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New Safrole Oxide Derivatives: Synthesis and *in vitro* Antiproliferative Activities on A549 Human Lung Cancer Cells

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A number of novel small molecules, safrole oxide derivatives **4a-c**, **6a-c**, **9a-h**, were synthesized by the reaction of safrole oxide with anilines **3** and **5**, or its alkyl allyl ether derivative **7** with alkyl bromide **8** in moderate yields. The antiproliferative effects of all the target molecules on A549 cell growth were investigated and it was found that the 14 novel compounds could suppress A549 lung cancer cell growth. Among them, compound **6b** was the most effective compound in inhibiting the proliferation of A549 cells.

Key Words : Safrole derivatives, A549 cells, MTT assay, Growth inhibition

Introduction

Lung cancer is the leading cause of cancer-related mortality in the developed countries, accounting for 26-28% of all cancer deaths.[1,2] Increasingly, lung cancer is becoming a global health problem, with greater than 1.3 million deaths annually attributed to lung cancer.[3] Based on the mechanism of action, the commonly used anticancer drugs can be grouped into four main classes: the agents directly acting on DNA (alkylating agents, platinum complexes, and topoisomerase inhibitors), the agents interfering with DNA synthesis (antimetabolites), the antimitotic agents (microtubule targeting agents), and the agents of protein kinases involved in signaling pathways as well as tumor angiogenesis (protein kinases inhibitors).[4] In spite of the impressive progress in diagnosis, surgery and therapy, the overall lung cancer mortality is still high and the medical need is largely unmet.[1,3]

It has been reported that safrole oxide induces apoptosis in A549 human lung cancer[5-7] and vascular endothelial cells (VECs), and inhibits angiogenesis.[8] According to the study of Zhao *et al.*, safrole oxide derivative, 1-ethoxy-3-(3,4-methylenedioxy phenyl)-2-propanol has a marked inhibition effect on cell proliferation of human lung cancer cell line A549.[9] Inspired by the discovery of the existing safrole derivatives and their inducement effect of apoptosis on human lung cancer cell line A549, we try to find out more safrole oxide derivatives, containing nitrogen atom or alkyl allyl ether moiety, which are expected to exhibit antiproliferative activity, to explore the diversity of small molecules as anticancer reagents. Since there are no reports on the synthesis and biological evaluation of these compounds, like 1-(benzo[*d*][1,3]dioxol-5-yl)-3-anilinopropan-2-ols (**4**), 3-(benzo[*d*][1,3]dioxol-5-yl)-2-anilinopropan-1-ols (**6**) and (*E*)-5-(3-alkyl/aryloxyprop-1-en-1-yl)benzo[*d*][1,3]dioxoles (**9**), we designed and synthesized these novel safrole oxide derivatives, and found that the 14 novel compounds could suppress A549 lung cancer cell growth.

Results and Discussions

The synthetic approach for safrole derivatives **4a-c** is shown in Scheme 1. Safrole oxide (**2**) was obtained from commercially available safrole (**1**) in the presence of *m*-CPBA in CCl_4 at room temperature. The reaction of safrole oxide (**2**) with substituted aniline **3** catalyzed by cesium carbonate in refluxing DMF, afforded 1-(benzo[*d*][1,3]dioxol-5-yl)-3-anilinopropan-2-ols (**4**) in good yields.

