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Antitubulin effects of aminobenzothiophene-substituted triethylated chromones

Yukiko Kobayashi [a], Yohei Saito [a], Masuo Goto [b,*], Kyoko Nakagawa-Goto [a,b,*]

[a] School of Pharmaceutical Sciences, College of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa 920-1192, Japan
[b] UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7568, United States

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ABSTRACT

In the course of our continuing studies on the 2-(benzo[*b*]thiophene-3′-yl)-6,8,8-triethyldesmosdumotin B (TEDB-TB) series, we designed and synthesized nine amino-TEDB-TB derivatives to improve pharmaceutical properties, identify structure activity relationships, and discover novel antitubulin agents. Among all newly synthesized amino-TEDB-TBs, the 5′- and 6′-amino derivatives, **6** and **7**, exhibited significant antiproliferative activity against five human tumor cell lines, including an MDR subline overexpressing P-gp. The IC_{50} values of 0.50–1.01 μM were 3–6 times better than those of previously reported hydroxy-TEDB-TBs. Compounds **6** and **7** inhibited tubulin polymerization, induced both depolymerization of interphase microtubules and multiple spindle formations, and caused cell arrest at prometaphase. Among all compounds, compound **7** scored best pharmaceutically with LogP 2.11 and biologically with greater antiproliferative activity and induction of cell cycle arrest at prometaphase.

Desmosdumotin B (**1**, Fig. 1)[1] was characterized as a unique flavonoid with a trimethylated A-ring structure. Our previous research revealed the following interesting bioactivities: a) **1** showed selective inhibition of MDR-tumor cell growth showing high collateral sensitivity (CS),[2,3] b) a triethylated analogue, TEDB-MDR (**2**), enhanced the selectivity more than 20-times,[4–6] c) its benzothiophene analogue, TEDB-TB (**3**), dramatically changed the bioactivity profile, and also demonstrated as a potent tubulin polymerization inhibitor binding to the colchicine site.[7] d) the hydroxylated analogues of TEDB-TB (**4** and **5**) efficiently induced cell cycle arrest at the G2/M phase with formation of immature multipolar spindles.[8] Despite the potency of TEDB-TB as an antitumor agent, its low solubility prevented subsequent *in vivo* investigation. In fact, the Clog P and the topological polar surface area (tPSA) values of **3** are 5.27 and 63.6 Å,[2] respectively.[9] The insertion of an OH group into **3**, as found in **4** and **5**, led to increased polarity as well as hydrogen bond formation with specific residues on tubulin; however, the inhibitory effects of such analogues against tumor cell growth were less than that of the parent **3**. Thus, our subsequent study of TEDB-TB series described herein was performed to optimize **3** toward *in vivo* screening.

The polar amino group (NH_2) is a classic bioisostere of the hydroxy group (OH). The replacement of a hydroxy group by an amino group often results in a significant change in molecular properties, not only the lipid-aqueous solubility and pKa of the compound, but also the chemical reactivity. With Clog P and tPSA values of 4.19 and 89.6 $Å^2$, respectively, amino derivatives of TEDB-TB, such as **6** and **7** (Scheme 1), could have better drug-like properties than **3**. Furthermore, various functional groups can be attached to an amine to produce alkylamines and amides, such as **8**–**14**, which might provide additional proton acceptor for hydrogen bonding with the biological target.

The desired compounds were synthesized via our established method starting from triethylated acetophloroglucinol **15**.[4] Claisen-Schmidt condensation of **15** with aminobenzothiophene aldehydes **16** and **17**, which were protected by *t*-butoxycarbonyl (BOC), under basic conditions generated chalcones **18** and **19** in 30% and 65% yields, respectively. The treatment of 5′-aminobenzothiophenylchalcone **18** with catalytic iodine and conc. H_2SO_4 in DMSO afforded the related flavones **20** and **21**, in which the BOC group was also removed. Demethylation of **20** with BBr_3 provided the desired 5′-NH_2 derivative **6** together with NBOC derivative **10** in 70% and 25% yields, respectively. The reaction temperature and time had to be carefully controlled; otherwise, decomposition occurred. In the same manner, 6′-aminobenzothiophenylchalcone

* Corresponding authors at: School of Pharmaceutical Sciences, College of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa 920-1192, Japan (K. Nakagawa-Goto). UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7568, United States (M. Goto).
E-mail addresses: goto@med.unc.edu (M. Goto), kngoto@p.kanazawa-u.ac.jp (K. Nakagawa-Goto).

