressed in many tissue types (e.g., intestine, kidney, liver, placenta, blood–brain barrier) and plays an important role in xenobiotic transport. Owing to its critical, protective role, a safer strategy would focus on targeting ABC transporters upregulated in CSCs and MDR cancers but whose

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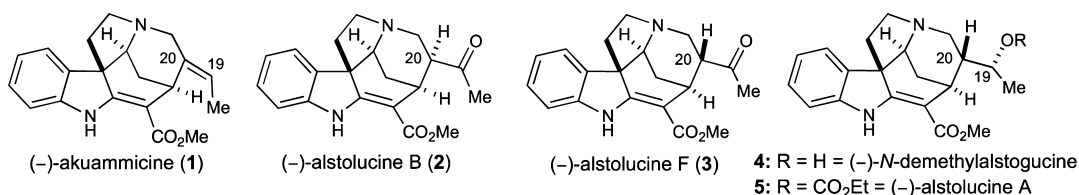
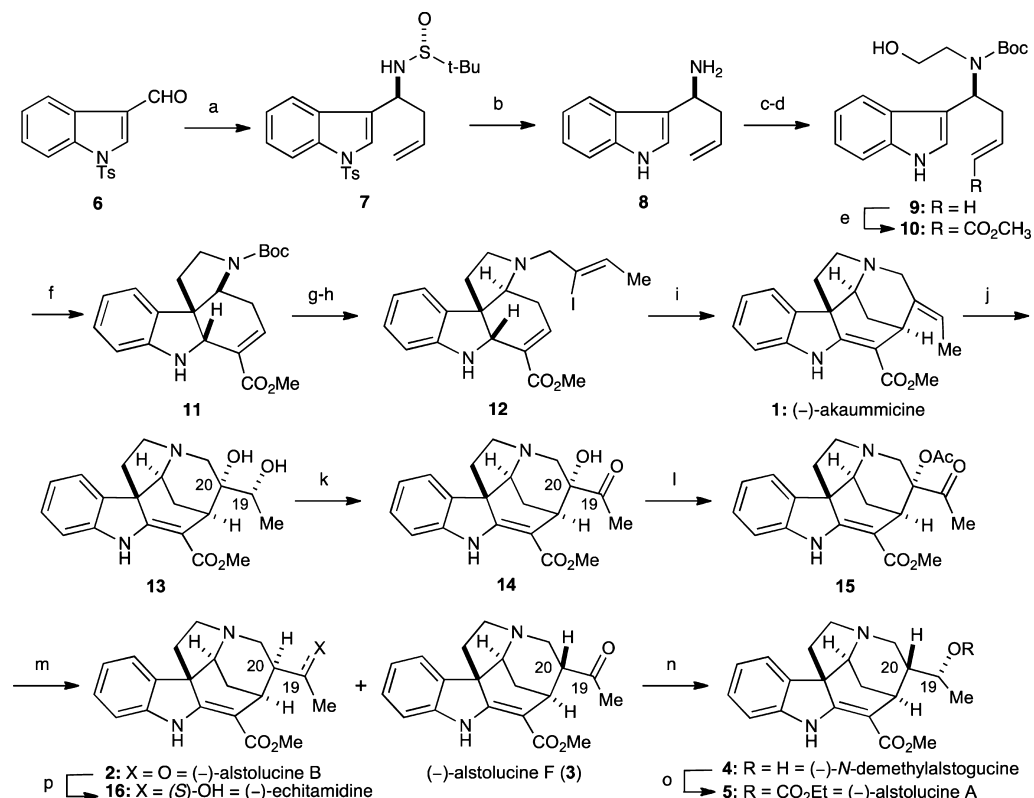


Figure 1. Structures of (-)-akuammicine (1) and novel pentacyclic *Strychnos* alkaloids (-)-alstolucine B (2), (-)-alstolucine F (3), (-)-*N*-demethylalstolucine (4), and (-)-alstolucine A (5).

Scheme 1. Total Synthesis of (-)-Akuammicine (1), (-)-Alstolucine B (2), (-)-Alstolucine F (3), (-)-*N*-Demethylalstolucine (4), and (-)-Alstolucine A (5)^a



