Accepted Manuscript

Synthesis, cytotoxicity against human oral cancer KB cells and structure–activity relationship studies of trienone analogues of curcuminoids

Thipphawan Chuprajob, Chatchawan Changtam, Ratchanaporn Chokchaisiri, Warangkana Chunglok, Nilubon Sornkaew, Apichart Suksamrarn

PII: S0960-894X(14)00462-4
DOI: http://dx.doi.org/10.1016/j.bmcl.2014.04.105
Reference: BMCL 21598

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 25 January 2014
Revised Date: 15 April 2014
Accepted Date: 25 April 2014

Please cite this article as: Chuprajob, T., Changtam, C., Chokchaisiri, R., Chunglok, W., Sornkaew, N., Suksamrarn, A., Synthesis, cytotoxicity against human oral cancer KB cells and structure–activity relationship studies of trienone analogues of curcuminoids, *Bioorganic & Medicinal Chemistry Letters* (2014), doi: http://dx.doi.org/10.1016/j.bmcl.2014.04.105

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Synthesis, cytotoxicity against human oral cancer KB cells and structure–activity relationship studies of trienone analogues of curcuminoids

Thipphawan Chuprajob [a], Chatchawan Changtam [b], Ratchanaporn Chokchaisiri [c], Warangkana Chunglok [d], Nilubon Sornkaew [a], Apichart Suksamrarn [a,*]

[a] *Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand*

[b] *Division of Physical Science, Faculty of Science and Technology, Huachiew Chalermprakiet University, Samutprakarn 10540, Thailand*

[c] *Department of Chemistry, School of Science, Phayao University, Phayao 56000, Thailand*

[d] *School of Allied Health Sciences and Public Health, Walailak University, Nakhon Si Thammarat 80160, Thailand*

* Corresponding author. Tel.: +66 2 319 0931; Fax: +66 2 319 0931.

E-mail addresses: s_apichart@ru.ac.th, asuksamrarn@yahoo.com (A. Suksamrarn).

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

ABSTRACT

A general method for the synthesis of substituted (1*E*,4*E*,6*E*)-1,7-diphenylhepta-1,4,6-trien-3-ones, based on the aldol condensations of substituted 4-phenylbut-3-en-2-ones and substituted 3-phenylacrylaldehydes, was achieved. The natural trienones **4** and **5** have been synthesized by this method, together with the trienone analogues **9**–**20**. These analogues were evaluated for their cytotoxic activity against human oral cancer KB cell line. The structure–activity relationship study has indicated that the analogues with the 1,4,6-trien-3-one function are more potent than the curcuminoid-type function. Analogues with *meta*-oxygen function on the aromatic rings are more potent than those in the *ortho*- and *para*-positions. Free phenolic hydroxy group is more potent than the corresponding methyl ether analogues. Among the potent trienones, compounds **11**, **18** and **20** were more active than the anticancer drug ellipticine. All compounds were also evaluated against the non-cancerous Vero cells and it was found that compounds **11**, **12** and **17** were much less toxic than curcumin (**1**); they showed high selectivity indices of 35.46, 33.46 and 31.68, respectively. These analogues are regarded as the potent trienones for anti-oral cancer study.

ACCEPTED MANUSCRIPT

Keywords:

Curcuminoid; Trienone analogue; Synthesis; Cytotoxicity; KB

ACCEPTED MANUSCRIPT

Curcuminoids are the major constituents of turmeric (*Curcuma longa* L., Zingiberaceae) and have been used for centuries as a dietary pigment, spice and traditional medicine in India and China. The major curcuminoid isolated from this plant species is curcumin (**1**), with demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**) as the minor constituents. Curcuminoids exhibited many interesting biological activities,[1] for example, antioxidant activity,[2,3] anti-inflammatory activity,[4,5] anticancer activity,[6,7] anti-protozoal activity[8] and anti-HIV activity.[9] In clinical report, curcuminoids could be orally taken up to 12 g/day without toxic effect in humans.[10] Therefore, many researchers have used this class of compounds to study different biological activities. Some of these studies investigated the role of α,β-unsaturated β-diketo moiety and it was concluded that this functional group is necessary for the biological activities.[11,12] Recently, a new curcuminoid analogue, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (**4**) (see Fig. 1), which is structurally related to the curcuminoid **3**, has been isolated as a minor component of *C. longa*.[13] This compound inhibited the production of TNF-α by lipopolysaccharide-activated macrophages.[14] Another new curcuminoid analogue, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one (**5**), which is structurally related to the curcuminoid **1**, has later been isolated.[15] It is interesting to note that these two compounds possess a 1*E*,4*E*,6*E*-heptatrien-3-one functionality, which is a rare group of naturally occurring curcuminoid analogue and has not much been studied, especially the biological activities of this type of compounds.

