Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5204–5209
Bioorganic & Medicinal Chemistry Letters

Antitumor agents. 258. Syntheses and evaluation of dietary antioxidant—taxoid conjugates as novel cytotoxic agents

Kyoko Nakagawa-Goto,[a] Koji Yamada,[a] Seikou Nakamura,[a] Tzu-Hsuan Chen,[a] Po-Cheng Chiang,[a] Kenneth F. Bastow,[b] Shao-Chun Wang,[c,d] Bill Spohn,[c,d] Mien-Chie Hung,[c,d] Fang-Yu Lee,[e] Fang-Chen Lee[e] and Kuo-Hsiung Lee[a,*]

[a]*Natural Products Research Laboratories, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA*
[b]*Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA*
[c]*Department of Molecular and Cellular Oncology, The University of Texas, MD Anderson Cancer Center, 1515 Holcombe Boulevard Box 108, Houston, TX 77030, USA*
[d]*Center for Molecular Medicine, China Medical University Hospital, Taichung, Taiwan*
[e]*Yung-Shin Pharmaceutical Co. Ltd, Ta-Chia, Taichung, Taiwan*

Received 14 March 2007; revised 21 June 2007; accepted 28 June 2007
Available online 4 July 2007

Abstract—Various dietary antioxidants, including vitamins, flavonoids, curcumin, and a coumarin, were conjugated with paclitaxel (**1**) through an ester linkage. The newly synthesized compounds were evaluated for cytotoxic activity against several human tumor cell lines as well as the corresponding normal cell lines. Interestingly, most tested conjugates selectively inhibited the growth of 1A9 (ovarian) and KB (nasopharyngeal) tumor cells without activity against other cell lines. Particularly, conjugates **16** and **20** were highly active against 1A9 (ED_{50} value of 0.005 μg/mL) as well as KB (ED_{50} values of 0.005 and 0.14 μg/mL, respectively) cells. Compound **22b**, the glycinate ester salt of vitamin E conjugated with **1**, appears to be a promising lead for further development as a clinical trial candidate as it exhibited strong inhibitory activity against Panc-1 (pancreatic cancer) with less effect on the related E6E7 (normal) cell line.

In current cancer therapy, undesirable side effects due to toxicity of antitumor drugs on normal tissues present an important problem to be solved. Therefore, a challenging research focus in cancer treatment is the discovery of efficient antitumor drugs with high therapeutic indexes, which will have selective activity against target tumors and reduced normal tissue damage. Among various strategies to improve drug selectivity, conjugation of cytotoxic drug components has proven to be a promising approach to enhance the activity as well as selectivity of an individual lead compound.[1] This concept is now accepted as an effective strategy for designing ligands, inhibitors, and other drugs that influence biological systems.[2] On the basis of this theory, some interesting results have been reported by our group[3] as well as others[4] in recent years. In our prior study, we explored the syntheses and evaluation of heterodimer conjugates, which combined two kinds of antitumor drugs through various linkages, as novel antitumor agents.[5]

Dietary anti-oxidants, such as flavonoids and vitamins A, C, and E that are found in various foods, can act as cancer preventive agents. These compounds are capable of neutralizing and deactivating reactive oxygen species, which can seriously damage DNA and other cellular molecules, thereby causing tumors.[6] Antioxidants act not only as cancer preventive agents, but also as therapeutic biologic response modifiers, and are able to directly induce apoptosis in established tumor cells. In addition, evidence shows that antioxidants can enhance chemotherapeutic antitumor effects.[7]

Therefore, conjugation of antitumor drugs with dietary antioxidants might provide new classes of antitumor drug candidates with tumor selectivity or activity

Keywords: Conjugation; Paclitaxel; Antioxidant; Cytotoxicity.
* Corresponding author. Tel.: +1 919 962 0066; fax: +1 919 966 3893; e-mail: khlee@unc.edu

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.06.083

against multi-drug resistant cancer cell lines. We chose paclitaxel (**1**)[8] as the antitumor base compound and various antioxidants as the conjugate partners as shown in Figure 1. Vitamins,[6] such as retinol (vitamin A, **2a**)[9,10] and α–tocopherol (vitamin E, **3**),[9,11] are well known dietary antioxidants. Compound **4** was also selected as a related vitamin E analog. Other known antioxidant agents, including curcumin (**5**)[6,12] found in turmeric, dehydrozingerone (**6**)[13] isolated from ginger, and its analog **7**, were also used. In addition, antioxidant flavonoids[6,14] and coumarins[6,15] are widely found in various vegetables, fruits, nuts, coffee, tea or wine, and representative compounds [galangin (**8**), chrysin (**9**), and 4-methylumbelliferone (**10**), respectively] were conjugated with **1** (Scheme 1).

