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Design and synthesis of de novo cytotoxic alkaloids through mimicking taxoid skeleton

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Abstract—Based on a common pharmacophore model and the hypothesis that the baccatin core of taxoids is a scaffold securing the proper orientation of the side chains, a bicyclic alkaloid scaffold was designed as a baccatin surrogate. Using this scaffold, two novel macrocyclic and open-chain 'taxoid-mimicking' compounds were synthesized. Two of these 'taxoid-mimics', **2** and **3**, were found to possess cytotoxicity with micromolar level IC_{50} values against human breast cancer cell lines.

1. Introduction

Paclitaxel (Taxol®) and docetaxel (Taxotere®) have met considerable clinical success in cancer chemotherapy, placing them as two of the most important anticancer drugs today.[1] These drugs act by promoting the polymerization of tubulin to form microtubules and by stabilizing the microtubules, thereby disrupting the dynamics required for mitosis and eventually inducing apoptosis.[2] This mechanism of action was found to be shared by several other naturally occurring compounds such as epothilones,[3] eleutherobin,[4] and discodermolide.[5]

We have proposed a plausible common pharmacophore for these microtubule-stabilizing agents.[6] This pharmacophore suggests that the role of the baccatin core may be to serve as a rigid 'scaffold' that secures the proper orientation of the C2, C3′, and C3′N moieties. If this is indeed the case, the baccatin core could be replaced by a much simpler scaffold that retains most of its three-dimensional features, but without its structural complexity. We describe here our approaches to the design of such baccatin-free 'taxoid-mimics', their syntheses and biological evaluations.

2. Design and Synthesis

Our common pharmacophore model for taxoids, epothilones, and eleutherobin makes it possible for us to design a bicyclic surrogate of the baccatin skeleton. We searched through chemical structure databases (ILIAD, TRIAD,[7] Cambridge Structural Database) to investigate various bicyclic structures bearing two hydroxyl groups that closely mimic the distance and proper dihedral angle of the C-2 and C-13 hydroxyl groups of baccatin III. Our search then singled out the indolizidine alkaloid skeleton **1**, which bears the two crucial hydroxyl groups at ca. 5 Å distance with a dihedral angle of $-40°$ to $-50°$, values similar to those of docetaxel in its X-ray structure (4.93 Å and $-39.7°$)[8] as well as those found in the energy-minimized models of paclitaxel and other taxoids. The presence of an olefin moiety in the molecule provides an additional handle for modifications by epoxidation and hydroxylation, etc. As Figure 1 shows, the indolizidinone scaffold **1** closely mimics the geometry of the two crucial hydroxyl groups at the C2 and C13 positions of the baccatin core as demonstrated by the overlay (Fig. 1B).

Based on this scaffold, we designed two types of de novo 'taxoid-mimicking' compounds as shown in Figure 2. Compound **2** bears a 19-membered macrocycle, which might provide additional conformational constraint to mimic a bioactive conformation of taxoids. Compound **2** is mimicking a second-generation taxoid that has a

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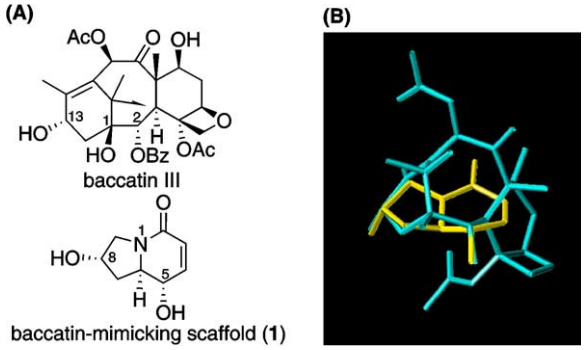


Figure 1. Indolizidinone scaffold mimicking the baccatin core. (A) Structures of the indolizidinone scaffold (**1**) and baccatin III; (B) The overlay of **1** (yellow) with baccatin III (cyan, C-2 side chain not shown for clarity).

