Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 3423–3428

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIPS OF NEW SECOND-GENERATION TAXOIDS

Iwao Ojima,*[a] Tao Wang,[a] Michael L. Miller,[a] Songnian Lin,[a] Christopher P. Borella,[a] Xudong Geng,[a] Paula Pera,[b] and Ralph J. Bernacki[b]

[a]*Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794–3400, U.S.A.;* [b]*Department of Experimental Therapeutics, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, U.S.A.*

Received 6 October 1999; accepted 1 November 1999

Abstract: A series of second-generation taxoids bearing a substituent on the C-2-benzoyl group and modifications at C-3'/C-10 positions was synthesized. These taxoids exhibited 2–3 orders of magnitude higher potency than that of paclitaxel against drug-resistant human breast cancer cell lines. It is also noteworthy that three taxoids showed almost no difference in activity against drug-resistant and drug-sensitive cell lines, which are categorized as "advanced second generation taxoids". © 1999 Elsevier Science Ltd. All rights reserved.

Taxol® (paclitaxel)[1] has proven to be one of the most exciting drugs for cancer chemotherapy in recent years. Paclitaxel exhibits potent antitumor activity against various cancers that have not been effectively treated by existing chemotherapeutic drugs through its unique anti-mitotic mechanism of action.[2] Paclitaxel was approved by the FDA for treatment of advanced ovarian cancer in 1992 and for treatment of breast cancer in 1994. Paclitaxel was also approved for the second-line treatment of AIDS related Kaposi's sarcoma in 1997 and is currently being tested for a series of other cancers. Docetaxel[3] was approved by the FDA for treatment of breast cancer in 1996, and is currently undergoing phase II and III clinical trials for breast and lung cancers worldwide. Although both paclitaxel and docetaxel possess potent antitumor activity, recent reports have shown that treatment with these drugs often results in various undesired side effects as well as multi-drug resistance (MDR). Therefore, it is important to develop new taxoid anticancer agents with fewer side effects, superior pharmacological properties, and improved activity against various classes of tumors, especially against drug-resistant human cancers.

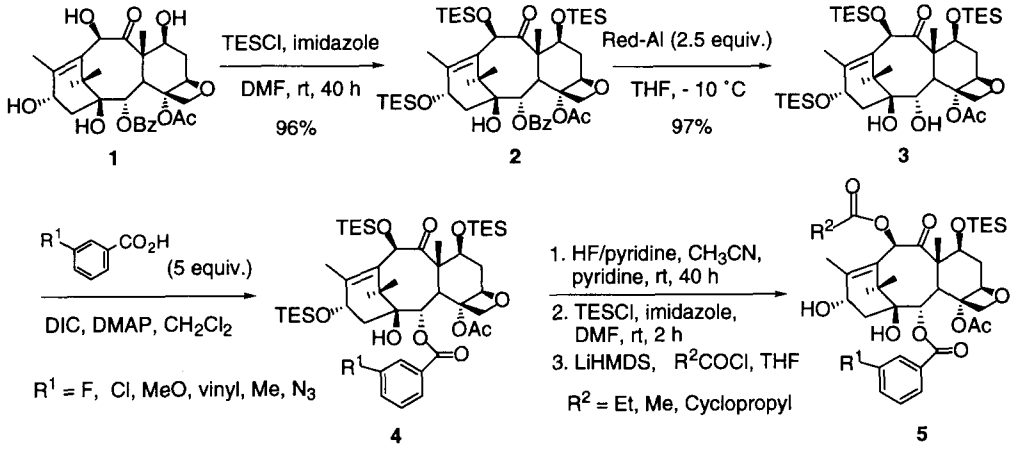
Extensive studies have been performed in different laboratories on the structure–activity relationships (SAR) of paclitaxel and their congeners, "taxoids".[3,4] As a part of our continuing SAR study of taxoids,[5,6] we have demonstrated that appropriate modification at the C-10 position and replacement of the phenyl group with an alkenyl or alkyl group at the C-3' position of taxoids provide the second generation taxoids which exhibit 1–2 orders of magnitude higher potency against drug-resistant cancer cell lines.[7,8] Kingston et al. reported that modifications at the 3-position of the C-2 benzoate with certain substituents (e.g., CN, N_3, MeO, and Cl)

PII: S0960-894X(99)00629-0

improved the anticancer activity significantly against the P-388 cell line.[9–11] We reasoned that a combination of these two modifications should provide a series of highly active antitumor taxoids. We report here the syntheses and SAR of these new second-generation taxoids.