Paclitaxel (**1**) was reacted first with succinic anhydride in pyridine to give **11**,[16] which has a succinate ester at the C-2′ position. However, esterification of **11** with **2a** provided an unstable compound; therefore, retinoic acid (**2b**, vitamin A acid) was reacted directly with **1** to afford **12**. Conjugations of **11** with other antioxidants, **3**–**10**, were carried out by a common esterification method using EDCI in the presence of DMAP to give **13**–**20**. Water solubility, which is connected with oral bioavailability, is always of concern for drug discovery and development. Accordingly, the resulting conjugates, **12**–**14** and **16**–**18**, were converted to the corresponding glycinate esters, **21**–**26**. These esters can be converted to various salts. As discussed later, the paclitaxel-vitamin E conjugate (**13**) showed good activity against a pancreatic cancer cell line with less effect against the related normal cell line. Therefore, in order to increase water-solubility, conjugate **13** was converted first to various amine (**22**, **27**, and **28**) or carboxylic (**29**) esters and then to the corresponding hydrochloride (**22a**, **27a**, and **28a**), methanesulfonate (**22b**, and **28b**), or triethylammonium (**29a**) salts as shown in Scheme 2.

The newly obtained conjugates were evaluated for cytotoxic activity against several human tumor cell lines. Interestingly, conjugates **12**, **14**, and **16**–**20** and the glycinate esters **21** and **23**–**26** selectively inhibited the growth of 1A9 and KB cells (data shown in Table 1), without inhibition of the remaining cell lines [lung carcinoma (A549), breast cancer (MCF-7), prostate carcinoma (LN-CAP, PC-3, DU-145) and multi-drug resistant variant expressing P-glycoprotein KB-VIN]. Conjugates **16** (paclitaxel-dehydrozingerone) and **20** (paclitaxel-coumarin) exhibited the highest potency against these two cell lines (1A9: ED_{50} 0.005 μg/mL; KB:ED_{50} 0.005 and 0.14 μg/mL, respectively). Moreover, against KB cells, glycinate ester **26** showed significantly increased cytotoxic activity (ED_{50} 0.09 μg/mL) compared to the related parent compound **18** (ED_{50} 0.26 μg/mL), even though glycinate esters **23**–**25** showed similar or lower activity compared with the parent compounds **14**, **16**, and **17**.

Conjugate **13**, its C-7 esters **22** and **27**–**29**, their corresponding salts, and conjugate **15** were evaluated for cytotoxic activity against A549, 1A9, colon adenocarcinoma

