Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1543–1546

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Cytotoxicity of 2α-Amido Docetaxel Analogues

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Received 18 October 2001; accepted 8 January 2002

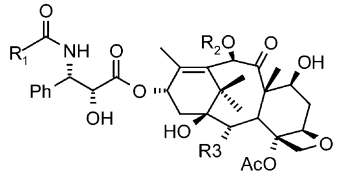
Abstract—Various 2-amido docetaxel analogues were prepared and evaluated for their cytotoxicities. Among them, *m*-methoxy and *m*-chlorobenzoylamido analogues were most active but not superior to docetaxel and paclitaxel, and D-seco analogues inactive. Change of 2-benzoate to 2-benzamide may not improve their activities to drug-resistant cell lines. © 2002 Published by Elsevier Science Ltd.

Paclitaxel (Taxol®) **1a**, a naturally occuring diterpenoid, and its semi-synthetic derivative docetaxel (Taxotere®) **1b** are now widely used in clinic for the treatment of breast, ovarian and lung cancers.[1] SAR studies hitherto indicated that C-2α acyl substitutions, which may be involved in the interaction of the taxoids with tubulin, are crucial to their antitumor activities.[2] A variety of C-2 aromoyl and alkanoyl esters have been prepared,[3] among which examples with enhancing cytotoxicities were frequently reported, such as 2-(*m*-azido)benzoyl, furfuryl and thienoyl analogues.

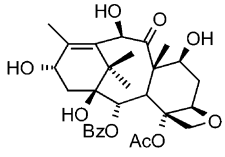
Recently, our group reported the synthesis and cytotoxicity of a 2α-benzoylamido docetaxel analogue **1c** through double inversion of C-2 configuration as a key step.[4] This compound, unlike C-13 amido paclitaxel analogues which are almost inactive, showed less or comparable cytotoxicities to paclitaxel in some tumor cell lines (KB, A549 and A2780).

To explore SAR of 2-amido analogues, a series of substituted benzoylamido analogues as well as some of their D-seco counterparts were synthesized and evaluated for their in vitro antitumor activities towards sensitive and drug-resistant tumor cell lines.

Synthetic approaches to 2α-benzoylamido-2-debenzoyloxy docetaxels included the synthesis of key intermediate 2α-azido-2-debenzoyloxy-7-TES-13-keto-baccatin III **3** which could be prepared by seven steps from 10-deacetylbaccatin III **2**.



1a R_1=Ph, R_2=Ac, R_3=OBz
1b R_1=*t*-BuO, R_2=H, R_3=OBz
1c R_1=*t*-BuO, R_2=Ac, R_3=NHBz



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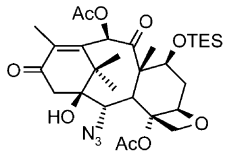

3

Having **3** at hand, many attempts were made to the preparation of 2-benzoylamido analogues. As depicted in Scheme 1, the azido group in **3** was easily reduced to amino compound **4** by catalytic hydrogenation in relatively high yield (80–85%). Benzoylation of **4** with benzoyl chloride and triethylamine gave 2-benzoylamido baccatin III analogue **5** as usual. Reduction of **5** with sodium borohydride in MeOH–THF yielded 13α product **6** as expected. The attachment of the C-13 side chain was achieved by the coupling of **6** with enantiopure β-lactam, producing 2-benzamido docetaxel analogues **7**, but the yield was rather poor (~15%). To investigate the possibility that the 2-amido group was