Insert Figure 1 ——————→

The structural diversity of the trienones **4** and **5** has prompted us to investigate their biological activities. However, the scarcity of these compounds in natural sources has prevented us from obtaining them for biological activity evaluations. In order to see whether this group of curcuminoid analogues exhibited more interesting biological activities than those of the curcuminoids **1**–**3**, we decided to synthesize the natural trienones **4** and **5**. Within the heptatrien-3-one framework, the analogues containing different hydroxy and methoxy substituents on the aromatic moieties would then be synthesized if the trienones **4** and **5** showed higher biological activity than the corresponding curcuminoids **3** and **1**.

Oral cancer is a major health problem in the economically developing countries. According to GLOBOCAN 2012, oral cancer was estimated to account for 2.7% of all cancers.[16] There were about 300,000 oral cancer cases in 2012 worldwide and about 50% of

ACCEPTED MANUSCRIPT

patients died from this type of cancer. The five-year relative survival rate of oral cancer patients is less than 35% in advanced stage of disease at initial diagnosis.[17] Late diagnosis, disease recurrence, metastasis and resistant to therapy may attribute to this poor survival rate. Conventional treatments, including surgical treatment combined with radiotherapy and chemotherapy or concomitant chemo-radiotherapy, have limited efficacy and result in adverse systemic and cytotoxic effects on normal cells. Chemoprevention is a promising treatment strategy for oral cancer. Appropriate chemopreventive agents should be inexpensive, nontoxic, and target important pathways involved in the development of this cancer. Combining chemopreventive agents and conventional therapeutic approaches may reduce toxicities and improving treatment outcomes. The addition of cetuximab, epidermal growth factor receptor (EGFR) targeted chemopreventive agent, to current conventional chemotherapeutic agents (cisplatin and 5-fluorouracil) represents a standard systemic treatment for recurrent or metastatic cancer.[18,19] However, the concurrent therapy also leads to substantial toxicities and the overall survival remains short. To date, various molecular targeted chemopreventive agents are actively under investigation, but the only pharmacologic strategy targeting the EGFR has been approved by regulatory agencies worldwide to treat this oral cancer.[20]

Insert Table 1 ⟶

The synthesis of the trienone **4** has been reported in seven steps in good yield.[21] Compound **5** has previously been prepared by a multi-step chemical modification of compound **1**.[22] However, we would like to have a general method for the synthesis of substituted trienones, so that a wide variety of this group of compounds could be prepared in case the trienones **4** and **5** exhibited better cytotoxic activity than the respective curcuminoids **3** and **1**. The retrosynthetic analysis of (1*E*,4*E*,6*E*)-1,7-diarylhepta-1,4,6-trien-3-ones by cross aldol condensation reaction has been proposed as shown in Scheme 1. Despite the fact that this type of condensation sometimes gives low yield of products,[23] it was nevertheless is a convenient and concise procedure for the synthesis of a large number of analogues for biological evaluation and structure–activity relationship study. Disconnection of the C4–C5 bond of the seven-carbon linker gave two key fragments, substituted 4-phenylbut-3-en-2-ones (substituted cinnamones) and substituted 3-phenylacrylaldehydes (substituted cinnamaldehydes). The resulting substituted 4-phenylbut-3-en-2-ones and substituted 3-

ACCEPTED MANUSCRIPT

phenylacrylaldehydes would then be further disconnected to the substituted benzaldehydes (Scheme 1).

Insert Scheme 1 ——————→

The key fragment substituted 4-phenylbut-3-en-2-ones and 3-phenylacrylaldehydes used for synthesis of the natural trienones (**4**, **5**) and analogues (**9**–**15** and **17**–**20**) were prepared as shown in Scheme 2. Condensation of the substituted benzaldehydes (**6a1**–**d1**, **6e** and **6f**) with excess acetone under basic aldol condensations at room temperature gave the substituted 4-phenylbut-3-en-2-one analogues **7a1**–**d1, 7e** and **7f**. The analogue **7c3** was obtained by methylation of **7c1** using methyl iodide in the presence of potassium carbonate in acetone. The synthesis of substituted 3-phenylacrylaldehydes **8a1**–**d1** were planned by coupling of the substituted benzaldehydes **6a1**–**d1** with acetaldehyde, using aldol condensation in the same method as that of substituted 4-phenylbut-3-en-2-one analogues. In this method, we were able to successfully generate only the *meta*-hydroxy-3-phenylacrylaldehyde analogue **8c1**. However, we failed to generate the *ortho*- and *para*-3-phenylacrylaldehydes **8a1, 8b1** and **8d1**. In view of these results, the *ortho*- and *para*-hydroxy groups of benzaldehydes **6a1, 6b1** and **6d1** were therefore protected as its tetrahydropyranyl (THP) ethers[24] **6a2, 6b2** and **6d2**, which were then condensed with acetaldehyde to yield the intermediates **8a2, 8b2** and **8d2**. Removal of the THP protecting group gave the corresponding 3-phenylacrylaldehydes **8a1, 8b1** and **8d1**, respectively. Methylation of **8c1** yielded the analogue **8c3**.

