Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 974–977

Syntheses and evaluation of novel fatty acid-second-generation taxoid conjugates as promising anticancer agents

Larissa Kuznetsova,[a] Jin Chen,[a] Liang Sun,[a] Xinyuan Wu,[a] Antonella Pepe,[a] Jean M. Veith,[b] Paula Pera,[b] Ralph J. Bernacki[b] and Iwao Ojima[a,*]

[a]*Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400, USA*
[b]*Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA*

Received 28 September 2005; revised 24 October 2005; accepted 26 October 2005
Available online 18 November 2005

Abstract—Polyunsaturated fatty acids such as docosahexaenoic acid (DHA), linolenic acid, and linoleic acid were linked to the C-2′ position of the second-generation taxoids that could overcome MDR caused by overexpressed ABC transporters. The new conjugates, tested in vivo, exhibited strong activity against drug-resistant colon cancer and drug-sensitive ovarian cancer xenografts in mice. Two of the new conjugates, DHA–SB-T-1214 and DHA–SB-T-1213, were found to achieve the total regression of drug-resistant and drug-sensitive tumors, respectively, in the animal models with substantially reduced systemic toxicity.

It is well known that the lack of tumor-specificity in conventional chemotherapeutic drugs treating cancer patients is a serious drawback, causing undesirable side effects. In order to solve this problem, considerable efforts have been made to find unique properties in tumor biochemistry that can be exploited for drugs to specifically target tumor cells. A promising approach along this line is the development of 'tumor-targeting prodrugs', based on a conjugate of a cytotoxic drug to a tumor-specific molecule. The basic premise of the 'tumor-targeting prodrugs' is that the drug is inactive until it is delivered to the target tumor cells by the tumor-specific molecule. At the target tumor cell the drug is internalized and released from the carrier to restore its original activity. 'Tumor-targeting prodrugs' can be classified into several groups based on the type of tumor-specific molecules, such as monoclonal antibodies (mAb), folic acids, hyaluronic acids (HA), and oligopeptides.[1,2] Some of these conjugates received increasing attention due to promising results in preclinical studies.[3–10] However, only limited successes have been achieved to date in clinical trials due to the inherent pitfalls of the tumor-specific molecules and other reasons.[11–13]

Polyunsaturated fatty acids (PUFAs) are ideal candidates as tumor-specific molecules. Representative naturally occurring PUFAs possess 18, 20, and 22 carbons, and 2–6 unconjugated *cis*-double bonds separated by one methylene, such as linolenic acid (LNA), linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).[14–19] These PUFAs are included in vegetable oils, cold-water fish, and meat. DHA is classified as a nutritional additive by the FDA in the US. Thus, DHA and its metabolites are considered to be safe to humans.[17,20,21] Perfusion studies of tissue isolated hepatomas with a single arterial inflow and a single venous outflow demonstrated that some PUFAs are taken up more rapidly by tumor cells than by normal cells, presumably for use as biochemical precursors and energy sources.[22–25]

Bradley et al.[26] applied this strategy and developed DHA–paclitaxel (Taxoprexin®) linking DHA to the C-2′ position of paclitaxel. Compared with paclitaxel, DHA–paclitaxel is stable in plasma and high concentrations are maintained in animal plasma for a long time, slowly releasing paclitaxel.[26] When tested at the optimum dose (20 mg/kg), paclitaxel caused neither complete nor partial regression in any of 10 mice in a Madison 109 sc lung tumor model, while DHA–paclitaxel achieved complete regression in 4 of 10 mice at 60 mg/kg.[26]

Keywords: Chemotherapy; Cancer; Anticancer agent; Fatty acid; Taxoid; Taxane; Conjugate; Tumor targeting.
* Corresponding author. Tel.: +1 631 632 7947; fax: +1 631 632 7942; e-mail: iojima@notes.cc.sunysb.edu

