Medicinal Chemistry

Nakagawa-Goto et al. Medchem 2011, 1:1
http://dx.doi.org/10.4172/2161-0444.1000101

Research Article Open Access

Antitumor Agents 291 Expanded B-Ring Modification Study of 6,8,8-Triethyl Desmosdumotin B Analogues as Multidrug-Resistance Selective Agents

Kyoko Nakagawa-Goto[1]*, Kenneth F Bastow[2], Emika Ohkoshi[1], Susan L Morris-Natschke[1] and Kuo-Hsiung Lee[1,3]*

[1]*Natural Products Research Laboratories, EshelmanSchool of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA*
[2]*Division of Medicinal Chemistry & Natural Products, EshelmanSchool of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA*
[3]*Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung, Taiwan*

Abstract

Drug usefulnessis frequently obstructed by the incidence of the multidrug resistance (MDR) phenotype and severe adverse effects. Exploiting collateral sensitive(CS)agents (in this case also called MDR-selective agents), which selectively target only MDR cells, is an emerging and novel approach to overcome MDR in cancer treatment. In prior studies, we found that 4'-methyl-6,6,8-triethyldesmosdumotin B (4'-Me-TEDB, **2**) is an MDR-selective synthetic flavonoid with significant *in vitro* anticancer activity against a MDR cell line (KB-Vin) but without activity against the parent cells (KB) as well as other non-MDR tumor cells.Our recent results suggest the absolute MDR-selectivity varies depending on the cell-line system. In order to explore this further and to better understand the critical pharmacophores, we have synthesized nine novel analogues of **2**, which containheteroaromatic as well ascycloalkyl B-rings. The new compounds were evaluated for cytotoxicity to explore the effect of B-ring modifications on MDR-selectivity. All analogues, except **7, 9** and **10**, were identified as significant MDR-selective compounds. This observation solidifies the importance of the 5-hydroxy-6,8,8-trialkyl-4*H*-chromene-4,7(8*H*)-dione skeleton (AC-ring system) for the pharmacological activity and establishes the B-ring as less critical for the broader spectrum MDR-selectivity. Notably, 3-furanyl (**3**)and 2-thiophenyl (**6**)analogues displayed substantial MDR–selectivity with KB/KB-Vin ratios of >12 and 16, respectively. Furthermore, **3** and 6 also exhibited MDR–selectivity in a second set of paired cell lines, theMDR/non-MDR hepatoma-cell system. Interestingly, a cyclohexyl analogue (**11**) showed moderate inhibition of A549, DU145, and PC-3 cell growth, while the other compoundswere inactive.These new findings are discussed in terms of current understanding of mechanism and structure–activity relationship (SAR) of our novel MDR-selective flavonoids.

Keywords: Triethyldesmosdumotin B; Multi-drug resistance; MDR-selectivity (collateral sensitivity); Heteroaromatic ring; Cycloalkyl ring

Abbreviations: TEDB: 6,6,8-Triethyldesmosdumotin B; MDR: multi-drug resistance/resistant; CS: Collateral sensitivity; P-gp: P-glycoprotein; SAR: Structure–activity relationship

Introduction

While chemotherapy is a valuable cancer treatment, its usefulness is frequently obstructed by the incidence of the multidrug resistance (MDR) phenotype and severe adverse effects [1,2]. MDR in tumor cells is often correlated with the overexpression of P-glycoprotein (P-gp, MDR1) [3], which belongs to the superfamily of ATP-binding-cassette (ABC) transporters [4,5,6]. Resistance to one drug often implies simultaneous resistance to structurally and mechanistically diverse anticancer drugs. The emergence of MDRcauses cancer drugs to be pumped out of the cell, thus reducing intracellular drug concentrations below cytotoxic levels. Thecurrent major pharmacological approaches to overcome MDR have focused on inhibition of the pump function, and/or down-regulation of pump over-expression or developing cancer drug candidates that are not pump substrates [7-10]. Manycompounds have been identified as MDR (P-gp) inhibitors (or modulators), and are generally classified as first, second,or third generation chemosensitizers. Third-generation agents, such as tariquidar, zusuquidar, and triarylimidazole ONT-093 have shown improved efficacy compared with early generation compounds, as well as higher potency and specificity for P-gp [11]. These compounds are currently in clinical trials; however, phase III trials with some of these agents have not been successful [12]. In addition, significant survival benefits using a P-gp inhibitor has yet to be demonstrated despite considerable efforts [13].