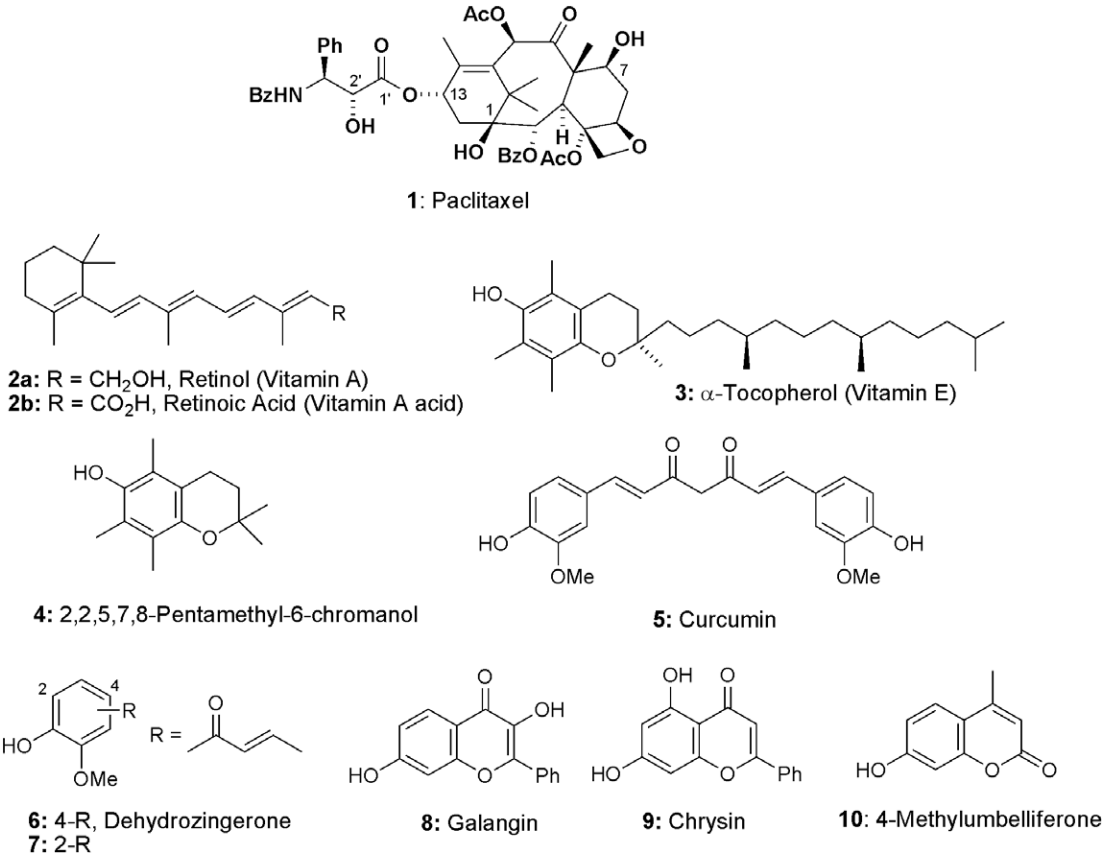
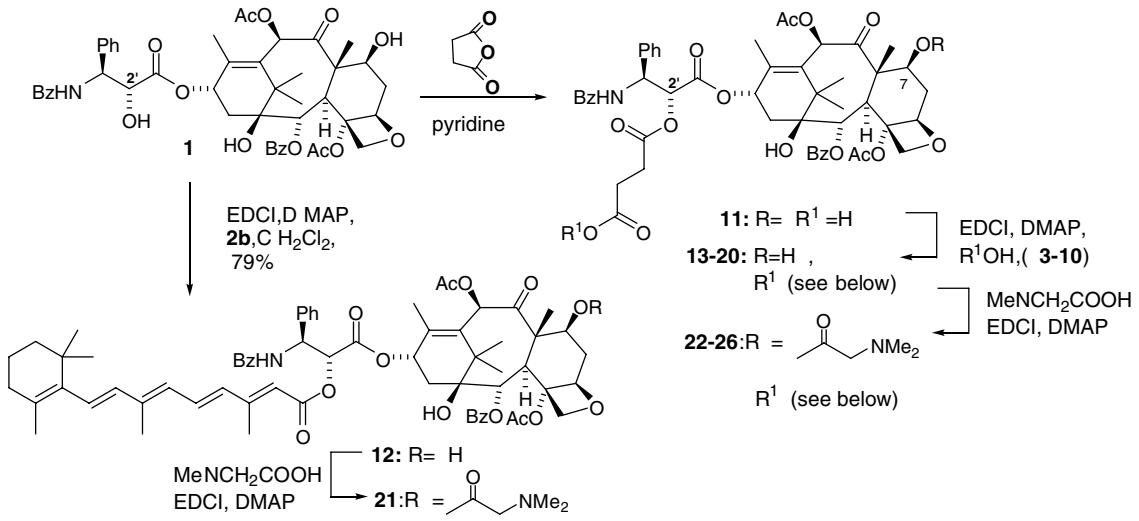