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.10.089

One of the drawbacks of paclitaxel is its lack of efficacy against drug-resistant tumors such as colon, pancreatic, melanoma, and renal cancers. Those tumor cells overexpress P-glycoprotein (Pgp), an ATP-binding cassette (ABC) transporter, effluxing out hydrophobic anticancer agents including paclitaxel and docetaxel. Although DHA–paclitaxel does not seem to be a good substrate for Pgp, paclitaxel, even when released slowly, will be caught by the efflux pump and eliminated from the cancer cells. In contrast, the second-generation taxoids developed in our laboratories exhibit 2–3 orders of magnitude higher cytotoxicity than paclitaxel against drug-resistant cancer cells expressing MDR phenotypes.[27] We hypothesized that the PUFA conjugates of the second-generation taxoids would be efficacious against drug-resistant tumors for which DHA–paclitaxel is not effective. Thus, in this study, the conjugates of DHA, LNA, and LA with the second-generation taxoids were synthesized and their efficacy assayed in vivo against ovarian and colon tumor xenografts.

Among the second-generation taxoids, SB-T-1213, SB-T-1214, SB-T-1216, and SB-T-1217 exhibit highly potent cytotoxicity (Table 1), 2 orders of magnitude better than paclitaxel and docetaxel against resistant breast cancer cells overexpressing the Pgp efflux pump. Those taxoids as well as SB-T-1103 and SB-T-1104 were selected for conjugation to PUFAs.

The synthesis of PUFA conjugates of the second-generation taxoids is straightforward. A free taxoid is coupled to a PUFA at the C-2′ hydroxyl group (Scheme 1) in the presence of DIC and DMAP, to afford the corresponding conjugates. Eight second-generation taxoids were synthesized following protocols previously published[27–29] starting from 10-deacetylbaccatin III (DAB) and conjugated to fatty acids. As shown in Table 2, the second-generation taxoids bear different substituents at the C-2, C-10, and C-3′ positions.

The PUFA–taxoid conjugates thus obtained were assayed for their efficacy against a drug-sensitive human ovarian tumor xenograft (Pgp−) A121 and a drug-resistant human colon tumor xenograft (Pgp+) DLD-1 in SCID mice (Tables 3 and 4).

As we anticipated, paclitaxel and DHA–paclitaxel were totally ineffective against the drug-resistant (Pgp+) DLD-1 tumor xenograft (Fig. 1). In contrast, DHA–SB-T-1214 achieved complete regression of the DLD-1 tumor in five of five mice at 80 mg/kg dose administered on days 5, 8, and 11 (total dose 240 mg/kg; tumor growth delay >187 days). This is a very promising result which promotes this compound as a lead candidate for further preclinical studies.

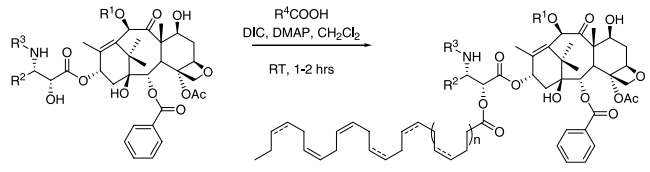
In the case of the drug-sensitive tumor A121 xenograft, the efficacy of DHA–paclitaxel reported by Bradley et al.[26] was confirmed by our results. DHA–paclitaxel

Table 1. Cytotoxicity of selected second-generation taxoids against human breast carcinoma cell lines[a]

Taxane	IC_{50} (nM)[b]	
	MCF7-S	MCF7-R
Paclitaxel	1.7	550
Docetaxel	1.0	723
SB-T-1103	0.35	5.1
SB-T-1104	0.51	7.9
SB-T-1213	0.18	4.0
SB-T-1214	0.20	3.9
SB-T-1216	0.13	7.4
SB-T-1217	0.14	9.7

[a] Human mammary tumor cell lines MCF7-S (Pgp−), MCF7-R (Pgp+), MDA-435-LCC6-WT (Pgp−), MDA-435-LCC6-MDR (mdr1 transfected line).

[b] Concentration of drug which inhibits cell growth by 50% (72 h continuous exposure).



Scheme 1. Synthesis of PUFA–taxoid conjugates.