^aReagents and conditions: (a) (*R*)-*N*-*tert*-butanesulfinamide, In(0), Ti(OEt)₄ and then allyl bromide, THF, 87% (dr = 10:1); (b) 4 M HCl in dioxane then Mg(0), MeOH, 75% over two steps; (c) ethyl glyoxaldehyde, 4 Å molecular sieves in THF and then lithium aluminum hydride, THF; (d) (Boc)₂O, *i*-Pr₂NEt, 57% over two steps; (e) methyl acrylate, 10 mol % Hoveyda–Grubbs second-generation catalyst [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(*o*-isopropoxyphenylmethylene)ruthenium], CH₂Cl₂, 80%; (f) PPh₃, DEAD, rt then DBU, toluene, 80 °C, 12 h, 56%; (g) TFA, CH₂Cl₂, quant.; (h) (*Z*)-2-iodobutenyl bromide, K₂CO₃, MeCN, 71%; (i) Pd(OAc)₂, PPh₃, Et₃N, 87%; (j) NMO, OsO₄, *t*-BuOH/MeOH/THF, 86%; (k) NCS, DMS, Et₃N, CH₂Cl₂, 66%; (l) Ac₂O, Et₃N, DMAP, CH₂Cl₂, 95%; (m) SmI₂, THF/MeOH, 71%; (n) NaBH₄, CeCl₃·7H₂O, MeOH, 83%; (o) EtCO₂Cl, Et₃N, CH₂Cl₂, 73%; (p) NaBH₄, MeOH, 85%.

inhibition would not prove toxic to the organism.¹⁰ Blockade or inhibition of these ABC transporters may prove to be novel targets to overcome chemoresistance.

The taxanes are a class of chemotherapeutic agents affected by CSCs and MDR that are widely used in the treatment of breast cancer.¹¹ Several ABC transporters, including P-gp and ABCC10, are known to efflux anticancer agents such as taxanes out of cancer cells.¹ Moreover, *in vitro* studies and a recent *in vivo* study have shown unambiguously that ABCC10 overexpression confers resistance to taxanes.^{12–14} Importantly, in recently published work, it was shown that ABCC10 is expressed in 100% of HER2-positive, 85% of HER2-negative, and 64% of triple-negative breast cancer tumor samples. Furthermore, it was shown that *Abcc10*-null mammary tumors are sensitized to taxanes and that there is a significant increase

in survival in *Abcc10*^{-/-} mice compared to wild-type counterparts following docetaxel treatment.¹⁵ Intriguingly, it was also shown that ABCC10 affects multiple parameters of breast tumor biology relevant to disease progression, including metastasis, proliferation, and migration. These data and another recently published report support the idea that ABC transporters impact tumor biology, which drives other mechanisms of resistance outside of drug efflux,¹⁶ including their roles in cancer initiation and propagation,¹⁷ thus supporting their validity as drug targets. Selective, potent inhibitors of ABCC10 that possess a lower affinity for P-gp would enable the resensitization of tumors to chemotherapeutic (e.g., taxanes). Currently, there are only a few inhibitors of ABCC10, the most potent inhibitor to date being cepharanthine. Moreover, none

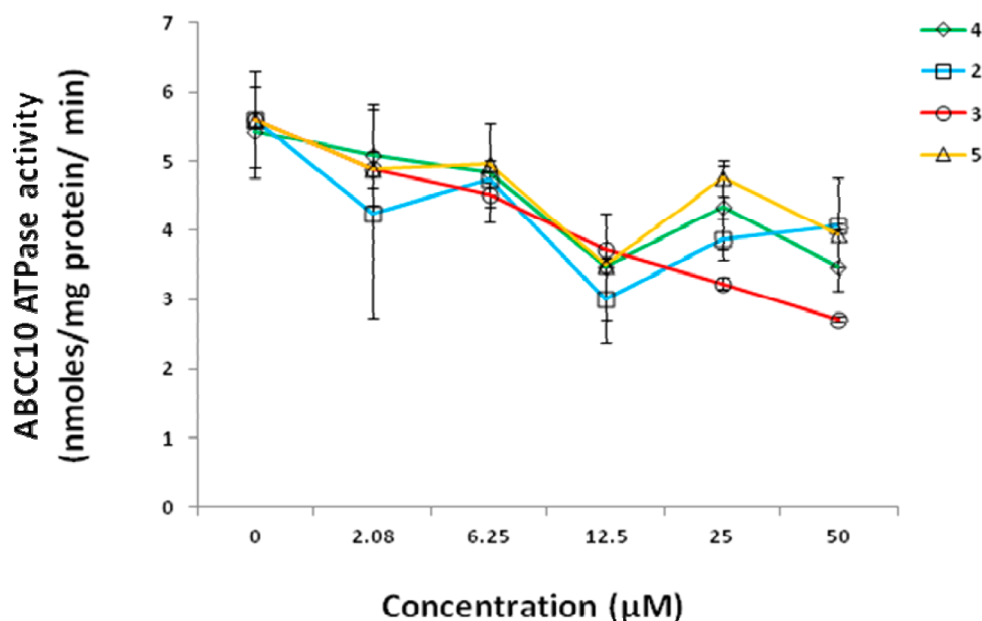


Figure 2. The effect of pentacyclic *Strychnos* alkaloids on ABCC10 ATPase activity. The ability of alstolucines and demethylalstogucine to inhibit ABCC10 ATPase activity was tested using a range of concentrations of alstolucine A (5), alstolucine B (2), alstolucine F (3), and *N*-demethylalstogucine (4). Alstolucine B (2) and alstolucine F (3) showed the best inhibition (~40–50%) at 12.5 and 50 μM , respectively. Experiments ($N = 3$) were performed with duplicate samples.