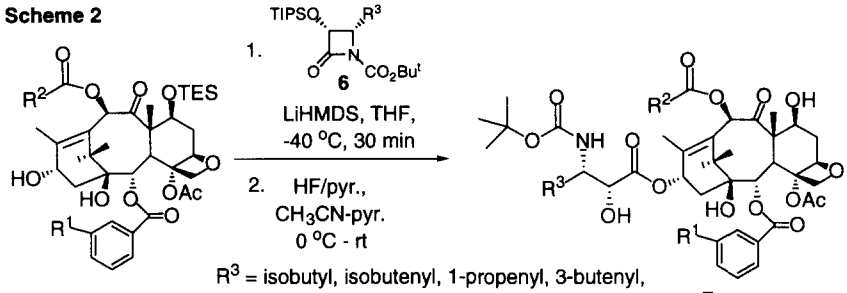
10-Deacetylbaccatin III (10-DAB, **1**) was first reacted with chlorotriethylsilane (TESCl) and imidazole in DMF to give 7,10,13-tri-TES-DAB[12] **2** in 96% yield. Removal of the 2-benzoyl group of baccatin **2** with Red-Al®[13] afforded diol **3** in 97% yield. Esterification of **3** with appropriate 3-substituted benzoic acids (R^1 = F, Cl, MeO, Me, vinyl) gave desired products **4** in 71–81% yields. Removal of all TES groups of **4** followed by selective protection of the C-7 hydroxyl group of baccatin and subsequent selective acylation at the C-10 position afforded the corresponding **5** in 71–95% yields (Scheme 1). However, the esterification of baccatin **3** with 3-azidobenzoic acid,[14,15] gave the desired product **4** ($R^1 = N_3$) as a minor product with D-ring opened compound(s) as the major product(s) that can be separated at a later stage. It is likely that the poor yield of this reaction is due to the steric hindrance of the 3-azido group. The baccatin **5** ($R^1 = N_3$, R^2 = Et) was obtained in 14% overall yield from **3**. Propanoyl, acetyl and cyclopropanecarbonyl groups were chosen as the 10-acyl substituents since these substituents showed excellent results in the SAR study of the second generation taxoids.[7,8]

Scheme 1



Enantiopure β-lactams **6** with various C-4 substituents were readily obtained through efficient chiral ester enolate-imine cyclocondensations, followed by removal of the *p*-methoxyphenyl (PMP) group and subsequent protection as their *tert*-butyl carbamates.[16] The coupling reactions of baccatin **5** with β-lactam **6** following our standard protocol[7,17] proceeded smoothly to give the corresponding new taxoids **7a–q** (see Table 1 for structures) after deprotection of the silyl groups (Scheme 2).

Scheme 2



Yield of **7** from **5** (two steps): **a:** 89%; **b:** 84%; **c:** 80%; **d:** 70%; **e:** 69%; **f:** 60%; **g:** 60%; **h:** 82%; **i:** 74%; **j:** 71%; **k:** 65%; **l:** 56%; **m:** 65%; **n:** 70%; **o:** 71%; **p:** 51%; **q:** 65%.

Cytotoxicity assay of the new second generation taxoids was performed on the human breast cancer cell lines, LCC6-WT and LCC-MDR, and selected taxoids were also assayed for their potency against MCF7 and MCF7-R.[18] As Table 1 shows, these new taxoids exhibit two to three times higher potency against drug-sensitive cancer cell lines (LCC6-WT, MCF7) as compared to that of paclitaxel. Moreover, these taxoids exhibit *2–3 orders of magnitude higher potency* against drug-resistant cancer cell lines (LCC6-MDR, MCF7-R) as compared to paclitaxel and docetaxel. The most characteristic feature of these new second generation taxoids is the very small difference in the IC_{50} values between drug-sensitive and drug-resistant cell lines (R/S = 0.89–7.0 except for **7g** and **7i** against MCF7 and MCF7–R). In some cases (**7e** and **7f**), the taxoids show even slightly better activity against the drug–resistant cell lines than that against the drug–sensitive cell line although it could be within experimental errors. This makes a sharp contrast with paclitaxel and docetaxel which show 2 orders of magnitude difference (R/S = 112–235) between the drug-sensitive and drug-resistant cell lines.