Table 2. PUFA–taxoid conjugates

Conjugate	R^1	R^2	R^3	R^4
DHA–paclitaxel	Ac	C_6H_5	C_6H_5	$C_{21}H_{31}$
DHA–docetaxel	OH	C_6H_5	*t*-Boc	$C_{21}H_{31}$
DHA–SB-T-1213	EtCO	Isobutenyl	*t*-Boc	$C_{21}H_{31}$
DHA–SB-T-1103	EtCO	Isobutyl	*t*-Boc	$C_{21}H_{31}$
DHA–SB-T-1214	*c*-PrCO	Isobutenyl	*t*-Boc	$C_{21}H_{31}$
DHA–SB-T-1104	*c*-PrCO	Isobutyl	*t*-Boc	$C_{21}H_{31}$
DHA–SB-T-1216	Me_2NCO	Isobutenyl	*t*-Boc	$C_{21}H_{31}$
DHA–SB-T-1217	MeOCO	Isobutenyl	*t*-Boc	$C_{21}H_{31}$
LA–SB-T-1213	EtCO	Isobutenyl	*t*-Boc	$C_{17}H_{31}$
LNA–SB-T-1213	EtCO	Isobutenyl	*t*-Boc	$C_{17}H_{29}$

Table 3. Antitumor effect of DHA–taxoid conjugates delivered iv to SCID mice bearing a Pgp+ human colon tumor xenograft, DLD-1

Treatment[a] (iv)	Total dose (mg/kg)	Growth delay (days)	Toxicity[b]	Cured mice[c] / group
Control	0	—	0	0/7
Vehicle-Crem	0	—	0	0/3
Vehicle-Tween	0	—	0	0/3
Paclitaxel	60	8	0	0/3
DHA–paclitaxel	240	4	0	0/5
DHA–SB-T-1213	75	54	0	0/5
DHA–SB-T-1103	75	4	0	0/5
DHA–SB-T-1214	240	>187	0	5/5
DHA–SB-T-1104	240	4	0	0/5
DHA–docetaxel	75	17	0	0/4
DHA–docetaxel	150	34	0	0/4

[a] Treatment given iv to SCID mice on days 5, 8, and 11 tumor implant, paclitaxel and DHA–paclitaxel formulated in Cremophor/EtOH; DHA–taxoid conjugates formulated in Tween/EtOH.

[b] Number of animals that either died or lost greater than 20% body weight.

[c] SCID mice with tumors less than 600 mm^3 after 201 days.

Table 4. Antitumor effect of DHA–taxoid conjugates delivered iv to SCID mice bearing a human ovarian tumor xenograft, A121

Treatment[a] (iv)	Total dose (mg/kg)	Growth delay (days)	Toxicity[b]	Cured mice[c]/ group
Control	0	—	0	0/10
Vehicle-Crem	0	3	0	0/5
Vehicle-Tween	0	3	0	0/5
Paclitaxel	60	83	0	0/5
DHA–paclitaxel	240	>186	0	2/5
DHA–SB-T-1216	90	>186	4	1/5
DHA–SB-T-1213	90	>186	1	4/5
DHA–SB-T-1104	240	115	0	0/5

[a] Treatment given iv to SCID mice on days 5, 8, and 11 after tumor implant. Paclitaxel and DHA–paclitaxel formulated in Cremophor/EtOH; DHA–taxoid conjugates formulated in Tween/EtOH.
[b] Number of animals that either died or lost greater than 20% body weight.
[c] SCID mice with no palpable tumor after 197 days.

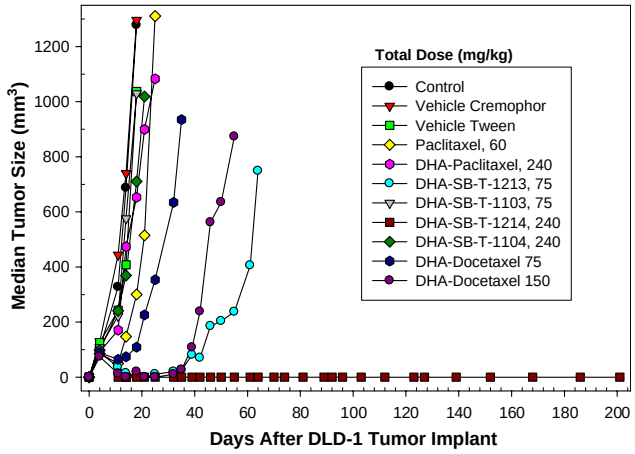


Figure 1. Effect of DHA–taxoid conjugates on human colon tumor xenograft (Pgp+) DLD-1.

