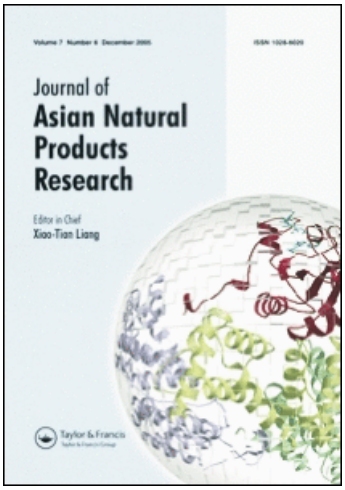


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Cancer preventive agents 10. Prenylated dehydrozingerone analogs as potent chemopreventive agents

Jin Tatsuzaki[a]; Kyoko Nakagawa-Goto[a]; Harukuni Tokuda[b]; Kuo-Hsiung Lee[a]
[a] Natural Products Research Laboratories, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC, USA [b] Department of Biochemistry, Kyoto Prefectural University of Medicine, Kyoto, Japan

Online publication date: 26 March 2010

To cite this Article Tatsuzaki, Jin , Nakagawa-Goto, Kyoko , Tokuda, Harukuni and Lee, Kuo-Hsiung(2010) 'Cancer preventive agents 10. Prenylated dehydrozingerone analogs as potent chemopreventive agents', Journal of Asian Natural Products Research, 12: 3, 227 — 232
To link to this Article: DOI: 10.1080/10286021003591617
URL: http://dx.doi.org/10.1080/10286021003591617

Journal of Asian Natural Products Research
Vol. 12, No. 3, March 2010, 227–232

ORIGINAL ARTICLE

Cancer preventive agents 10. Prenylated dehydrozingerone analogs as potent chemopreventive agents

Jin Tatsuzaki[a], Kyoko Nakagawa-Goto[a], Harukuni Tokuda[b] and Kuo-Hsiung Lee[a]*

[a]*Natural Products Research Laboratories, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7360, USA;* [b]*Department of Biochemistry, Kyoto Prefectural University of Medicine, Kyoto 602-0841, Japan*

(*Received 13 November 2009; final version received 4 January 2010*)

Dehydrozingerone analogs and related compounds were screened as potential antitumor promoters by using the *in vitro* short-term 12-*O*-tetradecanoylphorbol-13-acetate (TPA) -induced Epstein–Barr virus early antigen activation assay. Among the 40 synthesized compounds, the prenylated analogs **16** and **34**–**36** showed the most significant and promising activity (100% inhibition of activation at 1×10^3 mol ratio/TPA, and 82–80%, 37–35%, and 13–11% inhibition at 5×10^2, 1×10^2, and 1×10 mol ratio/TPA, respectively) in this screening. Their activity profiles were comparable to those of the reference standard curcumin. While a prenyl moiety conferred potent chemopreventive activity, an extended prenyl unit such as a farnesyl moiety did not improve activity. Because *in vitro* inhibitory effects in this assay generally correlate well with *in vivo* inhibitory effects on tumor promotion, our results strongly suggested that prenylated **16** and **34**–**36** are likely to be promising antitumor promoters.

Keywords: dehydrozingerone; antitumor-promoting effect; Epstein–Barr virus; two-stage carcinogenesis