of these inhibitors are specific for ABCC10 over other efflux pumps, such as P-gp (ABCB1).^{13,14,18–24}

Over 75% of chemotherapeutic agents employed are derived from natural products.^{25,26} In 2010, Kam and co-workers isolated novel pentacyclic *Strychnos* alkaloids alstolucines A–F, from *Alstonia spatulata* (Figure 1).²⁷ In addition, they were evaluated for their ability to resensitize vincristine-resistant KB cells, which overexpress P-gp. Structurally, the alstolucines are closely related to the classical *Strychnos* alkaloid akuammicine (1), which we have prepared by total synthesis in both racemic²⁸ and asymmetric form.²⁹ We reasoned that these complex pentacyclic *Strychnos* alkaloids, while structurally related, would inhibit ABCB1 (P-gp) and ABCC10 to varying extents, allowing us to obtain valuable structure–activity relationship data and accordingly develop potent and selective inhibitors for the latter over the former.

To determine the capacity of these novel alkaloids to reverse ABCC10 MDR and their affinity for P-gp, we prepared 2–5 by asymmetric total synthesis from (–)-1 and evaluated their biological activity. Specifically, we measured the inhibitory effect of 2–5 on (1) ABCC10 ATPase activity, (2) P-gp ATPase activity, and (3) the ability of 1–5 to resensitize ABCC10-transfected cell lines to paclitaxel.

RESULTS AND DISCUSSION

Chemistry. The asymmetric total syntheses of alkaloids 1–5 are outlined in Scheme 1. Utilizing the methodology of Yus and co-workers,³⁰ *N*-tosyl indole-3-carboxaldehyde (6) was condensed with (*R*)-*N*-*tert*-butanesulfinylamide in the presence of $\text{Ti}(\text{OEt})_4$ and $\text{In}(0)$.³¹ The addition of allyl bromide and in situ formation of an allylindium species enabled a stereoselective allylation of the preformed *N*-sulfinimine, which afforded homoallylic sulfinamide 7 in 87% yield (*dr* = 10:1). The sequential removal of the auxiliary and *N*-tosyl group was achieved by treating 7 with 4 M HCl in dioxane followed by $\text{Mg}(0)$ in MeOH to give 8 in 75% overall yield. The primary

amine was condensed with ethyl glyoxylate and reduced to the primary alcohol using lithium aluminum hydride. The resulting secondary amine was treated with Boc anhydride in the presence of Hünig's base to furnish 9 in 57% yield over two steps.

Cross-metathesis of 9 and methyl acrylate in the presence of 10 mol % Hoveyda–Grubbs second-generation catalyst gave enoate 10 in 80% yield.³² Inspired by Ellman's elegant work on (–)-aurantioclavine,³³ we employed a novel bis-cyclization protocol wherein the pentacyclic E-ring was closed using Mitsunobu conditions, followed by treatment with DBU to cyclize the C-ring and access tetracycle 11 in 56% yield.³⁴ Removal of the *N*-Boc group with TFA and subsequent alkylation with (*Z*)-2-iodobutenyl bromide^{35,36} and potassium carbonate afforded 12 in 71% yield. Heck cyclization of 12 using $\text{Pd}(\text{OAc})_2$, PPh_3 , and Et_3N , which was inspired by Rawal's elegant approach to the *Strychnos* alkaloids,³⁵ accessed the D-ring to deliver (–)-akuammicine (1) in 87% yield (10% overall yield in nine steps).²⁹

With 1 in hand, we were tempted to employ an anti-Markovnikov hydroboration/carbinol oxidation sequence to access alstolucine B (2) in two steps from 1. However, Levy and co-workers had shown that the hydroboration of 1 proceeds in a Markovnikov manner, affording the tertiary carbinol product.³⁷ In the light of this, we reasoned that a viable alternative route to 2–5 would feature (1) dihydroxylation of the C20–C19 double bond, (2) oxidation of the diol to install the requisite ketone functionality at C19, and (3) a deoxygenation of the acyloin intermediate to deliver the alstolucines. Toward this end, Upjohn dihydroxylation of 1 gave 19,20-dihydroxyakuammicine (13) in 86% yield.³⁸ Subjecting diol 13 to Corey–Kim conditions oxidized the C19 alcohol to afford 14 in 66% yield.³⁹ Acetylation of the tertiary C20 alcohol using standard conditions furnished acetate 15 in 95% yield. Reductive removal of the *O*-acetate moiety in 15 was realized using Molander's method of SmI_2 in THF at -78°C to afford alstolucine B (2, 44%) and alstolucine F (3,