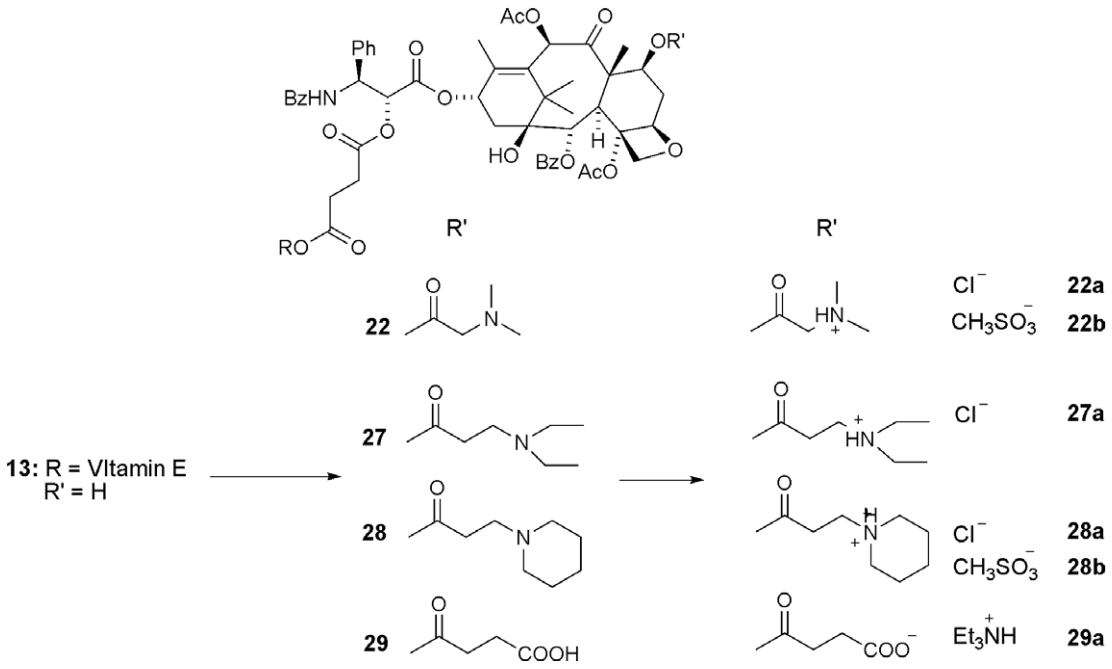
Figure 1. Structures of paclitaxel (**1**) and anti-oxidant conjugate partners (**2-10**).



Conjugate (R = -H)	Glycinate Ester (R = -$COCH_2NMe_2$)	R^1
13	**22**	R^2 =
14	**23**	R^2 = Me
15		
16	**24**	R^2 = 4-CH=CHCOCH$_3$
17	**25**	R^2 = 2-CH=CHCOCH$_3$
18	**26**	R^2 = OH, R^3 = H
19		R^2 = H, R^3 = OH
20		

Scheme 1. Synthesis of conjugates **12-26**.

(HCT-8), epidermoid skin carcinoma (A431), KB, and KB-VIN cell lines. The data are shown in Table 2. The ED_{50} values of **1**, **3** or **5**, and a 1:1 mixture of **1** with **3** or **5** are also shown for comparison. The 1:1 mixtures of **1** with **3** or **5** showed similar results to **1** itself against A549, A431 and 1A9 cells but were less potent against HCT-8 cells. However, the related conjugates **13** and **15** showed unique selectivity. Vitamin E conjugate **13** lost cytotoxic activity against most cell lines, and inhibited the growth of only the A431 cell line with an ED_{50} value of 0.1 μg/mL. In comparison, curcumin conjugate **15** showed similar potencies to **1** against A549, 1A9, A431, KB and KB-VIN cells, but lost activity against HCT-8 cells. Among the C-7 esters/salts of conjugate **13** (**22** and **27**–**29**), only the glycinate ester **22**, succinic ester **29**, and their salts displayed cytotoxic activity against the A431 cell line with similar ED_{50} values to the parent compound **13**.



Scheme 2. Synthesis of water soluble salts of conjugate **13**.

Table 1. Cytotoxic activity data for taxol conjugates **12**, **14**, **16**–**21**, **23**–**26** [ED_{50} (μg/mL)][a,b]

	Cell line	
	1A9[c]	KB[c]
Conjugate		
12	0.49	0.20
14	0.20	0.94
16	0.005	0.005
17	0.14	0.39
18	0.13	0.26
19	0.20	0.47
20	0.005	0.14
Glycinate ester		
21	4.47	7.65
23	1.01	9.79
24	0.63	0.70
25	0.19	0.34
26	0.11	0.09
Control		
1	0.001	0.002

[a] Cytotoxicity as ED_{50} values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay.
[b] All compounds tested did not reach 50% inhibition against human lung carcinoma (A549), breast cancer (MCF-7), human prostate carcinoma (LN-CAP, PC-3, DU-145), and multi-drug resistant KB variant expressing P-glycoprotein (KB-VIN).
[c] Human ovarian carcinoma (1A9), human epidermoid carcinoma of the nasopharynx (KB).