(80 mg/kg × 3 injections, total dose 240 mg/kg) exhibited greater than a twofold increase in tumor growth delay as compared with paclitaxel. One of the new DHA–taxoids exhibited even better activity, i.e., DHA–SB-T-1213 (30 mg/kg × 3) delayed the tumor growth for more than 186 days and caused complete regression of tumor in all surviving mice (four of five) even at the non-optimized dose (Fig. 2). DHA–SB-T-1213 and DHA–SB-T-1216 delayed the growth of the tumor xenograft for >186 days. DHA–paclitaxel also brought about a cure to two of five mice, but the tumor recurred after 150 days in three of five mice. (*Note*. The evaluation of DHA–SB-T-1214 for its efficacy against A121 and several tumor xenografts other than DLD-1 will be performed shortly and reported elsewhere.)

The impressive results obtained with DHA–taxoids prompted us to investigate the use of different PUFAs and their efficacy. The results are shown in Table 5 and Figure 3. We synthesized the conjugates of SB-T-1213 with DHA, LNA, and LA, and examined their efficacy against DLD-1 colon tumor xenograft (Pgp+). As Table 5 and Figure 3 show, LA–SB-T-1213 and

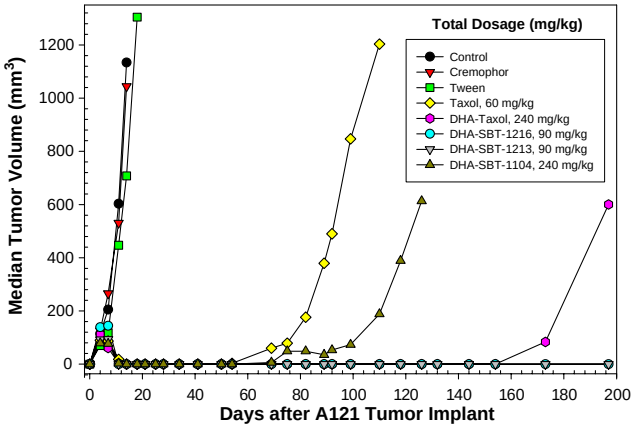


Figure 2. Effect of DHA–taxoid conjugates on human ovarian tumor xenograft (Pgp−) A121.

Table 5. Antitumor effect of PUFA–taxoid conjugates delivered iv to SCID mice bearing a Pgp+ human colon tumor xenograft, DLD-1

Treatment[a] (iv)	Total dose (mg/kg)	Growth delay (days)	Toxicity[b]	Cured mice[c]/ group
Control	0	—	0	0/7
Vehicle-Crem	0	—	0	0/4
Vehicle-Tween	0	—	0	0/4
Paclitaxel	75	9	0	1/5
DHA–SB-T-1213	75	54	0	0/5
LNA–SB-T-1213	75	>109	2	2/5
LA–SB-T-1213	75	21	1	0/5

[a] Treatment given iv to SCID mice on days 5, 8, and 11 after DLD-1 human colon tumor implant. Paclitaxel formulated in Cremophor/EtOH; DHA–taxoid conjugate, LNA–taxoid conjugate, and LA–taxoid conjugate formulated in Tween/EtOH.
[b] Number of animals that either died or lost greater than 20% body weight.
[c] SCID mice with no palpable tumor on day 120, end of experiment.

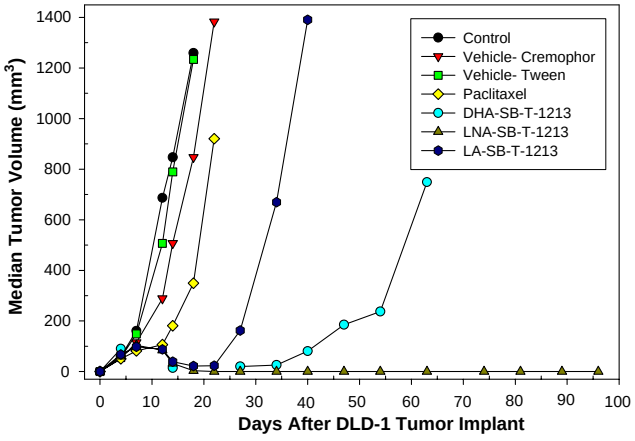


Figure 3. Antitumor effect of PUFA–taxoid conjugates delivered iv to SCID mice bearing a Pgp+ human colon tumor xenograft, DLD-1.