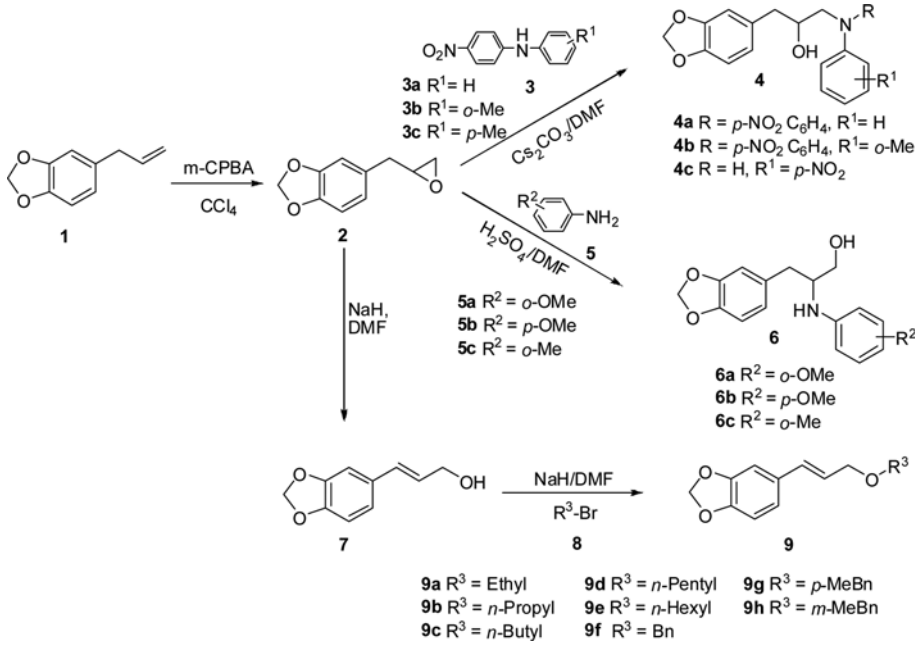
Similarly, synthesis of safrole oxide derivatives 3-(benzo[*d*][1,3]dioxol-5-yl)-2-anilinopropan-1-ol (**6a-c**) were achieved by the reaction of safrole oxide (**2**) with anilines **5** in the presence of sulphuric acid at 95 °C in DMF in moderate yields (Scheme 1).

As shown in Scheme 1, safrole oxide derivatives (*E*)-5-(3-alkyl/aryloxyprop-1-en-1-yl)benzo[*d*][1,3]dioxoles (**9a-h**) were synthesized by the reaction of (*E*)-3-(benzo[*d*][1,3]dioxol-5-yl) prop-2-en-1-ol (**7**) and alkyl bromide **8** in the presence of sodium hydride in DMF at room temperature. The compound **7** was conveniently prepared by the reaction of safrole oxide (**2**) in the presence of sodium hydride in DMF at 75 °C in good to moderate yields.

All of the compounds gave satisfactory spectral data. Representatively, the structures of **4a-c**, **6a-c** and **9a-h** were confirmed by HRMS, ^{1}H NMR and ^{13}C NMR data.

Effects of the Compounds on the Viability of A549 Lung Cancer Cells. The *in vitro* antiproliferative activity of all of the sixteen synthesized compounds was evaluated using MTT assay against A549 human lung cancer cells. Concentration of the test depends on the solubility of the compound.

The data obtained by MTT assay showed that compounds **2**, **4a-c**, and **6a-c** exhibited inhibitory effects on the pro-



Scheme 1. Synthesis of safrole oxide derivatives **4a-c**, **6a-c**, **9a-h**.

liferation of A549 cells in a concentration-dependant manner (Figure 1). The replacement of epoxyethane part in the structure of safrole oxide by alkyl allyl ether moiety significantly weakened the inhibitory activity, with the viability more than 50% at a high concentration of compound **7**, and **9a-h**, compared with less than 20% viability of safrole oxide **2** at the concentration of 400 μM. According to the experimental results, compound **4a-c** exhibited moderate antiproliferative activity at 50-200 μM after 48 h of treatment, while different moieties on the benzene group did not give significant influence on the inhibitory ability. For compound **6a-c**, the higher electronic density of anline containing electron-donating group at 3-(benzo[*d*][1,3]dioxol-5-yl)-2-aniline-propan-1-ols (**6**) contributed to a higher antiprofiferative activity, and the electron-donating group methoxy on *ortho* position of nitrogen atom suggested a stronger inhibition effect on the proliferation of human lung cancer cell line A549 than the compound with methoxy group on *para* position. Among compound **6a-c**, compound 3-(benzo[*d*][1,3]dioxol-5-yl)-2-(2-methoxyanilino)propan-1-ol (**6b**) showed the stronger inhibitory activity.

Experimental

Chemistry.

General: ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with tetramethylsilane as internal reference on a Bruker Advance FT spectrometer. Chemical shifts were reported in parts per million. MS detection was performed on an Agilent 6510 Q-TOF mass spectrometer with an ESI source. $CDCl_3$ was used as delivered from Sigma-Aldrich (St. Louis, MO, USA) or Adamas (Adamas reagent, Ltd). Silica gel (80-300 mesh) was used for flash column chromatography. All the reactions were monitored by TLC using 0.25 mm silica gel plates with UV indicator (Shanghai Jiapeng Technology Co., Ltd., China). Unless otherwise noted, other reagents were obtained from commercial suppliers and used without further purification.

