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Triethylated chromones with substituted naphthalenes as tubulin inhibitors

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ABSTRACT

Previously synthesized 2-(benzo[*b*]thiophene-3′-yl)-6,8,8-triethyldesmosdumotin B (**1**, TEDB-TB) and 2-(naphth-1′-yl)-6,8,8-triethyldesmosdumotin B (**2**) showed potent activity against multiple human tumor cell lines, including a multidrug-resistant (MDR) subline, by targeting spindle formation and/or the microtubule network. Consequently, ester analogues of hydroxylated naphthyl substituted TEBDs (**3**–**5**) were prepared and evaluated for their effects on tumor cell proliferation and on tubulin assembly. Among all new compounds, compound **6**, a 4′-acetoxynaphthalen-1′-yl derivative, displayed the most potent antiproliferative activity (IC_{50} 0.2–5.7 μM). Selected analogues were confirmed to be tubulin assembly inhibitors in cell-free and cell-based assays using MDR tumor cells. The new analogues partially inhibited colchicine binding to tubulin, suggesting their binding mode would be different from that of colchicine. This observation was supported by computational docking model analyses. Thus, the newly synthesized triethylated chromones with esterified naphthalene groups have good potential for development as a new class of mitotic inhibitors that target tubulin.

1. Introduction

The structural diversity of natural products (NPs) has contributed significantly to past drug discovery. In their recent review, Newman and Cragg stated that NPs are still playing a dominant role in the current discovery of leads and development of drugs for the treatment of human diseases.[1] The biosynthesis of NPs occurs efficiently via well-controlled reactions promoted by natural enzymes, while chemical syntheses of NPs are not always easy. Nonetheless, novel bioactive compounds that are biosynthetically not observed can be artificially produced as synthetic NP derivatives. Thus, the application of organic synthesis to core NP skeletons can supply richer structural variety to expand the possibility of potent drug leads. The chemical modification of lead natural products is also a useful strategy to improve the desired pharmacological activity and to reduce adverse clinical side effects. The differences in functional groups and their positions can affect various drug parameters, such as partition coefficient, electron density, structure conformation, bioavailability, and other pharmacokinetics factors involved in the interaction between ligand and cellular targets. We selected novel scaffolds that do not occur biosynthetically but can be converted to promising drug candidates, based on chemical modification of natural skeletons.

Aromatic ring systems are key scaffolds in medicinal chemistry, because their electron rich π systems and structurally rigid planar frameworks can often play a critical role in the interactions of ligands with their cellular targets. A phenyl group is the most common aromatic ring found in natural products. However, bicyclic aromatic systems, such as naphthalene, have expanded π orbitals, which can sometimes lead to dramatic changes in ligand–receptor interactions. It was reported previously that the biological profile of triethyldesmosdumotin B (TEDB) could be significantly changed based on the identity of the B-ring (Fig. 1).[2,3] When the pendant B-ring was a 6π-electron aromatic system, the TEDB analogue showed effective cytotoxicity only against P-glycoprotein (P-gp) overexpressing multi-drug resistant (MDR) cells, but no cytotoxicity against any chemosensitive tumor cell line.[2–5] In contrast, compounds with a 10π- rather than 6π-electron aromatic system, such

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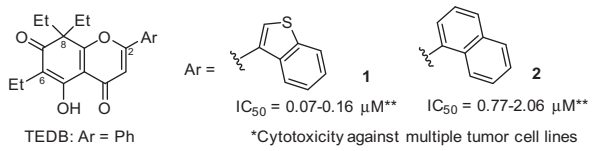


Figure 1. Structures of TEDB-TB analogues.

as 2-(benzo[*b*]thiophene-3′-yl)-6,8,8-triethyldesmosdumotin B (**1**, TEDB-TB) and 2-(naphth-1′-yl)-6,8,8-triethyldesmosdumotin B (**2**), exhibited potent antiproliferative activity against multiple human cancer cell lines, including MDR tumor cells, acting via inhibition of tubulin polymerization, in part through the colchicine site (CS).[6] It was also found that hydroxylated benzothiophene analogues efficiently induced cell cycle arrest at the G2/M phase, with formation of immature multipolar spindles.[7]

To explore the biological potential of this compound class, we have continued the investigation of TEDB derivatives. A hydroxy group provides a polar surface on the naphthalene molecule, which has no topological polar surface area (tPSA) by itself. Hydroxy naphthalene derivatives **3**–**5**, which were already in hand,[7] could be easily esterified to produce various ester derivatives. Acyl group might contribute to H-bond interactions with the target protein or could provide a spacer element. Furthermore, the functional group differences might alter the biological profile, as we previously described.[2–7]

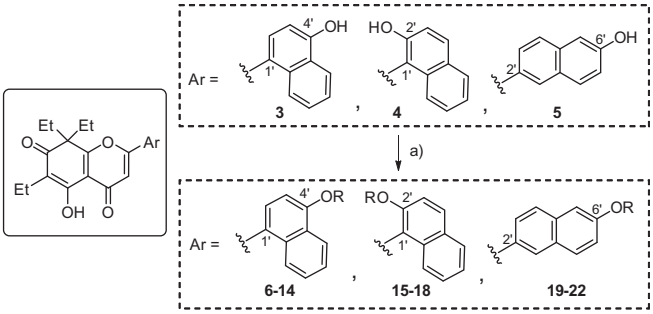
An ester group plays an important role in biological activity. A well-known instance is paclitaxel (PXL), an antitumor drug in clinical use. The ester side chain at position C-13, acetate at position C-4, and benzoate at position C-2 are essential for the antitubulin activity of PXL.[8] Analogues without ester groups at the above mentioned positions or with simplified side chains at C-13 have dramatically reduced activity.