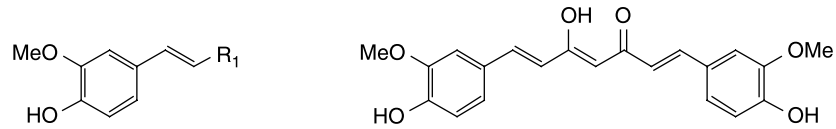
1. Introduction

The natural product dehydrozingerone (DZ, **1**) is the 'half analog' of curcumin (**2**) (Figure 1), which is known to have potent anti-oxidant, anti-inflammatory, and antitumor-promoting (chemopreventive) activities [1,2]. Curcumin inhibits epidermal inflammation in mice and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced tumor promotion in mouse skin [3]. Structure–activity relationship (SAR) correlations of curcumin analogs as antitumor-promoting agents have also been investigated [4,5]. DZ (**1**) and isoeugenol (**3**), which have similar catechol skeletons, but different alkenyl side chains, had stronger antitumor-promoting effects than curcumin [6]. In the search for antitumor promoters from natural sources, the antitumor-promoting properties of phenylpropanoids have also been reported [7–9]. The above evidence strongly supports the use of **1** as a lead to develop novel antitumor promoters and to further explore the structural features necessary for chemopreventive activity. Because certain natural products and synthetic compounds containing a prenyl moiety showed strong activity against the Epstein–Barr virus early antigen (EBV-EA) activation induced by TPA

*Corresponding author. Email: khlee@unc.edu

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286021003591617
http://www.informaworld.com



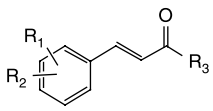
1: Dehydrozingerone $R_1 = COCH_3$ **2:** Curcumin
3: Isoeugenol $R_1 = CH_3$

Figure 1. Structures of DZ (**1**), curcumin (**2**), and isoeugenol (**3**).

in Raji cells [10–14], prenyl derivatives of **1** were thus synthesized and evaluated for *in vitro* inhibitory activity against EBV-EA. In this paper, we report the synthesis and SAR study of DZ analogs as chemopreventive agents.

2. Results and discussion

All compounds were previously synthesized [15]. Figure 2 shows the structures of analogs **4**–**11**, which are derivatives of **1**, and of the related chalcones **12**–**15**, in which the terminal methyl group is replaced by phenyl. Figure 3 shows the structures of analogs **16**–**33**, in which various types of alkyl and alkenyl groups were added to the C-4′ alcohol of **1** and **3**, as well as four additional prenyloxy derivatives **34**–**37**. The structures of methylated and prenylated curcumins (**38** and **39**, respectively) are shown in Figure 4. All analogs were evaluated *in vitro* as inhibitors of EBV-EA activation induced by TPA in Raji cells [16–18], and the inhibitory data are shown in Tables 1 and 2. Figure 5 indicates the typical fluorescent finding of EBV-EA activation in Raji cells through the fluorescence microscope.



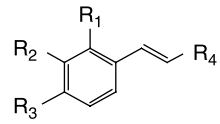
1, 4–11: R_3 = Me
12–15: R_3 = Ph

	R_1	R_2	R_3
1	3-OMe	4-OH	Me
4	2-OH	3-OMe	Me
5	3-OH	4-OMe	Me
6	2-OH	4-OMe	Me
7	H	H	Me
8	3-OEt	4-OH	Me
9	2-OH	3-OEt	Me
10	2-OH	3-F	Me
11	3-F	4-OMe	Me
12	3-OMe	4-OH	Ph
13	2-OH	3-OMe	Ph
14	3-OH	4-OMe	Ph
15	2-OH	4-OMe	Ph

Figure 2. Structures of DZ analogs.

Compounds with 3,4-disubstituted benzalacetone structures (**1**, **5**, and **8**) showed slightly better activity than compounds with other disubstitution patterns (e.g. 2,3- or 2,4-). The data for **12**–**15** indicated that a phenyl group at C4 decreased the activity. No remarkable difference was observed between methoxy and ethoxy groups (**1** vs. **8** and **4** vs. **9**). However, by comparing **10** and **11** with **1** and **4**–**9**, the presence of fluorine on the benzene ring might decrease the activity.