Procedure for the Synthesis of 5-(Oxiran-2-ylmethyl)-benzo[*d*][1,3]dioxole (2): To a solution of *m*-chloroperbenzoic acid (*m*-CPBA) (5.0 mmol, 1.0 equiv) in 150 mL CCl_4 was added safrole (6.0 mmol, 1.2 equiv) at room temperature. The reaction solution was stirred at the same temperature for 20 h. The solution was then washed with 3 × 30 mL 10% NaOH aqueous solution and with 2 × 20 mL water. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was separated on a silica gel chromatography column eluted with a mixture of petroleum/ethyl acetate 100/2 to provide compound **2**.

5-(Oxiran-2-yl-methyl)benzo[*d*][1,3]dioxole.[10]

Compound 2: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.77-6.68 (m, 3H, Ar*H*), 5.93 (s, 2H, OCH_2O), 3.14-3.06 (m, 1H, CH), 2.87-2.70 (m, 3H, CH_2 and $ArCH_2$), 2.53 (m, 1H, CH_2).

Representative Procedure for the Synthesis of 1-(Benzo[*d*][1,3]dioxol-5-yl)-3-aniline propan-2-ols (4). The solution of substituted aniline **3** (6.0 mmol, 1.2 equiv), compound **2** (5.0 mmol, 1.0 equiv) and Cs_2CO_3 (7.5 mmol, 1.5 equiv) in DMF (100 mL) was refluxed and monitored by TLC. After the completion of the reaction, the solvent was removed under vacuum and water (50 mL) was added into the residue. The mixture was then extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered and evaporated under vacuum to give the crude product **4**. The pure product **4** was obtained by column chromatography on silica gel (elute:ethyl acetate: petroleum = 1:20).

1-(Benzo[*d*][1,3]dioxol-5-yl)-3-(4-nitroaniline)propan-2-ol.