Table 2. Cytotoxic activity data of conjugates **13**, **15**, and related selected compounds

Compound	ED_{50} (μg/mL)					
	A549	1A9	HCT-8[a]	A431[a]	KB	KB-VIN
1	0.02	0.01	0.06	0.01	0.01	0.2
3 (Vitamin E)	NA[b]	NA	NA	0.75	NA	NA
13 (**1**+**3** Conjugate)	NA	NA	NA	0.1	NA	NA
1+**3** (1:1 Mixture)	0.02	0.01	0.20	0.01	0.01	0.1
22	NA	NA	NA	0.2	NA	NA
22a, **22b**	NA	NA	NA	0.1	NA	NA
27, **27a**, **28**, **28a**, **28b**	NA	NA	NA	NA	NA	NA
29	NA	NA	NA	0.1	NA	NA
29a	NA	NA	NA	0.2	NA	NA
5 (Curcumin)	NA	NA	NA	0.75	NA	NA
15 (**1**+**5** Conjugate)	0.03	0.03	NA	0.03	0.01	0.2
1+**5** (1:1 Mixture)	0.03	0.01	0.10	0.01	0.01	0.2

[a] Epidermoid skin carcinoma (A431), colon adenocarcinoma (HCT-8); for other cell lines, see Table 1.
[b] NA, not active.

Conjugate **13** and its related esters/salts were also screened against multiple human tumor as well as the corresponding normal cell lines. Figure 2 shows selected data after 48 h. Conjugate **13** demonstrated less growth inhibition of normal pancreatic cells (E6E7) than related tumor cells (Panc-1), whereas growths of other normal cells were inhibited more or as strongly than the related tumor cells. Glycinate ester salt **22b** also showed less inhibition of E6E7 growth and the highest potency against Panc-1, compared with the other esters/salts,

In conclusion, we have synthesized various **1**-antioxidant conjugates linked through an ester bond at the 2′-position of **1**. All conjugates were screened against various tumor cell lines and showed tumor-selective activity. Most conjugates selectively inhibited the growth of 1A9 and KB tumor cells and lacked activity against other tested cell lines. On the other hand, conjugate **13** showed cytotoxic activity only against A431, while conjugate **17** lacked any activity against HCT-8 while retaining similar activity to **1** against the other tumor cell lines. Salt **22b** exhibited inhibitory activity against Panc-1 with less effect on the related normal cell. Compound **22b** appears to be a promising new lead for