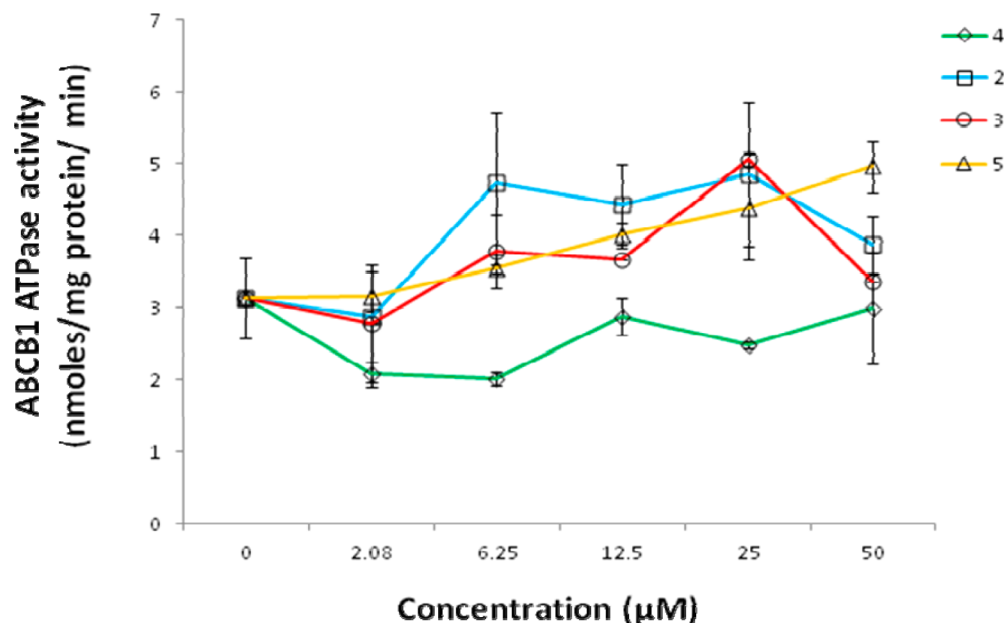


Figure 3. The effect of pentacyclic *Strychnos* alkaloids on ABCB1 ATPase activity. The ability of alstolucines and demethylalstogucine to inhibit ABCB1 ATPase activity was tested using a range of concentrations of alstolucine A (5), alstolucine B (2), alstolucine F (3), and *N*-demethylalstogucine (4). *N*-Demethylalstogucine (4) showed the only inhibition (~35–37%) at 2.08 and 6.25 μM , respectively. Experiments ($N = 3$) were performed with duplicate samples.

