Synthesis and Anti-tumor Activity Study of Shogaol Analogues

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Abstract: As one of the largest global public health problems, cancer greatly endangers human health. Researchers have been committed to discovering anticancer drugs with high efficiency and low toxicity. Shogaol, a kind of ingredient from ginger with high anti-tumor activity, has attracted our attention. This study was an investigation on the composition and structure-activity relationship of different analogues of shogaol, with the purpose to screen for shogaol compounds which had higher anti-tumor activity and could be easily synthesized. We chose zingerone as the starting material to synthesize shogaol through four-step reactions without protective group, as a result, 31 analogues were synthesized for anti-tumor activity detect. The biological test results showed that: the missing of the unsaturated ketone and the phenolic hydroxyl group reduced the anti-tumor activity of shogaol; the site of phenolic hydroxyl had no significant influence on the anti-tumor activity, the indicating that the larger the steric hindrance of substituent, the lower the activity, but the higher the activity of cyclobutyl substituted derivatives.

Key words: Shogaol, synthesis, analogues, anti-tumor activity.

1. Introduction

Cancer is among the greatest threat to global public health and only second to cardiovascular diseases in terms of harm. In a modernizing society and under adverse environmental impact, the prevalence of cancer has been rising. In 2000 there were 10.1 million newly diagnosed cancer patients worldwide, with 6.2 million deaths [1]. By 2008, the figures climbed to 12.66 million and 7.56 million, respectively [2]. It is estimated that, by 2015, there will be 15 million newly diagnosed cancer patients [3]. What aggravates the situation is that cancer is no longer confined to the developed countries. In 2008, the newly diagnosed cancer cases in the developing countries accounted for 56% of the total [2]. While 80% of the cancer cases were found in low to moderate-income countries in 2009, it is predicted that by 2015, about 9 million people in the developing countries will die from cancer [3]. To cope with the huge burden brought by cancer, looking for cheap and available anti-tumor drugs becomes increasingly urgent.

Shogaol (Fig. 1) is generated from gingerol by removing one molecule of water during storage or under heating. Shogaol is more pungent and displays a wider range of physiological activities, especially in the anti-tumor field [4]. 6-Shogaol can be used to treat gastric cancer [5], oral cancer [6], liver cancer [7], lung cancer [8] and colorectal cancer [9]. 6-Shogaol induces the apoptosis of liver cancer cells with low differentiation and P53 mutation (featured by high expression of drug resistance genes MDR-1 and Bcl-2) and enhances the sensitivity to chemotherapy [10]. Study has shown preliminarily that 6-shogaol is effective against acute leukemia. The phosphorylation of eIF2α and the subsequent caspase-dependent degradation are closely associated with the pro-apoptotic effect. This may be the basis for the use of 6-shogaol to treat hematological malignancies [11].

This study was an investigation on the chemical properties of shogaol as the anti-tumor agent, including its composition and the structure-activity relationship, in an attempt to screen for shogaol

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compounds which had higher activity and can be easily synthesized.

2. Synthesis of shogaol

(1) Researchers are devoted to the synthesis of shogaol to expand its application as a bioactive agent. In 1976 Mukaiyama [12] produced gingerol through the condensation of silyl enol ethers from zingerone and aliphatic aldehydes, then shogaol was obtained through further dehydration of gingerol in the presence of acid (Fig. 2). This strategy was widely

used in many situations [13]. Then in 1990, Sattar applied Horner–Wadsworth–Emmons reaction to prepare shogaol from vanillin [14] (Fig. 3). In 2010, Mase using vanillin as the starting material, synthesized shogaol through the Mannich-type condensation of the ketone donor with the aldehyde acceptor through the iminium cation intermediate reaction catalyzed by DIMCARB (dimethylammonium dimethylcarbamate) with aliphatic aldehydes [15] (Fig. 4).
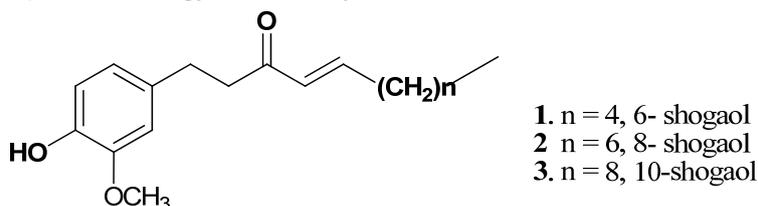
All the above mentioned pathways successfully

1. n = 4, 6- shogaol
2 n = 6, 8- shogaol
3. n = 8, 10-shogaol

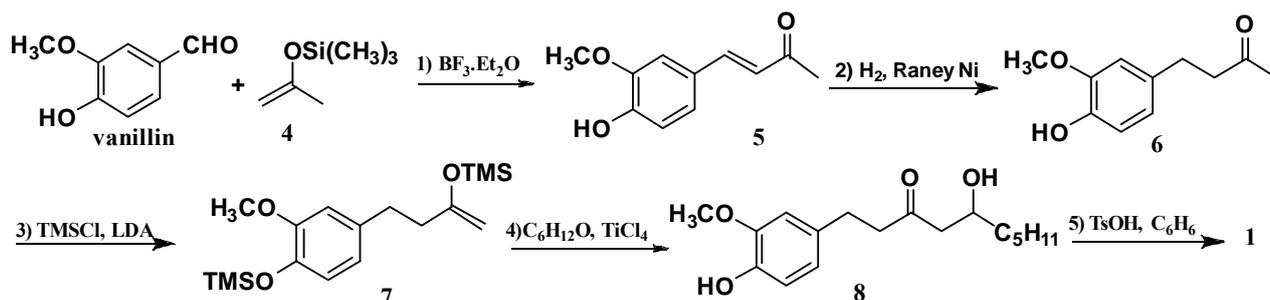**Fig. 1   Structural formula of shogaol.**

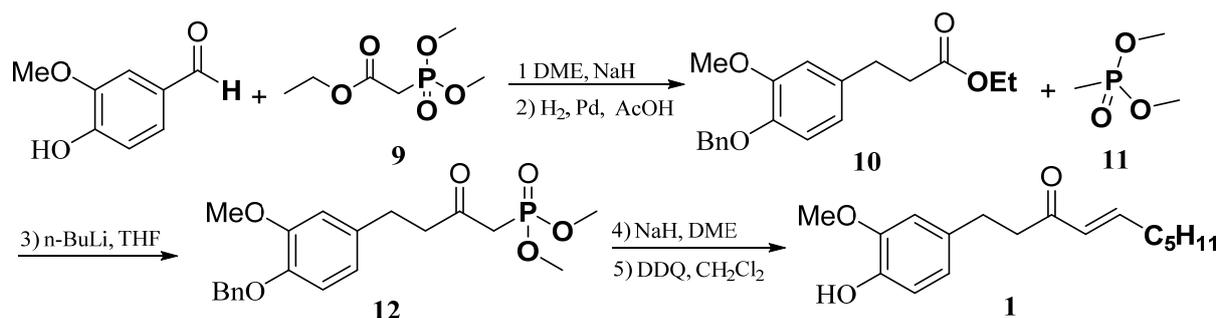**Fig. 2   Synthesis of shogaol by Teruaki Mukaiyama.**

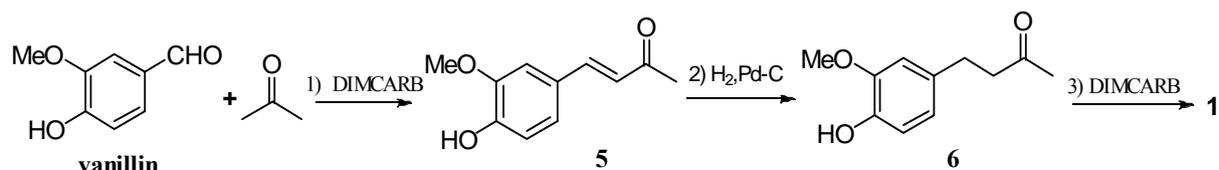**Fig. 3   Synthesis of shogaol by Sattar.**

Fig. 4   Synthesis of shogaol by Nobuyuki Mase.

synthesized shogaol by some expensive reagents or under the conditions of low temperature, water and oxygen free, in this paper we describe a cheaper and easier handle synthetic pathway.