The antiproliferative activities of all new TEBD derivatives against several cancer cell lines, including an MDR cell line, were studied. Selected analogues were investigated for potential inhibitory effects on tubulin assembly with purified tubulin and for effects on cell cycle progression in human tumor cells.

2. Results and discussion

2.1. Chemistry

The hydroxylated analogues **3**–**5** were synthesized previously (Scheme 1).[7] Esterifications of **3**–**5** were accomplished using the appropriate acyl chloride for **6**–**22**. The structures and purities of all synthesized compounds were confirmed by ^{1}H NMR, high resolution MS, and HPLC analysis.



Scheme 1. Syntheses of new analogues **6**–**22**. Reagents and conditions: (a) Et_3N, RCl [various acyl chlorides, such as acetyl chloride (R = CH_3CO), propionyl chloride (R = CH_3CH_2CO), butyryl chloride (R = $CH_3CH_2CH_2CO$), benzoyl chloride (R = PhCO), cinnamoyl chloride (R = PhCH=CHCO)].

2.2. Biological evaluation and structure–activity relationship

2.2.1. Antiproliferative activity

Newly synthesized analogues **3**–**22** were tested for antiproliferative activity against eight human tumor cell lines, A549 (lung carcinoma), HCT-8 (colon adenocarcinoma), Hep G2 (hepatocellular carcinoma), PC-3 (prostate cancer), DU 145 (prostate cancer), SK-BR-3 (HER2-overexpressing breast cancer), KB (originally isolated from epidermoid carcinoma of the nasopharynx), and KB-subline KB-VIN showing MDR phenotype with overexpression of P-gp (Table 1). The antiproliferative effects of compounds were determined by the sulforhodamine B (SRB) assay, and IC_{50} values were calculated from at least three independent experiments with duplication. The antiproliferative activities of the compounds **2**–**5** are also shown for comparison. A cytotoxic P-gp substrate, PXL, was used as an experimental control. The selected analogues were also tested with purified tubulin for inhibitory effects on its assembly and on the binding of [^{3}H]colchicine. The 50% effective concentration for inhibiting tubulin assembly (EC_{50}-ITA) and percent inhibition of colchicine binding to tubulin (ICB) in the presence of tested compounds are also presented in Table 1. Combretastatin A-4 (CA-4), a colchicine-type antitubulin agent, was used as a positive control for ITA (EC_{50} = 1.1 μM) and ICB (99% inhibition).

From the results with analogues **3** and **4**, hydroxylation at the 4′-position (**3**) of 2′-naphthyl-TEDB **2** effectively enhanced the cell growth inhibition in A549, Hep G2, KB and KB-VIN cell lines (IC_{50} 0.9, 0.4, 0.7, and 0.5 μM, respectively), while hydroxylation at the 2′-position (**4**) had less effect (IC_{50} 1.2, 1.6, 1.0, and 1.0 μM, respectively). The latter potencies were closer to those of the parent **2**.[7] Although the antiproliferative activities of **3** and **4** were slightly different, their ITA and ICB potencies using a cell free system were comparable. In contrast, the 6′-hydroxy-1′-naphthyl-TEDB analogue (**5**) exhibited marginal antiproliferative potency.

Except **22**, all tested compounds exhibited some antiproliferative activity (IC_{50} less than 20 μM) against KB-VIN, a P-gp-overexpressing MDR tumor cell line. Therefore, these analogues are not P-gp substrates and can be effective against MDR tumors. Notably, the antiproliferative activity of cinnamoyl ester **13** against KB-VIN was eightfold greater than that against KB, the parent non-MDR tumor cell line.

Esterifications of naphthols **3** and **4** successfully increased or preserved the antiproliferative effects in most cases. Especially, acetate **6** exhibited significantly improved antiproliferative activity against all tested cell lines, except DU 145 and SK-BR-3. The observed IC_{50} values of 0.2–0.5 μM were better than those of the parent **3**. Benzoate **12** and acetate **6** inhibited tumor cell growth with similar potency, but **12** was slightly less active than **6** against KB-VIN. Interestingly, benzoate **12** and propionate **7** did not inhibit tubulin assembly as opposed to **3** and acetate **6**, although all four compounds showed potent antiproliferative activity. Unlike other esters of naphthol **3**, compounds **9**–**11** displayed impressive activity against DU 145 and SK-BR-3. Among the ester analogues of **4**, acetate **15** demonstrated slightly better activity than the parent alcohol **4**. Other analogues showed similar antiproliferative activity, but benzoate **17** and cinnamate **18** did not inhibit tubulin assembly. All four ester analogues (**19**–**22**) of 6′-naphthol **5** showed no significant improvement in antiproliferative activity as compared with the parent compound.

Selected active compounds were tested for potential inhibition of tubulin assembly. Analogues **3**, **4**, **6**, and **16** inhibited tubulin assembly and modestly inhibited colchicine binding, while CA-4 caused 98% inhibition (data not shown) at the inhibitor concentration used. This observation suggested that these compounds might target tubulin in a different manner from CS-binding agents.