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The C8 side chain is one of the key functional group of Garcinol for its anti-cancer effects

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ABSTRACT

Garcinol from the fruit rind of *Garcinia indica* shows anti-carcinogenic and anti-inflammatory properties, but its mechanism and key functional groups were still need to be identified. Our previous computer modeling suggested that the C8 side chain of Garcinol is so large that it may influence the bioactivity of the compound. 8-Me Garcinol, a derivative of Garcinol in which the bulky side chain at the C8 position of Garcinol is replaced with a much smaller methyl group, was synthesized through a 12-step procedure starting from 1,3-cyclohexanedione. The antitumor activity of Garcinol and 8-Me Garcinol was evaluated *in vitro* by MTT, cell cycle and cell apoptosis assays. The results showed that 8-Me Garcinol had weaker inhibitory activity on cells proliferation, and little effects on cell cycle and apoptosis in oral cancer cell line SCC15 cells when compared with Garcinol. All of the results indicated 8-Me Garcinol exerts weaker antitumor activity than Garcinol, and the C8 side chain might be an important active site in Garcinol. Changing the C8 side chain will affect the inhibitory effect of Garcinol.

1. Introduction

Garcinol is a natural polyisoprenylated benzophenone [1] harvested from the dried rind of the fruit of *Garcinia indica*, a plant found extensively in tropical regions [2]. The dried rind of *Garcinia* is used as a food ingredient, garnish and medicine for treating inflammatory and infectious diseases in India and Southeast Asia [3–5]. The chemical structure of Garcinol is similar to that of a well-known antioxidant, curcumin, which contains both phenolic hydroxyl groups and a β-diketone moiety, and Garcinol shows strong antioxidant activity [6,7]. Other studies confirm the antioxidant activity of Garcinol, and reveal anti-inflammatory and anti-cancer activity as well [8–10]. Garcinol is an inhibitor of histone acetyltransferase p300, a transcriptional regulator [11]. In addition, Garcinol showed biological activities in cell culture studies, including anti-proliferation, induction of apoptosis, inhibition of COX-2, 5-LOX and iNOS expression [3,9,12]. However, the active sites and targets of Garcinol are unclear.

Our previous research showed that Garcinol had a chemopreventive effect on 7, 12-dimethylbenz [α] anthracene (DMBA)-induced acute inflammation and oral squamous cell carcinoma (OSCC) of hamster cheek pouch through inhibiting 5-lipoxygenase (5-LOX) [13]. Computer modeling predicts that Garcinol could combine with the 5-LOX active site, and that the 13,14-dihydroxy groups of Garcinol play an important role in inhibiting the activity of 5-LOX. Our recent research has confirmed that the 13, 14-dihydroxy groups are critical for the anti-cancer activity of Garcinol [14]. The computer model also indicates that the size of the C8 side chain in Garcinol is so large that the bulky group might cause poor permeability and reduce the anti-cancer effect of Garcinol [13].

By employing molecular pruning and classical isosterism approach, Milite et al. simplified the core structure of Garcinol to identify the minimal structural elements required for KAT inhibitory activity [15]. Their optimization led to the discovery of a benzylidenebarbituric acid derivative EML425, which is a potent and selective reversible inhibitor of two paralogue KAT3 acetyltransferases (CBP and p300), and endowed with good cell permeability.

In this study, 8-Me Garcinol (Scheme 1) was designed by replacing the bulky C8 side chain of Garcinol with a much smaller methyl group. Then we evaluated the new derivative against oral cancer

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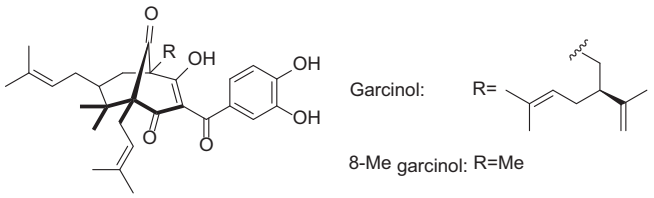