(2) Synthetic strategy 1

Drawing inspirations from Nobuyuki Mase's pathway, we attempted to directly prepare shogaol by condensation of zingerone and aliphatic aldehydes (e.g., hexaldehyde), using cheaper catalysts. First sodium hydroxide was chosen as the catalyst. In one equivalent of sodium hydroxide in methanol, zingerone was consumed rapidly with the appearance of 3 products, which were isolated by silica gel column chromatography. The primary product came from the condensation of hexaldehyde itself (14); the secondary product was 6-shogaol, the target product; the minor product was isomeric 6-shogaol (13) [16] (Fig. 5). The proportion of 6-shogaol (6S) to isomeric 6-shogaol (iso-6S) was 55:45, which indicated the

activity of methyl group and methylene group adjacent to carbonyl group was comparable. To raise the proportion of 6S, the conditions were optimized, including lowering the temperature and using larger-volume catalysts (e.g., triethylamine, pyridine and DBU), but the proportion of iso-6S was still high. Moreover, due to structural similarities between the 6S and iso-6S, it could be hardly separated by column chromatography which was not suitably for scale synthesis. Therefore, a second synthetic strategy was proposed.

(3) Synthetic strategy 2

The retrosynthetic analysis of strategy 2 was as follows (Fig. 6): formylated 6S was first prepared by Knoevenagel condensation reaction between formylated zingerone (7) and aliphatic aldehyde. Then 6S, the target product, was obtained by decarboxylation [17]. Formylated zingerone (7) can be prepared from zingerone.
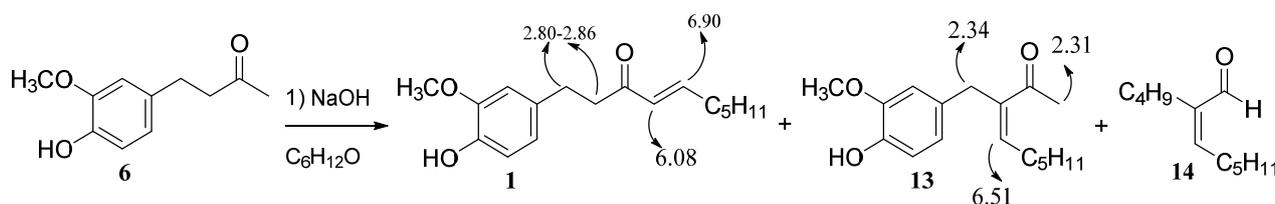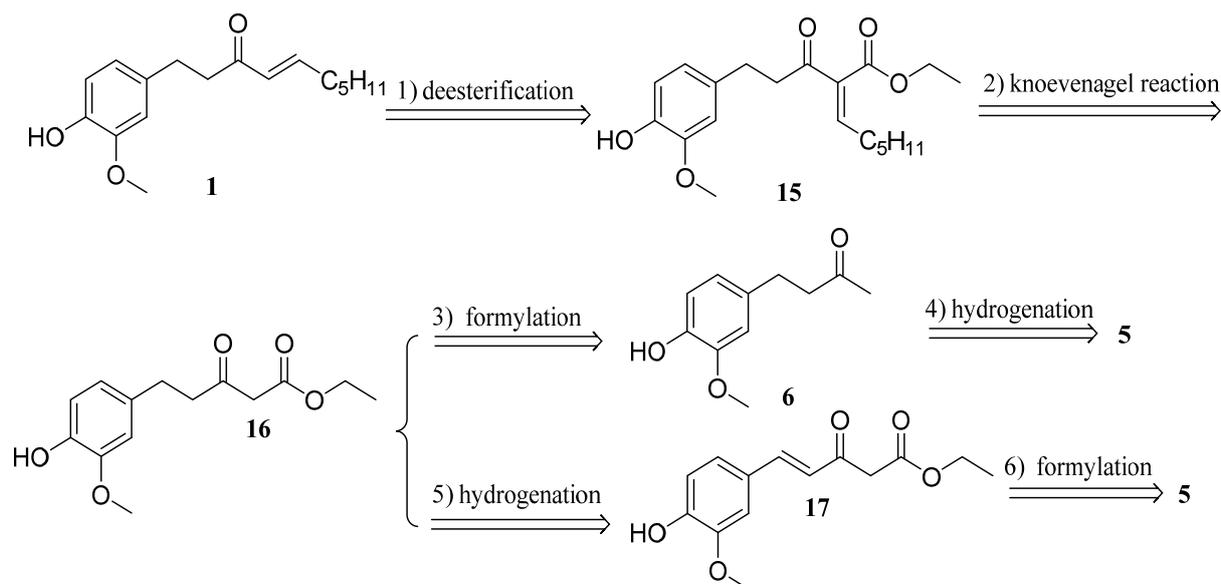

Fig. 5 Synthesis of Shogaol (strategy 1).

Fig. 6 Retrosynthetic analysis of 6S.

Formylated zingerone (7) was first tried to be synthesized from zingerone by formylation. Unfortunately, the products were very complex even we tried various conditions and the target product was hard to get. Therefore dehydrozingerone was used as the starting material. Under diethyl carbonate/sodium hydride system, the raw material was soon exhausted, and primary product appeared which was identified as the target formylated dehydrozingerone (17). Formylated dehydrozingerone (17) was then selective hydrogenated to formylated zingerone (7) by Raney Ni. When the important intermediate 7 was in hand, next we tried the key transformation to shogaol by knoevenagel condensation reaction and decarboxylation in one pot. As shown in Table 1, first, under classic knoevenagel condensation reaction (piperidine/EtOH), there is no shogaol appeared but only knoevenagel condensation product. Then we raised the reaction temperature to reflux, the products were very complicated the target was less than 10%. Based on the above experience we changed the piperidine to sodium hydroxide and made the reaction proceed in two stages: the initial temperature was 0 $^{\circ}$C, after the raw material was consumed out the temperature was 80 $^{\circ}$C. The way of adding hexaldehyde was also changed: if hexaldehyde was added in one time, the amount of (14) was very high, if the hexaldehyde was first dissolved in some solvent and the mixture was added into the reaction by syringe in a few minutes, (14) would greatly reduce. Besides, iso-6S was absent in the product. Through the above optimization, 6S was obtained from zingerone by 65% yield (Fig. 7).