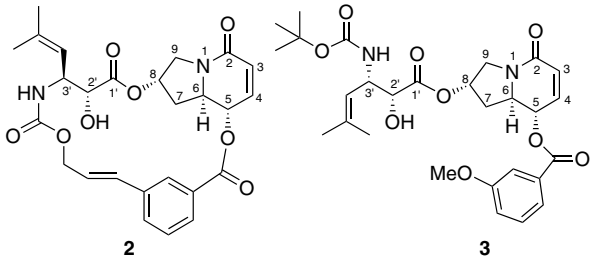
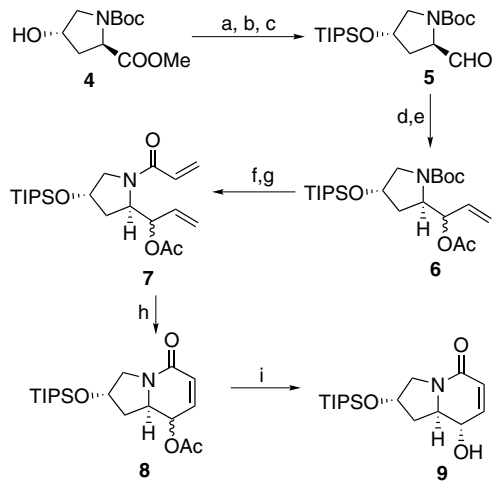


Figure 2. De novo 'taxoid-mimics'.



Scheme 1. Synthesis of indolizidine scaffold **9**. Reagents and conditions: (a) TIPSCl (2.0 equiv), imidazole (2.4 equiv), DMF overnight, 97%; (b) $LiBH_4$ (1.5 equiv), THF, 0 °C to rt, overnight, quant.; (c) SO_3–pyr. (3.0 equiv), Et_3N (7 equiv), DMSO, CH_2Cl_2, 0 °C, 1 h, 90%; (d) vinylmagnesiumchloride (1.5 equiv), THF, −78 °C, 4 h, 80%; (e) AcCl (2.0 equiv), Et_3N (4.0 equiv), DMAP, CH_2Cl_2, overnight, 85%; (f) TFA, CH_2Cl_2, 1 h, 0 °C; (g) acryloyl chloride (1.5 equiv), Et_3N (3.0 equiv), DMAP, CH_2Cl_2, overnight, 55% for two steps; (h) $Cl_2Ru{=}CHPh(PCy_3)_2$ (0.2 equiv), CH_2Cl_2, overnight, 90%; (i) K_2CO_3 (1.2 equiv) THF/H_2O, 1 h, then SiO_2 column, 75%.

(2-methylprop-1-enyl)isoserine moiety at the C13 position and a *meta*-substituted benzoate moiety at the C2 position, that is, the C5 and C8 positions of **2** correspond to the C2 and C13 positions of a taxoid, wherein these two positions are connected as part of the 19-membered macrocycle. This design also took into account the fact that the 'N-linked' macrocyclic taxoids[9] are generally more biologically active than the 'C-linked' counterparts[10] in our previous studies.

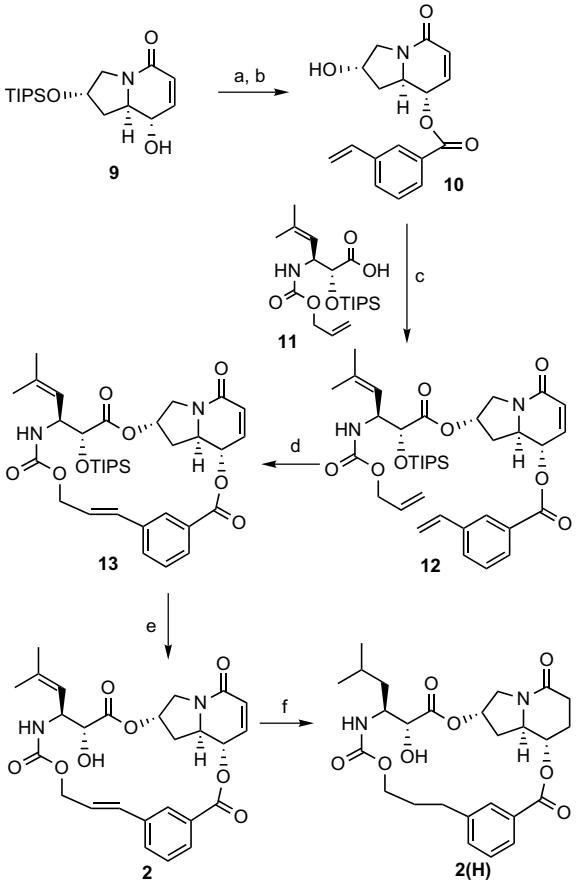
Compound **3** is a simpler mimic of a highly potent second-generation taxoid SB-T-121303,[11] that is, no macrocyclic tether is introduced. Thus, the side chains of SB-T-121303 at the C2 and C13 positions are introduced to the C5 and C8 positions of scaffold **1**. Since the unsaturated bonds in compounds **2** and **3** can be readily hydrogenated, we planned to synthesize compounds **2**(**H**) and **3**(**H**) that have saturated carbon frameworks except for the aromatic groups.