Compounds **16**–**24** (DZ analogs), **25**–**33** (isoeugenol analogs), **34**–**37** (prenylated analogs), and **38** and **39** (curcumin analogs) were also tested using an *in vitro* synergistic assay on EBV-EA activation induced by TPA. The inhibitory effects of tested compounds and the associated viability of Raji cells are shown in Table 2. DZ (**1**), isoeugenol (**3**), and curcumin (**2**) were used as positive controls. In this assay, all compounds showed inhibitory effects on EBV-EA activation without high cytotoxicity on Raji cells. At high concentrations (1×10^3 mol ratio), DZ (**16**–**24** and **34**–**37**), isoeugenol (**25**–**33**), and curcumin (**38** and



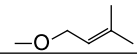
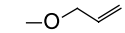
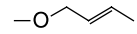
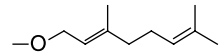
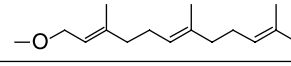
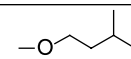
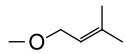
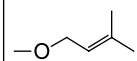
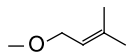
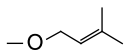
Dehydrozingerone R_4 = COMe	R_1	R_2	R_3	Isoeugenol R_4 = Me
16	H	OMe		**25**
17	H	OMe		**26**
18	H	OMe		**27**
19	H	OMe		**28**
20	H	OMe		**29**
21	H	OMe	OMe	**30**
22	H	OMe	OEt	**31**
23	H	OMe	OPr	**32**
24	H	OMe		**33**
34		OMe	H	-
35	H		OMe	-
36		H	OMe	-
37		F	H	-

Figure 3. Structures of DZ (**16**–**24**, **34**–**37**) and isoeugenol (**25**–**33**) analogs.

39) derivatives showed 100% inhibition, and at lower concentrations, they were as potent as or more potent than the parent compounds. The prenylated analogs, **16** and **25**, showed significant potency compared with other alkylated analogs in the respective series (see **16**–**24** for DZ analogs, and **25**–**33** for isoeugenol analogs). Prenylated DZ analogs **34**–**37** showed comparable activity with **16**, which showed the best activity in the alkylated series. These findings support the reported conclusions that a prenyl moiety is important for optimal inhibitory effects on EBV-EA activation [7–9]. Compound **37** was less active than **16** and **34**–**36**, indicating that fluorine does not affect the activity. Although analog **19** containing a geranyl group (two prenyl units) was more active than **20** and **29** with farnesyl groups (three prenyl units), it was less active compared with other analogs in the DZ

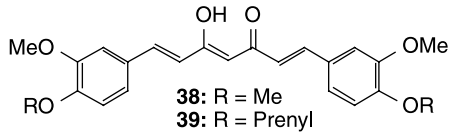


Figure 4. Structures of curcumin analogs **38** and **39**.

Table 1. Relative ratio[a] of EBV-EA activation with respect to positive control in the presence of DZ analogs.

	Percentage of EBV-EA positive cells				
	Compound concentration (mol ratio/TPA[b])				
Compound	1000	500	100	10	IC_{50}
DZ (**1**)[c]	0 (70)[d]	44.2	75.5	95.4	372
4	0 (70)	46.7	77.7	97.6	380
5	0 (70)	44.1	74.6	94.3	369
6	0 (70)	45.8	76.9	96.3	378
7	0 (70)	48.6	78.1	98.9	378
8	0 (70)	42.2	73.7	93.1	368
9	0 (70)	43.9	74.8	95.3	370
10	0 (60)	51.3	79.4	98.0	385
11	0 (60)	53.4	81.0	99.0	389
12	0 (60)	37.2	79.4	100	379
13	0 (60)	38.6	81.5	100	381
14	0 (60)	37.9	80.7	100	380
15	0 (60)	37.2	80.3	100	380

Notes: [a] Values represent percentages relative to the positive control value (100%).
[b] TPA concentration is 20 ng/ml (32 pmol/ml).
[c] Positive control.
[d] Values in parentheses are viability percentages of Raji cells.

series. Compounds **17**, **18**, **22**–**24** and **26**, **27**, **31**, **32**, which contain allyl, 2-butenyl, ethyl, propyl, and isopentyl substituents, respectively, showed similar activity, while methylated compounds **21** and **30** showed slightly lower activity. When analogs with structurally similar alkyl and alkenyl groups were compared (**16** vs. **24**, **17** and **18** vs. **23**), the presence of a double bond did not seem to affect the activity.