Because no chemotherapy is yet available to sufficiently overcome MDR phenotype, new agents possessing antitumor activity and unaffected by the MDR phenotype, which exploit the drug efflux phenomenon, are in high demand and would be valuable additions to the arsenal of newantitumor drugs.We are interested in both approaches and this report focuses on the latter-type.Thehypersensitivity of drug-resistant cancer cells to certain drugs, which selectively kill MDR cells relative to the non-MDR parental cells,is a specific type of "collateral sensitivity"(CS) [14]. Exploiting CS agents is an exciting emerging approach to overcome MDR in cancer. For example, a thiosemicarbazone derivative (NSC73306) was discovered as a CS agent through the US National Cancer Institute (NCI) anticancer drug screen as a drug lead for targeting MDR tumor cell populations [15]. Although this compoundwas toxic toward a diverse panel of P-gp-expressing tumor cell lines, its highest selectivity ratio [IC_{50} (non-MDR)/IC_{50} (MDR)] was 7.3 (KB-3-1/KB-V1) [16].

We previously reported the structurally unusual flavonoid,

***Corresponding authors:** Nakagawa-Goto k, Natural Products Research Laboratories, EshelmanSchool of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA, Tel: 919-843-5209; Fax: 919-966-3893; E-mail: goto@email.unc.edu

KH Lee, Natural Products Research Laboratories, EshelmanSchool of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA, Tel: 919-962-0066; Fax: 919-966-3893; E-mail: khlee@unc.edu

Received September 19, 2011; **Accepted** November 04, 2011; **Published** December 01, 2011

Citation: Nakagawa-Goto K, Bastow KF, Ohkoshi E, Morris-Natschke SL, Lee KH(2011) Antitumor Agents 291 Expanded B-Ring Modification Study of 6,8,8-Triethyl Desmosdumotin B Analogues as Multidrug-Resistance Selective Agents. Medchem 1:101. doi:10.4172/2161-0444.1000101

desmosdumotin B (**1**), as a MDR CS agent with selectivityratio of >20 (KB/KB-Vin) [17]. Further modification of **1** revealed that active compounds containing both a trialkylated non-aromatic A-ring and a 6π electronic B-ring inhibited only MDR-tumor cell growth. Compounds combining the same B-ring and a 10π electronic B-ring exhibited potent cytotoxicity against multiple tumor cells, acting at least in-partby inhibition of tubulin [18]. 4'-Methyl-6,6,8-triethyldesmosdumotin B (4'-Me-TEDB, **2**) displayed the most significant and unprecedented selectivity with a KB/KB-Vin ratio of 460 [19]. Results from our study indicated that the activity of **2** against KB-Vin was correlated with P-gp overexpression; however, **2** was not a P-gpinhibitor yet it interacted with the P-gp in a novel fashion [20,21].

To further investigate B-ring effects, we synthesized several TEDB analogues with heteroaromatic as well as cycloalkyl B-rings and evaluated their cytotoxicity against KB and KB-Vin to determine their MDR selectivity profiles. The active compounds were also evaluated using a second set of paired cell lines, in order to assess whether MDR selectivity to them was restricted or more generalized. Herein, we report the syntheses of the new analogues and their MDR-selective activity as well as a structure-activity relationship (SAR) study.

Material and Methods

Chemistry

All chemicals and solvents were used as purchased. All melting points were measured on a Fisher-Johns melting point apparatus without correction. ^{1}H NMR spectra were recorded on a Varian Inova (400 MHz) NMR spectrometer with TMS as the internal standard. All chemical shifts are reported in ppm. NMR spectra were referenced to the residual solvent peak, chemical shifts δ in ppm, apparent scalar coupling constants *J* in Hz. Mass spectroscopic data were obtained on a Shimazu LC-MS2010 instrument. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated aluminum silica gel sheets (Kieselgel 60 F-254). Isco Companion systems were used for flash chromatography. All target compounds were characterized anddetermined by ^{1}H-NMR, MS, and elemental analyses or high resolution MS.