Insert Scheme 2 ——————→

The trienones **4**, **5** and the analogues were synthesized as shown in Scheme 3. Compound **4** was synthesized by the following two different approaches. Condensation of **7a1** with **8a1** under aldol condition gave the trienone **4** in 29% yield. Alternatively, condensation of **7a1** with the intermediate 3-phenylacrylaldehyde **8a2** gave **4a** and subsequent THP deprotection with 3M HCl yielded the trienone **4**. The trienone **5** was similarly synthesized in 12% yield from **7b1** and **8b1**, or in 14% from **7b1** and 3-phenylacrylaldehyde **8b2** to yield the intermediate **5a** followed by deprotection of the THP group. All other trienone analogues, **9**–**15** and **17**–**20**, were similarly obtained from direct coupling of substituted hydroxy and/or methoxy 4-phenylbut-3-en-2-ones (**7a1**–**d1, 7e, 7f** and **7c3**) with substituted hydroxy or

ACCEPTED MANUSCRIPT

methoxy 3-phenylacrylaldehydes (**8a1**, **8c1, 8d1** and **8c3**) (Scheme 3). The trienone **16** was obtained by methylation of compound **11**. All synthesized analogues were purified by crystallization or column chromatography and characterized by NMR (^{1}H and ^{13}C NMR, and 2D COSY, HMQC and HMBC) and mass spectroscopy (see Supplementary data).

Insert Scheme 3 ——————→

The natural curcuminoids **1**–**3**, the trienone analogues **4** and **5**, and analogues **9**–**20** were subjected to cytotoxic activity evaluation against the KB cells using resazurin microplate assay for cancer cell growth inhibition[25] and the results are presented in Table 1. Compounds **1**–**3** exhibited weak cytotoxicity against this cancer cell line, with the IC_{50} values of 21.36, 26.45 and 21.44 μM, respectively, which was much less active than ellipticine, the reference anticancer drug, which exhibited cytotoxicity against KB cells at IC_{50} of 2.25 μM. The trienone analogues **4** and **5**, however, showed strong and moderate cytotoxic effects at IC_{50} of 5.75 and 12.23 μM, respectively. An increase in cytotoxicity of 1.7-fold from the curcuminoid **1** to the trienone **5**, and 3.7-fold from the curcuminoid **3** to the trienone **4** was very significant; it implied that the 1,4,6-trien-3-one moiety contributed to higher cytotoxicity against the KB cells than the 1,6-dien-3,5-dione moiety of the curcuminoids. This finding has prompted us to further investigate cytotoxicity of other trienone analogues. The strategy of was to modify the trienone structure by varying number and position of the oxygen functions (hydroxyl/methoxy groups). As the trienone **4** was about 2-fold more potent than the trienone **5**, it was used as the structure lead for analogues with higher cytotoxic activity. In order to see whether substitution pattern of the oxygen function on the aromatic rings affected the cytotoxicity of the trienone structure, compound **9**, the B-ring *meta*-hydroxy analogue of the trienone **4**, was synthesized and it was found that this analogue was approximately 2-fold more active than **4**, with the IC_{50} of 2.98 μM. In order to see the effect of oxygenation patterns on ring B compared to the *para*- and *meta*-hydroxy analogues **4** and **9**, the *ortho*-hydroxy analogue **10** was synthesized and it was found that its cytotoxicity (IC_{50} 8.16 μM) was lower than both the analogues **4** and **9**. The oxygen function at the *meta*-position of the B-ring is therefore essential for high cytotoxicity against KB cells. In order to see the effect of oxygenation patterns on ring A, the high cytotoxic compound **9** was further modified by moving the hydroxy group at the *para*-position on the A ring to the *meta*-position to give the trienone **11** and this analogue was found to be highly toxic against the KB cells; the IC_{50} of