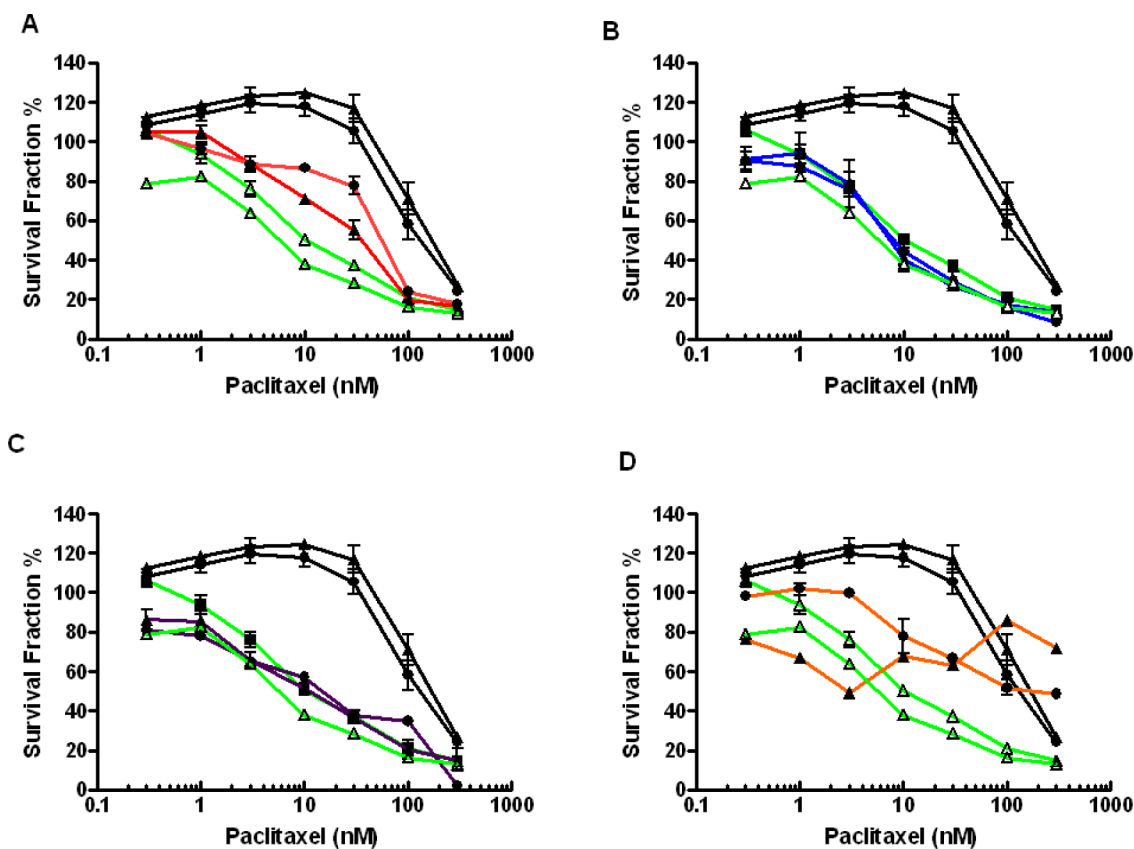


Figure 4. Alstolucines inhibit ABCC10-mediated paclitaxel resistance: (A) alstolucine F (3, red), (B) alstolucine B (2, blue), and (C) akummicine (1, purple) reverse ABCC10-mediated paclitaxel resistance in two ABCC10 transfectants (\blacktriangle , \bullet) to levels of nontransfected control cell lines (Δ). (D) Alstolucine A (5, orange) does not reverse ABCC10-mediated resistance. The green line represents the control nontransfected cell line. Black lines represent the survival of ABCC10 overexpressing cell lines in the absence of inhibitor. Compounds were tested at 12.5 μM .

27%) in a 1.6:1 ratio, which were separable by flash column silica gel chromatography.⁴⁰ Stereoselective Luche reduction of

alstolucine F (3) afforded *N*-demethylalstogucine (4) in 87% yield.⁴¹ Acylation of the newly formed alcohol using ClCO_2Et

and Et₃N furnished alstolucine A (5) in 73% yield. Alternatively, the stereoselective reduction of alstolucine B (2) yielded echitamidine (16) in 85%.

Biological Evaluation. To determine if compounds 2–5 and intermediate compounds inhibited ABCC10 ATPase activities, we performed ATPase assays using end point inorganic phosphate as previously described.¹⁸ As depicted in Figure 2, we have shown that alstolucines B (2) and F (3) inhibited ABCC10 40–50% at 12.5 and 50 μ M, respectively. All other compounds tested showed modest to no inhibition of ABCC10.

Further testing was conducted to determine selectivity of these novel compounds. ATPase activity assays were run in a similar matter to test inhibition of P-gp as depicted in Figure 3. It was determined that alstolucines A (5), B (2), and F (3) do not inhibit P-gp, while *N*-demethylalstogucine (4) inhibits P-gp (~35–37%) at 2.08 and 6.25 μ M. In comparison to cepharanthine (i.e., the best inhibitor of ABCC10 to date), alstolucines B (2) and F (3) are more potent inhibitors in ABCC10 ATPase assays (50% inhibition versus 20% inhibition); however, they selectively inhibit ABCC10 over P-gp, unlike cepharanthine and other known inhibitors of ABCC10.

We previously determined the resistance profile for ABCC10 using two HEK 293 cell lines, which overexpressed ABCC10, and a vector-only transfected line.¹² In this report, we use these previously described cell lines to assess if the alstolucines that inhibited ABCC10 in ATPase assays inhibited ABCC10 in vitro. Standard cellular proliferation assays were performed to determine if the alstolucines were able to modulate ABCC10's in vitro resistance capabilities for the taxane paclitaxel. As shown in Figure 4, both alstolucines B (2) and F (3) modulated ABCC10's resistance to taxanes. Alstolucines B (2) and F (3) completely reversed ABCC10-mediated resistance to paclitaxel by resensitizing the transfectants to levels similar to the vector-only transfected line.

CONCLUSION

We have accomplished the first asymmetric total syntheses of novel pentacyclic *Strychnos* alkaloids (–)-alstolucine B (2), F (3), and A (5) and *N*-demethylalstogucine (4) in an efficient manner, in addition to (–)-echitamidine (16). We have shown that ABCC10 plays an important role in modulating taxane resistance both in vitro and in vivo. Additionally, overexpression of ABCC10 confers ~3–10-fold resistance to paclitaxel and ~8–12-fold resistance to docetaxel. Results of in vitro and cell-based assays have identified alstolucine A (5), alstolucine B (2), and alstolucine F (3), at 12.5 μ M, as potent inhibitors of ABCC10 ATPase activity. Importantly, these compounds do not inhibit the function of P-gp ATPase activity, like current inhibitors of ABCC10. Furthermore, it was found that alstolucine B (2) and alstolucine F (3) at 10 μ M were able to resensitize the ABCC10-transfected cell lines to paclitaxel when compared to vector-transfected control lines to levels comparable to or greater than that of cepharanthine, the most potent inhibitor found to date.¹⁹ Altogether, these data justify the synthesis and evaluation of alstolucine analogs with increased potency and favorable physicochemical properties. Those results will be reported in due course.

