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DESIGN, SYNTHESIS AND ANTICANCER EVALUATION OF NEW 7-O-ALKYLATION GENISTEIN DERIVATIVES

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Genistein is a phytoestrogen compound which possesses multiple biological activities such as anti-cancer, while its application is limited by poor pharmacokinetic properties. Structural modification is an effective approach to get genistein derivatives with better activities and approved pharmacokinetic properties. Based on previous research work, we synthesized two series of genistein derivatives bearing halogen substituents, and evaluated their inhibitory effects on the cancer cell lines MCF-7, MDA-MB-231, and MDA-MB-435. Among these derivatives, compound **XI** displayed the best inhibitory activity against MCF-7, MDA-MB-231 and MDA-MB-435 cell lines *in vitro*, which is worth further studies.

Keywords: genistein derivatives; isoflavone; anticancer activity; MTT assay; synthesis.

1. INTRODUCTION

Breast cancer is the fifth leading cause of cancer-related death worldwide and the second leading cause of cancer death among women, accounting for approximately 626679 deaths in 2018 [1]. It is noteworthy that the breast cancer morbidity in some Asian countries such as China and Japan is on a relatively lower level [2]. This is probably related with Asian peoples having high consumption of beans that are rich in flavonoids. As a member of plant polyphenols, the flavonoid phenol groups in their structures may contribute to their anticancer activity [3].

Genistein (4′,5,7-trihydroxyisoflavone) is an isoflavonoid compound widely distributed in plants [4, 5], which had been emphasized by more than 3600 papers in the past decade because of its multiple biological effects such as the anticancer effect [6 – 9] and especially anti-breast cancer activity [19, 11]. Genistein is also a phytoestrogen due to its structural features similar to those of estradiol-17β estrogen [12]. However, the application of genistein is limited because of its structural characteristics, susceptibility to oxidation, *in vivo* instability, and poor solubility [13]. Up to now, numerous research works have studied the structure activity relationship of genistein and its derivatives, in the hope of getting potent anti-cancer agents through structural modification [14 – 16].

Our group had previously designed and synthesized several series of flavone derivatives and carried out their activity evaluation *in vitro* and *in vivo*, which showed that many of them possessed potent anticancer activity [17 – 19]. Inspired by the previous research work, we have designed and synthesized two series of genistein derivatives and evaluated their anticancer activity *in vitro* against MCF-7, MDA-MB-231, and MDA-MB-435 cell lines.

2. RESULTS AND DISCUSSION

2.1. Chemistry

All the new genistein derivatives were synthesized via a route presented in Fig. 1 under the indicated optimum conditions. Phloroglucinol and two kinds of benzacetonitrile (**Ia** and **Ib**) were used as starting compounds for the preparation of target genistein derivatives. The yield of **IIb** was significantly higher than that of **IIa**, mainly in the hydrolysis process of recrystallization. In step 2, we used icy water to quench the reaction, after which a pale yellow precipitate was obtained. In the presence of KI in step 3, the reaction