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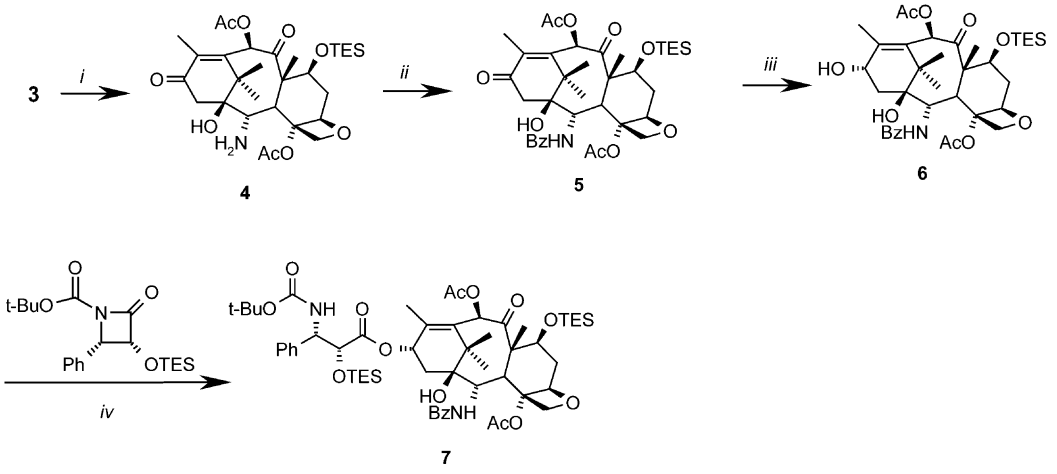
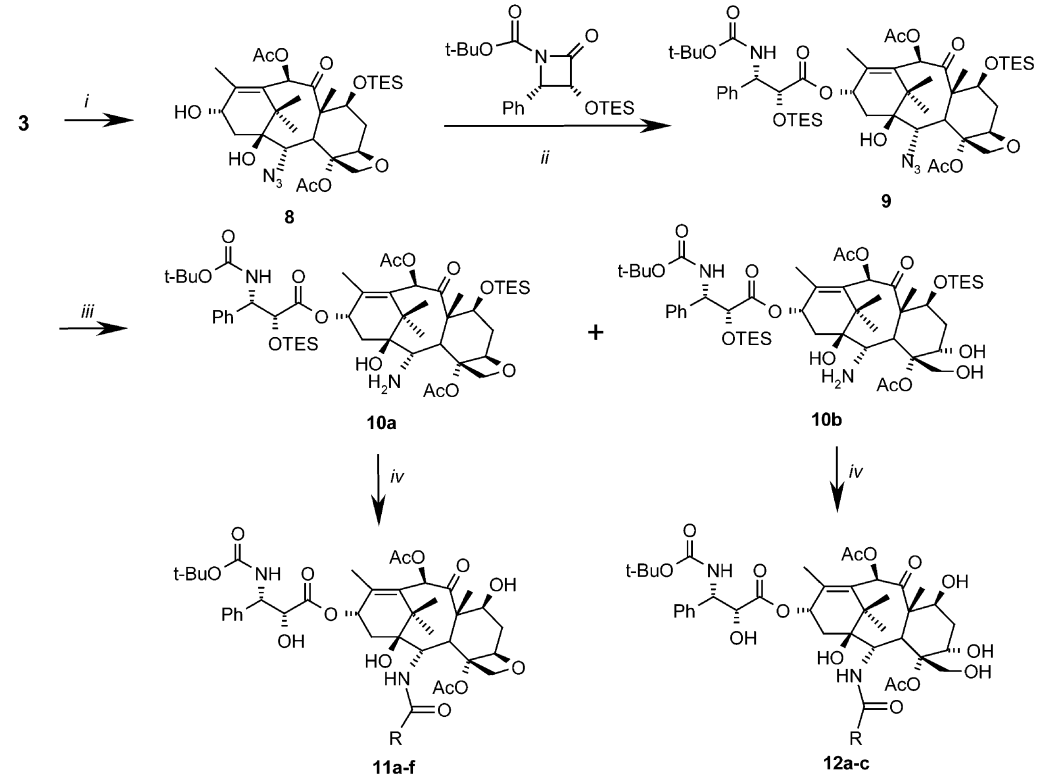
0960-894X/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0960-894X(02)00118-X

unstable in the coupling condition, protection of 2-NH in **5** with the Boc group was attempted. However, only 1-OH reacted with Boc_2O, while no 2-*N*-Boc product was detected.