General synthetic procedures for 3-9

A solution of **12** in EtOH–50% aq. KOH (1:1, v/v) and an excess of appropriate aromatic aldehyde (except 3-furaldehyde, 2- and 3-pyridinecarbaldehyde) was stirred at room temperature. After the reaction was complete by TLC analysis, the mixture was poured into ice-cold 1 NHCl, and then extracted with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel with EtOAc–hexane as eluent to afford the chalcone **13**. When 3-furaldehyde or 2- and 3-pyridinecarbaldehyde was used as the aromatic aldehyde, $Ba(OH)_2$ (2 eq. mole) or Cs_2CO_3 (4 eq. mole) was used as the base rather than KOH.The resulting compound **13** was dissolved in 1% H_2SO_4 in DMSO, then I_2 (0.1 eq. mole) was added and the reaction mixture heated at 90 ºC for 1.5–4 h. The mixture was quenched with ice-cold aq 10% $Na_2S_2O_3$ and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel eluting with EtOAc–hexane (1:4 to 1:0, v/v) to afford the related 7-methoxy analogue **14**, which was dissolved in anhydrous CH_2Cl_2. The mixture was cooled to -78 ºC. BBr_3 (3 eq. mole, 1.0 M solution in CH_2Cl_2) was added to the solution, which was warmed to 0 ºC spontaneously and stirred until the starting material was consumed. After addition of water, the reaction mixture was extracted three times with CH_2Cl_2. The extracts were combined, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel eluting with EtOAc–hexane (1:4) to obtain the target compound (3–9).

2-(Furan-3'-yl)-TEDB (3): Pale yellow prisms, mp 195–196 °C (EtOAc-hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.01 (s, 1H, 5-O*H*), 8.03 (s, 1H, *2′-H*), 7.61-7.57 (m, 1H, 5′-*H*), 6.72-6.68 ((m, 1H, 4′-*H*), 6.58 (s, 1H, *3-H*), 2.44 (q, 2H, *J* = 7.3 Hz, 6-C*H*$_2$CH$_3$), 2.28-2.16 (m, 2H, 8-C*H*$_2$CH$_3$), 1.96-1.84 (m, 2H, 8-C*H*$_2$CH$_3$), 1.03 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$*CH*$_3$), 0.66 (t, 6H, *J* = 7.4 Hz, 8-CH$_2$*CH*$_3$).MS (ESI$^+$) *m/z*: 328 (M$^+$).Anal. Calcd for $C_{19}H_{20}O_5$: C, 69.50; H, 6.14; O, 24.36. Found: C, 69.24; H, 6.07; O, 24.39.

2-(Thiophen-3'-yl)-TEDB (4): Pale yellow prisms, mp 196–197°C (EtOAc-hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.05 (s, 1H, 5-O*H*), 7.95 (dd, 1H, *J* = 1.3 and 3.0 Hz, *2′-H*), 7.54 (dd, 1H, *J* = 3.0 and 5.1 Hz,5′-*H*), 7.54 (dd, 1H, *J* = 1.3 and 5.1 Hz,4′-*H*), 6.73 (s, 1H, *3-H*), 2.45 (q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.31-2.19 (m, 2H, 8-C*H*$_2$CH$_3$), 2.02-1.90 (m, 2H, 8-C*H*$_2$CH$_3$), 1.04 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$*CH*$_3$), 0.67 (t, 6H, *J* = 7.4 Hz, 8-CH$_2$*CH*$_3$).MS (ESI$^+$) *m/z*: 344 (M$^+$).Anal. Calcd for $C_{19}H_{20}O_4S{\cdot}1/2H_2O$: C, 64.57; H, 5.99. Found: C, 64.75; H, 5.69.

2-(Furan-2'-yl)-TEDB (5): Pale yellow prisms, mp 183–184 °C (EtOAc-hexane).^{1}H NMR (400 MHz, $CDCl_3$) δ 13.06 (s, 1H, 5-O*H*), 7.68 (dd, 1H, *J* = 0.5 and 1.7 Hz, *5′-H*), 7.07 (d, 1H, *J* = 3.4 Hz, *3′-H*), 6.78 (s, 1H, *3-H*), 6.65 (dd, 1H, *J* = 1.7 and 3.4 Hz, *4′-H*), 2.44 (q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.27-2.16 (m, 2H, 8-C*H*$_2$CH$_3$), 1.98-1.86 (m, 2H, 8-C*H*$_2$CH$_3$), 1.03 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$*CH*$_3$), 0.66 (t, 6H, *J* = 7.4 Hz, 8-CH$_2$*CH*$_3$).MS (ESI$^+$) *m/z*: 328 (M$^+$).Anal. Calcd for $C_{19}H_{20}O_5$: C, 69.50; H, 6.14. Found: C, 69.22; H, 6.11.