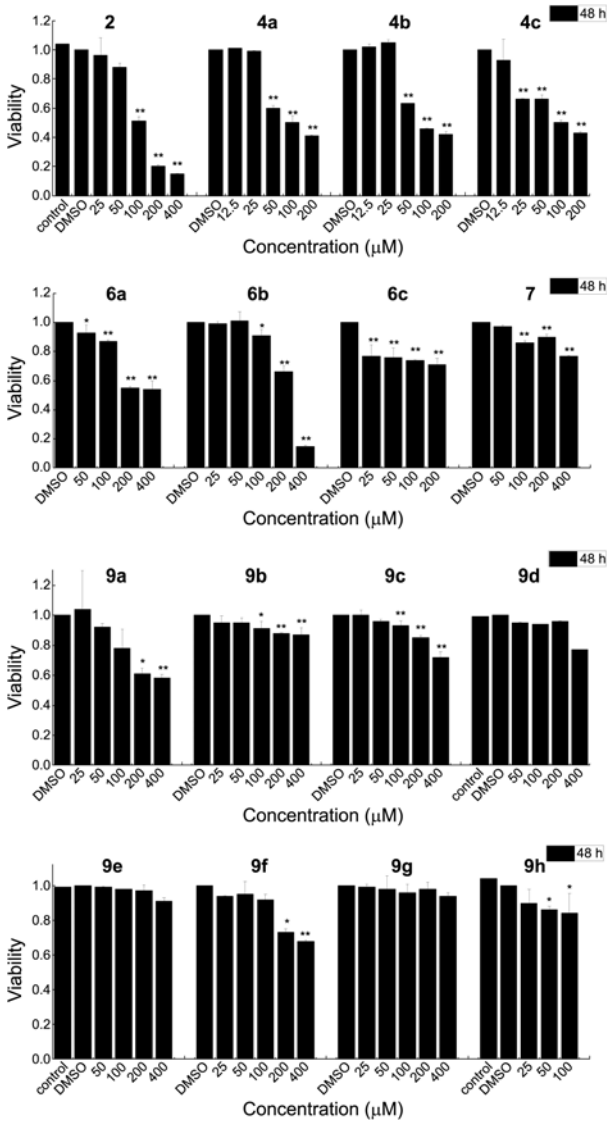


Figure 1. Effects of the safrole derivatives **2**, **4a-c**, **6a-c**, **7** and **9a-h** on the viability of A549 lung cancer cells. Control, the cells cultured in the medium without any derivatives. DMSO, the cells cultured in the medium containing DMSO 0.1% (v/v) or DMSO 0.2% (v/v) used as a vehicle control. Other bars show the viability of the cells treated with the 9 derivatives at the concentrations indicated for 48 h. Data are means ± SE from three independent experiments (*P < 0.05, **P < 0.01 *vs* the DMSO group).

Compound 4a: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, *J* = 9.4 Hz, 2H, Ar*H*), 7.46 (t, *J* = 7.8 Hz, 2H, Ar*H*), 7.32 (t, *J* = 7.4 Hz, 1H, Ar*H*), 7.28 (dd, *J* = 10.0, 2.6 Hz, 2H, Ar*H*), 6.75 (d, *J* = 7.9 Hz, 1H, Ar*H*), 6.62-6.67 (m, 4H, Ar*H*), 5.94 (s, 2H, OCH_2O), 4.11 (m, 2H, CHOH), 3.80-3.87 (m, 2H, CH_2), 2.64-2.79 (m, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.0 (C), 147.9 (C), 146.5 (C), 145.4 (C), 138.4 (C), 130.8 (C), 130.4 (CH), 127.5 (CH), 127.0 (CH), 125.7 (CH), 122.3 (CH), 113.3 (CH), 109.6 (CH), 108.5 (CH_2), 101.0 (CH), 70.4 (CH), 58.0 (CH_2), 41.3 (CH_2). HRMS (ESI): *m/z* 393.1440 (M+1).

1-(Benzo[*d*][1,3]dioxol-5-yl)-3-((4-nitrophenyl)(*o*-tolyl) amino)propan-2-ol.

Compound 4b: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 9.4 Hz, 2H, Ar*H*), 7.32-7.26 (m, 4H, Ar*H*), 6.75 (d, *J* = 7.9 Hz, 1H, Ar*H*), 6.68 (s, 1H, Ar*H*), 6.64 (d, *J* = 7.9 Hz, 1H, Ar*H*), 6.45 (d, 2H, *J* = 8.4 Hz, Ar*H*), 5.93 (s, 2H, OCH_2O), 4.13-4.09 (m, 1H, CH), 3.89-3.47 (m, 2H, CH_2), 2.79-2.62 (m, 2H, CH_2), 2.09 (s, 3H, CH_3). HRMS (ESI): *m/z* 407.1623 (M+1).

1-(Benzo[*d*][1,3]dioxol-5-yl)-3-(4-nitroaniline)propan-2-ol.

Compound 4c: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, *J* = 9.1 Hz, 2H, Ar*H*), 6.79 (d, *J* = 7.8 Hz, 1H, Ar*H*), 6.72-6.67 (m, 2H, Ar*H*), 6.54 (d, 2H, *J* = 9.1 Hz, Ar*H*), 5.96 (s, 2H, OCH_2O), 4.93 (s, 1H, OH), 4.05-4.06 (m, 1H, CH), 3.41-3.37 (m, 1H, CH_2), 3.18 (m, 1H, CH_2), 2.78 (m, 2H, $ArCH_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.3 (C), 148.1 (C), 146.7 (C), 138.3 (C), 130.5 (C), 126.4 (CH), 122.3 (CH), 111.4 (CH), 109.5 (CH), 108.6 (CH), 101.1 (CH_2), 71.0 (CH), 48.1 (CH_2), 41.4 (CH_2). HRMS (ESI): *m/z* 286.1444 (M-NO), 317.1136 (M+1).