(4) Synthesis of 6S analogues

In order investigate the SAR of shogaol on cancer cell, in this study we synthesized a variety of 6S analogues (Table 2).

The side chain of shogaols was regarded to play an important role for the shogaols' bioactivity [13], so three types of 6S analogues were prepared with aromatic, branched and circular side chains (19-30) (Fig. 8).

Phenolic hydroxyl group is regarded as having free radical clearing ability and antioxidative ability and

Table 1   Synthesis of 6S.

Entry	Hexaldehyde	Condition	1[c]	14[d]
1	0.9[a]	NaOH, 0 $^{\circ}$C, 1 h; reflux, 1 h	70%	5%
2	0.9[b]	NaOH, 0 $^{\circ}$C , 1 h; reflux, 1 h	60%	15%
3	0.9 [a]	NaOH, relux, 1 h	Complicated	-
4	1.2[a]	NaOH, 0 $^{\circ}$C, 1 h; reflux, 1 h	75%	15%
5	1.2[b]	NaOH, 0 $^{\circ}$C, 1 h; reflux, 1 h	70%	20%
6	1.2 [a]	NaOH, relux, 1 h	Complicated	-

[a] the hexaldehyde was dissolved in THF and added via syringe in 0.5 h; [b] the hexaldehyde was added in one time; [c] calculated by zingerone; [d] calculated by hexaldehyde.
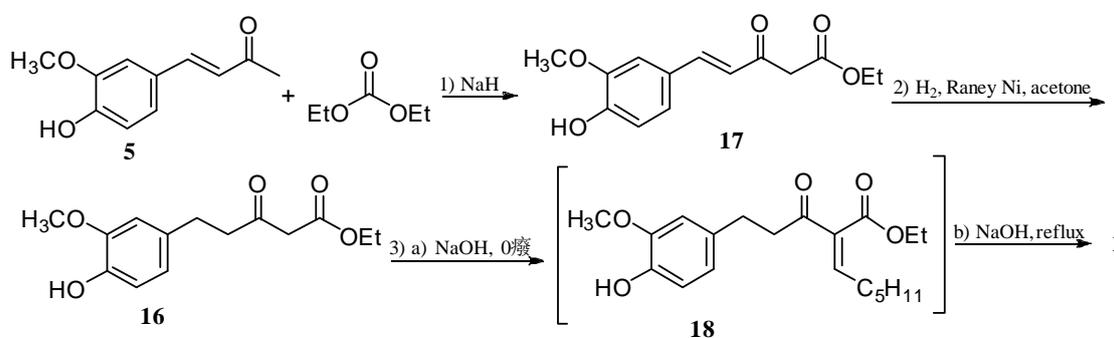

Fig. 7   Synthesis of 6S.

Table 2   Synthesis of 6S analogues.

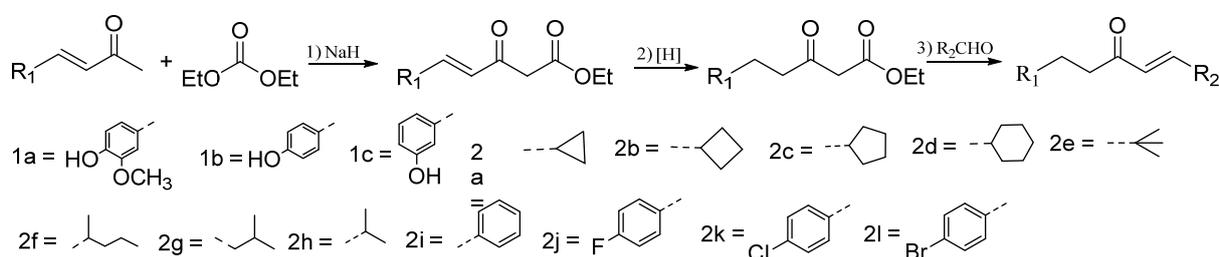| NO	R_1	R_2	Yield	NO	R_1	R_2	Yield	NO	R_1	R_2	Yield
19	1a	2a	60%	31	1b	2a	62%	39	1c	2a	60%
20	1a	2b	65%	32	1b	2b	65%	40	1c	2b	63%
21	1a	2c	68%	33	1b	2c	66%	41	1c	2c	66%
22	1a	2d	68%	34	1b	2d	65%	42	1c	2d	68%
23	1a	2e	70%	35	1b	2e	73%	43	1c	2e	72%
24	1a	2f	55%	36	1b	2f	58%	44	1c	2f	53%
25	1a	2g	50%	37	1b	2g	55%	45	1c	2g	55%
26	1a	2h	53%	38	1b	2h	55%	46	1c	2h	55%
27	1a	2i	70%					47			95%
28	1a	2j	72%					48			98%
29	1a	2k	75%					49			98%
30	1a	2l	70%								

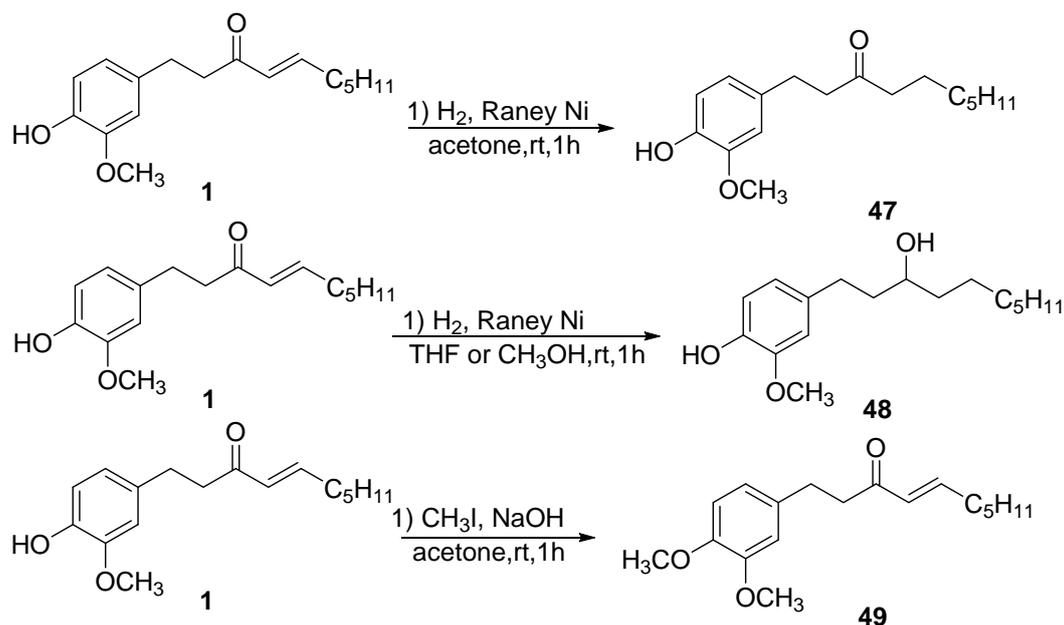**Fig. 8   General formula for synthesis of 6S analogues.**

Fig. 9   Synthesis of 6S analogues.

also an important structural domain with the anti-tumor effect. 6S analogues (31-46, 49) were synthesized to examine the effect of phenolic hydroxyl group on bioactivity (Fig. 8). Derivative (49) was prepared by methylation of 6S in acetone (Fig. 9).