In summary, prenylated DZ **16** and its analogs **34**–**36** showed the most significant and promising activity in this screening (100% inhibition of activation at 1×10^3 mol ratio/TPA, and 82–80%, 37–35%, and 13–11% inhibition at 5×10^2, 1×10^2, and 1×10 mol ratio/TPA, respectively). While a prenyl moiety conferred potent chemopreventive activity, an extended prenyl unit such as a farnesyl moiety did not improve activity. Hydrophobicity might be important for the inhibition of TPA-induced EBV-EA activation. Because *in vitro* inhibitory effects in this assay generally correlate well with *in vivo* inhibitory effects on tumor promotion [4,5,19,20], our results suggested that **16** and **34**–**36** are promising antitumor promoters and further *in vivo* investigations are now in progress.

3. Experimental

3.1 In vitro *EBV-EA activation experiments*

EBV-EA positive serum from a patient with nasopharyngeal carcinoma was a gift from Prof. H. Hattori, Department of Otorhinolaryngology, Kobe University. The EBV genome carrying lymphoblastoid cells (Raji cells derived from Burkitt's lymphoma) was cultured in 10% fetal bovine serum in RPMI-1640 medium (Sigma R8758, St Louis, MO, USA). Spontaneous activation of EBV-EA in our subline of Raji cells was less than 0.1%. The inhibition of EBV-EA activation was assayed using Raji cells (virus non-producer type) as described below. The cells were incubated at 37°C for 48 h in 1 ml of medium containing *n*-butyric acid (4 mM), TPA [32 pM = 20 ng in 2 μl

Table 2. Relative ratio[a] of EBV-EA activation with respect to positive control in the presence of DZ analogs and related compounds.

	Percentage of EBV-EA positive cells				
	Compound concentration (mol ratio/TPA[b])				
Compound	1000	500	100	10	IC_{50}
DZ (**1**)[c]	0 ± 0.5 (70)[d]	44.2 ± 1.9	75.5 ± 2.6	95.4 ± 0.2	372
16	0 ± 0.2 (60)	19.0 ± 1.3	65.2 ± 2.0	88.7 ± 0.4	216
17	0 ± 0.4 (60)	21.9 ± 1.5	67.8 ± 2.1	90.0 ± 0.2	234
18	0 ± 0.3 (60)	20.7 ± 1.5	66.9 ± 2.1	89.3 ± 0.3	220
19	0 ± 0.4 (60)	23.5 ± 1.5	71.0 ± 2.3	95.1 ± 0.1	242
20	0 ± 0.4 (60)	24.9 ± 1.3	73.9 ± 2.3	97.7 ± 0.2	250
21	0 ± 0.4 (60)	22.8 ± 1.3	70.9 ± 2.0	93.7 ± 0.3	239
22	0 ± 0.6 (60)	38.5 ± 1.7	72.0 ± 2.3	92.1 ± 0.2	368
23	0 ± 0.2 (60)	21.5 ± 1.2	68.9 ± 2.1	91.7 ± 0.3	238
24	0 ± 0.3 (60)	20.1 ± 1.3	66.2 ± 2.0	89.0 ± 0.3	220
Isoeugenol (**3**)[c]	16.7 ± 1.5 (70)	53.5 ± 1.9	81.0 ± 2.7	100 ± 0.1	490
25	0 ± 0.2 (60)	19.9 ± 1.1	66.0 ± 1.9	89.5 ± 0.4	219
26	0 ± 0.4 (60)	22.8 ± 1.4	68.0 ± 2.2	92.7 ± 0.2	236
27	0 ± 0.3 (60)	21.8 ± 1.3	67.3 ± 2.1	90.3 ± 0.3	234
28	NA[e]				–
29	0 ± 0.5 (60)	26.3 ± 1.4	75.3 ± 2.3	98.8 ± 0.1	261
30	0 ± 0.4 (60)	23.6 ± 1.5	72.0 ± 2.3	95.6 ± 0.2	246
31	NA				–
32	0 ± 0.4 (60)	22.7 ± 1.3	69.3 ± 2.3	92.8 ± 0.1	240
33	0 ± 0.4 (60)	21.2 ± 1.4	67.3 ± 2.1	91.5 ± 0.2	237
34	0 ± 0.2 (60)	19.1 ± 1.1	64.8 ± 2.0	88.0 ± 0.4	222
35	0 ± 0.2 (60)	18.0 ± 1.0	63.2 ± 2.0	86.8 ± 0.3	207
36	0 ± 0.2 (60)	19.7 ± 1.1	63.2 ± 2.0	86.8 ± 0.3	219
37	0 ± 0.2 (60)	22.8 ± 1.3	67.7 ± 2.1	90.5 ± 0.3	231
Curcumin (**2**)[c]	0 ± 0.4 (60)	21.1 ± 1.1	80.1 ± 2.4	100 ± 0.1	379
38	0 ± 0.2 (60)	19.6 ± 1.1	76.5 ± 2.4	98.5 ± 0.1	253
39	0 ± 0.1 (60)	17.3 ± 0.9	72.6 ± 2.1	92.4 ± 0.3	240