Representative Procedure for the Synthesis of 3-(benzo [*d*][1,3]dioxol-5-yl)-2-(phenyl amino)propan-1-ols (6). The reaction mixture of 5-(oxiran-2-ylmethyl)benzo[*d*][1,3] dioxole (**2**) (5.0 mmol, 1.0 equiv), substituted phenylamine (**5**) (6.0 mmol, 1.2 equiv), H_2SO_4 (0.4 mL) in 40 mL DMF was stirred at 95 °C and monitored by TLC. After completion of the reaction, the solvent was removed under vacuum. To the residue it was added 40 mL water and then extracted by ethyl acetate (3 × 40 mL), and the combined organic layers were dried over $MgSO_4$. Removal the $MgSO_4$ and evaporation of the solvent at reduced pressure gave the crude product. The pure product **6** was obtained by column chromatography on silica gel (elute:ethyl acetate:petroleum = 1:20).

3-(Benzo[*d*][1,3]dioxol-5-yl)-2-(4-methoxyaniline)propan-1-ol.

Compound 6a: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.78 (d, *J* = 9.0 Hz, 2H, Ar*H*), 6.76-6.72 (m, 2H, Ar*H*), 6.68 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar*H*), 6.64 (d, *J* = 8.9 Hz, 2H, Ar*H*), 5.94 (s, 2H, OCH_2O), 4.01 (m, 1H, CH), 3.75 (s, 3H, OCH_3), 3.24 (m, 1H, CH_2), 3.01 (m, 1H, CH_2), 2.77-2.71 (m, 2H, $ArCH_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.6 (C), 147.8 (C), 146.3 (C), 142.3 (C), 131.5 (C), 122.3 (CH), 114.9 (CH), 114.8 (CH), 109.7 (CH), 108.4 (CH), 101.0 (OCH_2O), 71.2 (CH_2), 55.8 (CH), 50.6 (OCH_3), 41.3 (CH_2). HRMS (ESI): *m/z* 302.1384 (M+1).

3-(Benzo[*d*][1,3]dioxol-5-yl)-2-(2-methoxyaniline)propan-1-ol.

Compound 6b: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.85 (m, 1H, Ar*H*), 6.78-6.67 (m, 5H, Ar*H*), 6.60 (dd, *J* = 7.8, 1.1 Hz, 1H, Ar*H*), 5.93 (s, 2H, OCH_2O), 4.02-4.07 (m, 1H, CH), 3.84 (s, 3H, OCH_3), 3.28 (m, 1H, CH_2), 3.11 (m, 1H, CH_2), 2.78 (m, 2H, $ArCH_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.8 (C), 147.2 (C), 146.3 (C), 138.1 (C), 131.7 (C), 122.3 (CH), 121.3 (CH), 117.1 (CH), 110.4 (CH), 109.7 (CH), 109.6 (CH), 108.4 (CH), 101.0 (OCH_2O), 71.2 (CH_2), 55.4 (CH), 49.3 (OCH_3), 41.3 (CH_2). HRMS (ESI): *m/z* 302.1386 (M+1).

3-(Benzo[*d*][1,3]dioxol-5-yl)-2-(*o*-tolylamino)propan-1-ol.

Compound 6c: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (d, *J*

= 7.5 Hz, 1H, Ar*H*), 7.08-7.04 (m, 1H, Ar*H*), 6.76-6.62 (m, 4H, Ar*H*), 6.59 (d, *J* = 8.1 Hz, 1H, Ar*H*), 5.93 (s, 2H, OCH_2O), 4.07-4.04 (m, 1H, CH_2O), 3.32 (d, *J* = 12.6 Hz, 1H, CH_2O), 3.07 (m, 1H,CH), 2.79-2.70 (m, 2H, $ArCH_2$), 2.15 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.9 (C), 146.4 (C), 146.1 (C), 131.4 (C), 130.2 (CH), 127.1 (CH), 122.6 (CH), 122.3 (CH), 117.5 (C), 110.2 (CH), 109.7 (CH), 108.4 (CH), 101.0 (CH_2), 71.1 (CH_2), 49.3 (CH), 41.4 (CH_2), 17.5 (CH_3). HRMS (ESI): *m/z* 286.1441 (M+1).