As for the SAR of these taxoids on the 3(*meta*)-position of the C-2 benzoate, the cytotoxicity decreases in the order: F > Cl > MeO > N_3 > Me> $CH{=}CH_2$ against drug-sensitive cell line LCC6-WT, while the order is MeO > N_3, F, Cl > Me > $CH{=}CH_2$ against drug-resistant cell line LCC6-MDR. A similar trend is observed against MCF7 and MCF7-R. Although it is known that 3-azidobenzoyl analog possesses uniquely the highest potency in the SAR of paclitaxel,[9,10] 3-methoxybenzoyl taxoids in these new second-generation taxoids are more potent than 3-azidobenzoyl taxoids, especially against MCF7-R (compare **7e**, **7f** vs **7n**, **7o**). As observed earlier for the first series of the second generation taxoids,[7] introduction of an*n*-propanoyl group at the C-10 position is effective against drug-resistant cell lines (**7f** vs **7l**, **7m**) . In contrast to the previous observation that 3'-(2-prop-1-enyl) taxoids generally show better activity than 3'-(2-propyl) taxoids,[7] 3'-(2-propyl) taxoids (**7a**, **7c**, **7e** and **7n**) and 3'-(2-prop-1-enyl) taxoids (**7b**, **7d**, **7f**, and **7o**) in the new series possess essentially the same potency.

Table 1. Cytotoxicity of taxoids **7a–q** (IC_{50} nM)[a]

Taxoid	R^1	R^2	R^3	LCC6-WT[b]	LCC6-MDR[c]	R/S[d]	MCF7[e]	MCF7-R[f]	R/S[d]
Paclitaxel	–	–	–	3.1	346	112	1.7	300	177
Docetaxel	–	–	–	1.0	120	120	1.0	235	235
7a	F	Et	$CH_2CH(CH_3)_2$	0.4	2.4	6.0			
7b	F	Et	$CH{=}C(CH_3)_2$	0.5	2.1	4.2			
7c	Cl	Et	$CH_2CH(CH_3)_2$	0.8	2.9	3.6			
7d	Cl	Et	$CH{=}C(CH_3)_2$	0.8	1.3	1.6			
7e	MeO	Et	$CH_2CH(CH_3)_2$	**0.9**	**0.8**	**0.89**	**0.36**	**0.43**	**1.19**
7f	MeO	Et	$CH{=}C(CH_3)_2$	**1.0**	**0.9**	**0.90**	**0.36**	**0.33**	**0.92**
7g	MeO	Et	CF_2H	1.5	4.7	3.1	0.4	4.3	10.8
7h	MeO	Et	$CH_2CH_2CH{=}CH_2$	0.9	5.4	6.0	2.0	7.7	3.9
7i	MeO	Et	$CH_2CH{=}CH_2$	1.2	8.4	7.0	0.8	8.7	10.9
7j	MeO	Et	(*S*)-2,2-dimethyl-cyclopropyl	0.48	1.1	2.3	0.6	1.5	2.5
7k	MeO	Et	(*E*)-$CH{=}CHCH_3$	1.2	4.1	3.4			
7l	MeO	Me	$CH{=}C(CH_3)_2$	0.6	2.7	4.5	0.8	2.3	2.9
7m	MeO	*c*-Pr	$CH{=}C(CH_3)_2$	1.0	2.9	2.9			
7n	N_3	Et	$CH_2CH(CH_3)_2$	1.1	2.4	2.2	1.0	2.1	2.1
7o	N_3	Et	$CH{=}C(CH_3)_2$	**0.9**	**1.2**	**1.3**	**0.9**	**1.1**	**1.2**
7p	$CH{=}CH_2$	Et	$CH{=}C(CH_3)_2$	2.9	7.1	2.4			
7q	Me	Me	$CH{=}C(CH_3)_2$	1.5	5.8	3.9	0.8	5.0	6.3

[a]The concentration of compound which inhibits 50% (IC_{50}, nM) of the growth of human tumor cell line after 72 h drug exposure.[18] [b]LCC6-WT: human breast carcinoma. [c]LCC6-MDR: MDR1 transduced line. [d]Ratio of activities, drug-resistant (R) vs. drug-sensitive (S) cell lines. [e]MCF7: human breast carcinoma. [f]MCF7-R: multi-drug resistant human breast carcinoma.

