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## Synthesis of ester derivatives of Rhein and their *in vitro* antitumor activities on cervical cancer cells (Hela)

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Taking Rhein as the lead compound, ten esters of rhein were synthesized by esterification. For synthesis of smaller steric hindrance alcohols,  $\text{SOCl}_2$  was used as a catalyst to synthesize methyl, ethyl and butyl esters. For larger steric hindrance alcohols, dicyclohexyl carbodiimide (DCC) was used as dehydrating agent, and 4-dimethylaminopyridine (DMAP) was used as the catalyst to synthesize isopropyl, isobutyl, tertbutyl, isoamyl, benzyl, 2-phenyl ethyl, 2-chloroethyl esters. Structural characterization of the target compounds were done using melting point,  $^1\text{H NMR}$ ,  $^{13}\text{C NMR}$  and HRMS. Five out of the 10 compounds were new. All the compounds were evaluated for antitumor activities *in vitro* against Hela human cervical carcinoma cell lines. Study found that all the ten compounds showed differences in their growth inhibitory effect on tumor cells. Compound with benzyl groups improved the antitumor activity. Results showed that compound 3b exhibited maximum activity against Hela cell lines at  $100 \mu\text{g mL}^{-1}$  ( $\text{IC}_{50}$  value) with an inhibition rate of 70%, while the derivative 3i showed the lowest inhibitory activity ( $\text{IC}_{50} < 64.3 \mu\text{g mL}^{-1}$ ).

**Keywords:** Anticancer, Antitumor, DMAP, Ester derivatives, Esterification, Rhein

For humans, cancer is one of the most important life threatening diseases. As much as 8.1 million people are reported to be suffering from cancer that accounts for 16% of total deaths worldwide. Asia leads both in cancer incidence (57.3%) and cancer mortality (48.4%). It is reported that United States would approximately record 1.81 million new cancer cases and 0.61 million deaths in 2020<sup>1-3</sup>. In drug research, plants serve as the natural reservoir of innumerable bioactive components exhibiting various toxic as well as beneficial properties. Newman *et al.*<sup>4</sup> found that more than 60% new chemical entities of small molecules are drugs sourced from the wild plants.

Rhein, a main constituent of rhubarb isolated from *Rheum spiciforme*, belongs to the hydroxyl group, and

its anthraquinone derivatives represent a wide range of anti-inflammatory, antioxidant, antiviral, hypoglycemic, lipid-lowering effects and pharmacological activities<sup>5-10</sup>. More importantly, rhein has significant resistance, tumor activity and synergistic anti-tumor potential<sup>11-13</sup>. Numerous reports show that anthraquinone derivatives can be used as antitumor drugs<sup>5,7,14</sup>. Drugs with anthraquinone structure as pharmacophore have become an important class of clinical anticancer drugs, such as doxorubicin, mitoxantrone, etc. (Fig. 1).

Doxorubicin mainly affects topoisomerase enzyme II activity to show its anti-tumor effects. Mitoxantrone is a DNA intercalator, which can intercalate into DNA bases and blocks DNA synthesis and transcription<sup>15</sup>. Intermittently, doxorubicin can inhibit DNA topoisomerase II, causes unwinding of genomic DNA. Therefore, Rhein can be used as a potential lead in finding anti-tumor drugs. Available research is mainly focused on pharmacological properties of rhein, and its derivatization at 3-position structure, and the simultaneous derivatization of its 1,8- and 3- position<sup>16,17</sup>. The current study involves derivatization of 1,8 position of rhein. Besides, it also focuses on its tumor activity and ability to interact with DNA which are expected to enhance the series solubility of the drug and relieve the cytotoxicity of the drug, and improve its bioavailability rate.