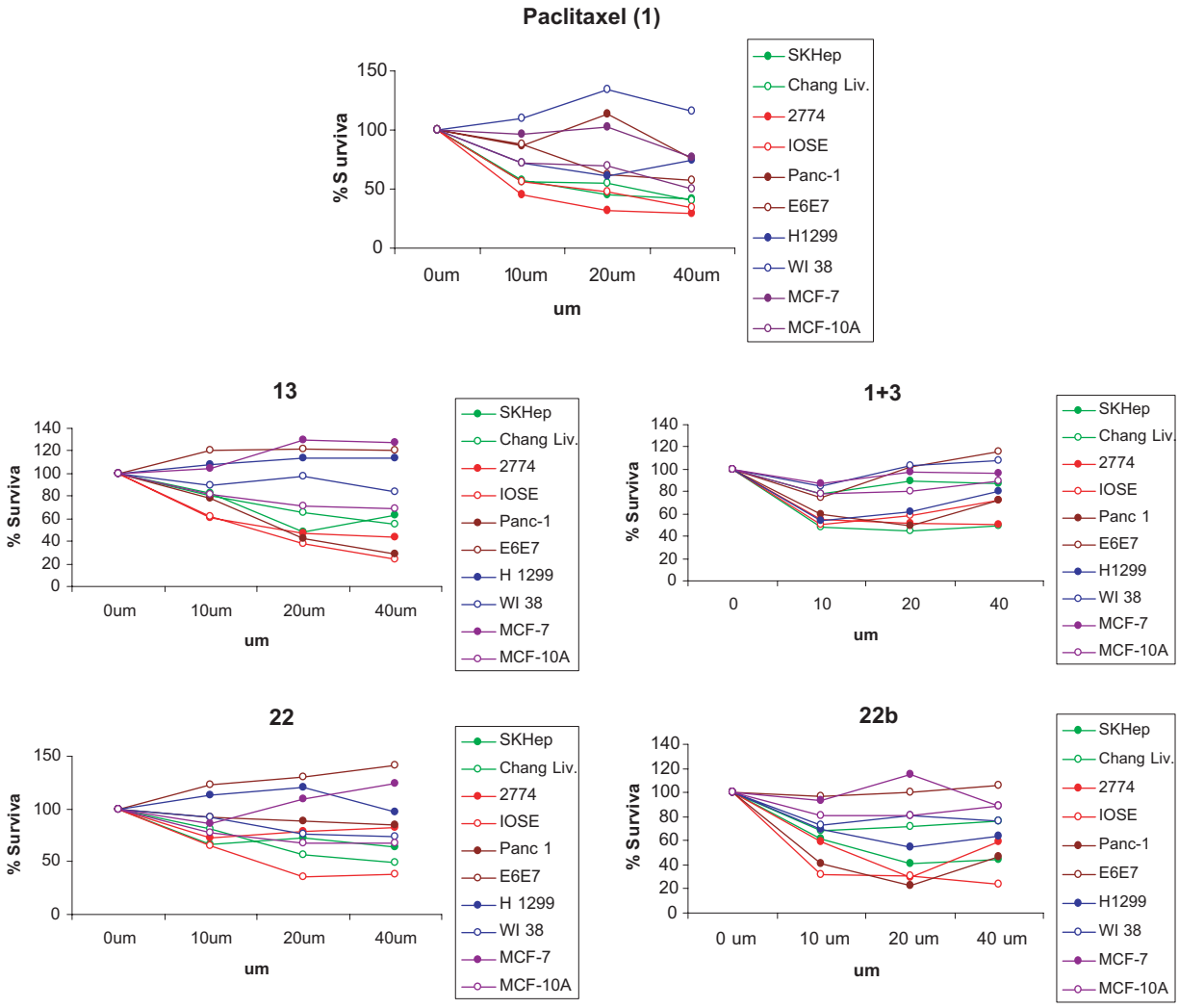


Figure 2. Liver human hepatomacarcinoma (SKHep), normal liver epithelial cell (Chang Liv), human ovarian carcinoma (2774), normal surface ovarian epithelial cell (IOSE), human pancreatic cancer cell (Panc 1), normal ovarian epithelial cell (E6E7), human lung cancer cell (H1299), normal human fibroblast (W138), breast cancer (MCF-7) and normal breast epithelial cell (MCF-10A).

further development into a clinical trial candidate. In summary, the conjugation of **1** with dietary antioxidants enhanced tumor selectivity dependant on the identity of the partner compound.

Acknowledgment

This study was supported by Grant CA17525 from the National Cancer Institute, NIH, awarded to K.H.L.