Representative Procedure for the Synthesis of (*E*)-5-(3-alkyl/aryloxyprop-1-en-1-yl)benzo[*d*][1,3]dioxole (9). The solution of compound **2** (5.0 mmol, 1.0 equiv) and NaH (7.5 mmol, 1.5 equiv) in 40 mL DMF was stirred at 75 ºC and monitored by TLC. After the reaction was completed, it was cooled to room temperature, compound **8** (5.0 mmol, 1.0 equiv) and NaH (7.5 mmol, 1.5 equiv) were added to the reaction mixture. The reaction was stirred at room temperature until it was completed (monitored by TLC). The reaction mixture was added to 50 mL water at 0-5 °C. The solvents were removed under vacuum. Water (40 mL) was added to the residue and the obtained mixture was extracted with EtOAc (3 × 40 mL). The combined organic layers were dried over $MgSO_4$. Removal of the solvent under reduced pressure gave the crude product. The pure product **9** was obtained by column chromatography on silica gel (elute: ethyl acetate:petroleum = 1:100).

(*E*)-3-(Benzo[*d*][1,3]dioxol-5-yl)prop-2-en-1-ol.[11]

Compound 7: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (s, 1H, Ar*H*), 6.79 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.74 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.50 (d, *J* = 15.8 Hz, 1H, =CHAr), 6.18 (m, 1H, =CH), 5.94 (s, 2H, OCH_2O), 4.27 (d, *J* = 5.7 Hz, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.1 (C), 147.4 (C), 131.3 (C), 130.9 (=CH), 126.9 (=CH), 121.3 (CH), 108.4 (CH), 105.8 (CH), 101.2 (OCH_2O), 63.7 (OCH_2).

(*E*)-5-(3-Ethoxyprop-1-en-1-yl)benzo[*d*][1,3]dioxole.

Compound 9a: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92 (s, 1H, Ar*H*), 6.80 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.73 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.50 (d, *J* = 15.9 Hz, 1H, ArCH=), 6.12 (m, 1H, ArCH=), 5.92 (s, 2H, OCH_2O), 4.09 (d, *J* = 6.0, 2H, CH_2), 3.52 (q, *J* = 7.0 Hz, 2H, CH_2), 1.23 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.4 (C), 148.6 (C), 133.3 (CH), 132.7 (C), 125.9 (CH), 122.5 (CH), 109.6 (CH), 107.2 (CH), 102.4 (CH), 72.6 (CH_2), 67.0 (CH_2), 16.6 (CH_3). HRMS (ESI): *m/z* 161.0604 (M-OC_2H_5).

(*E*)-5-(3-Propoxyprop-1-en-1-yl)benzo[*d*][1,3]dioxole.

Compound 9b: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94 (s, 1H, Ar*H*), 6.82 (dd, *J* = 8.0, 0.9 Hz, 1H, Ar*H*), 6.75 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.51 (d, *J* = 15.8 Hz, 1H, =CHAr), 6.14 (dt, *J* = 15.8, 6.1 Hz, 1H, =CH), 5.94 (s, 2H, OCH_2O), 4.09 (d, *J* = 6.0, 2H, =CHC*H*$_2$O), 3.43 (t, *J* = 6.9 Hz, 2H, CH_2CH_2O), 1.67-1.58 (m, 2H, CH_2), 0.95 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.4 (C), 148.6 (C), 133.2 (CH), 132.7 (C), 126.0 (CH), 122.5 (CH), 109.6 (CH), 107.2 (CH), 102.4 (CH), 73.5 (OCH_2), 72.8 (CH_2O), 24.4 (CH_2), 12.0 (CH_3). HRMS (ESI): *m/z* 161.0607 (M-OC_3H_7).

(*E*)-5-(3-Butoxyprop-1-en-1-yl)benzo[*d*][1,3]dioxole.

Compound 9c: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94-6.74 (m, 3H, Ar*H*), 6.52-6.09 (m, 2H, CH=CH), 5.95 (s, 2H, OCH_2O), 4.09-4.10 (m, 2H, CH_2O), 3.47 (t, *J* = 6.8 Hz, 2H, OCH_2), 1.58-1.63 (m, 2H, CH_2), 1.36-1.42 (m, 2H, CH_2), 0.93 (t, *J* = 3.6 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.4 (C), 148.6 (C), 133.2 (CH), 132.7 (C), 126.0 (CH), 122.5 (CH), 109.6 (CH), 107.2 (CH), 102.4 (CH), 72.8 (OCH_2), 71.6 (CH_2O), 33.3 (CH_2), 20.8 (CH_2), 15.3 (CH_3). HRMS (ESI): *m/z* 161.0602 (M-OC_4H_9).