The synthesis of the indolizidinone scaffold **9** (i.e., 8-TIPS-**1**) is illustrated in Scheme 1. Methyl *N*-*t*-Boc-*trans*-hydroxy-D-prolinate (**4**)[12] was treated with TIPSCl and imidazole to protect the hydroxyl group. The subsequent reduction of the methyl ester and the oxidation of the resulting alcohol afforded aldehyde **5**. Grignard reaction of **5** using vinylmagnesium chloride followed by acetylation gave acetate **6** in a 5:1 diastereomer ratio, favoring the desired isomer. The *t*-Boc protecting group was removed by using trifluoroacetic acid (TFA) and the resulting amine salt was treated with triethylamine and acryloyl chloride to afford diene **7** in 55% yield for two steps. Finally, diene **7** was subjected to ring closing metathesis (RCM) catalyzed by $Cl_2Ru{=}CHPh(PCy_3)_2$[13] to afford indolizidinone **8** in 90% yield (a 5:1 diastereomer mixture). After removal of the acetyl group by hydrolysis, the two resulting alcohol isomers were readily separated by column chromatography on silica gel to afford the enantiopure indolizidinone scaffold **9** in 75% yield.

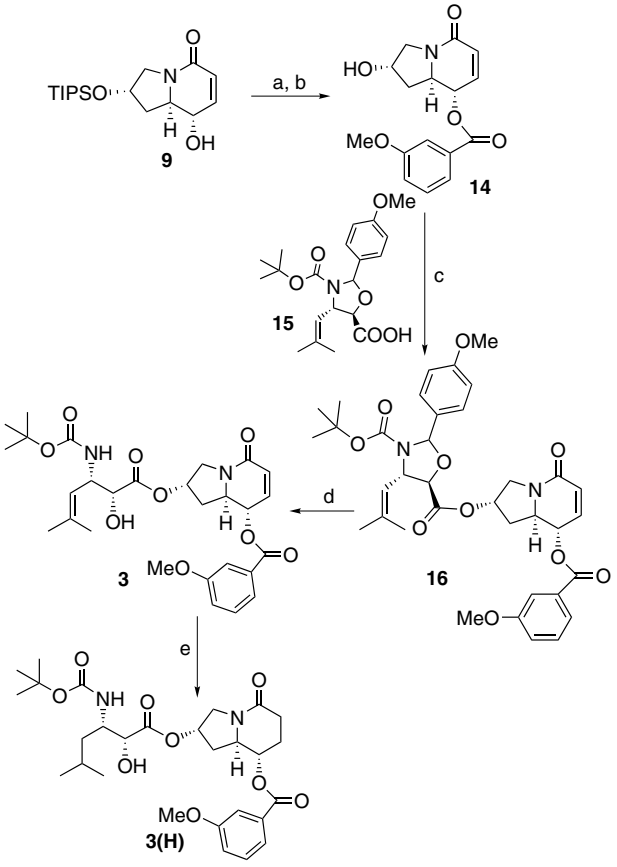
We decided to use RCM to construct the macrocycle in compound **2**. Accordingly, alcohol **9** was reacted with 3-vinylbenzoic acid in the presence of DIC and DMAP, followed by TIPS deprotection to give **10**. Hydroxyindolizidinone **10** was then coupled with *N*-alloc-*O*-TIPS-isoserine **11** (prepared from the corresponding enantiopure isoserine) using DIC and 4-pyrrolidin-1-ylpyridine[14] to afford diene **12** in 80% yield.

The RCM reaction of diene **12** catalyzed by $Cl_2Ru{=}CHPh(PCy_3)_2$ afforded macrocycle **13** in 90% yield (*E*-isomer only). Deprotection of TIPS using HF–pyridine gave macrocyclic compound **2**[15] in 90% yield. Compound **2** was then subjected to hydrogenation over Pd/C to afford compound **2**(**H**) in quantitative yield (Scheme 2).