EXPERIMENTAL SECTION

General. All reactions containing water- or air-sensitive reagents were performed in oven-dried glassware under nitrogen or argon.

Tetrahydrofuran and dichloromethane were passed through two columns of neutral alumina. Toluene was passed through one column of neutral alumina and one column of Q5 reactant. Triethylamine was distilled from calcium hydride prior to use, and 4 Å molecular sieves were activated by flame-drying under vacuum. Methanol was distilled from magnesium. Methyl acrylate was distilled prior to use. Compounds 1 and 7–12 were prepared according to the procedures of Andrade.²⁹ (*Z*)-2-Iodobutenyl bromide was prepared according to the procedure of Cook.³⁶ For cross-metathesis reactions, CH₂Cl₂ was deaerated by bubbling argon (1 min/mL). All other reagents were purchased from commercial sources and used without further purification. All solvents for workup procedures were used without further purification. Flash column chromatography was performed with ICN Silitech 32-63 D 60 Å silica gel with the indicated solvents. Thin-layer chromatography was performed on Analtech 60F₂₅₄ silica gel plates. Detection was performed using UV light, KMnO₄ stain, PMA stain, and subsequent heating. ¹H and ¹³C NMR spectra were recorded at the indicated field strength in CDCl₃ at room temperature (rt). The purity of each compound tested was determined on an Agilent 1200 LC/MS instrument using a Kinetex 2.6u C18 column (30 × 2.1 mm, with a flow rate of 1 mL/min and detection at 254 nm) employing a 5–100% acetonitrile/water/0.1% formic acid gradient. All compounds tested were >95% pure.

(–)-19,20-Dihydroxyakuammicine (13). *N*-Methylmorpholine *N*-oxide (607 mg, 5.18 mmol) was added to 1 (334 mg, 1.04 mmol). The compounds were dissolved in a mixture of *t*-BuOH/THF/H₂O (3:2:1). A catalytic amount of OsO₄ (4 wt % solution in H₂O, 0.15 mL) was added dropwise to the solution. The reaction was stirred at rt for 18 h. The reaction was quenched by adding 40% Na₂SO₃ solution (25 mL). The reaction mixture was extracted using CHCl₃ (3 × 10 mL). The combined organic phases were dried over Na₂SO₄ and concentrated under vacuum. The residue was purified by flash column chromatography eluting with MeOH/CH₂Cl₂/NEt₃ (9:1:0.05) to give 316 mg of 13 (86%) as a white foam: [α]_D²⁰ –100.5 (*c* 0.9, CHCl₃); IR (neat) 3355, 3053, 2948, 1669, 1540, 1474, 790 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ 9.10 (s, 1H), 7.18 (d, *J* = 7.4 Hz, 1H), 7.13 (td, *J* = 7.7, 1.2 Hz, 1H), 6.91 (td, *J* = 7.5, 0.9 Hz, 1H), 6.81 (d, *J* = 7.8 Hz, 1H), 3.86 (s, 1H), 3.77 (s, 3H), 3.59 (q, *J* = 6.2 Hz, 1H), 3.15–3.00 (m, 2H), 3.01–2.80 (m, 3H), 2.72–2.60 (m, 1H), 2.48 (d, *J* = 12.7 Hz, 1H), 1.87 (dd, *J* = 13.2, 6.6 Hz, 1H), 1.39 (d, *J* = 6.2 Hz, 3H), 1.20–1.13 (m, 1H); ¹³C NMR (126 MHz, CDCl₃) δ 172.17, 168.21, 144.04, 135.51, 127.79, 121.38, 119.90, 109.75, 100.57, 73.95, 72.74, 60.17, 56.57, 54.11, 52.59, 51.05, 42.77, 33.89, 25.91, 17.40; HRMS (FAB) calcd for C₂₀H₂₄N₂O₄ + H = 357.1982, found 357.1808.