α, β-unsaturated ketone is considered the anti-tumor domain in many natural metabolites. By Michael addition with large molecules, α, β-unsaturated ketone can induce endoplasmic reticulum stress and hence tumor cell death [18] or kill tumor cells by damaging

the microtubules [19]. We synthesized the selective reductive compound (47) and the fully reductive compound (48) by hydrogenating in acetone or THF.

3. *In vitro* Screening for Shogaol Derivatives with High Anti-tumor Activity

Materials

a. Reagents

HeLa cervical cancer cells (ATCC medium), pancreatic cancer cells ASPC-1 and PANC-1 (Nanjing KeyGen Biotech Co., Ltd.), human umbilical vein endothelial cells HUVECs (ATCC medium). DMEM medium (Nanjing KeyGen Biotech Co., Ltd., for culturing pancreatic cancer cells, ATCC medium for culturing HUVECs), fetal bovine serum (GIBCO, USA), MTT solution (Sigma, USA), DMSO (Sinopharm Group Co., Ltd.), Matrigel (BD Corporation, USA). 6S was isolated at our laboratory. All 6S analogues were prepared into 100 μM stock solution using DMSO.

b. Equipments

Biosafety cabinet, cell incubator, microplate reader.

c. Method

MTT assay:

Log phase cells were harvested, made into a cell suspension and inoculated to a 96-well plate at the density of 5,000 cells/mL for 200 μL per well. The cells were placed at 37 ^{o}C into a 5% CO_2 humidified incubator for 24 h and the drugs of different concentrations were added into the 96-well plate. MTT solution (5 mg/mL) was added into the 96-well plate for 20 μL per well and incubated at 37 ^{o}C for 4 h. The supernatant was collected and 200 μL DMSO was added into each well. The crystals were fully dissolved on a low-speed shaker for 10 min. Absorbance was measured in each well using a microplate reader at 570 nm. IC_{50} value was calculated using Graphpad software.

In vitro vascular lumen formation assay:

(1) A total of 50 μL matrigel was thawed and added into the 96-well plate precooled at 4 ^{o}C for curing at 37 ^{o}C for 30 min.

(2) Three groups were set up, namely, blank control group, negative control group and drug treatment group, with 5 replicates in each group. Into the blank control group 100 μL of the culture medium containing 3×10^4 HUVECs was added. For the negative control group, 100 μL of culture medium containing 3×10^4 HUVECs and DMOS was added. For the drug treatment group, the drug of different concentrations was added along with the culture medium containing 3×10^4 HUVECs.

(3) The plates were cultured at 37 ^{o}C in a 5% CO_2 incubator for 6 h. Images were taken under the inverted microscope at 200× magnification.

Evaluation criteria: The effect of the drugs on vascular lumen formation was assessed by counting vascular lumens within the visual field. The more the vascular lumens, the greater the effect of the drugs on vascular lumen formation.

4. Results

4.1 *Effects of 6S and Its Analogues on Tumor Cell Proliferation*

As shown in Table 3, the reactivity of analogues with aromatic side chains was weaker than that of 6S on Hela cells. The reactivity decreased with the reduction in electron cloud density of the benzene ring (27 < 28 < 29 < 30). But this variation pattern did not apply to pancreatic cancer cells Aspc-1 and Panc-1.

Of 12 analogues with circular side chains (19-22, 31-34, 39-42), the activity of analogues with cyclobutyl groups on the side chains (20, 32, 40) was stronger compared to the other 6 derivatives. The activity of analogues with cyclohexyl groups on the side chain (22, 34, 42) came next and resembled that of 6S. The activity of analogues with cyclopentyl groups on the side chains was weaker than that of 6S. The activity of analogues with cyclopropyl groups on the side chains (19, 31, 39) was the lowest. In terms of activity, analogues with cyclopropyl groups < analogues with cyclohexyl groups < analogues with

Table 3   Proliferation toxicity of 6S analogues on Hela cells and IC$_{50}$ (μM).

Comp.	Hela	Aspc-1	Panc-1	Comp.	Hela	Aspc-1	Panc-1
19	> 80.0	84.7	79.6	34	37.1	58.6	61.3
20	10.0	34.3	24.9	35	> 80	> 100	> 100
21	19.0	31.4	48.3	36	11.8	30.9	18.9
22	20.0	48.8	60.0	37	> 80	38.3	43.0
23	80.0	> 100	> 100	38	37.1	46.8	49.8
24	30.0	43.4	52.1	39	38.5	> 100	> 100
25	18.5	43.4	45.7	40	26.7	61.0	62.5
26	25.0	31.8	60.0	41	21.8	36.5	42.7
27	65.0	31.3	45.1	42	35.2	54.3	50.8
28	45.2	42.0	47.1	43	60.0	> 100	88.8
29	33.0	35.5	49.4	44	15.0	26.2	21.6
30	20.8	39.3	37.9	45	20.2	40.0	49.2
31	21.0	> 100	> 100	46	16.3	34.0	37.8
32	19.4	51.9	51.5	47	> 80	> 100	> 100
33	18.8	40.1	48.6	48	> 80	> 100	> 100
1	15.0	34.2	40.7	49	> 80	> 100	> 100

cyclopentyl groups < analogues with cyclobutyl groups.

Of 12 analogues with branched side chains (23-26, 35-38, 43-46), the activity of trimethylacetaldehyde derivatives (23, 35, 43) on all three cell lines was weaker to that of 6S; the activities of other 9 analogues were comparable to that of 6S.

Comparison between analogues 19-26, 31-38 and 39-46 indicated no substantial difference in bioactivity with the same side chains, that is, the missing of methyl ether had no impact on bioactivity. Comparison between analogues 31-38 and 39-46 indicated that substitution of phenolic hydroxyl group at different positions also had limited impact on bioactivity. But after methyl etherification of phenolic hydroxyl group, the bioactivity of compound 48 greatly reduced, as opposed to no obvious impact from the position of substitution of phenolic hydroxyl group. The reason is probably that the methyl etherification of phenolic hydroxyl group results in the loss of free radical clearing ability and hence the decline of anti-tumor effects.

Compounds 47 and 48, the products from the reduction of 6S, were almost ineffective against Hela, Aspc-1 and Panc-1 cells based on IC$_{50}$ value, probably due to the failure of Michael addition between the reduction products and the large molecules inside the organisms.

Compared with pancreatic cancer cells ASPC-1 and PANC-1, Hela cells were more sensitive to 6S, indicating different working mechanism of 6S on the three cell lines.

4.2 Effects of 6S Analogues on the Proliferation of HUVECs and Vascular Lumen Formation

The proliferation toxicity of 50 μM 6S analogues on HUVECs was assessed using MTT assay. The results showed that the toxicity of compounds 23, 35, 32, 43, 39 and 51 on HUVECs was smallest at 50 μM concentration (Fig. 10). These 6 compounds were further tested in vascular lumen formation experiment.