Under such circumstances, another approach was applied (Scheme 2). Reduction of the 13-keto group in **3** yielded **8**, to which the β-lactam side chain was successfully attached. It was then found that the 2-azido docetaxel analogue **9**[9] was resistant to a lot of reducing agents. Reduction of the azido group with 1,3-propane dithiol[5] and triarylphosphine[6] failed to give desired amino product **10a**. Only catalytic hydrogenation with a heavy load of catalysts (20% w/w of 10% palladium on carbon or palladium hydroxide on carbon) transformed **9** to **10a** in moderate yield. D-seco byproduct **10b** was also obtained and its yield increased with prolonged reaction time. Compound **10a** was applied to the Schotten–Bauman reaction condition and subsequent desilylation furnished different amido products **11a–f**. D-seco products **12a–c** could be prepared with the same procedure as **11a–f**.



Scheme 1. Reagents and conditions: (i) H_2, 10% Pd/C, EtOAc, 85%; (ii) BzCl, Et_3N, CH_2Cl_2, 0 °C, 56%; (iii) $NaBH_4$, THF–MeOH, 0 °C, 80%; (iv) LHMDS, THF, −45→−15 °C, 15%.



Scheme 2. Reagents and conditions: (i) $NaBH_4$, THF–MeOH, −15→0 °C, 83%; (ii) LHMDS, THF, −45 °C, 75% on 80% conversion; (iii) H_2, 10% Pd/C, EtOAc–EtOH, 40% for **10a**, 24% for **10b**; (iv) RCOCl, aq $NaHCO_3$–EtOAc, rt, then 40% aq HF, py-CH_3CN, rt, 40% for **11a**, 35% for **11b**, 37% for **11c**, 48% for **11d**, 39% for **11e**, 43% for **11f**, 45% for **12a**, 39% for **12b**, 41% for **12c** (overall).

Table 1. In vitro antitumor evaluation results for compounds **11a–f** and **12a–c**

Compd	Substituents R	IC_{50} (μM)		IC_{50} (μM)		IC_{50} (μM)	
		KB	KB/VCR[a]	A549	A549/Taxol[b]	MCF-7	MCF-7/Adr[c]
1a (paclitaxel)		0.0091	1.7	0.012	0.96	0.0055	>6.0
1b (docetaxel)		0.0044	0.2	0.0041	0.098	0.0079	4.2
1c	Ph	0.15	—	0.25	—	—	—
11a	$PhCH_2O$	>0.1	>1	>0.1	>1	—[d]	—
11b[9]	*m*-MeO–Ph	0.074	1.1	0.083	1.0	0.073	>4.6
11c	*m*-Cl–Ph	0.078	>1	0.095	1.1	0.11	>4.5
11d	*p*-MeO–Ph	>0.1	>1	>0.1	>1	—	—
11e	*p*-Cl–Ph	>0.1	>1	>0.1	>1	—	—
11f	*p*-NO_2–Ph	>0.1	>1	>0.1	>1	—	—
12a	Ph	>0.1	>1	>0.1	>1	—	—
12b	*m*-Cl–Ph	>0.1	>1	>0.1	>1	—	—
12c	*p*-Cl–Ph	>0.1	>1	>0.1	>1	—	—

[a]Vincristine-resistant KB nasophyrnx cancer cell lines.
[b]Taxol-resistant A549 lung cancer cell lines.
[c]Adriamycin-resistant MCF-7 breast cancer cell lines.
[d]Not determined.

All compounds were subjected to MTT assays with sensitive tumor cell lines KB (nasopharyx) and A549 (lung), and their drug-resistant counterparts KB/VCR and A549/Taxol. Two analogues with good potencies were also evaluated on MCF (breast) and MCF-7/Adr cell lines.