## Materials and Methods

### Instruments and reagents

Bruker Avance III HD 400 nuclear magnetic resonance instrument ( $\text{CDCl}_3$  or  $\text{DMSO}-d_6$  is the solvent; AL104 analytical balance WRS-1B digital

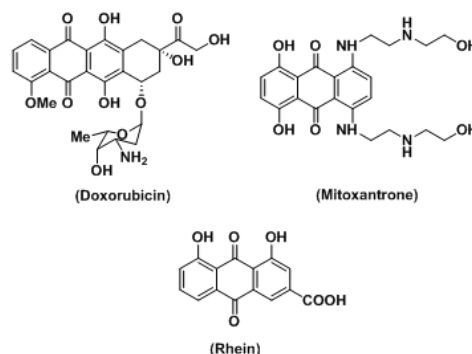


Fig. 1 — Chemical structures of doxorubicin, mitoxantrone and rhein.

melting point instrument (Shanghai Precision Scientific Instrument Co., Ltd. Division); Full Automatic polarimeter (Rudolph Autopol V); silica gel for column chromatography (200~300 mesh, Qingdao Ocean Chemical Co., Ltd.); and IC<sub>50</sub> concentration calculation software is Origin 9.0 were used in the study. All chemicals of analytical grade were purchased from Yu Aladdin Reagents (Shanghai) Co., Ltd. and Adamas reagent company Ltd. (Shanghai).

### Synthesis of compounds

Dissolve rhein (5 mmol) in absolute ethanol 20 mL as a solvent and add 5 mL of SOCl<sub>2</sub> and DMAP as a catalyst in a 100 mL round bottom flask and heat at 80-90°C. After 24 h, the raw material was completely converted. Reaction solution was poured directly into 500 mL of distilled water, followed by suction filtration, and washed the solid with a large amount of distilled water, solid was fully dried in a vacuum drying oven to obtain 1.464 g of compound 2 (yield 95%). Then compound 2 was added in 100 mL round bottom flask (1.0 mmol, 312 mg), DMF 50 mL, then slowly NaH (5 mmol, 120 mg) was added after changing the color of reaction. Excess amount of iodine/bromoalkane hydrocarbon (6 mmol) was added carefully. The reaction liquid is heated to 100-120°C to react to the raw material point disappears and it was monitoring by TLC. After the reaction, the 30mL (1.0 mol/L) of HCl was added to the reaction mixture, and the aqueous solution was extracted using dichloromethane, and the organic phase was collected. The product obtained by column chromatography [Eluent (hexane): (ethyl acetate) = 5:1] and the solid product was obtained after rotary evaporation under reduced pressure. In 50 mL RBF, the crude product was collected and 20 mL (1.0 mol/L) NaOH aqueous solution was added at room temperature (25°C) to 60°C. Stir and monitor the progress of the reaction by TLC till completion of hydrolysis. Then, 1.0 mol/L HCl was used to adjust the pH of the aqueous solution (5-6 pH). After reaching pH 5-6 the product was precipitated and filter using suction, washed the obtained solid product with deionized water, and dried it. The yellow solid obtained (3a-3j) having the yield is 46~86%.

### In vitro anti-tumor activity test

Cells at about 10.5 mg/mL density were seeded in 96-well plates, each well was inoculated with 100 µL and incubate in a CO<sub>2</sub> incubator until the logarithmic

growth phase. The samples were tested as per concentration gradient, and test was repeated three times for each gradient. The control group was added an equal volume of solvent for dissolving the sample. After 48 h of incubation, 20 µL of MTT (5 mg/mL) were added to each well, and then place at 37°C for 4 h for incubation. After removing the supernatant, 100 µL of DMSO was added to each well and shaken well. The precipitate was allowed to dissolve for 10 min, and then checked for optical density (OD) value with a microplate reader at a wavelength of 490 nm. Following formula was used to find the cell survival rate at a certain concentration of the sample:

$$\text{Survival rate (\%)} = \frac{\text{Average OD value of the sample group}}{\text{Average OD of the control group value}} \times 100$$

The cell survival rate was plotted against the logarithm of the drug concentration, and drawing method was used to calculate IC<sub>50</sub> value for each sample.