ACCEPTED MANUSCRIPT

this compound was 1.72 μM, or 3.3-fold more active than the trienone **4**. It is worth noting that the trienone **11** was 1.3-fold more active than ellipticine. In order to see the effect of replacement of the *meta*-hydroxy group on the A-ring of compound **11** by the *ortho*-hydroxy group, compound **12** was synthesized and its cytotoxic activity was 1.4-fold less active than **11**; its IC_{50} value was 2.35 μM, which was almost as active as ellipticine. As expected, placement of the hydroxy groups at the *ortho*-positions on the A- and B-rings in analogue **13** resulted in decrease in activity compared with the *meta*- or *para*-dihydroxy analogue; it exhibited moderate cytotoxicity with IC_{50} of 11.19 μM, that is 6.5- and 2-fold less active than compounds **11** and **4**, respectively. The results have indicated that the presence of hydroxy group at the *meta*-position of the A-ring was also responsible for the increase in cytotoxicity against KB cells. The contribution of free hydroxy group on cytotoxic activity was demonstrated by the methyl ether **14** that showed 2.6-fold decrease in activity when compared with compound **11**. Sharp decrease in cytotoxicity was observed in the isomeric B-ring methyl ether **15**; it was 11-fold less active than compound **11**. As expected, full methylation of **11** resulted in the analogue **16** which showed weak cytotoxic activity.

In order to see the effect of an extra oxygen function on the aromatic ring, the A-ring 3-hydroxy-4-methoxy analogue **17** was evaluated for cytotoxicity and this compound exhibited 1.7-fold less active than compound **11**. The isomeric A-ring 4-hydroxy-3-methoxy analogue **18**, however, exhibited unexpectedly high activity; it showed comparable cytotoxic potency (IC_{50} 1.70 μM) to that of compound **11**. In order to compare the B-ring *para*-hydroxy analogue of compound **18** and to make a complete set of the trienone analogue of demethoxycurcumin (**2**), the trienone **19** was synthesized and cytotoxicity was evaluated. As expected, it was more active (IC_{50} 7.57 μM) than the curcuminoid **2** and was less active than its isomeric B-ring *meta*-hydroxy analogue **18**. Removal of the methyl group from either compound **17** or **18** gave the corresponding trihydroxy analogue **20**. Surprisingly, compound **20** exhibited the most active cytotoxicity among the trienones tested; its IC_{50} was 1.36 μM, or 1.7-fold more active than ellipticine.

The results have indicated that the analogues with the 1,4,6-trien-3-one function are more potent than the curcuminoids with the 1,6-dien-3,5-dione function. Analogues with *meta*-oxygen function on both aromatic rings are more potent than those in the *ortho*- and *para*-positions. Free phenolic hydroxy group is more potent than the corresponding methyl ether analogues.

To assess whether the cytotoxic activity described above should be attributed to general toxicity, rather than specific cytotoxic activity of compounds to the cancer cells, these

ACCEPTED MANUSCRIPT

analogues were also tested for their effect on the normal cells (Vero cells, the African green monkey kidney cells) using green fluorescent protein (GFP) detection method[26] and the results are included in Table 1. The results have indicated that most of the trienones were toxic against Vero cells and were more toxic than curcumin (**1**), which was regarded as weakly toxic, except compounds **11**, **12**, **16** and **17**, which were approximately 2- to 3-fold less active than the curcuminoid **1**. The selectivity indices (SIs, Table 1) of the toxic trienones were between 0.55 and 6.17, whereas those of **1** and ellipticine were 1.64 and 2.88, respectively. In contrast, the SIs of the non-toxic or less toxic trienones, **11**, **12** and **17**, were 35.46, 33.46 and 31.68, which were relatively very high. Among these three highly active trienones with high SIs, the trienone **11** seems to be the most suitable compound for further in-depth study.

In conclusion, we report the general method for the synthesis of the (1*E*,4*E*,6*E*)-1,7-diarylhepta-1,4,6-trien-3-one based on the aldol condensations of substituted 4-phenylbut-3-en-2-ones and substituted 3-phenylacrylaldehydes. The natural trienones **4** and **5** have been synthesized by this method, together with the trienone analogues **9**–**20**. These analogues were evaluated for their cytotoxic effects against KB cell line. The results have indicated that the analogues with the 1,4,6-trien-3-one function are more potent than the curcuminoids. Analogues with *meta*-oxygen function on the aromatic rings are more potent than those in the *ortho*- and *para*-positions. Free phenolic hydroxy group is more potent than the corresponding methyl ether analogues. Among the potent trienones, compounds **11**, **18** and **20** were more active than the anticancer drug ellipticine. All compounds were also evaluated against the non-cancerous Vero cells and it was found that compounds **11**, **12** and **17** were much less toxic than curcumin (**1**); they showed high selectivity indices of 35.46, 33.46 and 31.68, respectively.

Acknowledgments

This work was supported by The Thailand Research Fund. Financial support from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education is gratefully acknowledged.