2-(Thiophen-2'-yl)-TEDB (6): Pale yellow prisms, mp162–163°C (EtOAc-hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.02 (s, 1H, 5-O*H*), 7.70-7.63 (m, 2H, *3′*- and 5'-*H*), 7.23 (dd, 1H, *J* = 3.9 and 5.1 Hz,4′-*H*), 6.74 (s, 1H, *3-H*), 2.45 (q, 2H, *J* = 7.5 Hz, 6-C*H*$_2$CH$_3$), 2.29-2.18 (m, 2H, 8-C*H*$_2$CH$_3$), 2.02-1.90 (m, 2H, 8-C*H*$_2$CH$_3$), 1.04 (t, 3H, *J* = 7.5 Hz, 6-CH$_2$*CH*$_3$), 0.68 (t, 6H, *J* = 7.5 Hz, 8-CH$_2$*CH*$_3$).MS (ESI$^+$) *m/z*: 344 (M$^+$).Anal. Calcd for $C_{19}H_{20}O_4S{\cdot}1/4H_2O$: C, 65.40; H, 5.92. Found: C, 65.41; H, 5.56.

2-(5'-Methylthiophen-2'-yl)-TEDB (7): Yellow prisms, mp 129–131 °C (EtOAc-hexane).^{1}H NMR (400 MHz, $CDCl_3$) δ 13.13 (s, 1H, 5-O*H*), 7.48 (d, 1H, *J* = 3.9 Hz,3'-*H*), 6.89 (d, 1H, *J* = 3.9 Hz,4′-*H*), 6.63 (s, 1H, *3-H*), 2.59 (s, 3H, -C*H*$_3$), 2.44 (q, 2H, *J* = 7.3 Hz, 6-C*H*$_2$CH$_3$), 2.27-2.16 (m, 2H, 8-C*H*$_2$CH$_3$), 2.00-1.88 (m, 2H, 8-C*H*$_2$CH$_3$), 1.03 (t, 3H, *J* = 7.3 Hz, 6-CH$_2$*CH*$_3$), 0.67 (t, 6H, *J* = 7.3 Hz, 8-CH$_2$*CH*$_3$).MS (ESI$^+$) *m/z*: 358 (M$^+$).Anal. Calcd for $C_{20}H_{22}O_4S{\cdot}1/2H_2O$: C, 65.37; H, 6.31. Found: C, 65.68; H, 5.89.

2-(Pyridin-2'-yl)-TEDB (8): Yellow prisms, mp 160–161 °C (EtOAc-hexane).^{1}H NMR (400 MHz, $CDCl_3$) δ 13.06 (s, 1H, 5-O*H*), 8.79-8.74 (m, 1H, 5'-*H*), 7.96-7.86 (m, 2H,3′- and 4'-*H*), 8.09-8.03 (m, 1H, 3'-*H*), 7.56 (s, 1H, *3-H*), 7.53-7.46 (m, 1H, 5'-*H*), 2.46 (q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.33-2.22 (m, 2H, 8-C*H*$_2$CH$_3$), 2.05-1.94 (m, 2H, 8-C*H*$_2$CH$_3$), 1.05 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$*CH*$_3$), 0.68 (t, 6H, *J* = 7.4 Hz, 8-CH$_2$*CH*$_3$).MS (ESI$^+$) *m/z*: 339 (M$^+$).HRMS (*m/z*): [M+H]$^+$Calcd for $C_{20}H_{22}NO_4$, 340.1543, Found: 340.1542.

2-(Pyridin-3'-yl)-TEDB (9): Yellow prisms, mp 149–150 C (EtOAc-hexane).^{1}H NMR (400 MHz, $CDCl_3$) δ 12.80 (s, 1H, 5-O*H*), 9.08 (d, 1H, *J* = 2.2 Hz,2'-*H*), 8.83 (dd, 1H, *J* = 1.3 and 4.6 Hz,6′-*H*), 8.09-8.03 (m, 1H, 3'-*H*), 7.53 (dd, 1H, *J* = 4.6 and 8.1 Hz,5'-*H*), 6.94 (s, 1H, *3-H*), 2.46 (q, 2H, *J* = 7.4 Hz, 6-C*H*$_2$CH$_3$), 2.32-2.21 (m, 2H, 8-C*H*$_2$CH$_3$), 2.04-1.92 (m, 2H, 8-C*H*$_2$CH$_3$), 1.05 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$*CH*$_3$), 0.69 (t, 6H, *J* = 7.4 Hz, 8-CH$_2$*CH*$_3$).MS (ESI$^+$) *m/z*: 339

(M^+).HRMS (*m/z*): $[M+H]^+$Calcd for $C_{20}H_{22}NO_4$, 340.1543, Found: 340.1570.