## Results and Discussion

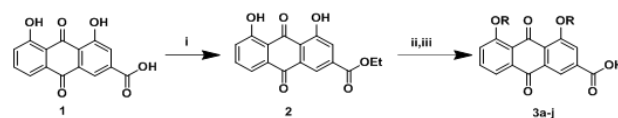
### Compound synthesis and their structure

As shown in Scheme 1 for the synthesis of targeted molecules, rhein (**1**) was used as the starting material, SOCl<sub>2</sub>/DMAP as a catalyst in ethanol as a solvent under reflux conditions for esterification reaction<sup>18</sup>. Highly substituted or halogenated alkanes ring gave more yield and stability in the presence of DMAP with respect to SOCl<sub>2</sub>. Ethyl rhein **2** was obtained, which reacted with different halogenated hydrocarbons in the presence of strong base sodium hydride. Ether was formed under the hydrolysis of the ester to obtain 1,8-dialkoxy rhein (**3a-j**). The structure of the target molecules were characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR, and HRMS.

### Analysis of target compound (3a-j)

#### 3a. 4,5-dimethoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid

Yellow solid, yield 84%. mp 218.8~221.7°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 12.74 (s, 1 H), 7.95 (s, J =



R = methyl, ethyl, butyl, benzyl, isopropyl, isobutyl, tertbutyl, isoamyl, 2-phenyl ethyl, 2-chloroethyl.

Scheme 1: Synthesis route of the target compound [reagents and conditions: (i) SOCl<sub>2</sub>, EtOH, reflux; (ii) R<sub>1</sub>X, NaH, DMF, 100-120°C; (iii) 1mmol/L DCC (aq.), RT to 60°C; then 1 mol/L HCl (aq.), RT

1.5 Hz, 1 H), 7.82 (d,  $J = 7.6$ , Hz, 1 H), 7.67 (s,  $J = 1.5$  Hz, 1 H), 7.32 (d,  $J = 7.5$  Hz, 1 H), 7.84 (t,  $J = 7.4$  Hz, 1 H), 3.90 (s,  $J = 7.6$ , 3 H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz): d 182.2, 182.2, 159.8, 160.1, 120.5, 125.4, 131.2, 135.8, 134.5, 123.0, 119.1, 120.4, 117.6, 133.2, 169.3, 55.4, 55.4 and HRMS  $[\text{M} + \text{H}]^+$  312.0347.

**3b. 4, 5-diethoxy-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid**

Yellow solid, yield 84%. mp 218.8~223.7°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  12.74 (s, 1 H), 7.95 (s,  $J = 1.5$  Hz, 1 H), 7.82 (d,  $J = 7.6$ , Hz, 1 H), 7.67 (s,  $J = 1.5$  Hz, 1 H), 7.32 (d,  $J = 7.5$  Hz, 1 H), 7.84 (t,  $J = 7.4$  Hz, 1 H), 4.5 (t,  $J = 8.0$ , 2 H), 1.34 (m,  $J = 8.0$ , 3 H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz): d 182.2, 182.2, 158.3, 157.8, 124.7, 125.4, 130.6, 135.4, 134.3, 123.3, 118.4, 119.7, 117.9, 132.4, 169.3, 64.7, 64.7, 14.7, 14.7 and HRMS  $[\text{M} + \text{H}]^+$  340.0653.