These results confirm that the cytotoxicity of taxoids is highly sensitive to the structure of substituents at the C-3', C-10 and the C-2 benzoate positions. It is noteworthy that three of the newly developed second-generation taxoids, **7e**,[19] **7f**,[20] and **7o**,[21] show virtually no difference in activity against drug-resistant and drug-sensitive cell lines. These taxoids can be categorized as "advanced second generation taxoids", which are highly promising candidates for cancer chemotherapy.

Acknowledgment: This work has been supported by a grant from the National Institutes of Health (NIGMS). Generous support from Indena SpA, is gratefully acknowledged.

References and Notes

1. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325–2327.
2. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665–667.
3. Guénard, D.; Guéritte-Vogelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160–167.
4. Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. In *Taxol®: Science and Applications*; M. Suffness, Ed.; CRC Press: New York, 1995; pp 317–375.
5. Ojima, I.; Lin, S.; Wang, T. *Curr. Med. Chem.* **1999**, *6*, 927–954.
6. Ojima, I.; Kuduk, S. D.; Chakravarty, S. In *Adv. Med. Chem.*; B. E. Maryanoff and A. B. Reitz, Ed.; JAI Press: Greenwich, CT, 1998; Vol. 4; pp 69–124.
7. Ojima, I.; Slater, J. C.; Michaud, E.; Kuduk, S. D.; Bounaud, P.-Y.; Vrignaud, P.; Bissery, M.-C.; Veith, J.; Pera, P.; Bernacki, R. J. *J. Med. Chem.* **1996**, *39*, 3889–3896.
8. Ojima, I.; Lin, S. *J. Org. Chem.* **1998**, *63*, 224–225.
9. Kingston, D. G. I. In *Taxane Anticancer Agents: Basic Science and Current Status; ACS Symp. Ser. 583*; G. I. Georg; T. T. Chen; I. Ojima and D. M. Vyas, Ed.; American Chemical Society: Washington, D. C., 1995; pp 203–216.
10. Chaudhary, A. G.; Gharpure, M. M.; Rimoldi, J. M.; Chordia, M. D.; Gunatilaka, A. A. L.; Kingston, D. G. I.; Grover, S.; Lin, C. M.; Hamel, E. *J. Am. Chem. Soc.* **1994**, *116*, 4097–4098.
11. Kingston, D. G. I.; Chaudhary, A. G.; Chordia, M. D.; Gharpure, M.; Gunatilaka, A. A. L.; Higgs, P. I.; Rimoldi, J. M.; Samala, L.; Jagtap, P. G.; Giannakakou, P.; Jiang, Y. Q.; Lin, C. M.; Hamel, E.; Long, B. H.; Fairchild, C. R.; Johnston, K. A. *J. Med. Chem.* **1998**, *41*, 3715–3726.
12. Marder-Karsenti, R.; Dubois, J.; Bricard, L.; Guénard, D.; Guéritte-Voegelein, F. *J. Org. Chem.* **1997**, *62*, 6631–6637.
13. Chen, S.-H.; Farina, V.; Wei, J.-M.; Long, B.; Fairchild, C.; Mamber, S. W.; Kadow, J. F.; Vyas, D.; Doyle, T. W. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 479–482.
14. Carnazzi, E.; Aumelas, A.; Barberis, C.; Guillon, G.; Seyer, R. *J. Med. Chem.* **1994**, *37*, 1841–1849.
15. Georg, G. I.; Boge, T. C.; Park, H.; Himes, R. H. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 615–620.
16. Ojima, I.; Zucco, M.; Duclos, O.; Kuduk, S. D.; Sun, C.-M.; Park, Y. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2479–2482.
17. Ojima, I.; Sun, C. M.; Zucco, M.; Park, Y. H.; Duclos, O.; Kuduk, S. D. *Tetrahedron Lett.* **1993**, *34*, 4149–4152.
18. Skehan, P.; Streng, R.; Scudierok, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1107–1112.