General synthetic procedures for 10 and 11

A solution of **12** in anhydrous THF was cooled to -78°C under Ar. LiHMDS (5 eq. mol, 1.0M solution in THF) was slowly added and the mixture was gradually warmed to 0°C over 1.5 h. After stirring additional 2 h at 0°C, the mixture was cooled to -78°C. The appropriate acyl chloride (2 eq. mole) was added and stirred at -78°C for 1 h. The whole was warmed to rt spontaneously, and keep stirring overnight. The mixture was poured onto ice-cold 2NHCl, stirred for 1h and extracted with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc–hexane as eluent to afford the related **14** and **16**. Compound **16** was dissolved in benzene and refluxed with a catalytic amount of pTsOH for 2 days. The volatile solvent was removed in vacuo. The residue was chromatographed on silica gel eluting with EtOAc–hexane to afford the intermediate**14**. The resulting **14** was treated with BBr_3 as described above to obtain the target compounds (10 and 11).

2-Cyclopropyl-TEDB (10): Colorless prisms, mp 124–125 °C (EtOAc-hexane).^{1}H NMR (400 MHz, $CDCl_3$) δ 13.15 (s, 1H, 5-O*H*), 6.32 (s, 1H, *3-H*), 2.41 (q, 2H, *J* = 7.3 Hz, 6-CH_2CH$_3$), 2.20-2.08 (m, 2H, 8-CH_2CH$_3$), 1.97-1.89 (m, 1H, 1'-*H*), 1.80-1.70 (m, 2H, 8-CH_2CH$_3$), 1.24-1.18, (m, 2H), 1.11-1.06 (m, 2H), 1.01 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$CH_3), 0.61 (t, 6H, *J* = 7.4 Hz, 8-CH$_2$CH_3).MS (ESI$^+$) *m/z*: 302(M^+).HRMS (*m/z*): $[M+H]^+$Calcd for $C_{18}H_{23}O_4$, 303.1591, Found: 303.1518.

2-Cyclohexyl-TEDB (11): Colorless prisms, mp 112–113 C (EtOAc-hexane).^{1}H NMR (400 MHz, $CDCl_3$) δ 13.07 (s, 1H, 5-O*H*), 6.27 (s, 1H, *3-H*), 2.62-2.49 (m, 1H, 1'-*H*), 2.42 (q, 2H, *J* = 7.4 Hz, 6-CH_2CH$_3$), 2.22-2.10 (m, 2H, 8-CH_2CH$_3$), 2.05-1.95 (m, 2H, Cyclohexyl-*H*), 1.94-1.64 (m, 4H, 8-CH_2CH$_3$and Cyclohexyl-*H*), 1.48-1.20, (m, 6H, Cyclohexyl-*H*), 1.02 (t, 3H, *J* = 7.4 Hz, 6-CH$_2$CH_3), 0.61 (t, 6H, *J* = 7.4 Hz, 8-CH$_2$CH_3).MS (ESI$^+$) *m/z*: 344 (M^+). HRMS (*m/z*): $[M+H]^+$Calcd for $C_{21}H_{29}O_4$, 345.2060, Found:345.2016.