LNA–SB-T-1213 exhibited strong antitumor activity, while paclitaxel is ineffective. LNA–SB-T-1213 exhibited the complete regression in two of five mice tested

against drug-resistant human colon tumor xenografts (Pgp+) DLD-1 (tumor growth delay >109 days). Although the toxicity of LNA–SB-T-1213 to the animals was higher than that of DHA–SB-T-1213, LNA–SB-T-1213 exhibited better overall activity than DHA–SB-T-1213 at the dose examined, which was not optimized. LA-SB-T-1213 did not show meaningful efficacy in the same assay, which revealed the marked difference between *n*-3 PUFA (LNA, DHA) and *n*-6 PUFA (LA). These results suggest that DHA is not the only PUFA that can be used for the PUFA–taxoid conjugates.

It should be noted that in these preliminary studies, the doses of the PUFA–taxoid conjugates were based on our experience dealing with paclitaxel and second-generation taxoids in animal models in the past, and thus the results shown above are not at their optimal doses, except for DHA–paclitaxel that was used as the standard at its known optimal dose for comparison purposes. Further investigations are actively underway in these laboratories to determine the optimal doses for those PUFA–taxoid conjugates.

The exceptional efficacy of PUFA–taxoid conjugates against drug-resistant and drug-sensitive human tumor xenografts provides bright prospects for further investigations for the applications of those conjugates in cancer chemotherapy.

Acknowledgments

This research was supported by grants from the National Cancer Institute (CA 103314 to I.O. and CA 73872 to R.J.B.) and the New York State Office of Science, Technology and Academic Research (NYSTAR) (to I.O.).