19. White solid; mp 132–134 °C; $[\alpha]^{20}_D$ -114.3 (*c* 0.07, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.95 (m, 6H), 1.13 (s, 3H), 1.28 (m, 8H), 1.66 (m, 6H), 1.88 (s, 3H), 2.37 (m, 6H), 2.52 (m, 4H), 3.21 (bs, 1H), 3.80 (d, *J* = 6.9 Hz, 1H), 3.86 (s, 3H), 4.12 (m, 2H), 4.30 (d, *J* = 8.4 Hz, 1H), 4.40 (dd, *J* = 10.6, 6.8 Hz, 1H), 4.57 (d, *J* = 9.6 Hz, 1H), 4.96 (d, *J* = 8.1 Hz, 1H), 5.63 (d, *J* = 7.0 Hz, 1H), 6.16 (t, *J* = 8.4 Hz, 1H), 6.30 (s, 1H), 7.13 (d, *J* = 7.9 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.62 (s, 1H), 7.68 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 9.0, 9.6, 14.9, 21.8, 22.5, 23.3, 24.7, 26.6, 27.6, 28.1, 35.5, 41.2, 43.2, 45.6, 51.2, 55.3, 58.5, 72.2, 72.6, 73.0, 75.4, 75.5, 76.2, 79.1, 79.7, 81.1, 84.4, 114.1, 120.4, 122.7, 129.6, 130.4, 132.9, 142.5, 155.4, 159.6, 166.8, 169.9, 174.0, 174.6, 203.8. HRMS (FAB): *m/e* calcd for $C_{45}H_{63}O_{16}N{\cdot}H^+$: 874.4225. Found: 874.4224 (Δ = 0.1 ppm).

20. White solid; mp 130–132 °C; $[\alpha]^{20}_D$ -75.0 (*c* 0.08, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (s, 3H), 1.28 (m, 8H), 1.33 (s, 9 H), 1.66 (m, 3H), 1.73 (s, 3H), 1.75 (s, 3H), 1.89 (m, 5H), 2.37 (m, 6H), 2.52 (m, 3H), 3.80 (d, *J* = 6.9 Hz, 1H), 3.86 (s, 3H), 4.12 (m, 2H), 4.32 (d, *J* = 8.5 Hz, 1H), 4.40 (dd, *J* = 10.6, 6.8 Hz, 1H), 4.72 (m, 2H), 4.96 (d, *J* = 8.3 Hz, 1H), 5.30 (d, *J* = 7.6 Hz, 1H), 5.64 (d, *J* = 7.0 Hz, 1H), 6.16 (t, *J* = 8.6 Hz, 1H), 6.30 (s, 1H), 7.13 (d, *J* = 7.9 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.62 (s, 1H), 7.68 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 9.0, 9.5, 14.9, 18.5, 21.8, 22.4, 25.7, 26.6, 27.5, 28.2, 35.5, 43.2, 45.6, 51.5, 55.3, 58.5, 72.2, 72.3, 73.7, 75.1, 75.4, 76.2, 79.1, 79.9, 81.1, 84.4, 114.6, 120.1, 120.6, 122.5, 129.6, 130.4, 132.9, 137.8, 142.5, 155.4, 159.6, 166.8, 170.0, 174.0, 174.6, 203.8. HRMS (FAB): *m/e* calcd for $C_{45}H_{61}O_{16}N{\cdot}H^+$: 872.4069. Found: 872.4072 (Δ = - 0.4 ppm).

21. White solid: mp 128–130 °C; $[\alpha]^{20}_D$ - 70.5 (*c* 0.44, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.14 (s, 3H), 1.25 (m, 9H), 1.34 (s, 9H), 1.66–1.73 (m, 12H), 1.89 (s, 3H), 2.37 (m, 5H), 2.52 (m, 4H), 3.32 (bs, 1H), 3.81 (d, *J* = 6.9 Hz, 1H), 4.12 (m, 2H), 4.30 (d, *J* = 8.1 Hz, 1H), 4.40 (dd, *J* = 10.6, 6.8 Hz, 1H), 4.74 (m, 2H), 4.96 (d, *J* = 8.2 Hz, 1H), 5.29 (m, 1H), 5.64 (d, *J* = 7.0 Hz, 1H), 6.13 (t, *J* = 9.0 Hz, 1H), 6.31 (s, 1H), 7.23 (d, *J* = 7.5 Hz, 1H), 7.46 (t, *J* = 7.9 Hz, 1H), 7.78 (s, 1H), 7.86 (d, *J* = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 9.0, 9.5, 15.0, 18.6, 21.8, 22.5, 25.7, 26.6, 27.6, 28.2, 35.5, 43.2, 45.6, 51.6, 58.6, 72.2, 72.6, 73.7, 75.4, 76.4, 79.2, 81.0, 84.5, 87.3, 120.1, 120.5, 124.3, 126.8, 130.2, 132.7, 140.8, 166.0, 170.1, 174.6, 203.8. HRMS (FAB): *m/e* calcd for $C_{44}H_{58}O_{15}N_4{\cdot}H^+$: 883.3977. Found: 883.3987 (Δ = - 1.1 ppm).