The 96-well plate was coated with 50 μL Matrigel, and 100 μL of the culture medium containing the drugs, 3×10^4 HUVECs and DMSO was added. After the plate was placed at 37 °C in a 5% CO$_2$ incubator for 6 h, vascular lumen formation was observed under the inverted fluorescence microscope. The photos are shown below (Fig. 11). All six compounds tested did not inhibit vascular lumen formation. In other words, they had neither cytotoxicity nor proliferation toxicity on HUVECs.
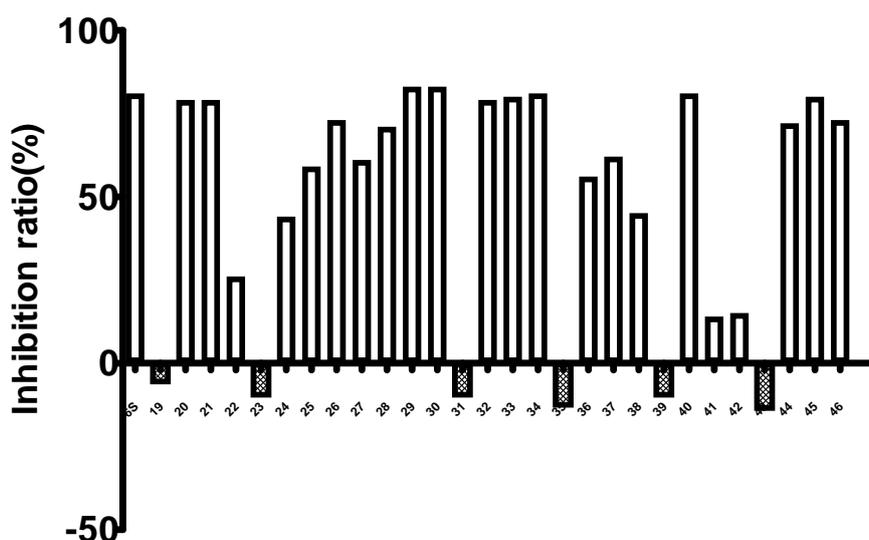
Fig. 10   Screening for 6S analogues with no proliferation toxicity on HUVECs by MTT assay.
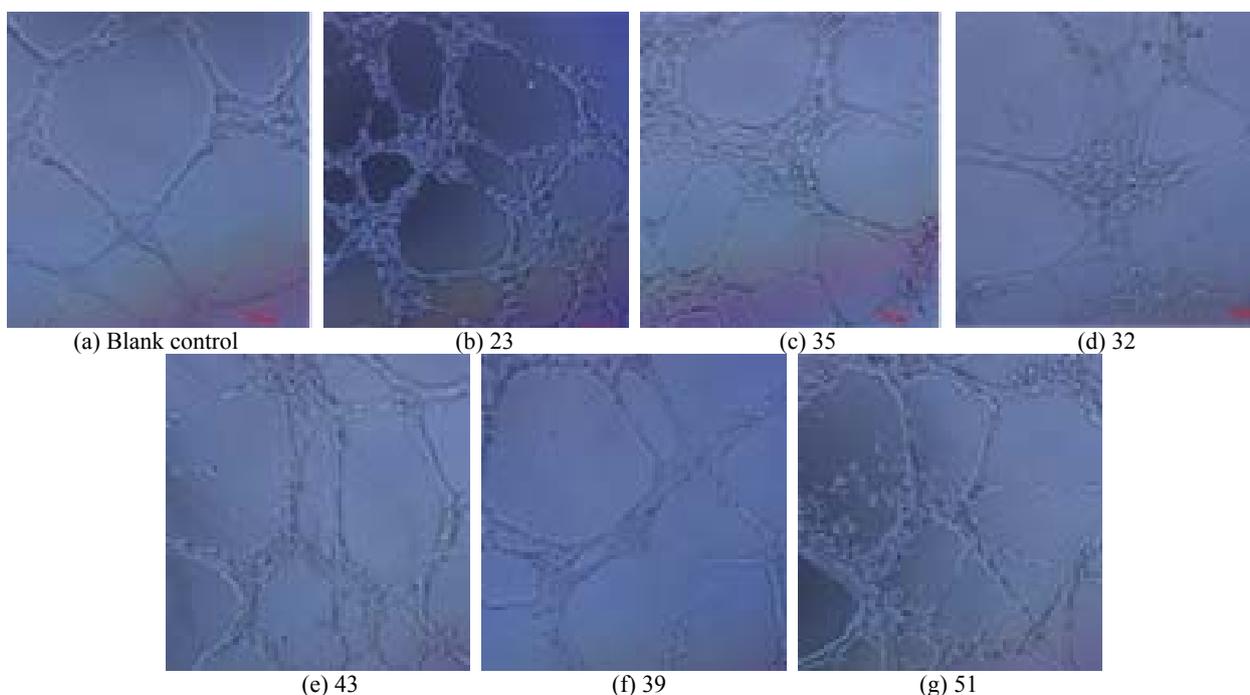

(a) Blank control   (b) 23   (c) 35   (d) 32

(e) 43   (f) 39   (g) 51

Fig. 11   Effects of 6S analogues on vascular lumen formation of HUVECs.

5. Discussion

Abnormalities of cell proliferation and differentiation are important reasons for canceration. As cancer develops, more nutrients will be needed to form microvessels. Efforts to develop anti-tumor drugs are mainly concentrated on the inhibition of tumor cell proliferation and angiogenesis. 6S is an important active ingredient from ginger and believed to have anti-tumor activity, but not all tumor cells are sensitive to 6S. Cervical cancer and pancreatic cancer are highly malignant and by preliminary screening, we found that 6S significantly inhibited the proliferation of cervical cancer cell line Hela and pancreatic cancer cells ASPC-1 and PANC-1. However, the IC_{50} of 6S on the three cells was high. Therefore, 6S analogues

were further synthesized to assess the anti-proliferative effect. The result showed that compared with ASPC-1 and PANC-1 cells, Hela cells were more sensitive to 6S, indicating that different working mechanisms existed for 3 cell lines. Compounds 20 and 36 were more inhibitory on the proliferation of Hela cells than 6S, for ASPC-1 cells, compounds 44 displayed superior inhibitory performance, for PANC-1 cells, compounds 20, 44 exhibited superior inhibitory performance.

6. Conclusions

In this paper, we describe an efficient process to synthesize 6S and analogues through 4-step reactions. In vitro anti-tumor activities test, some of the above analogues showed a strong potential for cancer therapy.

Acknowledgments

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Supplementary data
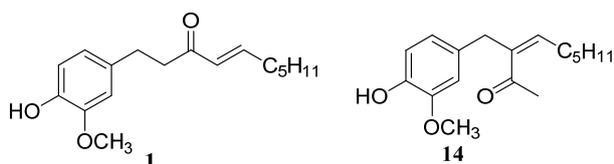
1: ^{1}H-NMR (CDCl$_3$, 500 MHz): 6.83, m, 1H; 6.77, s, 1H; 6.68, d(J=8.0), 1H; 6.61, d(J=7.0), 1H; 6.10, d(J=7.0), 1H; 15.1; 3.82, s, 3H; 2.79-2.88, m, 4H; 2.21, dd(J=15.6, 7.0), 2H; 1.46, m, 2H; 1.31, m, 4H; 0.91, t (J=7.2),3H; MS (*m/z*): [M+H]$^+$277.