(*E*)-5-(3-(Pentyloxy)prop-1-en-1-yl)benzo[*d*][1,3]dioxole.

Compound 9d: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.93 (s, 1H, Ar*H*), 6.81 (d, *J* = 7.9 Hz, 1H, Ar*H*), 6.74 (d, *J* = 7.9 Hz, 1H, Ar*H*), 6.50 (d, *J* = 15.8 Hz, 1H, ArCH=), 6.12 (m, 1H, =CH), 5.93 (s, 2H, OCH_2O), 4.09 (d, *J* = 6.0 Hz, 2H, =CHC*H*$_2$O), 3.46 (t, *J* = 6.6 Hz, 2H, OCH_2), 1.60 (m, 2H, CH_2), 1.34 (m, 4H, CH_2), 0.90 (m, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.4 (C), 148.6 (C), 133.2 (CH), 132.7 (C), 126.0 (CH), 122.5 (CH), 109.6 (CH), 107.2 (CH), 102.4 (CH_2), 72.8 (CH_2), 71.9 (CH_2), 30.9 (CH_2), 29.8 (CH_2), 24.0 (CH_2), 15.4 (CH_3). HRMS (ESI): *m/z* 161.0606 (M-OC_5H_{11}).

(*E*)-5-(3-(Hexyloxy)prop-1-en-1-yl)benzo[*d*][1,3]dioxole.

Compound 9e: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91 (s, 1H, Ar*H*), 6.79 (dd, *J* = 8.0, 0.9 Hz, 1H, Ar*H*), 6.71 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.48 (d, *J* = 15.9 Hz, 1H, ArCH=), 6.11 (dt, *J* = 15.9, 6.0 Hz, 1H, =CH), 5.90 (s, 2H, OCH_2O), 4.07 (d, *J* = 6.0 Hz, 2H, =CHC*H*$_2$O), 3.44 (t, *J* = 6.6 Hz, 2H, OCH_2), 1.30 (m, 8H, CH_2 CH_2 CH_2 CH_2), 0.89 (t, *J* = 6.5 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.4 (C), 148.6 (C), 133.2 (CH), 132.7 (C), 126.0 (CH), 122.5 (CH), 109.6 (CH), 107.1 (CH), 102.4 (CH_2), 72.8 (CH_2), 71.9 (CH_2), 33.1 (CH_2), 31.2 (CH_2), 27.3 (CH_2), 24.0 (CH_2), 15.4 (CH_3). HRMS (ESI): *m/z* 161.0607 (M-OC_6H_{13}).

(*E*)-5-(3-(Benzyloxy)prop-1-en-1-yl)benzo[*d*][1,3]dioxole.

Compound 9f: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.24 (m, 5H, Ar*H*), 6.93 (s, 1H, Ar*H*), 6.81 (d, *J* = 7.9 Hz, 1H, Ar*H*), 6.74 (d, *J* = 7.9 Hz, 1H, Ar*H*), 6.53 (1H, d, *J* = 15.8 Hz, ArCH=), 6.20-6.11 (m, 1H, =CH), 5.93 (s, 2H, OCH_2O), 4.56 (s, 2H, $ArCH_2O$), 4.16 (d, *J* = 5.9 Hz, 2H, CH_2O). ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.1 (C), 147.3 (C), 138.3 (C), 132.3 (CH), 131.2 (C), 128.5 (CH), 127.9 (CH), 127.7 (CH), 124.3 (CH), 121.2 (CH), 108.3 (CH), 105.8 (CH), 101.1 (CH_2), 72.2 (CH_2), 70.8 (CH_2). HRMS (ESI): *m/z* 161.0613 (M-$OCH_2C_6H_5$).

(*E*)-5-(3-((4-Methylbenzyl)oxy)prop-1-en-1-yl)benzo[*d*][1,3]dioxole.