ACCEPTED MANUSCRIPT

Supplementary data

Supplementary data (^{1}H, ^{13}C NMR and DEPT spectra and mass spectroscopic data) associated with this article, can be found, in the online version, at ….

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

References and notes

1. Maheshwari, R. K.; Singh, A. K.; Gaddipatti, J.; Srimal, R. C. *Life Sci.* **2006**, *78*, 2081.
2. Ruby, A. J.; Kuttan, G.; Babu, K. D.; Rajasekharan, K. N.; Kuttan, R. *Cancer Lett.* **1995**, *94*, 79.
3. Grinberg, L. N.; Shalev, O.; Tønnesen, H. H.; Rachmilewitz, E. A. *Int. J. Pharmaceut.* **1996**, *132*, 251.
4. Chan, M. M.-Y.; Ho, C.-T.; Huang, H.-I. *Cancer Lett.* **1995**, *96*, 23.
5. Chan, M. M.-Y.; Huang, H.-I.; Fenton, M. R.; Fong, D. *Biochem. Pharmacol.* **1998**, *55*, 1955.
6. Inano, H.; Onoda, M.; Inafuku, N.; Kubota, M.; Kamada, Y.; Osawa, T.; Kobayashi, H.; Wakabayashi, K. *Carcinogenesis* **2000**, *21*, 1835.
7. Thapliyal, R.; Maru, G. B. *Food Chem. Toxicol.* **2001**, *39*, 541.
8. Araujo, C. A. C.; Alegrio, L. V.; Gomes, D. C. F.; Lima, M. E. F.; Gomes-Cardoso, L.; Leon, L. L. *Mem. Inst. Oswaldo. Cruz.* **1999**, *94*, 791.
9. Mazumder, A.; Raghavan, K.; Weinstein, J.; Kohn, K. W.; Pommier, Y. *Biochem. Pharmacol.* **1995**,*49*, 1165.
10. Liang, G.; Shao, L.; Wang, Y.; Zhao, C.; Chu, Y.; Xiao, J.; Zhao, Y.; Li, X.; Yang, S. *Bioorg. Med. Chem.* **2009**, *17*, 2623.
11. Pan, M.-H.; Lin-Shiau, S.-Y.; Lin, J.-K. *Biochem. Pharmacol.* **2000**, *60*, 1665.
12. Ohtsu, H.; Xiao, Z.; Ishida, J.; Nagai, M.; Wang, H.-K.; Itokawa, H.; Su, C.-Y.; Shih, C.; Chiang, T.; Chang, E.; Lee, Y. F.; Tsai, M.-Y.; Chang, C.; Lee, K.-H. *J. Med. Chem.* **2002**, *45*, 5037.
13. Nakayama, R.; Tamura, Y.; Yamanaka, H.; Kikuzaki, H,; Nakatani, N. *Phytochemistry* **1993**, *33*, 501.
14. Jang, M. K.; Sohn, D. H.; Ryu, J.-H. *Planta Med.* **2001**, *67*, 550.
15. Park, S.-Y.; Kim, D. S. H. L. *J. Nat. Prod.* **2002**, *65*, 1227.
16. Ferlay, J.; Soerjomataram, I.; Ervik, M.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D. M.; Forman, D.; Bray, F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed on 4/4/2014.
17. Siegel, R.; Naishadham, D.; Jemal, A. *CA Cancer J. Clin*. **2012**, *62*, 10.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

18. Peyrade, F.; Cupissol, D.; Geoffrois, L.; Rolland, F.; Borel, C.; Ciais, C.; Faivre, S.; Guigay, J. *Oral Oncol.* **2013**, *49*, 482.
19. Vermorken, J. B.; Licitra, L.; Stöhlmacher-Williams, J.; Dietz, A.; Lopez-Picazo, J. M.; Hamid, O.; Hossain, A. M.; Chang, S. C.; Gauler, T. C. *Eur. J. Cancer* **2013**, *49*, 2877.
20. Du, Y.; Peyser, N. D.; Grandis, J. R. *Pharmacol. Ther.* **2014**, *142*, 88.
21. Da, S. J.; Huang, C. S.; Li, X. H.; Li, Y. L. *Chin. Chem. Lett.* **1997**, *8*, 291.
22. Changtam, C.; De Koning, H. P.; Ibrahim, H.; Sajid, M. S.; Gould, M. K.; Suksamrarn, A. *Eur. J. Med. Chem.* **2010**, *45*, 941.
23. (a) Shen, L.; Sun, D. *Tetrahedron Lett.* **2011**, *52*, 4570; (b) Dhuru, S.; Bhedi, D.; Gophane, D.; Hirbhagat, K.; Nadar, V.; More, D.; Parikh, S.; Dalal, R.; Fonseca, L. C.; Kharas, F.; Vadnal, P. Y.; Vishwakarma, R. A.; Sivaramakrishnan, H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3784.
24. (a) Munro, T. A.; Duncan, K. K.; Xu, W.; Wang, Y.; Liu-Chen, L.-Y.; Carlezon, W. A., Jr.; Cohen, B. M.; Béguin, C. *Bioorg. Med. Chem.* **2008**, *16*, 1279; (b) Banaszak, E.; Xu, L.-W.; Bardeau, J.-F.; Castanet, A.-S.; Mortier, J. *Tetrahedron* **2009**, *65*, 3961.
25. Brien, J. O.; Wilson, I.; Orton, T.; Pognan, F. *Eur. J. Biochem.* **2000**, *267*, 5421.
26. Hunt, L.; Jordan, M.; De Jesus, M.; Wurm, F. M. *Biotechnol. Bioeng.* **1999**, *65*, 201.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Captions