14: ^{1}H-NMR (CDCl$_3$, 500 MHz): 6.89 t (J=7.5), 1H; 6.69, s, 1H; 6.65, d(J=2.0), 1H; 6.53, d(J=7.0), 1H; 3.78, s, 3H; 2.34, m, 2H; 2.31, s, 3H; 1.46, t (J=2), 2H; 1.31, m,4H; 0.88, t (J=2), 3H. MS (*m/z*): [M+H]$^+$277.
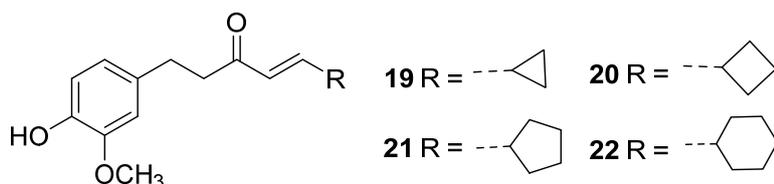

19: H-NMR (CDCl$_3$, 500 MHz): 0.63, m, 2H; 0.97, m, 2H; 1.50, m, 1H; 2.75-2.87,m, 4H;3.87, s, 3H; 6.20, d(J=15.5), 1H; 6.27, d(J=10), 1H; 6.66-6.83, m, 3H; ^{13}C- NMR(CDCl$_3$, 75 MHz): 8.9, 8.9, 14.6, 29.8, 42.1, 55.7, 111.0, 114.0, 120.6, 127.1, 133.1, 146.3, 150.1, 152.8, 198.8. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{15}$H$_{17}$O$_3$, 245.1183; found, 245.1179.

20: H-NMR (CDCl$_3$, 500 MHz): 1.85-2.0, m, 6H; 2.73, d(J=7.5), 1H, 2.80-2.86, m, 4H; 3.86, s, 3H; 6.00, dd(J=15.9, 1.2), 1H; 6.65-6.83, m, 4H; ^{13}C-NMR(CDCl$_3$, 75 MHz): 19.0, 28.9, 28.9, 30.7, 35.7, 42.0, 55.7, 111.1, 114.0, 120.5, 127.8, 131.8, 143.6, 146.3, 149.0, 199.5. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{16}$H$_{19}$O$_3$, 259.1304; found, 259.1311.

21: H-NMR (CDCl$_3$, 500 MHz): 1.35, m, 2H; 1.61, m, 2H; 1.65, m, 2H; 1.82, m, 2H; 2.55, m, 1H; 2.88, m, 4H; 3.87, s, 3H; 6.06, d(J=15.6), 1H; 6.67-6.83, m, 4H; ^{13}C-NMR(CDCl$_3$, 75 MHz): 25.2, 25.6, 29.8, 32.4, 33.2, 41.9, 43.0, 55.7, 111.0, 114.2, 120.6, 128.3, 133.1, 143.6, 146.3, 151.9, 200.0. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{17}$H$_{21}$O$_3$, 273.1496; found, 273.1500.
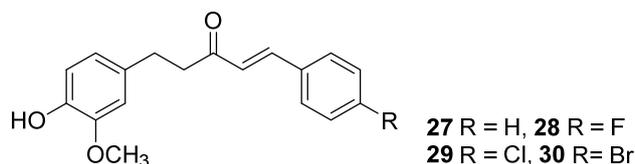
22: H-NMR (CDCl$_3$, 500 MHz): 1.18-1.32, m, 5H; 1.65-1.75, m, 5H; 2.10, m, 1 H; 2.82, m, 4H; 3.86, s, 3H; 6.12, dd(J=15.6, 1.5), 1H; 6.66-6.82, m, 4H; ^{13}C-NMR(CDCl$_3$, 75 MHz): 25.5, 25.7, 29.8, 31.6, 31.6, 40.4, 41.8, 55.7, 111.1, 114.2, 120.6, 133.0, 143.8, 146.3, 152.5, 200.2. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{18}$H$_{23}$O$_3$, 287.1653; found, 287.1649.
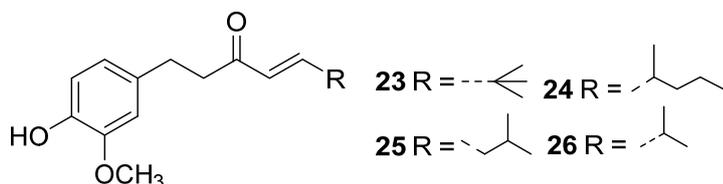
23: H-NMR (CDCl$_3$, 500 MHz): 1.06, s, 9H; 2.86, s, 4H; 6.00, d(J=16.2), 2H; 6.67-6.84, m, 3H; ^{13}C-NMR(CDCl$_3$, 75 MHz): 28.5, 28.5, 28.5, 29.8, 33.6, 42.1, 55.8, 111.1, 114.3, 120.7, 125.4, 133.1, 143.8, 146.4, 157.2, 200.3. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{18}$H$_{17}$O$_3$, 281.1183; found, 281.1182

25: H-NMR (CDCl$_3$, 500 MHz): 0.91, d(J=6.6), 6H; 1.73, m, 1H; 2.08, d(J=7.5), 2H; 2.85, m, 2H; 5.52, b.s., 1H; 6.08, dt(J=15.9, 1.2,), 1H; 6.66-6.84, m, 4H; ^{13}C-NMR(CDCl$_3$, 75 MHz):22.2, 22.2, 27.8, 29.8, 41.6, 41.9,55.7, 111.1, 114.2, 120.7, 128.4, 133.1, 143. 8, 146.5, 153.0, 200.0. HRMS (m/z): [M-H]$^-$ calcd. for C$_{17}$H$_{23}$O$_3$, 275.1653 ; found, 275.1649.

26: H-NMR (CDCl$_3$, 500 MHz): 1.05, d(J=6.9), 6H; 2.43, m,1H; 2.85, m, 4H; 3.87, s, 3H; 5.49, s, 1H; 6.04, dd(J=15.9, 1.2), 1H; 6.67-6.84, m, 4H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 21.2, 21.2, 29.8, 31.1, 42.0, 55.8, 111.1, 114.9, 120.7, 127.5, 133.2, 143.8, 146.4, 153.7, 200.1. HRMS (m/z): [M-H]$^-$ calcd. for C$_{15}$H$_{19}$O$_3$, 247.1340; found, 247.1340.

HO—[aryl]—CH$_2$CH$_2$—C(=O)—CH=CH—[aryl]—R, OCH$_3$ **27** R = H, **28** R = F **29** R = Cl, **30** R= Br
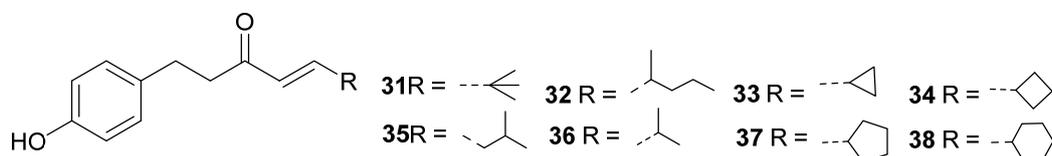
27: H-NMR(CDCl$_3$, 500 MHz): 2.94, m, 4H; 3.87, s, 3H; 5.51, bs, 1H; 6,65-6.85, m, 4H; 7.37-7.56, m,6H; ^{13}C-NMR(CDCl$_3$, 75 MHz): 29.8, 42.7, 55.8, 111.1, 114.3, 120.8, 126.1, 128,2, 128.2, 128.9, 128.9, 130.4, 133.0, 133.0, 134.4, 142.7, 143.9, 146.4, 199.5. HRMS (m/z): [M-H]$^-$ calcd. for C$_{18}$H$_{17}$O$_3$, 281.1183; found, 281.1182.