**3c. 4, 5-dibutoxy-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid**

Yellow solid, yield 84%. mp 215.8~220.7°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  12.74 (s, 1H), 7.95(s,  $J = 1.5$  Hz, 1 H), 7.82 (d,  $J = 7.6$ , Hz, 1 H), 7.67 (s,  $J = 1.5$  Hz, 1 H), 7.32 (d,  $J = 7.5$  Hz, 1 H), 7.84 (t,  $J = 7.4$  Hz, 1 H), 4.5 (t,  $J = 7.1$  2 H), 1.74 (m,  $J = 7.1$ , 2 H), 1.47 (m,  $J = 7.1$ -8.0 2 H), 0.96 (t,  $J = 8.0$ , 3 H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz): d 182.3, 182.3, 158.0, 157.7, 124.6, 125.3, 130.6, 135.4, 134.2, 123.5, 118.2, 119.7, 117.8, 132.7, 169.5, 68.2, 68.2, 31.7, 31.7, 19.3, 19.3, 14.1, 14.1 and HRMS:  $[\text{M} + \text{H}]^+$  396.1537.

**3d. 4, 5-bis(benzyloxy)-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid**

Yellow solid, yield 84%. mp 217.8~224.7°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  12.74 (s, 1 H), 7.95 (s,  $J = 1.5$  Hz, 1 H), 7.82 (t,  $J = 7.6$ , Hz, 1 H), 7.67 (s,  $J = 1.5$  Hz, 1 H), 7.47(d,  $J = 7.5$ -1.5 Hz, 1 H), 7.3 (d,  $J = 7.5$ -1.5 Hz, 1 H), 7.3 (t,  $J = 7.4$  Hz, 1 H), 7.40 (tt,  $J = 7.5$ -1.5, 1 H), 7.34 (t,  $J = 7.5$ -1.5, 1 H), 5.16 (s, 3 H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz): d 182.2, 182.2, 161.4, 161.2, 120.4, 125.5, 131.2, 135.9, 134.5, 136.7, 136.6, 133.0, 119.4, 120.2, 127.3, 127.3, 133.2, 128.8, 128.9, 128.8, 128.9, 127.6, 127.6, 169.2, 70.8, 70.8 and HRMS  $[\text{M} + \text{H}]^+$  464.1217.

**3e. 4, 5-diisopropoxy-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid**

Yellow solid, yield 84%. mp 218.8~225.7°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  12.74 (s, 1 H), 7.95(s,  $J = 1.5$  Hz, 1 H), 7.82 (d,  $J = 7.6$ , Hz, 1 H), 7.67 (s,  $J = 1.5$  Hz, 1 H), 7.32 (d,  $J = 7.5$  Hz, 1 H), 7.85 (t,  $J =$

7.4 Hz, 1 H), 4.69 (m,  $J = 6.8$ , 1 H), 1.31 (dd,  $J = 6.8$ , 3 H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz): d 182.2, 182.0, 158.2, 157.8, 121.5, 125.2, 130.1, 135.8, 134.9, 123.1, 118.6, 119.7, 117.2, 132.7, 169.7, 75.8, 75.8, 22.3, 22.3, 23.3, 23.3 and HRMS  $[\text{M} + \text{H}]^+$  368.1219.

**3f. 4, 5-diisobutoxy-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid**

Yellow solid, yield 84%. mp 216.2~221.3°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  12.74 (s, 1 H), 7.95(s,  $J = 1.5$  Hz, 1 H), 7.82 (d,  $J = 7.6$ , Hz, 1 H), 7.67 (s,  $J = 1.5$  Hz, 1 H), 7.32 (d,  $J = 7.5$  Hz, 1 H), 7.84 (t,  $J = 7.4$  Hz, 1 H), 3.86 (d,  $J = 7.0$ , 2 H), 1.82 (m,  $J = 6.8$ , 1 H), 0.61 (d,  $J = 6.8$ , 3H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz): d 182.4, 182.4 158.3, 157.8, 124.6, 125.8, 130.6, 135.4, 134.6, 123.8, 118.4, 119.8, 117.4, 132.6, 169.3, 74.6, 74.6, 28.3, 19.6, 19.6, 19.6, 19.6 and HRMS  $[\text{M} + \text{H}]^+$  396.1534.