Figure 1. Structures of the natural curcuminoids, curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**), and the natural trienones **4** and **5**.

Scheme 1. Retrosynthesis of (1*E*,4*E*,6*E*)-1,7-diphenylhepta-1,4,6-trien-3-one analogues.

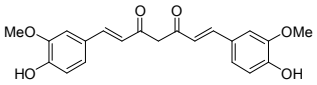
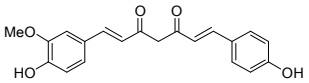
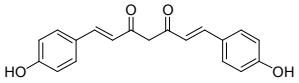
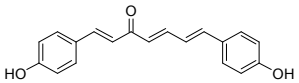
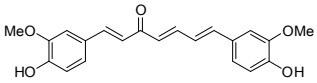
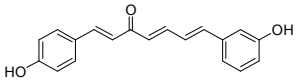
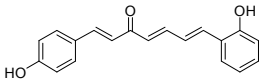
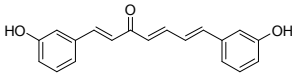
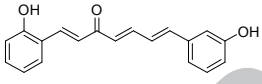
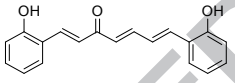
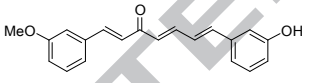
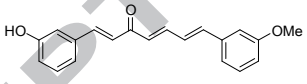
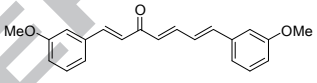
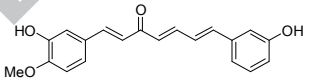
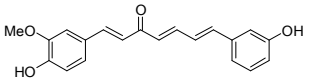
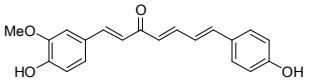
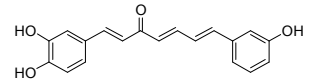
Scheme 2. Synthesis of the substituted 4-phenylbut-3-en-2-ones **7a**–**f** and the substituted 3-phenylacrylaldehydes **8a**–**d** from the aldehydes **6a**–**f**. Reagents and conditions: (i) excess acetone, 20% aq. NaOH, EtOH; (ii) 3,4-dihydro-*2H*-pyran, *p*-TsOH, THF; (iii) MeI, K_2CO_3, acetone, rt; (iv) excess acetaldehyde, 20% aq. NaOH, EtOH, 0–10 ^oC; (v) *p*-TsOH, MeOH–H_2O.

Scheme 3. Synthesis of the trienones **4**, **5**, **9**–**15** and **17**–**20** from aldol condensation of the substituted 4-phenylbut-3-en-2-ones **7a**–**f** and the substituted 3-phenylacrylaldehydes **8a**–**d**, and methylation of the trienone **11** to **16**. Reagents and conditions: (i) 20% aq. NaOH, EtOH, 0 ^oC–rt; (ii) 3M HCl; (iii) MeI, K_2CO_3, acetone, rt.