28: H-NMR CDCl$_3$, 500 MHz): 2.94, m, 4H; 3.87, s, 3H; 5.46, bs, 1H; 6.67-6.84, m, 4H; 7.34,-7.49, m, 5H; ^{13}C-NMR(CDCl$_3$, 75 MHz): 29.8, 42.9, 55.8, 111.1, 114.3,115.9, 116.2, 120.8, 125.8, 125.8, 130.0,130.1, 133.0, 141.3, 143.9, 146.4,165.5, 199.2, HRMS (m/z): [M-H]$^-$ calcd. for C$_{18}$H$_{16}$O$_3$F, 299.1089; found, 299.1087.

29: H-NMR (CDCl$_3$, 500 MHz): 2.94, m, 4H; 3.87, s, 3H; 5.46, bs, 1H; 6.68-6.73, m, 3H; 1H; 6.83, d(J=8), 1H; 7.37, d(J=8.5), 2H; 7.46, d(J=16), 1H; 7.52, d(J=8.5), 2H; ^{13}C-NMR(CDCl$_3$, 75 MHz): 29.7, 42.9, 55.8, 111.1, 114.3, 120.7, 126.4, 129.1, 129.1, 129.3, 129.3, 131.0, 132.9, 135.2, 141.1, 143.9, 146.4, 199.2. HRMS (m/z): [M-H]$^-$ calcd. for C$_{18}$H$_{16}$O$_3$Cl, 315.0793; found, 315.0800.

30: H-NMR (CDCl$_3$, 500 MHz): 2.94, m, 4H; 3.87, s, 3H; 5.45, b.s., 1H; 6.67-6.73, m, 3H; 6.83, d(J=8), 1H; 7.36, d(J=8), 2H; 7.44-7.49, m, 3H; ^{13}C-NMR(CDCl$_3$, 75 MHz): 29.8, 42.9, 55.8, 111.1, 114.3 120.8, 124.7, 126.6, 129.6,129.9 132.1,132.1, 133.0, 133.7, 141.2, 143.9, 146.4, 199.2. HRMS (m/z): [M-H]$^-$ calcd. for C$_{18}$H$_{16}$O$_3$Br, 359.0300; found, 359.0300.

HO—[aryl]—CH$_2$CH$_2$—C(=O)—CH=CH—R **31**R = (isobutenyl) **32** R = (sec-butyl) **33** R = (cyclopropyl) **34** R = (cyclobutyl) **35**R = (isopropyl) **36** R = (isobutyl) **37** R = (cyclopentyl) **38** R = (cyclohexyl)

31: H-NMR (CDCl$_3$, 500 MHz): 1.05, s, 9H; 2.86, s, 4H; 5.28, s, 1H; 6.00, d(J=15.9), 1H; 6.75-6.84, m, 3H; 7.03, d(J=8.4), 1H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 28.5, 28.5, 28.5, 29.4, 33.7, 42.0, 115.3, 115.3, 125.3, 132.6, 132.6, 154.3,158.0, 201.4. HRMS (m/z): [M-H]$^-$ calcd. for C$_{15}$H$_{19}$O$_2$, 231.1391; found, 231.1388.

32: H-NMR (CDCl$_3$, 500 MHz): 0.87, t(J= 6.6),3H; 1.14, d(J=6.6), 3H; 1.18-1.40, m, 4H; 2.85, m, 4H; 6.06, d(J=15.9), 1H; 6.68-6.78, m, 3H; 7.03, d(J= 8.4), 2H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 13.8, 19.2, 120.2, 29.3, 36.4, 38.0, 41.7, 115.3, 115.3, 128.3, 129.2, 129.2, 132.3, 153.8, 154.5, 201.2. HRMS (m/z): [M-H]$^-$ calcd. for C$_{16}$H$_{21}$O$_2$, 245.1454; found, 245.1547.

33: H-NMR(CDCl$_3$, 500 MHz): 0.90, d(J=6.9), 6H; 1.75, m, 1H; 2.08, t (J=7.2), 2H; 2.84, m, 4H; 6.13, d(J=15.9), 1H; 6.68-7.03, m, 5H; ^{13}C-NMR (CDCl$_3$,75 MHz): 22.2, 22.2, 27.7, 29.4, 41.6, 41.8, 115.2, 115.2, 129.1, 129.1, 131.1, 132.5, 147.4, 154.1, 200.5. HRMS (m/z): [M-H]$^-$ calcd. for C$_{15}$H$_{19}$O$_2$, 231.1391; found, 231.1389.

34: H-NMR(CDCl$_3$, 500 MHz): 1.03, d(J= 6.9), 6H; 2.47, m, 1H; 2.78-2.85, m, 4H; 6.05, d(J= 15.9), 1H; 6.70-7.03, m, 5H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 21.1, 21.1, 29.4, 30.1, 42.9, 115.3, 115.3, 127.3, 129.3, 129.3, 132.5, 152.1, 154.7, 201.5. HRMS (m/z): [M-H]$^-$ calcd. for C$_{14}$H$_{17}$O$_2$, 217.1234; found, 217.1234.

35: H-NMR(CDCl$_3$, 500 MHz): 0.55-0.67, m, 2H; 0.92, m, 2H; 1.54, m, 1H; 2.62-2.90, m, 4H; 6.18-6.35, m, 2H; 6.75-7.08, m, 4H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 8.75, 8.75, 14.1, 29.4, 41.8, 115.3, 115.3, 126.9, 129.2, 129.2, 132.3, 154.0, 154.4, 200.2. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{14}$H$_{17}$O$_2$, 215.1078; found, 215.1076.

36: H-NMR(CDCl$_3$, 500 MHz): 1.81-1.98, m, 3H; 2.10-2.25, m, 3H; 2.84, m, 4H; 3.08, m, 1H; 6.02, d(*J*=15.9), 1H; 6.68-7.02, m, 5H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 18.2, 27.2, 27.2, 28.9, 29.3, 37.7, 41.8, 115.2, 115.2, 127.7, 129.8, 129.8, 132.5, 151.8, 154.3, 201.3. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{15}$H$_{17}$O$_2$, 229.1234; found, 21235.

37: H-NMR(CDCl$_3$, 500 MHz):1.36-1.40, m, 2H; 1.52-1.64, m, 2H; 1.65-1.90, m, 2H; 2.10, m, 1H; 2.84, m, 4H; 6.12, d(*J*=15.9), 1H; 6.67-7.02, m, 5H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 25.2, 25.2, 29.4, 32.3, 32.3, 41.7, 43.9, 115.2, 115.2, 128.1, 129.2, 129.2, 132.4, 153.0, 154.3, 201.4. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{16}$H$_{19}$O$_2$, 243.1391 found, 243.1390.