**3g. 4, 5-di-tert-butoxy-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid**

Yellow solid, yield 84%. mp 219.8~226.5°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  12.74 (s, 1 H), 7.96 (s,  $J = 1.5$  Hz, 1 H), 7.82 (d,  $J = 7.6$ , Hz, 1 H), 7.65 (s,  $J = 1.5$  Hz, 1 H), 7.32 (d,  $J = 7.5$  Hz, 1 H), 7.84 (t,  $J = 7.4$  Hz, 1 H), 1.43(s,  $J = 7.6$ , 3 H), 71.43 (s,  $J = 7.6$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz): d 182.2, 182.2, 158.2, 157.7, 120.9, 125.7, 130.3, 135.6, 134.2, 123.6, 118.7, 119.2, 117.3, 132.7, 169.5, 86.0, 86.0, 27.8, 27.8, 27.8, 27.8 and HRMS  $[\text{M} + \text{H}]^+$  396.1539.

**3h. 4, 5-bis(isopentyloxy)-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid**

Yellow solid, yield 84%. mp 217.5~222.7°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  12.74 (s, 1 H), 7.96 (s,  $J = 1.5$  Hz, 1 H), 7.83 (d,  $J = 7.6$ , Hz, 1 H), 7.65 (s,  $J = 1.5$  Hz, 1 H), 7.32 (d,  $J = 7.5$  Hz, 1 H), 7.84 (t,  $J = 7.4$  Hz, 1 H), 4.05 (t,  $J = 7.1$ , 2 H), 1.81 (m,  $J = 7.0$ , 3 H), 1.66 (m,  $J = 7.1$  Hz, 2 H), 0.97 (d,  $J = 6.8$  Hz, 3 H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz): d 182.3, 182.3, 158.2, 157.8, 124.5, 125.3, 130.6, 135.4, 134.7, 123.2, 118.5, 119.3, 117.6, 132.4, 169.2, 65.8, 65.8, 24.3, 24.3, 39.4, 39.4, 23.1, 23.1, 23.1 and HRMS $[\text{M} + \text{H}]^+$  396.1543.

**3i. 9, 10-dioxo-4, 5-diphenethoxy-9, 10-dihydroanthracene-2-carboxylic acid**

Yellow solid, yield 84%. mp 214.2~220.7°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  12.74 (s, 1 H), 7.96 (s,  $J = 1.5$  Hz, 1 H), 7.82 (d,  $J = 7.6$ , Hz, 1 H), 7.65 (s,  $J = 1.5$  Hz, 1 H), 7.21 (d,  $J = 7.5$  Hz, 1 H), 7.32 (d,  $J = 7.5$  Hz, 1 H), 7.21 (d,  $J = 7.5$  Hz, 1 H), 7.84 (t,  $J = 7.4$  Hz, 1 H), 7.23 (t,  $J = 7.5$ , 1 H), 7.23 (t,  $J = 7.5$ , 1 H) 7.19 (t,  $J = 7.5$ , 1 H), 4.27 (t,  $J = 7.1$  Hz, 2 H), 3.10 (t,  $J = 7.1$  Hz, 2

H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz): d 182.1, 182.1, 158.3, 157.7, 124.5, 125.8, 130.6, 135.4, 134.3, 138.4, 138.4, 123.6, 115.3, 119.5, 127.6, 127.6, 117.9, 127.5, 127.5, 132.7, 128.5, 128.5, 128.5, 128.5, 125.7, 125.7, 169.3, 67.2, 67.2, 35.3, 35.3 and HRMS  $[\text{M} + \text{H}]^+$  379.9835.