Notes: [a] Values represent percentages relative to the positive control value (100%).
[b] TPA concentration is 20 ng/ml (32 pmol/ml).
[c] Positive control.
[d] Values in parentheses are viability percentages of Raji cells.
[e] Not applicable.

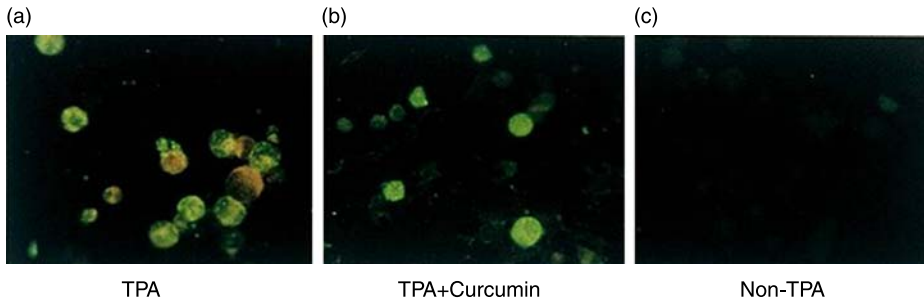


Figure 5. Typical fluorescent findings of EBV-EA activation. (a) EBV-EA expression by treatment with TPA, (b) EBV-EA expression was reduced by curcumin and (c) no EBV-EA expression was observed without TPA.

dimethyl sulfoxide (DMSO)] and various amounts of the test compounds dissolved in 2 μl of DMSO. Smears were made from the cell suspension. The EBV-EA-inducing cells were stained by means of an indirect immunofluorescence technique. In each assay, at least 500 cells were counted, and the number of stained cells (positive cells) was recorded. Triplicate assays were performed for each compound. The average EBV-EA induction of the test compound was expressed as a ratio relative to the control experiment (100%), which was carried out with *n*-butyric acid (4 mM) plus TPA (32 pM). EBV-EA induction was ordinarily around 35%. The viability of treated Raji cells was assayed by the trypan blue staining method. The cell viability of the TPA positive control was greater than 80%. Therefore, only those compounds that induced less than 80% (% of control) of the EBV-active cells (those with a cell viability of more than 60%) were considered to be able to inhibit the activation caused by promoter substances.

Acknowledgements

This investigation was supported in part by a grant CA 17625 from the National Cancer Institute awarded to K.H.L. This study was also supported in part by a grant from the Ministry of Education, Sciences, Sports and Cultures, and Ministry of Health and Welfare, Japan (Kyoto).

