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

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Abstract

Drug usefulness is 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. Prior studies, we found that 4'-methyl-6,6,8-triethyl desmosdumotin B (4'-Me-TEDB, 2) is an MDR-selective synthetic flavonoid with significant in vitro anticancer activity against A tumor 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 contain heteroaromatic as well as cycloalkyl 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-triethyl-4H-chromene-4,7(8H)-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, the MDR/non-MDR hepatoma-cell system. Interestingly, a cyclohexyl analogue (11) showed moderate inhibition of A549, DU145, and PC-3 cell growth, while the other compounds were 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: Triethyl desmosdumotin B; Multi-drug resistance; MDR-selectivity (collateral sensitivity); Heteroaromatic ring; Cycloalkyl ring

Abbreviations: TEDB: 6,6,8-Triethyl desmosdumotin 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 MDR causes cancer drugs to be pumped out of the cell, thus reducing intracellular drug concentrations below cytotoxic levels. The current 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]. Many compounds 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, zosuquidar, and triatrylimidazole 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 new antitumor drugs. We are interested in both approaches and this report focuses on the latter type. The hypersensitivity 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 compound was toxic toward a diverse panel of P-gp-expressing tumor cell lines, its highest selectivity ratio [IC50 (non-MDR)/IC50 (MDR)] was 7.3 (KB-3-I/KB-V) [16].

We previously reported the structurally unusual flavonoid,
desmosdumotin B (1), as a MDR CS agent with selectivity ratio of

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>20 (KB/KB-Vin) [17]. Further modification of 1 revealed that

active compounds containing both a trialkylated non- aromatic A-ring and a 6t electronic B-ring inhibited only MDR-tumor cell growth. Compounds combining the same B-ring and a 10t electronic B-ring exhibited potent cytotoxicity against multiple tumor cells, acting at least in-part by inhibition of tubulin [18]. 4’-Methyl-6,8-

triyldesmosdumotin 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-gp-inhibitor 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. 1H 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

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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 of 1.0M solution in THF) was slowly added and the mixture was gradually warmed to 0°C over 1 h. After stirring additional 2 h at 0°C, the mixture was cooled to -78°C. The appropriate acyl chloride (2 eq. mol) was added and stirred at -78°C for 1 h. The mixture was poured onto ice-cold 2N HCl, stirred for 1 h and extracted with CH₂Cl₂. The extract was washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was chromatographed on silica gel with EtOAc–hexane as eluent to afford the related intermediates (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 BBr₃ as described above to obtain the target compounds (10 and 11).

2-Cyclopropyl-TEDB (10): Colorless prisms, mp 124–125 °C (EtOAc-hexane). ¹H NMR (400 MHz, CDCl₃) δ 13.15 (s, 1H, 5-OH), 0.63 (t, 6H, J = 7.4 Hz, 6-CH₂), 2.22–2.10 (m, 2H, 8-CH₂), 1.11–1.06 (m, 2H), 1.01 (t, 3H, J = 7.4 Hz, 6-CH₂), 0.61 (t, 6H, J = 7.4 Hz, 8-CH₂). MS (ESI +): m/z 302 (M⁺). HRMS (m/z): [M+H]⁺Calcd for C₂₁H₂₉O₄, 345.2060, Found:345.2016.

2-Cyclohexyl-TEDB (11): Colorless prisms, mp 112–113 °C (EtOAc-hexane). ¹H NMR (400 MHz, CDCl₃) δ 7.07 (s, 1H), 0.87 (t, 3H, J = 7.4 Hz, 6-C₂H₂), 2.22–2.10 (m, 2H, 8-C₂H₂), 1.11–1.06 (m, 2H), 1.01 (t, 3H, J = 7.4 Hz, 6-C₂H₂), 0.61 (t, 6H, J = 7.4 Hz, 8-C₂H₂). MS (ESI +): m/z 340.1570. Found:340.1570.