The open-chain taxoid-mimics **3** and **3**(**H**) were synthesized using a similar protocol. Esterification of alcohol **9** with 3-methoxybenzoic acid followed by deprotection of the silyl group afforded alcohol **14** in 60% yield. Alcohol **14** was then coupled with oxazolidinecarboxylic acid **15** (prepared from the corresponding enantiopure β-lactam) using EDC hydrochloride to give the coupling product **16** in 96% yield. The removal of the aminal protecting group with *p*-toluenesulfonic acid (*p*-TSA)



Scheme 2. Syntheses of macrocyclic 'taxoid-mimics' **2** and **2(H)**. Reagents and conditions: (a) 3-vinylbenzoic acid (1.2 equiv), DIC (2 equiv), DMAP, CH_2Cl_2, 1 d, 90%; (b) HF–pyr., CH_3CN/pyr., 17 h, 80%; (c) **11** (2 equiv), DIC (2 equiv), 4-pyrrolidin-1-yl-pyridine (0.5 equiv), CH_2Cl_2, 1 d, 80%; (d) $Cl_2Ru{=}CHPh(PCy_3)_2$ (0.2 equiv), CH_2Cl_2, 2 d, 90%; (e) HF–pyr., CH_3CN/pyr., 17 h, 90%; (f) Pd/C, H_2, EtOAc, overnight, quant.



Scheme 3. Syntheses of open-chain 'taxoid-mimics' **3** and **3(H)**. Reagents and conditions: (a) 3-methoxybenzoic acid (1.2 equiv), DIC (2 equiv), DMAP, CH_2Cl_2, 1 d; (b) HF–pyr., CH_3CN, pyridine, overnight, 60% for two steps; (c) **15** (1.8 equiv), EDC·HCl (2 equiv), CH_2Cl_2, DMAP (0.5 equiv), 1 d, 96%; (d) *p*-TSA (0.2 equiv), MeOH, overnight, 86%; (e) H_2, Pd/C, overnight, 91%.

afforded compound **3**[16] in 86% yield. Hydrogenation of **3** on Pd/C gave compound **3(H)** in 91% yield (Scheme 3).

3. Biological activity

The four 'taxoid-mimics' thus synthesized were evaluated for their in vitro cytotoxicities against sensitive and resistant cancer cell lines. The results are summarized in Table 1. As Table 1 shows, compound **2** and **3** exhibit micromolar level IC_{50} values against LCC6-WT and MCF-7 human breast cancer cell lines in spite of their drastically simplified structures. The hydrogenated congeners, **2(H)** and **3(H)**, showed much lower cytotoxicities. It should be noted that these two compounds appear not to be affected by P-glycoprotein-based multidrug resistance. It is also worth mentioning that against the human breast carcinoma cell line MCF7 (wild type and MDR expressing), compound **3** was found to

Table 1. In vitro cytotoxicities (IC_{50}, μM[a]) of 'taxoid-mimics'

Taxoid	LCC6-WT[b]	LCC6-MDR[c]	MCF7-S[d]	MCF7-R[e]
Paclitaxel	0.004	0.379	0.002	1.185
Cisplatin	—	—	3.97 ± 0.49	3.53 ± 0.30
2	14 ± 1.0	>20	8.1 ± 0.22	13 ± 0.6
2(H)	>20	>20	>20	>20
3	8.9 ± 0.22	10 ± 0.56	5.7 ± 0.26	8.7 ± 0.18
3(H)	>20	>20	14 ± 3.4	>20

[a] The concentration of compound which inhibits 50% of the growth of human tumor cell line after 72 h drug exposure.
[b] LCC6-WT, human breast carcinoma.
[c] LCC6-MDR, MDR1 transduced line.
[d] MCF7-S: human breast carcinoma.
[e] MCF7-R: MDR phenotype human breast carcinoma.

possess cytotoxic activities comparable to cisplatin in the same assay.

The fact that the hydrogenated congeners **2(H)** and **3(H)** are much less active or do not show appreciable cytotoxicity may suggest that a certain rigidity of the scaffold is crucial for the biological activities. On the other hand, the result that the open-chain 'taxoid-mimic' **3** is a little more potent than the macrocyclic 'taxoid-mimic' **2** may imply an advantage of rather flexible side chains to accommodate favorable conformation(s) for bioactivity.