**3j. 4, 5-bis(2-chloroethoxy)-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid**

Yellow solid, yield 84%. mp 219.1~227.7°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  12.74 (s, 1 H), 7.96 (s,  $J = 1.5$  Hz, 1 H), 7.82 (d,  $J = 7.6$  Hz, 1 H), 7.65 (s,  $J = 1.5$  Hz, 1 H), 7.32 (d,  $J = 7.5$  Hz, 1 H), 7.84 (t,  $J = 7.4$  Hz, 1 H), 4.09 (t,  $J = 7.1$ , 2 H), 4.22 (t,  $J = 7.0$ , 2 H),  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz): d 182.2, 182.2, 158.2, 157.8, 124.5, 125.7, 130.6, 135.4, 134.1, 123.2, 118.4, 119.5, 117.5, 132.5, 42.6, 42.6, 169.2, 75.1, 75.1 and HRMS  $[\text{M} + \text{H}]^+$  492.1537.

**Biological activity of pure molecules using MTT assay and *in vitro* anti-tumor activity test**

**Anti-tumor activity based on structural analysis**

The colorimetric method of tetramethyl azozole (MTT) was used to colour the target compound (3a-j) and were tested for *in vitro* antitumor activity. Cisplatin and amycin were used as the positive control. The substrate scope of the target compounds is shown in Table 1.

All the derivatives did not show noticeable resistance to tumor activity. The isobutyl (3f) and isoamyl (3h) showed significant antitumor activity compared to other derivatives in the same series. For the compound with benzyl groups, the antitumor activity of all derivatives was further improved and the entire series showed general inhibitory activity in Hela human cervical carcinoma cell lines. Tikhomirov *et al.*<sup>19</sup> found that anthraquinone derivative (anthracene-9,10-dione) represent an exceptionally valuable class in anticancer drug development. It was also established that chemical structure modifications helps in optimization of the anticancer properties of natural compounds.

***In vitro* anti-tumor activity basis of  $\text{IC}_{50}$  value**

Compounds 3a-e showed  $\text{IC}_{50} > 100 \mu\text{g mL}^{-1}$ , while as compounds 3f-j showed  $\text{IC}_{50} < 100 \mu\text{g mL}^{-1}$ . Results showed that compound 3b exhibited high activity against Hela cell line at  $\text{IC}_{50}$  value  $> 100 \mu\text{g mL}^{-1}$  and an inhibition rate is of 70%. The derivative 3i showed lowest inhibitory activity ( $\text{IC}_{50} < 64.3 \mu\text{g mL}^{-1}$ ) when tested on Hela human cervical carcinoma cell lines. The  $\text{IC}_{50}$  value for each sample is shown in Table 2.

Table 1 — Substrate scope of the target Rhein derivatives (3a-j)

Compound	R	Catalyst
3a	-CH <sub>3</sub>	SOCl <sub>2</sub>
3b	-CH <sub>2</sub> CH <sub>3</sub>	SOCl <sub>2</sub>
3c	-CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	SOCl <sub>2</sub>
3d	Ph -CH <sub>2</sub> -	DMAP
3e	-CH(CH <sub>3</sub> ) <sub>2</sub>	DMAP
3f	-CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	DMAP
3g	-C(CH <sub>3</sub> ) <sub>3</sub>	DMAP
3h	-CH <sub>2</sub> CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	DMAP
3i	Ph -CH <sub>2</sub> -CH <sub>2</sub>	DMAP
3j	-CH <sub>2</sub> CH <sub>2</sub> Cl	DMAP

Table 2 — *In vitro* anti-tumor activity of ten Rhein derivatives

Compound	Hela cell line $\text{IC}_{50}$ ( $\mu\text{g mL}^{-1}$ )	Compound	Hela cell line $\text{IC}_{50}$ ( $\mu\text{g mL}^{-1}$ )
3a	>100	3f	69.8
3b	>100	3g	66.2
3c	>100	3h	69.1
3d	>100	3i	64.3
3e	>100	3j	68.4

**Conclusions**

The current study used rhein as raw material, and carried out its esterification, alkylation, hydrolysis, condensation in different steps. Then, ten esters derivatives of rhein were designed and synthesized. Structural conformation was carried out for this series of compounds using different analytical methods including  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and HRMS. MTT assay was used to study the effect of compounds on cervical cancer cells (Hela). Results showed that compound 3b exhibited high activity against Hela cell line at  $\text{IC}_{50}$  value  $> 100 \mu\text{g mL}^{-1}$  and an inhibition rate is of 70%, while as compound 3i showed lowest inhibitory activity ( $\text{IC}_{50} < 64.3 \mu\text{g mL}^{-1}$ ). Study found that all the ten compounds showed differences in their growth inhibitory effect on tumor cells. Compound with benzyl groups showed improved the antitumor activity.