ACCEPTED MANUSCRIPT

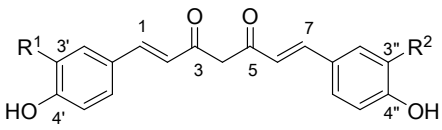
ACCEPTED MANUSCRIPT

Table 1
The structure and cytotoxicity of trienones against KB and Vero cells and selectivity index

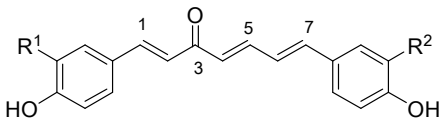
	Compound/Structure	Cytotoxicity (IC_{50}, μM)		SI[c]
		KB[a]	Vero[b]	
1		21.36	35.05	1.64
2		26.45	CD[d]	-
3		21.44	CD[d]	-
4		5.75	9.65	1.68
5		12.23	6.78	0.55
9		2.98	10.81	3.63
10		8.16	12.18	1.49
11		1.72	60.99	35.46
12		2.35	78.64	33.46
13		11.19	19.60	1.75
14		4.47	8.00	1.79
15		19.06	5.68	0.30
16		20.10	54.37	2.70
17		2.90	91.86	31.68
18		1.70	10.49	6.17
19		7.57	14.36	1.90
20		1.36	6.97	5.13
Ellipticine		2.25	6.48	2.88

[a] Cancer of the oral cavity.
[b] Green African monkey kidney cells.
[c] Selectivity index = IC_{50} for normal cell line/IC_{50} for cancerous cell line.
[d] Could not be determined due to fluorescence behavior of the compound.

ACCEPTED MANUSCRIPT



1: $R^1 = R^2 = OMe$
2: $R^1 = OMe$, $R^2 = H$
3: $R^1 = R^2 = H$

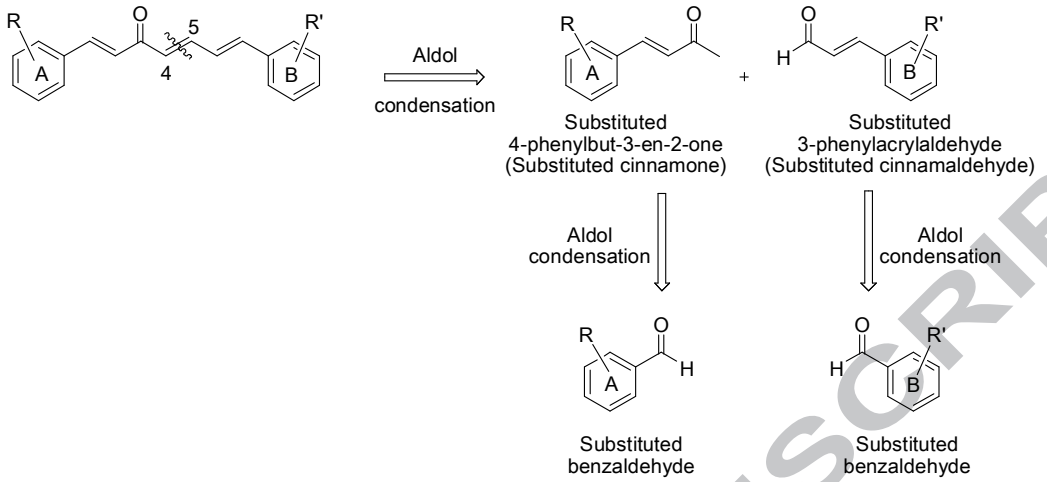


4: $R^1 = R^2 = H$
5: $R^1 = R^2 = OMe$

Figure 1. Structures of the natural curcuminoids, curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**), and the natural trienones **4** and **5**.