References

[1] Y. Sugiyama, S. Kawakishi, and T. Osawa, *Biochem. Pharmacol.* **52**, 519 (1996).
[2] A.J. Rudy, G. Kuttan, K.D. Babu, K.N. Rajasekharan, and R. Kuttan, *Cancer Lett.* **94**, 79 (1995).
[3] M.T. Huang, R.C. Smart, C.Q. Wong, and A.H. Conney, *Cancer Res.* **48**, 5941 (1988).
[4] J. Ishida, M. Kozuka, H. Wang, T. Konoshima, H. Tokuda, M. Okuda, X.Y. Mou, H. Nishino, N. Sakurai, K.H. Lee, and M. Nagai, *Cancer Lett.* **159**, 135 (2000).
[5] J. Ishida, M. Kozuka, H. Tokuda, H. Nishino, S. Nagumo, and K.H. Lee, *Bioorg. Med. Chem.* **10**, 3361 (2002).
[6] N. Motohashi, C. Yamagami, H. Tokuda, T. Konoshima, Y. Okuda, T. Mukainaka, H. Nishino, and Y. Saito, *Cancer Lett.* **134**, 37 (1998).
[7] C. Ito, M. Itoigawa, H. Furukawa, E. Ichiishi, T. Mukainaka, M. Okuda, M. Ogata, H. Tokuda, and H. Nishino, *Cancer Lett.* **142**, 49 (1999).
[8] C. Ito, M. Itoigawa, T. Otsuka, H. Tokuda, H. Nishino, and H. Furukawa, *J. Nat. Prod.* **63**, 1344 (2000).
[9] M. Itoigawa, C. Ito, H. Tokuda, F. Enjo, H. Nishino, and H. Furukawa, *Cancer Lett.* **214**, 165 (2004).
[10] C. Ito, M. Itoigawa, T. Takakura, N. Ruangrungsi, F. Enjo, H. Tokuda, H. Nishino, and H. Furukawa, *J. Nat. Prod.* **66**, 200 (2003).
[11] C. Ito, M. Itoigawa, N. Kojima, H. Tokuda, T. Hirata, H. Nishino, and H. Furukawa, *J. Nat. Prod.* **67**, 1125 (2004).
[12] T. Akihisa, H. Tokuda, M. Ukiya, M. Iizuka, S. Schneider, K. Ogasawara, T. Mukainaka, K. Iwatsuki, T. Suzuki, and H. Nishino, *Cancer Lett.* **201**, 133 (2003).
[13] C. Ito, M. Itoigawa, Y. Miyamoto, S. Onoda, K.S. Rao, T. Mykainaka, H. Tokuda, H. Nishino, and H. Furukawa, *J. Nat. Prod.* **66**, 206 (2003).
[14] M. Itoigawa, C. Ito, T.S. Wu, F. Enjo, H. Tokuda, H. Nishino, and H. Furukawa, *Cancer Lett.* **193**, 133 (2003).
[15] J. Tatsuzaki, K.F. Bastow, K. Nakagawa-Goto, S. Nakamura, H. Itokawa, and K.H. Lee, *J. Nat. Prod.* **69**, 1445 (2006).
[16] M. Takasaki, T. Konoshima, K. Fujitani, S. Yoshida, H. Nishimura, H. Tokuda, H. Nishino, A. Iwashima, and M. Kozuka, *Chem. Pharm. Bull. (Tokyo)* **38**, 2737 (1990).
[17] H. Tokuda, H. Ohigashi, K. Koshimizu, and Y. Ito, *Cancer Lett.* **33**, 279 (1986).
[18] G. Henle and W. Henle, *J. Bacteriol.* **91**, 1248 (1966).
[19] T. Konoshima, M. Takasaki, T. Tatsumoto, M. Kozuka, R. Kasai, O. Tanaka, R. Nie, H. Tokuda, H. Nishino, and A. Iwashima, *Biol. Pharm. Bull.* **17**, 668 (1994).
[20] N. Sakurai, M. Kozuka, H. Tokuda, Y. Nobukuni, J. Takayasu, H. Nishino, A. Kusano, G. Kusano, M. Nagai, Y. Sakurai, and K.H. Lee, *Bioorg. Med. Chem.* **11**, 1137 (2003).