**Conflict of interest**

The authors declare no conflict of interests in this study.

**References**

- 1 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA & Jemal A, *CA Cancer J Clin*, 68 (2018) 394.
- 2 Siegel RL, Miller KD & Jemal A, *CA Cancer J Clin*, 7 (2020) 7.
- 3 American Cancer Society, *Global Cancer Facts & Figures 4<sup>th</sup> Edn.*, (American Cancer Society, Atlanta, USA), 2018. <https://www.cancer.org/content/dam/cancerorg/research/cancer-facts-and-statistics/global-cancer-factsand-figures/global-cancer-facts-and-figures-4th-edition.pdf>.

- 4 Newman DJ, & Cragg GM, *J Na. Prod*, 70 (2007) 461.
- 5 Agarwal SK, Lakshmi V, Verma S & Kumar S, *J Sci Ind Res*, 60 (2001) 1.
- 6 Singh SS, Pandey SC, Singh R & Agarwal SK, *Indian J Chem B*, 44B (2005) 1494.
- 7 Bhat AH, Dar KB, Sofi MA, Dar SA, Masood A & Ganie SA, *Indian J Exp Biol*, 56 (2018) 556.
- 8 Liu X, Cheng J, Zheng XC, Chen YG, Wu C, Li B, Fu JF, Cao HW, Lu YL, Li J, Zheng J & Zhou H, *Curr Vasc Pharmacol*, 7 (2007) 293.
- 9 Yu L, Xiang H, Fan J W, Wang DC, Yang F, Guo N, Jin Deng & QXM, *J Biotechnol*, 135 (2008) 304.
- 10 Yadav A, Bhardwaj R & Sharma RA, *Res J Med Plant*, 7 (2013) 150.
- 11 Fernand VE, Losso JN, Truax RE, Villar EE, Bwambok DK, Fakayode SO, Lowry M & Warner IM, *Chem Biol Interact*, 192 (2011) 220.
- 12 Hsia TC, Yang JS, Chen GW, Chiu TH, Lu HF, Yang MD, Yu FS, Liu KC, Lai KC, Lin CC & Chung JG, *Anticancer Res*, 29 (2009) 309.
- 13 Lai WW, Yang JS, Lai KC, Kuo CL, Hsu CK, Wang CK, Chang CY, Lin JJ, Tang NY, Chen PY, Huang WW & Chung JG, *In Vivo*, 23 (2009) 309.
- 14 Marković V, Debeljak N, Stanojković T, Kolundzija B, Sladić D, Vujčić M, Janović B, Tanić N, Perović M, Tesić V & Antić Joksović JMD, *Eur J Med Chem*, 89 (2015) 401.
- 15 Barthel BL, Mooz EL, Wiener LE, Koch GG & Koch TH, *J Med Chem*, 59 (2016) 2205.
- 16 Zhu X, Ye X, Song L, Luo Y, Tang Q, Jin Y & Li X, *Med Chem Res*, 22 (2013) 2228.
- 17 Yuan YF, Hu XY, He Y & Deng JG, *Nat Prod Commun*, 7 (2012) 207.
- 18 Changjian Z, Jianwei X, Jie Z & Bin D, *Chinese J Org Chem*, 37 (2017) 122.
- 19 Tikhomirov AS, Shtil AA & Shchekotikhin AE, *Recent Pat Anticancer Drug Discov*, 13 (2018) 159.