INTRODUCTION

Multi-Drug Resistance (MDR) in leukemia due to elevated expression of P-glycoprotein (P-gp) renders many established therapies inactive and remains a serious clinical problem. In this study, natural plant products were investigated for their cytotoxicity in leukemia cells and their ability to re-sensitize P-gp overexpressing cells to the P-gp substrate vincristine. Brucine (1), brucine A (2), (-)-hydrocinnamyl (3), kopsinine Nα-oxide (4), and (8S)-(-)-N-methylactinidine (5) (Fig. 1) were evaluated using the acute lymphoblastic leukemia (ALL) cell line 697 and its multidrug resistant variant 697R. Brucine (1) and brucine A (2), quassinoids isolated from Brucia species, demonstrated potent cytotoxicity against multiple solid tumor cell lines (ED50 < 30 nM and 100 nM, respectively). In addition, 1 showed in vitro cytotoxicity in a panel of 11 leukemia cell lines and in vivo activity in B16 melanoma, P388 lymphocytic leukemia, and L1210 lymphoid leukemia in mice. Phase I and II clinical trials were conducted with 1, but no significant activity and in some cases severe toxicity was observed, and trials were discontinued. The flavonolignans, (-)-hydrocinnamyl (3), has been isolated from Brucia javanica, Hydnocarpus wightiana, Verbascum sinaticum, and Berberis species. (-)-Hydrocinnamyl (3), kopsinine Nα-oxide (4) and (8S)-(-)-N-methylactinidine (5) were isolated from an alkoidal extract of Tabernaemontana patula (Apocynaceae) (unpublished results). Kopsinine Nα-oxide (4), an adsporflavonone isotope alkaloid, was first isolated from Pleiocarpa muschla in the 1960s. and later from Kopsinia spp. Analogs of 4 have been shown to possess antiinflammatory and antimalarial activities. In addition, analogs of this alkaloid have successfully reversed MDR in vincristine-resistant nasopharynx carcinoma cells. (8S)-(-)-N-methylactinidine (5) is a new monoterpene alkaloid containing a cyclopenta[d]pyridine skeleton, and is a derivative of (-)-actinidine. (-)-Actinidine was first isolated from the roots of Actinidia deliciosa. (-)-Actinidine has shown interesting antifungal (e.g., Aspergillus spp.) and antibacterial (e.g., Bacillus and Streptococcus spp.) activities. Actinidine was also isolated from the salivary secretion of beetles and stick insects.

RESULTS

Cell lines used included the acute lymphoblastic leukemia cell line 697 and its MDR derivative 697-R. 697-R cells have previously been shown to achieve the MDR phenotype via highly increased P-gp expression. Both cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1-glutamine, and penicillin/streptomycin. 697-R cells were cultured with bi-weekly addition of 80 nM silvestrol to maintain the MDR phenotype. Cell viability was monitored by trypan blue dye exclusion. All cells were confirmed to be at least 90% viable before in vitro testing.

MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt) assays, which monitor mitochondrial activity as a surrogate for cell growth, were used to evaluate cytotoxicity and re-sensitization. Fluorescence-activated cell sorter (FACS) analysis was performed in all assays. MTS is a colorimetric method for determining the viability of viable cells. The ability of (-)-hydrocinnamyl (3) to inhibit P-gp function was then tested by measuring the efflux of rhodamine 123 from 697-R cells that express high levels of P-gp. In these experiments, 3 at 10 μM did not re-sensitize rhodamine 123 dye, resulting in a large increase in fluorescence relative to cells treated with an inactive agent. As before, verapamil was included as a positive control for P-gp inhibition.

CONCLUSIONS AND DISCUSSION

Brucine (1) and brucine A (2) exhibited potent cytotoxicity in the parental 697 cell line (IC50 = 26 nM and 16 nM, respectively). These agents showed similar cytotoxicity in the P-gp expressing 697-R cells (IC50 = 28 nM (1) and 22 nM (2); Fig. 2). These compounds were therefore not tested further for P-gp inhibitory activity.

REFERENCES