(–)-19-Hydroxyalstolucine B (14). To a solution of *N*-chlorosuccinimide (80.4 mg, 0.401 mmol) in CH₂Cl₂ (4 mL) at –20 °C was added dimethyl sulfide (47 mg, 0.602 mmol). The reaction was stirred for 20 min at –20 °C, a solution of the diol 13 (143 mg, 0.401 mmol) in dry CH₂Cl₂ (4 mL) was added to the reaction, and the mixture stirred for 1.5 h. At this time, triethylamine (81 mg, 0.802 mmol) was added. The reaction was allowed to warm to rt, while being stirred for 1 h. The reaction was quenched with satd aq NaHCO₃ (3 mL) and diluted with CH₂Cl₂ (25 mL). The organic layer was washed with satd aq NaHCO₃ (10 mL) and brine (10 mL). The crude product was dried over Na₂SO₄ and concentrated under vacuum. The residue was purified by flash column chromatography eluting with MeOH/CH₂Cl₂ (0.2:9.8) to afford 94 mg of 14 (66%) as a white foam: [α]_D²⁰ –610.7 (*c* 0.09, CHCl₃); IR (neat) 3355, 3052, 2948, 2877, 1705, 1675, 1238, 962, 748 cm^{–1}; ¹H NMR (400 MHz, CDCl₃) δ 8.96 (s, 1H), 7.18 (d, *J* = 7.3 Hz, 1H), 7.14 (td, *J* = 7.7, 1.1 Hz, 1H), 6.91 (td, *J* = 7.5, 0.7 Hz, 1H), 6.81 (d, *J* = 7.7 Hz, 1H), 3.94 (s, 1H), 3.74–3.69 (q, 1H), 3.68 (s, 3H), 3.13 (d, *J* = 13.2 Hz, 1H), 3.07 (s, 3H), 2.81 (dd, *J* = 14.8, 9.0 Hz, 2H), 2.64 (d, *J* = 13.3 Hz, 1H), 2.36 (s, 3H), 1.96–1.88 (m, 1H), 1.23 (t, *J* = 7.0 Hz, 1H); ¹³C NMR (101 MHz, CDCl₃) δ 204.65, 170.90, 167.43, 144.03, 142.47, 127.97, 121.57, 119.92, 109.99, 96.87, 77.48, 77.16, 76.84, 59.96, 53.93, 51.17, 45.87, 43.30, 34.17, 29.75, 25.70, 24.48, 21.66; HRMS (FAB) calcd C₂₀H₂₂N₂O₄ + H = 355.4128, found 355.1580.

(–)-19-Acetoxyalstolucine B (15). Acetic anhydride (45 mg, 0.444 mmol) and triethylamine (49 mg, 0.484 mmol) were added to a

solution of hydroxyl ketone (143 mg, 0.403 mmol) in dry CH_2Cl_2 (3 mL). A catalytic amount of DMAP (4.9 mg, 0.040 mmol) was added and the reaction was stirred at rt for 4 h. The reaction was quenched with a saturated NaHCO_3 solution (5 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (0.1:9.9) to afford 152 mg of **15** (95%) as a white foam: $[\alpha]_{\text{D}}^{20} -487.8$ (c 0.1, CHCl_3); IR (neat) 3352, 3053, 2949, 2879, 1731, 1590, 1474, 1462, 1435, 1240, 866, 734 cm^{-1} ; ^1H NMR (500 MHz): δ 9.0 (s, 1H), 7.16 (d, $J = 5.0$ Hz, 1H), 7.14–7.11 (m, 1H), 6.92–6.89 (m, 1H), 6.80 (d, $J = 10.0$ Hz, 1H), 3.85 (s, 1H), 3.68 (s, 1H), 3.30 (dd, $J = 15.5$ Hz, 1.5 Hz, 1H), 3.22 (s, 1H), 3.09–2.98 (m, 3H), 2.84 (m, 1H), 2.68 (td, $J = 16.5$ Hz, 3.0 Hz, 1H), 2.22 (s, 3H), 2.17 (s, 3H), 1.86–1.85 (m, 1H), 1.28–1.25 (m, 1H); ^{13}C NMR (101 MHz, CDCl_3) δ 204.2, 172.6, 170.5, 167.3, 144.0, 135.4, 127.7, 121.3, 119.7, 109.7, 96.9, 85.1, 59.8, 57.0, 53.9, 50.8, 45.9, 43.2, 34.2, 25.7, 24.3, 21.4; HRMS (FAB) calcd $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_5 + \text{H} = 397.1234$, found 397.1749.

(–)-Alstolucine B (2) and (–)-Alstolucine F (3). To a round-bottomed flask containing **15** (87 mg, 0.219 mmol) were added deaerated THF (7 mL) and MeOH (3 mL) under inert atmosphere. The solution was cooled to -78 °C, and a solution of SmI_2 (10.9 mL, 0.1 M, 1.10 mmol), prepared using Molander's method, was added slowly.⁴⁰ The reaction mixture was stirred at -78 °C for 30 min, warmed to rt, and stirred overnight. The reaction was quenched with a satd aq solution of Na_2CO_3 (3 mL) and extracted with EtOAc (3×10 mL). The combined organic phases were washed with brine (10 mL), concentrated under reduced pressure, and purified by flash column chromatography eluting with MeOH/DCM (0:10 \rightarrow 0.2:9.8) to give 33 mg of **2** (44%) as a white foam and 20 mg of **3** (27%) as a yellow oil. ^1H and ^{13}C NMR spectra were identical with reported literature values; however, the optical rotation for synthetic **2** was $[\alpha]_{\text{D}}^{20} -464$ (c 0.35, CHCl_3), whereas the reported value was $[\alpha]_{\text{D}}^{20} -515$ (c 1.28, CHCl_3). The optical rotation for synthetic **3** was $[\alpha]_{\text{D}}^{20} -307$ (c 0.29, CHCl_3), whereas the reported value was $[\alpha]_{\text{D}}^{20} -371$ (c 0.15, CHCl_3).²⁷