References and notes

1. Chen, J.; Jaracz, S.; Zhao, X.; Chen, S.; Ojima, I. *Expert Opin. Drug Deliv.* **2005**, *2*, 873, and references therein.
2. Jaracz, S.; Chen, J.; Kuznetsova, L. V.; Ojima, I. *Bioorg. Med. Chem.* **2005**, *13*, 5043, and references therein.
3. Trail, P. A.; Willner, D.; Lasch, S. J.; Henderson, A. J.; Hofstead, S.; Casazza, A. M.; Firestone, R. A.; Hellstrom, I.; Hellstrom, K. E. *Science* **1993**, *261*, 212.
4. Doronina, S. O.; Toki, B. E.; Torgov, M. Y.; Mendelsohn, B. A.; Cerveny, C. G.; Chace, D. F.; DeBlanc, R. L.; Gearing, R. P.; Bovee, T. D.; Siegall, C. B.; Francisco, J. A.; Wahl, A. F.; Meyer, D. L.; Senter, P. D. *Nat. Biotechnol.* **2003**, *21*, 778.
5. Liu, C. N.; Tadayoni, B. M.; Bourret, L. A.; Mattocks, K. M.; Derr, S. M.; Widdison, W. C.; Kedersha, N. L.; Ariniello, P. D.; Goldmacher, V. S.; Lambert, J. M.; Blattler, W. A.; Chari, R. V. J. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8618.
6. Chari, R. V. J.; Kristine, A.; Boyrret, L. A.; Derr, S. M.; Tadayoni, B. M.; Mattocks, K. M.; Shah, S. A.; Liu, C.; Blattler, W. A.; Goldmacher, V. S. *Cancer Res.* **1995**, *55*, 4079.
7. Ojima, I.; Geng, X.; Wu, X.; Qu, C.; Borella, C. P.; Xie, H.; Wilhelm, S. D.; Leece, B. A.; Bartle, L. M.; Goldmacher, V. S.; Chari, R. V. J. *J. Med. Chem.* **2002**, *45*, 5620.
8. Leamon, C. P.; Reddy, J. A. *Adv. Drug Deliv. Rev.* **2004**, *56*, 1127.
9. Goren, D.; Horowitz, A. T.; Tzemach, D.; Tarshish, M.; Zalipsky, S.; Gabizon, A. *Clin. Cancer Res.* **2000**, *6*, 1949.
10. Nagy, A.; Schally, A. V.; Halmos, G.; Armatis, P.; Cai, R.-Z.; Csernus, V.; Kovacs, M.; Koppan, M.; Szepeshazi, K.; Kahan, Z. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1794.
11. Hamann, P. R.; Hinman, L. M.; Hollander, I.; Beyer, C. F.; Lindh, D.; Holcomb, R.; Hallett, W.; Tsou, H. R.; Upeslacis, J.; Shochat, D.; Mountain, A.; Flowers, D. A.; Bernstein, I. *Bioconjug. Chem.* **2002**, *13*, 47.
12. Tolcher, A. W.; Ochoa, L.; Hammond, L. A.; Patnaik, A.; Edwards, T.; Takimoto, C.; Smith, L.; de Bono, J.; Schwartz, G.; Mays, T.; Jonak, Z. L.; Johnson, R.; DeWitte, M.; Martino, H.; Audette, C.; Maes, K.; Chari, R. V. J.; Lambert, J. M.; Rowinsky, E. K. *J. Clin. Oncol.* **2003**, *21*, 211.
13. Wahl, A. F.; Donaldson, K. L.; Mixan, B. J.; Trail, P. A.; Siegall, C. B. *Int. J. Cancer* **2001**, *93*, 590.
14. Widmer, C., Jr.; Holman, R. T. *Arch.. Biochem.* **1950**, *25*, 1.
15. Hardman, W. E. *J. Nutr.* **2002**, *132*, 3508S.
16. Tapiero, H.; Nguyen Ba, G.; Couvreur, P.; Tew, K. D. *Biomed. Pharmacother.* **2002**, *56*, 215.
17. Moser, U. *J. Appl. Cosmet.* **2002**, *20*, 137.
18. Huang, Y.-S.; Pereira, S. L.; Leonard, A. E. *Biochimie* **2004**, *86*, 793.
19. Morimoto, K. C.; Van Eenennaam, A. L.; DePeters, E. J.; Medrano, J. F. *J. Dairy Sci.* **2005**, *88*, 1142.
20. Heird, W. C.; Lapillonne, A. *Annu. Rev. Nutr.* **2005**, *25*, 549.
21. Harris, W. S. In *Encyclopedia of Dietary Supplements*; Coates, P. M., Ed.; Marcel Decker: New York, 2005; pp 493–504.
22. Sauer, L. A.; Dauchy, R. T. *Biochem. Soc. Trans.* **1990**, *18*, 80.
23. Sauer, L. A.; Dauchy, R. T. *Br. J. Cancer* **1992**, *66*, 297.
24. Sauer, L. A.; Nagel, W. O.; Dauchy, R. T.; Miceli, L. A.; Austin, J. E. *Cancer Res.* **1986**, *46*, 3469.
25. Sauer, L. A.; Stayman, J. W., III; Dauchy, R. T. *Cancer Res.* **1982**, *42*, 4090.
26. Bradley, M. O.; Webb, N. L.; Anthony, F. H.; Devanesan, P.; Witman, P. A.; Hemamalini, S.; Chander, M. C.; Baker, S. D.; He, L.; Horwitz, S. B.; Swindell, C. S. *Clin. Cancer Res.* **2001**, *7*, 3229.
27. Ojima, I.; Slater, J. C.; Michaud, E.; Kuduk, S. D.; Bounaud, P.-Y.; Vrignaud, P.; Bissery, M.-C.; Veith, J. M.; Pera, P.; Bernacki, R. J. *J. Med. Chem.* **1996**, *39*, 3889.
28. Ojima, I.; Kuduk, S. D.; Chakravarty, S.. In *Advances in Medicinal Chemistry*; Maryanoff, B. E., Reitz, A. B., Eds.; JAI Press: Greenwich, CT, 1999; Vol. 4, p 69.
29. Ojima, I.; Wang, T.; Miller, M. L.; Lin, S.; Borella, C. P.; Geng, X.; Pera, P.; Bernacki, R. J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3423.