References and notes

1. (a) Langer, M.; Kraz, F.; Rothen-Rutishause, B.; Wunderli-Alemnspach, H.; Beck-Sickinger, A. G. *J. Med. Chem.* **2001**, *44*, 1341; (b) Safavy, A.; Bonner, J. A.; Waksal, H. W.; Buchsbaum, D. J.; Gillespie, G. Y.; Khazaeli, M. B.; Arani, R.; Chen, D. T.; Carpenter, M.; Raisch, K. P. *Bioconjugate Chem.* **2003**, *14*, 302.
2. Choi, S. K. *Synthetic Multivalent Molecules*; Wiley-Interscience: New York, 2004.
3. (a) Bastow, K. F.; Wang, H. K.; Cheng, Y. C.; Lee, K. H. *Bioorg. Med. Chem.* **1997**, *5*, 1481; (b) Chang, J. Y.; Guo, X.; Chen, H. X.; Jiang, Z.; Fu, Q.; Wang, H. K.; Bastow, K. F.; Zhu, X. K.; Guan, J.; Lee, K. H.; Cheng, T. C. *Biochem. Pharm.* **2000**, *59*, 497; (c) Shi, Q.; Wang, H. K.; Bastow, K. F.; Tachibana, Y.; Chen, K.; Lee, F. Y.; Lee, K. H. *Bioorg. Med. Chem.* **2001**, *9*, 2999; (d) Ohtsu, H.; Nakanishi, Y.; Bastow, K. F.; Lee, F. Y.; Lee, K. H. *Bioorg. Med. Chem.* **2003**, *11*, 1851.
4. For example (a) Milas, L.; Mason, K. A.; Hunter, N.; Li, C.; Wallace, S. *Int. J. Radiat. Oncol. Biol. Phys.* **2003**, *55*, 707; (b) Kuznetsova, L.; Chen, J.; Sun, L.; Wu, X.; Pepe, A.; Veith, J. M.; Pera, P.; Bernacki, R. J.; Ojima, I. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 974; (c) Danieli, B.; Giardini, A.; Lesma, G.; Passarella, D.; Peretto, B.; Sacchetti, A.; Silvani, A.; Pratesi, G.; Zunino, F. *J. Org. Chem.* **2006**, *71*, 2848.
5. Nakagawa-Goto, K.; Nakamura, S.; Nyarko, A.; Peng, C. Y.; Bastow, K. F.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2894.
6. Recent reviews for dietary antioxidants and cancers: (a) Drisko, J. A.; Chapman, J.; Hunter, V. J. *Gynecol. Oncol,* **2003**, *88*, 434; (b) Borek, C. *Integr. Cancer Ther.* **2004**, *3*, 333; (c) Collins, A. R. *Eur. J. Cancer* **2005**, *41*, 1923; (d)

Kaliora, A. C.; Dedoussis, G. V. Z.; Schmit, H. *Atherosclerosis* **2006**, 187.
7. (a) Park, C. H.; Amare, M.; Savin, M. A.; Hoogstraten, B. *Cancer Res.* **1980**, *40*, 1062; (b) Chinery, R.; Brockman, J. A.; Peeler, M. O.; Shyr, Y.; Beauchamp, R. D.; Coffey, R. J. *Nat. Med.* **1997**, *3*, 1233; (c) Prasad, K. N.; Kumar, A.; Kochupillai, V.; Cole, W. C. *J. Am. Coll. Nutr.* **1999**, *18*, 13; (d) Conklin, K. A. *Nutr. Cancer* **2000**, *37*, 1.
8. (a) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; MacPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1971**, *93*, 2325; (b) Itokawa, H.; Lee, K. H. *Taxus*; Taylor & Francis: London and New York, 2003.
9. Debier, C.; Larondelle, L. *British J. Nutr.* **2005**, *93*, 153.
10. Napoli, J. L. *Encyclopedia Biol. Chem.* **2004**, *4*, 354.
11. Niki, E. *Oxid. Stress Disease* **2006**, *21*, 217.
12. (a) Anto, R. J.; Kuttan, G.; Babu, K. V. D.; Rajasekharan, K. N.; Kuttan, R. *Int. J. Pharm.* **1996**, *131*, 1; (b) Ahsan, H.; Parveen, N.; Khan, N. U.; Hadi, S. M. *Chem. Biol. Interact.* **1999**, *121*, 161; (c) Sakano, K.; Kawanishi, S. *Arch. Biochem. Biophys.* **2002**, *405*, 223.
13. (a) De Bernardi, M.; Vidari, G.; Vita-Finzi, P. *Phytochemistry* **1976**, *15*, 1785; (b) Motohashi, N.; Ashihara, Y.; Yamaguchi, C.; Saito, Y. *Mutat. Res.* **1997**, *377*, 17.
14. (a) Woodman, O. L.; Chan, E. C. H. *Clin. Exp. Pharmacol. Physiol.* **2004**, *31*, 786; (b) Moon, Y. J.; Wang, X.; Morris, M. E. *Toxicol. in vitro* **2006**, *20*, 187.
15. (a) Lacy, A.; O'Kennedy, R. *Curr. Pharmaceut. Design* **2004**, *10*, 3797; (b) Irena, K. *Mini Rev. Med. Chem.* **2006**, *6*, 365.
16. Deutsh, H. M.; Glinski, J. A.; Hernandez, M.; Haugwits, R. D.; Narayanan, V. L.; Suffness, M.; Zalkow, L. H. *J. Med. Chem.* **1989**, *32*, 788.