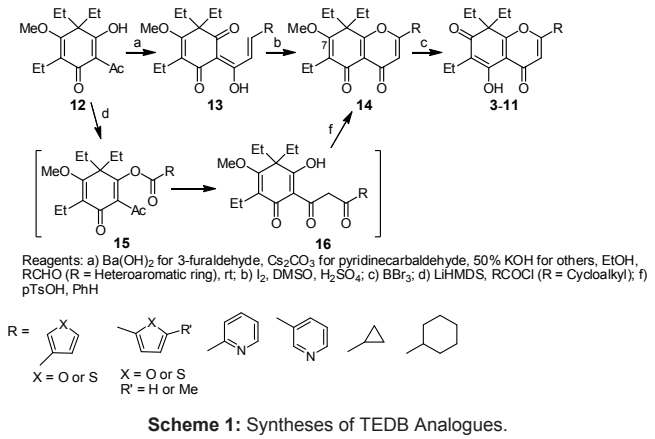
Cytotoxic Activity Assay

All human tumor cell lines were cultured in RPMI-1640 medium supplemented with 25mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 μg/mL kanamycin in 5% CO_2 and 95% air at 37˚C.Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1500-7500 cells per well with compounds added from DMSO-diluted stock. After three days in culture, attached cells were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B (SRB). The absorbency at 515 nm was measured using a microplate reader (ELx800, Bio-Tek) after solubilizing the bound SRB dye in 10mM Tris-base. The mean IC_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent determinations that were reproducible and statistically significant. The following human tumor cell lines were used in the assay: KB (nasopharyngeal carcinoma), KB-Vin (vincristine-resistant KB subline), A549(lung carcinoma), PC-3 and DU145 (prostate cancer),SKBR-3 (breast cancer), HCT-8 (human colon adenocarcinoma), HepG2 (hepatocellular carcinoma), and HepG2-Vin (vincristine-resistant HepG2 subline). All cell lines were obtained from Lineberger Cancer Center (UNC-CH) or from ATCC (Rockville, MD), except KB-Vin, which was a generous gift of Professor Y.-C. Cheng, Yale University, and HepG2-Vin. HepG2-Vin was established from a parental HepG2 by gradually increasing the concentration of vincristine from 0.5 to 2 μM with 20% increments at each treatment according to previously described method [22]. The established 2 μM vincristine-resistant HepG2 (HepG2-Vin) were also tolerant to paclitaxel at IC_{50} of 2.4 μM compare with parental HepG2 at IC_{50} of 0.63 μM. Increased P-gp expression in HepG2-Vin was confirmed by calcein-AM assay (data not shown).

Results

Analogues **3**–**6** were preparedthrough a three-step sequence, Claisen-Schmidt condensation of **12** with the corresponding aromatic aldehyde (RCHO) and 50% aq. KOH, cyclization, and C-7 demethylation of 14 with BBr_3 according to the reported method [12,14] (Scheme 1). Claisen-Schmidt condensation with 3-furaldehyde and pyridinecarbaldehyde were carried out using $Ba(OH)_2$, and Cs_2CO_3, respectively, rather than KOH. For analogues **10** and **11**, the intermediates (**14**) were obtained by the treatment of **12** with the appropriate cycloalkyl acid chloride in the presence of LiHMDS, followed by treatment with acid.

All synthesized analogues **3**–**11**were evaluated *in vitro* against two human tumor cell lines, the KB-VIN cell line, an MDR P-gp expressing cloned subline stepwise selected using vincristine, and its parental non-MDR KB cell line. The cytotoxic activity data including KB/KB-VIN selectivity are listed in Table 1. While none of the **2**-analogues with a hetero aromatic B-ring (**3**–**9**) inhibited the non-MDR tumor cell (KB) growth, most of them did significantly (**3**–**6**) or moderately (**8**) inhibit the MDR tumor cell (KB-VIN) growth. The inhibitory effects of **7** and **9** against KB-Vinwere moderate at best and likely insignificant. The detailed SAR shows 2-(furan-3'-yl)-TEDB (**3**) and 2-(thiophen-2'-yl)-TEDB (**6**)displayed KB/KB-VIN hypersensitivity of >12 and 16 with IC_{50} values of 5.2 and 3.2 μM against KB-VIN, respectively, which is greater than the thiosemicarbazone prototype NSC73306. The non-substituted furanyl and thiophenyl B-ring (five-membered



Scheme 1: Syntheses of TEDB Analogues.

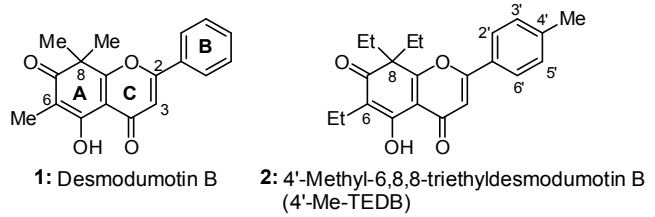
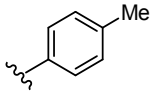
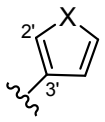
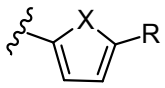
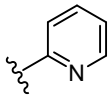
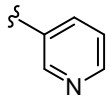
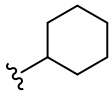


Figure 1:

				ED_{50} (μM)[a]		Selectivity
		X	R	KB	KB-VIN	KB/KB-VIN
1	Desmosdumotin B	-	-	>135	6.8	>20
2		-	-	39.2	0.08	460
3		O	-	>61	5.2	>12
4		S	-	>58	8.7	>6.7
5		O	H	>61	7.9	>7.7
6		S	H	50.9	3.2	16
7		S	Me	37.7	27.4	1.4
8		-	-	51.6	15.6	3.3
9		-	-	>59.0	42.8	>1.4
10		-	-	>33	>33	1
11		-	-	30.8	8.3	3.7
NSC73306[b]				10.65 (KB-3-1)	1.47	7.3
Paclitaxel				0.007	1.77	0.004

[a]Cytotoxicity as ED_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay. Epidermoid carcinoma of the nasopharynx (KB), and MDR line overexpressing P-glycoprotein (KB-VIN).[b]The data were cited from ref [10]

Table 1: Activity of 1–11against KB and KB-VIN.