38: H-NMR(CDCl$_3$, 500 MHz): 1.04-1.34, m, 4H; 1.64-1.74, m, 4H; 2.09, m, 1H; 6.04, d(*J*=15.9), 1H; 6.71-7.03, m, 5H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 25.5, 25.5, 25.7, 29.3, 31.5,31.5, 40.5, 41.7, 111.3, 115.3, 127.6, 129.2, 129.2, 132.5, 153.5, 154.3, 201.5. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{17}$H$_{21}$O$_2$, 257.1547; found, 257.1544.
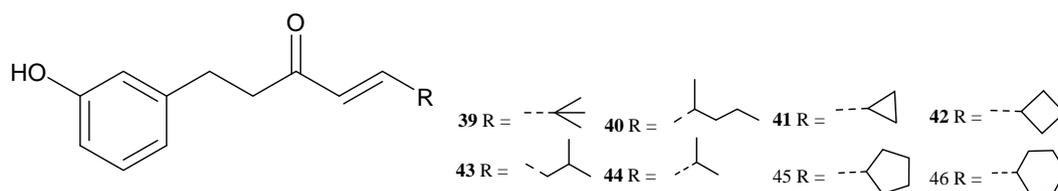
39: H-NMR(CDCl$_3$, 500 MHz): 1.05, s, 9H; 2.88, s, 4H; 6.00, d(*J*=16.2), 1H; 6.68-6.75, m, 3H; 6.81, d(*J*=16.2), 1H; 7.12, t(*J*=8.1), 1H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 28.6, 28.6, 28.6, 30.0, 33.7, 41.5, 113.2, 115.4, 120.3, 125.3, 129.5, 142.8, 156.0, 158.1, 201.2. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{15}$H$_{19}$O$_2$, 231.1394; found, 231.1391.

40: H-NMR(CDCl$_3$, 500 MHz): 0.85, t(*J*=6.3), 3H; 1.01, d(*J*=6.6), 3H; 1.26-1.35, m, 4H; 2.28, m, 1H; 2.80, m, 2H; 6.06, d(*J*=16.2), 1H; 6.68-6.76, m, 4H; 7.11, t(*J*=8.1), 1H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 13.6, 19.2, 20.2, 30.0, 36.5, 38.1, 41.3, 113.2, 115.2, 120.2, 128.3, 129.5, 142.7, 154.1, 156.1, 201.1. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{16}$H$_{21}$O$_2$, 245.1454; found, 245.1543.

41: H-NMR(CDCl$_3$, 500 MHz): 0.91, d(*J*=6.6), 6H; 1.62-1.82, m, 4H; 2.86, m, 4H; 5.27, s, 1H; 6.14, d(*J*=15.9), 1H; 6.64-7.12, m, 5H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 22.2, 22.2, 27.7, 30.0, 41.3, 41.6, 112.9, 115.3, 120.1, 129.4, 131.1, 140.9, 142.6, 156.1, 200.3. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{15}$H$_{19}$O$_2$, 231.1391; found, 231.1389.

42: H-NMR(CDCl$_3$, 500 MHz): 1.03, d(*J*=6.9), 6H; 2.42, m, 1H; 2.86, m, 4H; 6.04, d(*J*=15.9), 1H; 6.56-7.25 m, 5H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 21.1, 21.1, 30.0, 31.1, 41.3, 113.0, 115.3, 120.1, 127.3, 129.3, 142.6, 154.8, 156.1, 201.3. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{14}$H$_{17}$O$_2$, 217.1234; found, 217.1231.

43: H-NMR(CDCl$_3$, 500 MHz): 0.61, m, 4H; 0.95, m, 1H; 2.83, m, 4H; 6.14-6.35, m, 2H; 6.65-6.77, m, 2H; 7.04-726, m, 2H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 9.05, 9.05, 14.7, 30.0, 41.4, 112.9, 115.3, 120.0, 126.9, 129.4, 142.6, 154.0, 156.4, 199.9. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{14}$H$_{15}$O$_2$, 215.1074; found, 215.1078.

44: H-NMR(CDCl$_3$, 500 MHz): 1.82-2.00, m, 6H; 2.86, m, 3H; 3.11, 3.40, m, 1H; 6.00, d(*J*=15.9), 1H; 6.23-7.14, m, 5H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 18.5, 30.0, 30.0, 31.0, 37.9, 41.4, 113.1, 115.3, 120.3, 127.7, 129.5, 150.3, 151.8, 156.1, 200.9. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{15}$H$_{17}$O$_2$, 229.1234; found, 229.1233.

45: H-NMR(CDCl$_3$, 500 MHz): 1.35, m, 2H; 1.62, m, 2H; 1.83, m, 2H; 2.86, m, 4H; 5.28, s, 1H; 6.68-6.84, m, 4H; 7.13, t (*J*=8.1), 1H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 25.5, 25.5, 29.8, 32.3, 32.7, 41.3, 43.0, 113.1, 115.3, 120.0, 121.1, 129.4, 142.1, 151.1, 156.2, 200.7. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{16}$H$_{19}$O$_2$, 243.1391; found, 243.1389.

46: H-NMR(CDCl$_3$, 500 MHz): 1.14-1.42, m, 6H; 1.56-1.78, m, 4H; 2.10, m, 1H; 2.86, m, 4H; 6.04, dd(*J*= 1 5.9, 1.2), 1H; 6.67-6.75, m, 4H; 7.12, m, 1H; ^{13}C-NMR (CDCl$_3$, 75 MHz): 25.5, 25.2, 25.6, 29.9, 31.5, 31.5, 40.5, 41.2, 113.0, 115.3, 120.2, 127.6, 129.4, 142.8, 150.7, 156.0, 200.9. HRMS (*m/z*): [M-H]$^-$ calcd. for C$_{17}$H$_{21}$O$_2$, 257.1547; found, 257.1548.
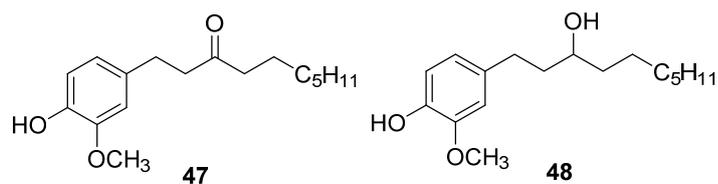
47：H-NMR(CDCl$_3$, 500 MHz): 0.85, t(J=6.9), 3H; 1.25, bs, 8H; 1.57, m, 2H; 2.36, t(J=7.5), 2H; 2.66-2.85, m, 4H; 3.86, s, 3H; 6.64-6.83, m, 3H.

48：H-NMR(CDCl$_3$, 500 MHz): 0.89, t(J=6.5), 3H; 1.25, bs, 10H; 1.45, m, 4H; 1.74, m, 2H; 2.60, 2.72, m, 2H; 3.61, s 1H; 3.87, s, 3H; 6.68-6.70, m, 2H; 6.82, d(J=8), 1H.
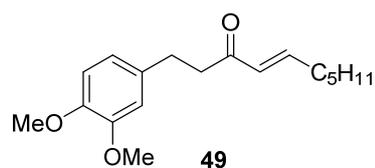
49：H-NMR(CDCl$_3$, 500 MHz): 0.88, t(J=9.6), 3H; 1.25-1.34, m, 6H; 2.20, m, 2H; 2.85, m, 4H; 3.83, 3.85, s, 各 3H; 6.14, dt(J= 16.5, 1.5), 1H; 6.67-6.87, m, 4H.