Compound 9g: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, *J* = 7.9 Hz, 2H, Ar*H*), 7.16 (d, *J* = 7.7 Hz, 2H, Ar*H*), 6.92 (s, 1H, Ar*H*), 6.81 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.74 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.52 (d, *J* = 15.9 Hz, 1H, =CH), 6.14 (m, 1H, =CH), 5.93 (s, 2H, OCH_2O), 4.51 (s, 2H, $ArCH_2O$), 4.13 (d, *J* = 5.7 Hz, 2H, =CHC*H*$_2$O), 2.34 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.4 (C), 148.7 (C), 138.7 (C), 136.6 (C), 133.6 (CH), 132.7 (CH), 130.5 (CH), 129.3 (CH), 125.8 (CH), 122.6 (CH), 109.7 (CH), 107.2 (CH), 102.5 (CH_2), 73.4 (CH_2), 72.0 (CH_2), 22.6 (CH_3). HRMS (ESI): *m/z* 161.0601 (M-*p*-$MeC_6H_4CH_2$ O).

(*E*)-5-(3-((3-Methylbenzyl)oxy)prop-1-en-1-yl)benzo[*d*][1,3]dioxole.

Compound 9h: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.23 (m, 1H, Ar*H*), 7.19 (s, 1H, Ar*H*), 7.16 (d, *J* = 7.4 Hz, 1H, Ar*H*), 7.11 (d, *J* = 7.5 Hz, 1H, Ar*H*), 6.94 (d, *J* = 1.2 Hz, 1H, Ar*H*), 6.82 (dd, *J* = 8.0, 1.2 Hz, 1H, Ar*H*), 6.75 (d, *J* = 8.0, 1H, Ar*H*), 6.53 (d, *J* = 15.9 Hz, 1H, ArCH=), 6.16 (d, *J* = 15.8 Hz, 1H, =CH), 5.95 (s, 2H, OCH_2O), 4.52 (s, 2H, $ArCH_2O$), 4.16 (m, 2H, CH_2O), 2.36 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.0 (C), 147.3 (C), 138.2 (C), 138.1 (C), 132.3 (CH), 131.2 (C), 128.6 (CH), 128.4 (CH), 128.3 (CH), 124.9 (CH), 124.3 (CH), 121.2 (CH), 108.3 (CH), 105.8 (CH), 101.1 (CH_2), 72.2 (CH_2), 70.8 (CH_2), 21.4 (CH_3). HRMS (ESI): *m/z* 161.0552 (M-*m*-$MeC_6H_4CH_2O$).

MTT Assay.

Materials and Methods:

Materials – RPMI 1640 medium, bovine calf serum, MTT (dimethyl thiazolyl tetrazolium bromide) and 96-well plates were purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd. A549 lung cancer cells were kindly provided by professor Xiao-Yan Zhao (Southwest university, China). All the chemicals were of analytical grade or biological reagent.

Cell Culture: A549 lung cancer cells were cultured in RPMI 1640 medium at 37 ºC with 5% CO_2, and 95% air, supplemented with 10% (v/v) bovine calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin.

Cell Viability Assay: The antiproliferative effect of compounds on A549 cells was determined by MTT assay according to Price *et al*.[2] A549 cells were seeded in 96-well plates at a density of 4~6 × 10^4 cells per well. After overnight incubation, the cells were treated with different concentrations (12.5, 25, 50, 100, 200, 400 μM) of the desired safrole oxide derivatives for 48 h. After the treatment, MTT (5 μg/mL) was added to each well, and incubation was then continued for 4 h at 37 ºC, the cultures were removed and the formazan crystals were dissolved by adding 200 μL DMSO. Cell viability was detected by measuring the absorbance at 570 nm with ELISA plate reader (BioTek). Here, OD_0 and OD_1 indicated the optical density of MTT in DMSO after the cells were incubated only in DMSO and with the safrole oxide derivatives, respectively.

Conclusions

In summary, we have described a facile approach to prepare safrole oxide derivatives **4a-c**, **6a-c** and **9a-h**, and we found 14 novel interesting compounds that could suppress A549 lung cancer cell growth according to the pharmacological experiments. Compound **6b** was the most effective safrole oxide derivative in suppressing A549 cell growth among all the synthesized compounds, giving approximately equal survival cell numbers to the control safrole oxide (**2**) at a concentration of 400 μm. Unfortunately, the detailed structure-activity relationship (SAR) could not be established at present due to the limited variety of compounds. The further SAR of the safrole oxide derivatives in order to determine in detail the structural requirements will be studied in our laboratory.

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