(–)-Echitamidine (16). To a solution of **2** (20 mg, 0.059 mmol) in MeOH (1.5 mL) at 0 °C was added NaBH_4 (3.9 mg, 0.103 mmol). The reaction was warmed to rt and stirred for 1 h. The reaction was quenched with a satd aq solution of NaHCO_3 (3 mL) and extracted with CH_2Cl_2 (3×10 mL). The solution was concentrated under reduced pressure, and the residue was purified by flash column chromatography eluting with $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (0.7:9.3). ^1H and ^{13}C NMR spectra were identical with reported literature values; however, the optical rotation for synthetic (–)-echitamidine (**16**) was $[\alpha]_{\text{D}}^{20} -509$ (c 0.10, CHCl_3), whereas the reported literature value was $[\alpha]_{\text{D}}^{16} -515$ (c 5.0, EtOH).⁴²

(–)-*N*-Demethylalsogucine (4). To a stirred solution of **3** (8 mg, 0.023 mmol) in MeOH (1 mL) was added cerium(III) chloride heptahydrate (9.6 mg, 0.026 mmol) at rt. After stirring for 10 min, the solution was cooled to 0 °C, NaBH_4 (2.1 mg, 0.057 mmol) was added, and the solution was stirred for 1 h at 0 °C. The reaction was quenched with a half-saturated solution of NaHCO_3 (1 mL) and extracted with ethyl acetate (3×10 mL). The combined organic extractions were washed with brine (10 mL) and dried over Na_2SO_4 , and the solvent was concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (0.4:9.6 \rightarrow 0.6:9.4) to give 6.7 mg of **4** (87%). ^1H and ^{13}C NMR spectra were identical with reported literature values; however, the optical rotation for synthetic material was $[\alpha]_{\text{D}}^{20} -382$ (c 0.10, CHCl_3), whereas the literature value was $[\alpha]_{\text{D}}^{20} -399$ (c 0.33, CHCl_3).²⁷

(–)-Alstolucine A (5). Triethylamine (0.028 mL, 0.295 mmol) and ethyl chloroformate (0.04 mL, 0.295 mmol) were added to a stirred solution of **4** (20 mg, 0.059 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction was warmed to room temperature and stirred for 1 h. The reaction was quenched with saturated NH_4Cl (3 mL). The reaction mixture was extracted with CH_2Cl_2 (3×10 mL) and dried over Na_2SO_4 . The solvent was concentrated under reduced pressure, and

the residue was purified by flash column chromatography eluting with $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (0.4:9.6) to give 18 mg of **5** (73%). ^1H and ^{13}C NMR spectra were identical with reported literature values; however, the optical rotation of synthetic material was $[\alpha]_{\text{D}}^{20} -413$ (c 0.40, CHCl_3), whereas the literature value was $[\alpha]_{\text{D}}^{20} -438$ (c 0.12, CHCl_3).

Analysis of Drug Sensitivity.¹² Drug sensitivity was analyzed using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt/phenazine methosulfate microtiter plate assay (Cell-Titer 96 Cell Proliferation Assay; Promega, Madison, WI). HEK293-pcDNA3 and MRP7-transfected cell lines HEK293-MRP7-C17 and HEK293-MRP7-C18 were seeded in triplicate at 5000 cells/well in 96-well dishes in DMEM containing 10% fetal bovine serum. The following day, drugs were added at various concentrations to the growth medium. Growth assays were performed after 72 h of incubation in the presence of drug. Paclitaxel was purchased from Sigma Chemical Co. (St. Louis, MO).

Measurement of ATPase Activity.⁸ ABC10 specific activity was recorded as BeFx-sensitive ATPase activity. The amount of inorganic phosphate released over 20 min at 37 °C was measured; 2X ATPase assay buffer (100 mM Tris-HCl pH 7.5, 1 M KCl, 0.25 M sodium azide, 0.125 M EGTA, 1 mM ouabain, 1 M DTT) was combined with 2 M MgCl_2 , 5–10 μg of membrane protein, and various drugs or substrates for a 5 min preincubation at 37 °C. The reaction was initiated by 5 mM ATP addition and quenched with 100 μL of 5% SDS. The amount of Pi released was quantitated using the colorimetric method of Lanzetta.⁴³

■ ASSOCIATED CONTENT

📄 Supporting Information

^1H NMR and ^{13}C NMR spectra of all compounds synthesized in this work. 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

ABC, adenosine 5'-triphosphate binding cassette; ATPase, adenosine triphosphatase; Boc, *tert*-butoxycarbonyl; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DEAD, diethyl azodicarboxylate; DMAP, 4-(*N,N*-dimethylamino)pyridine; DMS, dimethyl sulfide; MDR, multidrug resistance; NCS, *N*-chlorosuccinimide; NMO, *N*-methylmorpholine *N*-oxide; TFA, trifluoroacetic acid.

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