	IC_{50} (μM)[a]		Selectivity
	HepG2	HepG2-Vin	HepG2/ HepG2-Vin
3	>61	16.0	>3.8
6	43.8	2.4	18.3
Paclitaxel	0.63	2.4	0.26

[a]Cytotoxicity as ED_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay. Hepatocellular carcinoma (HepG2), and its MDR line (HepG2-Vin)

Table 2: MDR-selectivity of **3** and **6** against hepatocellular carcinoma.

ring) analogs, **3**–**6**, displayed greater cytotoxicity against KB-Vin than the pyridinyl B-ring (six-membered ring) analogs (**8** and **9**), but it is really not clearthat the ring size or the nature of the heteroatom contributed to the activity. Among five-membered ring derivatives, the nature of the heteroatom and the connection position to C-ring influenced both the relative and absolute activity against the KB-cell system. When the heteroaromatic B-ring was attached through its C-3' position to C-ring, oxygen was preferred to sulfur (**3** vs **4**). However, when B-ring was connected through the C-2' position to C-ring, sulfur was favorable (**5** vs **6**). The presence of a methyl group on a thiophenyl B-ringsignificantly reduced the MDR-selectivity (**6** vs **7**), while the presence of a methyl group at the para-position ofa phenyl B-ring (**2**) led to extremely high MDR-selectivity.

Compounds **10** and **11** contain cyclopropyl and cyclohexyl B-rings, respectively. Compound **11** exhibited moderate cytotoxicity against KB-Vin with a 3.7 ratio of MDR selectivity, while **10** did not show significant cytotoxicity or any selectivity. Interestingly, compound 11 exhibited moderate cytotoxicity against other non-MDR tumor cells, such as A549 (IC_{50} = 18.1 μM), DU145 (IC_{50} = 15.4 μM), and PC-3 (IC_{50} = 17.8 μM), despite the fact that other 2-analogues, including **1** and **2**, did not show any cytotoxicity against these three cell lines.

The most active analogues **3** and **6** were also evaluated *in vitro* against a human hepatoma MDR cell system, and the data are shown in Table 2. HepG2-Vin cells are P-gp-expressing and selected from the parent non-MDR HepG2 cell line using vincristine. The MDR-selectivity ratios measured for **3** and **6** were 3.8 and 18.3, respectively. This finding shows that the MDR-selectivity displayed by **3** and **6**is not limited to a single MDR-cell line.

The results in this and our prior studies indicate that MDR-selectivity of **2**-analoguesis not critically dependent on the type of B-ring. Although the structural features of the pendant B-ring can affect the activity as well as selectivity, compounds with acyclic B-ring, including mono-phenyl, heteroaromatic, and cycloalkylrings, but not bicyclic ring systems, acted as MDR-selective agents. Thus, the 5-hydroxy-6,8,8-trialkyl-4H-chromene-4,7(8H)-dione skeleton (A/C-ring system in **1**) appears to be the crucial factor for the MDR selectivity. It is intriguing that structural variation of the B-ring is well tolerated. This suggests that interaction with P-gp, the presumed target, is relaxed, just as it is for substrates of the enzyme, including flavonoids, where structural variation is well tolerated. A similar finding was recently reported for new thiosemicarbazone-derived MDR-selective agents [13], although these actives are not thought to be enzyme substrates or inhibitors.

Detailed mechanism of action studies coupled with the development of new probe compounds designed to study target interaction will be key to further understand and develop **2**-analogs as drug candidates in future studies. Such work is planned and will be worthwhile in order to fully exploit MDR and overcome the resistance barrier of cancer chemotherapy.

Acknowledgement

This study was supported by grant CA-17625 from the National Cancer Institute, NIH, awarded to K. H. L and by a grant from the University Research Council, awarded to K.N.G.