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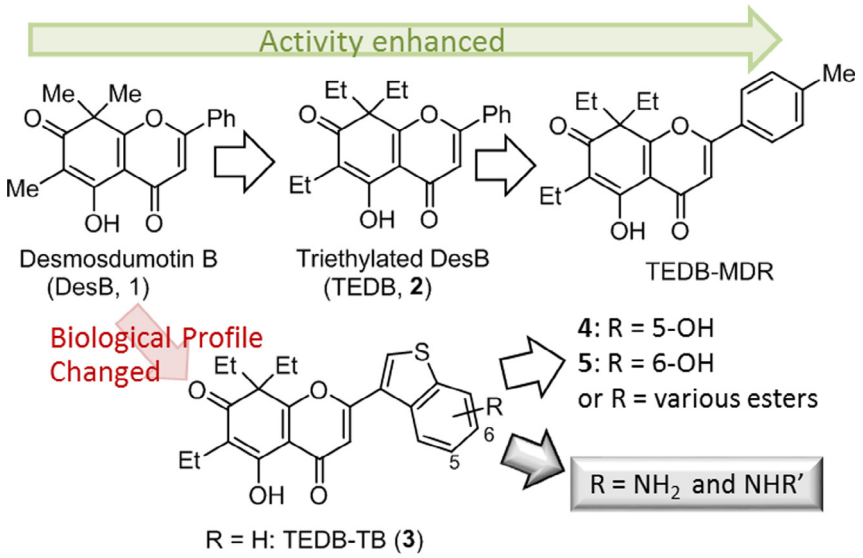
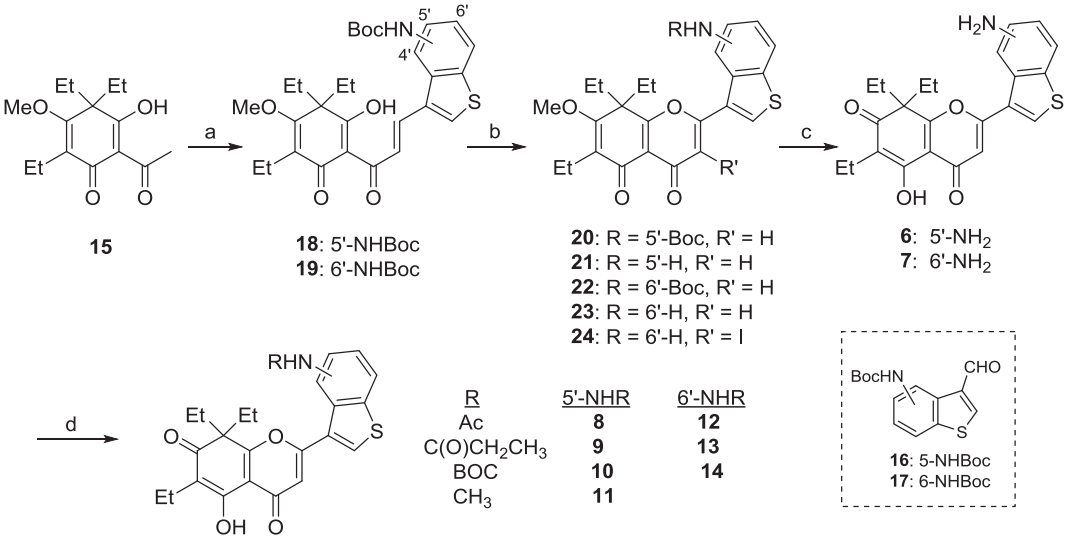


	R = H	R = OH	R = NH_2
Clog P	5.267	4.759	4.19
tPSA	63.6	83.83	89.62

Fig. 1. Summary of Desmosdumotin B Analogues.



Scheme 1. Syntheses of new TEDB-TB analogues. Reagents and conditions: (a) **16** or **17**, 50% KOH, EtOH, rt, overnight, 30% for **18**, 65% for **19**; (b) I_2, cH_2SO_4 (cat.), DMSO, 90 °C, 34% for **20**, 21% for **21**, 35% for **22**, 9% for **23**, 3% for **24**; (c) BBr_3, CH_2Cl_2, −10 °C to 0 °C, 70% for **6**, 95% for **7**; (d) Ac_2O, CH_2Cl_2, rt for **8** and **12**, 98% for **8**, 100% for **12**; propionyl chloride, $NaHCO_3$, EtOAc, H_2O, rt for **9** and **13**, 94% for **9**, 100% for **13**; Boc_2O, K_2CO_3, THF, H_2O, 40 °C for **10** and **14**, 100% for **10**, 77% for **14**; Me_2SO_4, K_2CO_3, acetone, rt for **11**, 41%.

19 was converted to the related flavones **22** and **23**. In this reaction, a trace amount of 3-iodo derivative **24** was also isolated. BOC protected flavone **22** was treated carefully with BBr_3 to obtain 6′-NH_2-TEDB-TB (**7**) in 95% yield. Amino-TEDB-TBs **6** and **7** were acylated with Ac_2O and propionated with propionyl chloride to obtain acetylamides **8** and **12**, as well as propylamides **9** and **13**, respectively. *tert*-Butyl carbamates **10** and **14** were also prepared by the treatment of **6** and **7** with Boc_2O in the presence of K_2CO_3. Furthermore, secondary amino-TEDB-TB **11** was prepared by adding a methyl group, the smallest alkyl group, to primary amino-TEDB-TB **6** to determine its biological effect.

Scheme 2 shows the preparation of 5- and 6-NHBoc benzo[*b*]thiophene aldehyde, **16** and **17**, which were obtained from 3- and 4-nitrobenzenethiol (**25**[10] and **26**[11]) by known methods. The introduction of a propynyl group to thiols **25** and **26**, followed by oxidation of the resulting **27** and **28** with hydrogen peroxide provided the related propargyl sulfoxides, **29** and **30**, respectively.[12] The tandem [2,3]/[3,3] thio-Claisen rearrangement followed by cyclization of 1-nitro-4-(prop-2-yn-1-ylsulfinyl)benzene (**30**) under acidic conditions generated (5-nitrobenzo[*b*]thiophen-3-yl) methanol (**32**). The nitro group was reduced to an amine and protected with BOC to produce **36**.[13] The cyclization of 3-nitro **29** produced **31** and **33** in 48% and 30% yields, respectively, depending on the cyclization position. (6-Nitrobenzo[*b*]thiophen-3-yl)methanol (**33**) was converted to **37** in the same manner as **32** to **36**. Alcohols **36** and **37** were oxidized with 2-iodoxybenzoic acid to obtain