It was found, however, that none of the 'taxoid-mimics' showed appreciable activity in promoting the formation of microtubules in the standard in vitro tubulin polymerization assay.

Accordingly, it is very likely that these two compounds, albeit bearing taxoid side chains (macrocyclic or open-chained), derive cytotoxic activities through a different mechanism of action from that of paclitaxel and taxoids. More specifically, these results suggest that (i) the isoserine moiety at the C13 position and the benzoate moiety at the C2 position of taxoids, although crucial for achieving extremely strong cytotoxicity, have to be combined with a close mimic of the baccatin scaffold, and (ii) oversimplified baccatin core mimics might miss some of the key baccatin–tubulin interactions crucial for potent tubulin binding activity (C9 carbonyl orientation, C and D rings).

Nevertheless, compounds **2** and **3**, possessing micromolar level cytotoxicity, could certainly serve as leads for the development of de novo anticancer agents, considering the numerous functionalization possibilities in their simple structure. Further studies on the molecular target of compounds **2** and **3**, SAR, and optimization of these compounds as well as exploration of new baccatin surrogate scaffolds are actively underway in these laboratories.

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15. *Macrocycle* ***2***: A white solid, mp 148–150 °C; $[\alpha]_D^{20}$ +34.0 (*c* 0.05, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.74 (s, 3H), 1.82 (s, 3H), 2.02–2.12 (m, 1H), 2.41–2.42 (m, 1H), 3.20 (br s, 1H), 3.76–3.82 (m, 1H), 3.95–4.32 (m, 4H), 4.22 (m, 1H), 4.76 (m, 1H), 5.06 (d, $J = 9.4$ Hz, 1H), 5.28 (d, $J = 10.3$ Hz, 1H), 5.34 (d, $J = 12.0$ Hz, 1H), 5.58 (br s, 1H), 6.02 (d, $J = 8.0$ Hz, 1H), 6.63 (d, $J = 16.0$ Hz, 1H), 6.59–6.70 (m, 1H), 7.39 (m, 2H), 7.87 (d, $J = 6.7$ Hz, 1H), 8.18 (br s, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 18.4, 18.8, 25.6, 25.8, 29.7, 38.3, 39.2, 50.3, 51.2, 51.5, 57.4, 57.9, 65.1, 73.0, 73.1, 73.3, 73.7, 77.2, 122.1, 126.1, 127.9, 128.1, 128.4, 129.1, 131.3, 132.5, 136.4, 137.5, 139.6, 155.9, 162.3, 164.9, 172.8. HRMS (FAB) *m/e* calcd for $C_{26}H_{29}N_2O_8H^+$: 497.1924, found: 497.1926 ($\Delta = -0.4$ ppm).
16. *Compound* ***3***: A white solid, mp 78–80 °C; $[\alpha]_D^{20}$ −10.9 (*c* 1.8, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.23 (s, 9H), 1.71 (br s, 6H), 2.04–2.17 (m, 1H), 2.59–2.63 (m, 1H), 3.21 (d, $J = 5.7$ Hz, 1H), 3.80 (m, 2H), 3.85 (s, 3H), 4.10 (br s, 1H), 4.21 (m, 1H), 4.69–4.78 (m, 2H), 5.22 (d, $J = 7.2$ Hz, 1H), 5.48 (br s, 1H), 5.78 (d, $J = 11.4$ Hz, 1H), 6.03 (d, $J = 9.9$ Hz, 1H), 6.53 (d, $J = 9.9$ Hz, 1H), 7.13 (d, $J = 6.0$ Hz, 1H), 7.36 (t, $J = 7.8$ Hz, 1H), 7.57 (s, 1H), 7.66 (d, $J = 7.5$ Hz, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 18.6, 25.6, 28.1, 37.8, 51.1, 51.2, 55.5, 58.2, 58.5, 73.0, 73.6, 76.5, 79.4, 114.5, 117.6, 120.1, 121.3, 122.4, 125.9, 129.5, 130.3, 132.0, 137.1, 140.5, 155.1, 159.6, 162.3, 165.6, 172.3, 172.4. HRMS calcd for $C_{28}H_{36}N_2O_9Na^+$ 567.2319, found 567.2320 ($\Delta = -0.3$ ppm).