ACCEPTED MANUSCRIPT

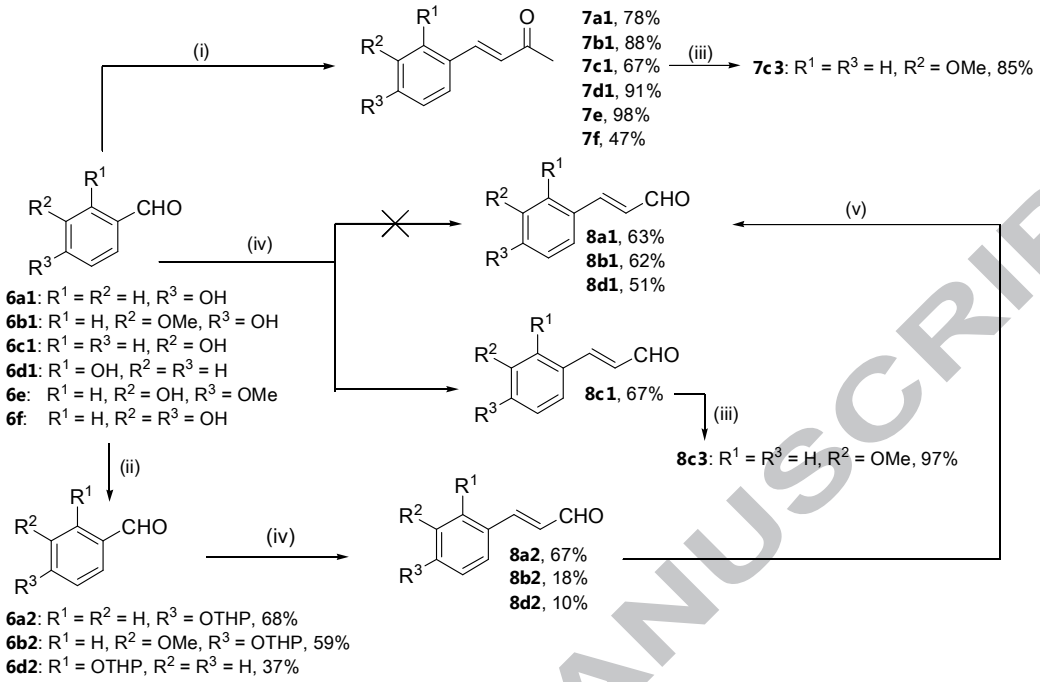
ACCEPTED MANUSCRIPT



Scheme 1. Retrosynthesis of (1*E*,4*E*,6*E*)-1,7-diphenylhepta-1,4,6-trien-3-one analogues.

ACCEPTED MANUSCRIPT

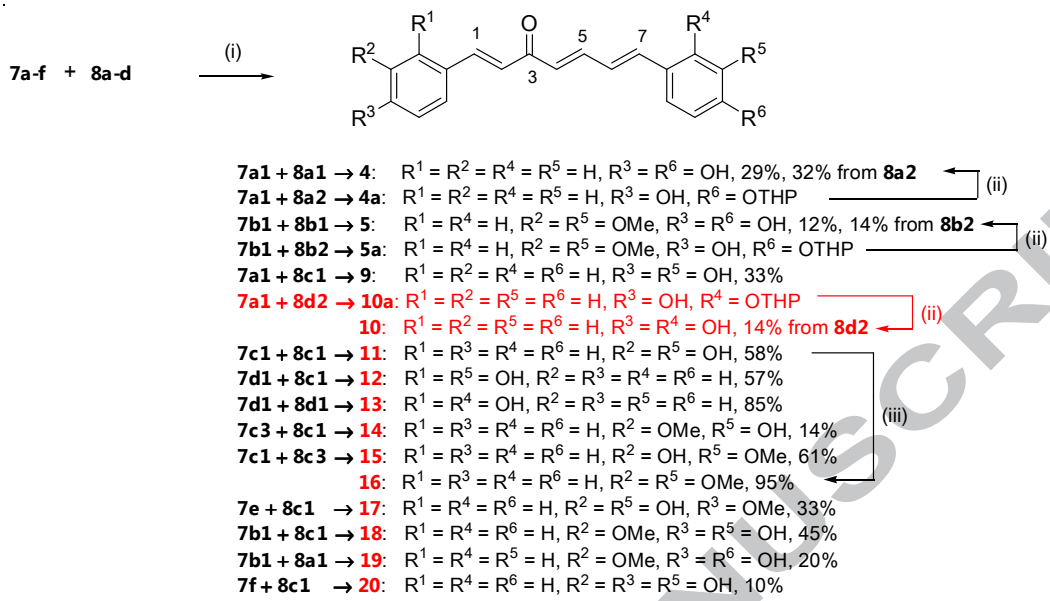
ACCEPTED MANUSCRIPT



Scheme 2. Synthesis of the substituted 4-phenylbut-3-en-2-ones **7a–f** and the substituted 3-phenylacrylaldehydes **8a–d** from the aldehydes **6a–f**. Reagents and conditions: (i) excess acetone, 20% aq. NaOH, EtOH; (ii) 3,4-dihydro-*2H*-pyran, *p*-TsOH, THF; (iii) MeI, K_2CO_3, acetone, rt; (iv) excess acetaldehyde, 20% aq. NaOH, EtOH, 0–10 ^oC; (v) *p*-TsOH, MeOH–H_2O.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



Scheme 3. Synthesis of the trienones **4**, **5**, **9**–**15** and **17**–**20** from aldol condensation of the substituted 4-phenylbut-3-en-2-ones **7a**–**f** and the substituted 3-phenylacrylaldehydes **8a**–**d**, and methylation of the trienone **11** to **16**. Reagents and conditions: (i) 20% aq. NaOH, EtOH, 0 ^{o}C–rt; (ii) 3M HCl; (iii) MeI, K_2CO_3, acetone, rt.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Graphical_abstract_REVISED

Synthesis, cytotoxicity against human oral cancer KB cells and structure–activity relationship studies of trienone analogues of curcuminoids

Thipphawan Chuprajob, Chatchawan Changtam, Ratchanaporn Chokchaisiri, Warangkana Chunglok, Nilubon Sornkaew, Apichart Suksamrarn*