References

1. Lage H (2008) An overview of cancer multidrug resistance: a still unsolved problem. Cell Mol Life Sci 65: 3145-3167.
2. Perez-Tomas R (2006) Multidrug resistance: retrospect and prospects in anti-cancer drug treatment. Curr Med Chem 13: 1859-1876.
3. Endicott JA, Ling V (1989) The biochemistry of P-glycoprotein-mediated multidrug resistance. Annu Rev Biochem 58:137-171.
4. Fletcher JI, Haber M, Henderson MJ, Norris MD (2010) ABC transporters in cancer: more than just drug efflux pumps. Nat Rev Cancer 10: 147-156.
5. Eckford PD, Sharom FJ (2009) ABC efflux pump-based resistance to chemotherapy drugs. Chem Rev 109: 2989-3011.
6. Sharom FJ (2008) ABC multidrug transporters: Structure, function and role in chemoresistance. Pharmacogenomics 9:105-127.
7. Takara K, Sakaeda T, Okumura K. (2006) An update on overcoming MDR1-mediated multidrug resistance in cancer chemotherapy. Curr Pharm Des 12: 273-286.
8. Avendaño C, Menéndez JC (2004) Recent advances in multidrug resistance modulators. Med Chem Rev 1: 419-444.
9. Kawase M, Motohashi N (2003) New multidrug resistance reversal agents. Curr Drug Targets 4: 31-43.

10. Lehne G (2000) P-glycoprotein as a drug target in the treatment of multidrug resistant cancer. Curr Drug Targets1: 85-99.

11. Krishna R, Mayer LD (2001) Modulation of P-glycoprotein (PGP) mediated multidrug resistance (MDR) using chemosensitizers: recent advances in the design of selective MDR modulators. Curr Med Chem Anticancer Agents 1: 163-174.

12. Coley HM (2010) Overcoming multidrug resistance in cancer: clinical studies of P-glycoprotein inhibitors. Methods Mol Biol 596: 341-358.

13. Ozben T (2006) Mechanisms and strategies to overcome multiple drug resistance in cancer. FEBS Lett 580: 2903–2909.

14. Hall MD, Handley MD, Gottesman MM (2009) Is resistance useless? Multidrug resistance and collateral sensitivity. Trends Pharmacol Sci 30: 546-556.

15. Ludwig JA, Szakács G, Martin SE, Chu BF, Cardarelli C, et al. (2006) Selective toxicity of NSC73306 in MDR1-positive cells as a new strategy to circumvent multidrug resistance in cancer. Cancer Res 66: 4808-4815.

16. Hall MD, Brimacombe KR, Varonka MS, Pluchino KM, Monda JK, et al. (2011) Synthesis and structure-activity evaluation of isatin-β-thiosemicarbazones with improved selective activity toward multidrug-resistant cells expressing P-glycoprotein. J Med Chem 54: 5878-5889.

17. Nakagawa-Goto K, Bastow KF, Wu JH, Tokuda H, Lee KH (2005) Total synthesis and bioactivity of unique flavone desmosdumotin B and its analogs. Bioorg Med Chem Lett 15: 3016-3019.

18. Nakagawa-Goto K, Wu PC, Lai CY, Hamel E, Zhu H, et al. (2011) Antitumor agents. 284. New desmosdumotin B analogues with bicyclic B-ring as cytotoxic and antitubulin agents. J Med Chem 54: 1244-1255.

19. Nakagawa-Goto K, Bastow KF, Chen TH, Morris-Natschke SL, Lee KH (2008) Antitumor agents 260. New desmosdumotin B analogues with improved in vitro anticancer activity. J Med Chem 51: 3297-3303.

20. Nakagawa-Goto K, Chang PC, Lai CY, Hung HY, Chen TH, et al. (2010) Antitumor agents. 280. Multidrug resistance-selective desmosdumotin B analogues. J Med Chem 53: 6699-6705.

21. Kuo TC, Chiang PC, Yu CC, Nakagawa-Goto K, Bastow KF, et al. (2011) A unique P-glycoprotein interacting agent displays anticancer activity against hepatocellular carcinoma through inhibition of GRP78 and mTOR pathways. Biochem Pharmacol 81:1136-1144.

22. Takano M, Otani Y, Tanda M, Kawami M, Nagai J, et al. (2009) Paclitaxel-resistance conferred by altered expression of efflux and influx transporters for paclitaxel in the human hepatoma cell line, HepG2. Drug Metab Pharmacokinet 24:418-427.