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

Thipphawan Chuprajoba, Chatchawan Changtamb, Ratchanaporn Chokchaisiric, Warangkana Chungl, Nilubon Sornkaewa, Apichart Suksamrarna

A general method for the synthesis of substituted (1E,4E,6E)-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-phenylacrylic aldehydes, 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.

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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 2.1% of all cancers. There were about 300,000 oral cancer cases in 2012 worldwide and about 50% of 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. 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 improve 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. 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.

The synthesis of the trienone 4 has been reported in seven steps in good yield. Compound 5 has previously been prepared by a multi-step chemical modification of compound 1. 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 (1E,4E,6E)-1,7-dialdehyde-heta-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, it was nevertheless 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-phenylacrylaldehydes would then be further disconnected to the substituted benzaldehydes (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 analogues 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 tetrahydro-pyranal (THP) ethers 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.

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 3 M 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 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 (1H and 13C NMR, and 2D COSY, HMQC and HMBC) and mass spectroscopy (see Supplementary data).

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 and the results are presented in Table 1. Compounds 1–3 exhibited weak cytotoxicity against this cancer cell line, with the IC50 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 IC50 of 2.25 μM. The trienone analogues 4 and 5, however, showed strong and moderate cytotoxic effects at IC50 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...
### Table 1
The structure and cytotoxicity of trienones against KB and Vero cells and selectivity index

<table>
<thead>
<tr>
<th>Compound/structure</th>
<th>Cytotoxicity (IC_{50}, µM)</th>
<th>SI</th>
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<tr>
<td></td>
<td>KB(^a)</td>
<td>Vero(^b)</td>
</tr>
<tr>
<td>1</td>
<td>21.36</td>
<td>35.05</td>
</tr>
<tr>
<td>2</td>
<td>26.45</td>
<td>CD(^f)</td>
</tr>
<tr>
<td>3</td>
<td>21.44</td>
<td>CD(^f)</td>
</tr>
<tr>
<td>4</td>
<td>5.75</td>
<td>9.65</td>
</tr>
<tr>
<td>5</td>
<td>12.23</td>
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of other trienone analogues. The strategy was to modify the trienone structure by varying number and position of the oxygen functions (hydroxy/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 IC₅₀ 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 (IC₅₀ 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 IC₅₀ of 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 IC₅₀ 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 IC₅₀ 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 (IC₅₀ 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 (IC₅₀ 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 IC₅₀ 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 curcuminoïds with the 1,4,6-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 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 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 toxic than the curcuminoid 1. The selectivity indices (SIs, Table 1) of the toxic trienones were between 0.30 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 (1E,4E,6E)-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-phenylacylaldehydes. 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 curcuminoïds. 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.

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Supplementary data

Supplementary data (1H, 13C NMR and DEPT spectra and mass spectroscopic data) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.105.

References and notes