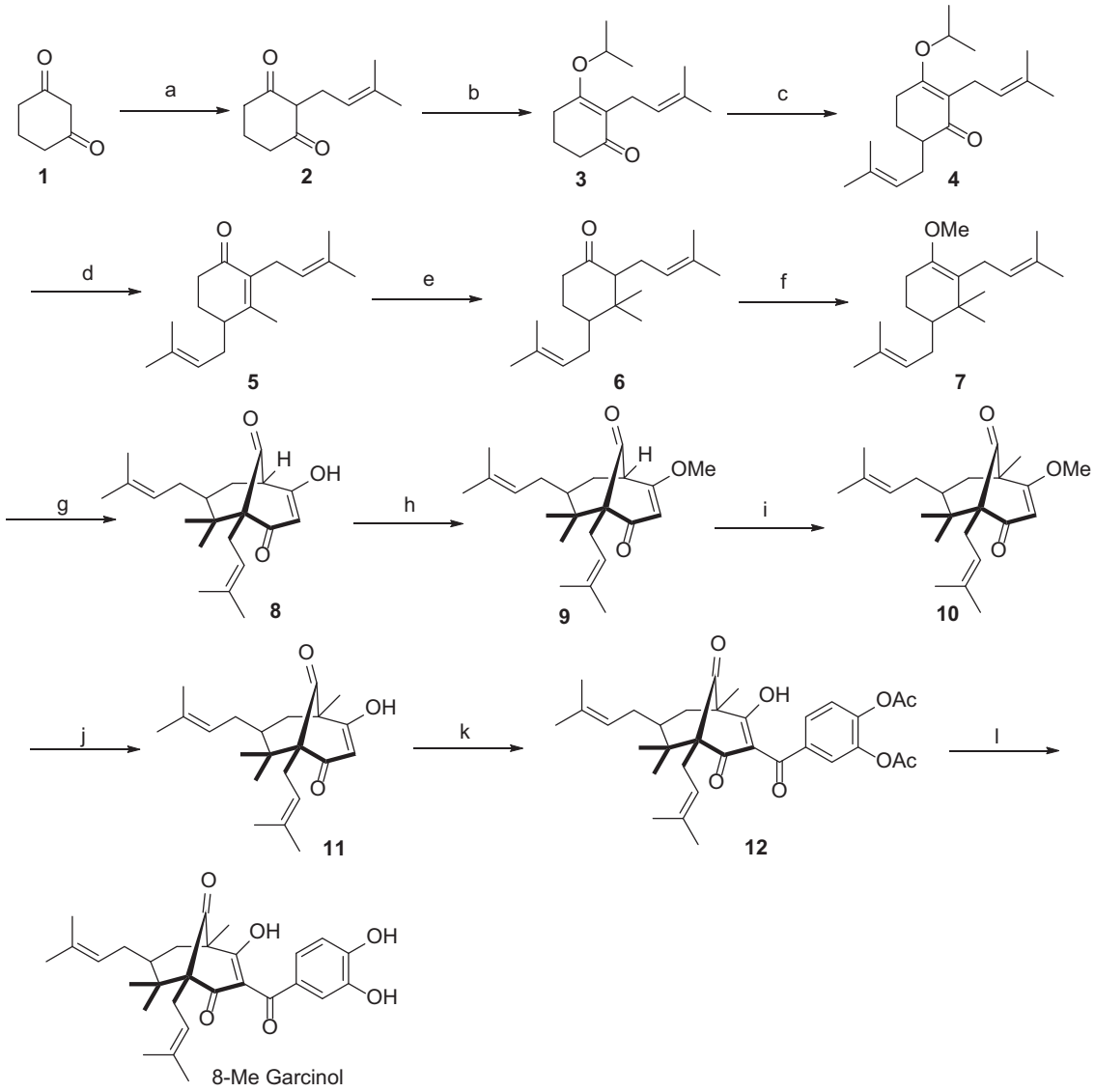
Scheme 1. Chemical structure of Garcinol and 8-Me Garcinol.

cell line SCC15 for cell proliferation, cell cycle, and apoptosis in oral squamous carcinoma cells.

2. Results and discussion

2.1. Synthesis of 8-Me Garcinol

The synthetic route for 8-Me Garcinol is outlined in Scheme 2, starting from (**1**), 3-cyclohexanedione. Alkylation of the starting material with 3,3-dimethylallyl bromide gave 2-(3-methylbut-2-en-1-yl) cyclohexan-1, 3-dione (**2**). A second 3,3-dimethylallyl group was introduced to the 6-position after one of the carbonyl groups was protected as isopropoxy enol (**3**), and then alkylated with 3,3-dimethylallyl bromide in the presence of the strong base LDA, resulting in formation of 3-isopropoxy-2,6-bis(3-methylbut-2-en-1-yl)cyclohex-2-en-1-one (**4**). Addition of methyllithium to the carbonyl group in (**4**) and followed by acidic hydrolysis and elimination generated methyl cyclohexanone derivative (**5**). The copper-mediated Michael addition of methylmagnesium bromide to (**5**) gave 3, 3-dimethylcyclohexanone derivative (**6**) in 86% yield. After (**6**) was converted into methyl cyclohexenol ether (**7**) by reaction with dimethyl sulfate, (**7**) was cyclized with malonyl dichloride by the catalysis of benzyltriethylammonium chloride to produce a tricyclic intermediate (**8**)[16]. In order to introduce a methyl group at the C8 position, the hydroxy group was protected by treating with trimethyl orthoformate, and the resulting methyl enol ether (**9**) was methylated at the C8 position with iodomethane in the presence of LDA, giving the key intermediate (**11**) after basic



Scheme 2. Synthetic route for 8-Me Garcinol. Reagents and conditions: (a) 3,3-dimethylallyl bromide, *N,N*-diisoproyl ethylamine (DIEA), H_2O, RT, 12 h, 60%; (b) *i*-PrI, K_2CO_3, acetone, 60 °C, 4 h, 93%; (c) LDA, 3,3-dimethylallyl bromide, −78 °C, 1 h, 71%; (d) MeLi, THF, 0 °C-rt, 1 h; 1 N HCl, RT, 30 min, 70%; (e) CuBr·MeS, MeMgBr, HMPA, TMSCl, THF, −78 °C, 2 h, 86%; (f) Me_2SO_4, *t*-BuOK, DMSO, RT, 1 h, 72%; (g) malonyl dichloride, benzyltriethylammonium chloride, ether, −20 °C, 24 h, 27%; (h) trimethyl orthoformate, TsOH, MeOH, 50 °C, 16 h, 81%; (i) MeI, LDA, THF, −78 °C, 1 h, 72%; (j) LiOH, dioxane, H_2O, 90 °C, 4 h, 71%; (k) 4-(cyanocarbonyl)-1,2-phenylene diacetate, Et_3N, THF, RT, 16 h, 73%; (l) K_2CO_3, MeOH, RT, 1 h, 64%.

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