As summarized in Table 1, SAR patterns for 2-amido docetaxel analogues were similar to those of paclitaxel in that oxetane D ring was crucial to the activity, and different from those of paclitaxel since *m*-methoxybenzamido analogue **11b** and *m*-chlorobenzamido analogue **11c** was equipotent to that of 2-benzamido,[4] while *m*-chlorobenzoyl and *m*-methoxybenzoyl analogues could improve potencies hundreds of times for 2-benzoate paclitaxel analogues.[3a] Extension of substitution length (for **11a**) will lead to the loss of cytotoxicity as well. Based on the preliminary molecular modeling study results (data not shown), we reasoned that the binding of these 2-amido analogues to the receptors is similar in some ways, but the amido groups may alter the microenvironment of the binding sites so that different SAR patterns were observed.

In drug-resistant cell lines (KB/VCR), only **11c** was slightly more active than paclitaxel. Change of 2-benzoate into 2-benzamide may not improve their activities to drug-resistant tumor cells. It has been reported that a subtle change of substituted groups, especially those at C-10, enhanced activities toward MDR tumor cell lines.[7] It is worthy of exploring whether 2-amido analogues with different C-10 groups will improve their potencies in drug-resistant tumors.

In order to understand why C-13 amido analogues[8] are almost inactive while C-2 amido analogues remained active, a comprehensive structure–activity relationship study of 2-nitrogen analogues and molecular modeling studies are being undertaken in our lab. The results will be reported in due time.

Acknowledgements

The author (W.-S. Fang) would like to thank financial support by funding to Outstanding Doctor Thesis Authors of Chinese Universities (grant No. 199949) from Ministry of Education of PR China.

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9. ^{1}H NMR (400 MHz) for **9**: 7.37–7.22 m (5H), 6.39 s (1H), 6.18 t (1H, J=8.7 Hz), 5.51 d (1H, J=10.2 Hz), 5.24 d (1H, J=10.2 Hz), 4.51 d (1H, J=8.1 Hz), 4.61 brs (2H), 4.42 d (1H, J=10.2, 6.9 Hz), 3.97 t (1H, J=6.0 Hz), 3.54 d (1H, J=6.9 Hz), 2.66 m (1H), 2.51 ddd (1H, J=14.1, 9.3, 6.0 Hz), 2.38 s (3H), 2.29 d (1H, J=5.4 Hz, D_2O exchanged), 2.15 s (3H), 2.15 m (1H, overlapped), 1.97 s (3H), 1.91 m (1H), 1.62 s (3H), 1.41 s (9H), 1.24 s (3H), 1.14 s (3H), 0.91 t (9H, J=8.1 Hz), 0.78 t (9H, J=8.1 Hz), 0.55 q (6H, J=8.1 Hz), 0.42 m (6H).
^{1}H NMR (300 MHz) for **11b**: 7.31–7.41 m (7H), 7.19 brd (1H, J=7.5 Hz), 7.07 dd (1H, J=7.8, 3.0 Hz), 6.29 s (1H),

6.16 brs (1H), 6.14 t (1H, $J=9.0$ Hz), 5.29 d (1H, $J=9.3$ Hz), 5.16 d (1H, $J=8.7$ Hz), 4.82 brs (1H), 4.70 d (1H, $J=9.3$ Hz), 4.63 d (1H, $J=9.3$ Hz), 4.55 brd (1H, $J=3.3$ Hz), 4.42 m (1H), 4.32 brs (1H, D_2O exchanged), 3.84 s (3H), 3.61 d (1H, $J=6.3$ Hz), 3.31 brd (1H, $J=6.0$ Hz, D_2O exchanged), 2.87 m (1H), 2.56 ddd (1H, $J=15.6$, 9.6, 6.6 Hz), 2.40 m (2H), 2.23 s (6H), 1.92 ddd (1H, $J=14.1$, 11.1, 2.4 Hz), 1.87 s (3H), 1.67 s (3H), 1.38 s (3H), 1.36 s (9H), 1.17 s (3H).