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% CO₂ and 95% air at 37˚C. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at 1000 cells/well. After 24 h, medium was renewed, 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 IC₅₀ 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 IC₅₀ of 2.4 µM compare with parental HepG2 at IC₅₀ of 0.63 µM. Increased P-gp expression in HepG2-Vin was confirmed by calcine-AM assay (data not shown).

Results

Analogues 3–6 were prepared through 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 BBr₃ according to the reported method [12,14] (Scheme 1). Claisen-Schmidt condensation with 3-furaldehyde and pyridinecarboxaldehyde were carried out using Ba(OH)₂ and Cs₂CO₃, 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-analalogues 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/VIN hypersensitivity of >12 and 16 with IC₅₀ values of 5.2 and 3.2 µM against KB-VIN, respectively, which is greater than the theoisomerasein protease NSC73306. The non-substituted furanyl and thiophenyl B-ring (five-membered hetero aromatic B-ring (9)) displayed KB/KB-VIN hypersensitivity of >12 and 16 with IC₅₀ values of 5.2 and 3.2 µM against KB-VIN, respectively.
Hepatocellular carcinoma (HepG2), and its MDR line caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay. Epidermoid carcinoma of the nasopharynx (KB), and caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay. Paclitaxel 0.63 2.4 0.26

The results in this and our prior studies indicate that MDR-selectivity of 2-analogues is not critically dependent on the type of B-ring. Although the structural features of the pendant B-ring can influence both the relative and absolute activity against the KB-cell line, the presence of a methyl group at the para-position of a phenyl B-ring is really not clear that the ring size or the nature of the heteroatom contributed to the activity. Among five-membered ring derivatives, the activity was favorable. The presence of a methyl group on a thiophenyl B-ring significantly reduced the MDR-selectivity (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

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<th>Compounds</th>
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<th>Selectivity</th>
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<td>1 Desmosdumotin B</td>
<td>X R KB KB-VIN KB/KB-VIN</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>- - &gt;135 6.8 &gt;20</td>
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<tr>
<td>3</td>
<td>O - 39.2 0.08 460</td>
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<td>4</td>
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<td>5</td>
<td>O H &gt;61 5.2 &gt;12</td>
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<td>6</td>
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<td>7</td>
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<tr>
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</tr>
<tr>
<td>10</td>
<td>- - &gt;33 &gt;33 1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>- - 30.8 8.3 1.4</td>
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Table 1: Activity of 1–11 against KB and KB-VIN.

The most active analogues and 3 & 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 & 6 were 3.8 and 18.3, respectively. This finding shows that the MDR-selectivity displayed by 3 & 6 is not limited to a single MDR-cell line.

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 (IC50 = 18.1 µM), DU145 (IC50 = 15.4 µM), and PC-3 (IC50 = 17.8 µM), despite the fact that the other 2-analogue, including 1 and 2, did not show any cytotoxicity against these three cell lines.

The results in this and our prior studies indicate that MDR-selectivity of 2-analogues is not critically dependent on the type of B-ring. Although the structural features of the pendant B-ring can influence both the relative and absolute activity against the KB-cell line, the presence of a methyl group on a phenyl B-ring is really not clear that the ring size or the nature of the heteroatom contributed to the activity. Among five-membered ring derivatives, the activity was favorable. The presence of a methyl group on a thiophenyl B-ring significantly reduced the MDR-selectivity (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

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<th>Compounds</th>
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<tr>
<td>3</td>
<td>&gt;61 16.0 &gt;3.8</td>
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<tr>
<td>6</td>
<td>43.8 2.4 18.3</td>
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</tr>
<tr>
<td>Paclitaxel</td>
<td>0.63 2.4 0.26</td>
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* Cytotoxicity as IC50 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 its MDR line overexpressing P-glycoprotein (KB-VIN).*The data were cited from ref [10]

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

* Cytotoxicity as IC50 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)

References