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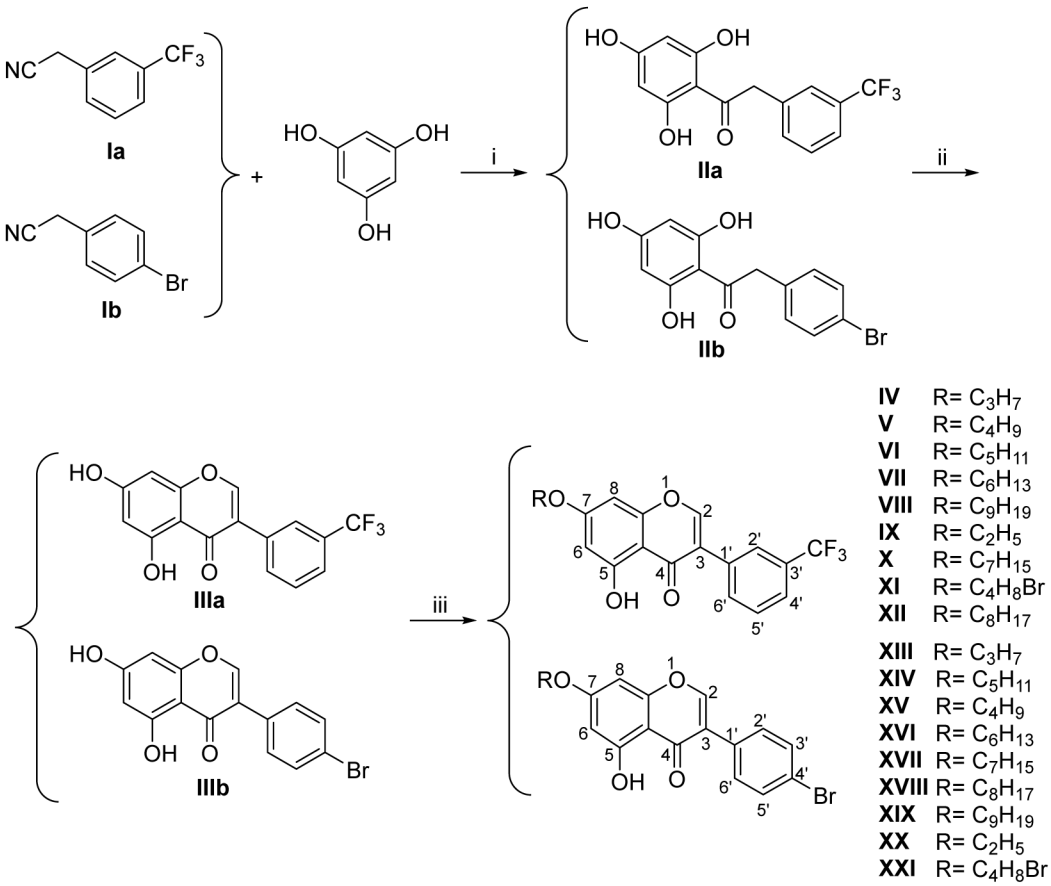

Fig. 1. Synthetic route for compounds **II** – **XXI**. Reagents and conditions: (i) $ZnCl_2$, HCl (g), Et_2O, 0°C; (ii) PCl_5, $BF_3.Et_2O$, DMF; (iii) K_2CO_3, KI, RBr, acetone, reflux.

time could be greatly shortened, and the reflux temperature could also be lowered [20].

All halogenated hydrocarbons used in the alkylation reaction were bromo-alkanes. Due to the effect of hydrogen bonding between 7-OH group and 4-carbonyl, the alkylation of genistein only occurred at the 7-OH group. All products were accurately analyzed by spectroscopic techniques, and the results were in full accordance with the proposed structures (Fig. 2).

2.2. Virtual Screening

Based on the structure of estrogen receptor and the previous structure activity relationship study, we applied CADD (computer-aided drug design) to the rational design of the genistein derivatives, and used the SYBYL-Surflex docking to test the compounds' *in silico* affinity for the estrogen receptor.

According to the virtual screening results (Table 1), compounds with longer alkyl side chains were expected to be stronger estrogen blockers, the probable reason being that the long lipophilic side chains are preferred for binding to the hydrophobic pocket of estrogen receptor. However, it seems that compounds show decreased total score when the alkyl side chains become too long (≥ 9 atoms) as can be explained by their steric hindrances.

From the binding mode of these compounds with estrogen receptor (Fig. 3), it can be seen that the alkyl side chain is located in the hydrophobic pocket, forming hydrophobic interaction with the receptor.

2.3. Biological Activity

Totally 18 genistein derivatives were evaluated for their antiproliferative activity against MCF-7, MDA-MB-231, and MDA-MB-435 cell lines. The results are summarized in Table 2. From these results, we preliminarily concluded that some of the synthesized compounds displayed cellular activity comparable with the positive control drugs cisplatin (DDP) and Tamoxifen (TAM). Among all the drugs tested, compounds **IX**, **XI**, **XIV** and **XV** showed moderate antiproliferative activity. Compound **XI** showed the best activity, while other compounds did not exhibit evident inhibitory effect.

As was mentioned above, the virtual screening results showed that compounds with longer alkyl side chains were

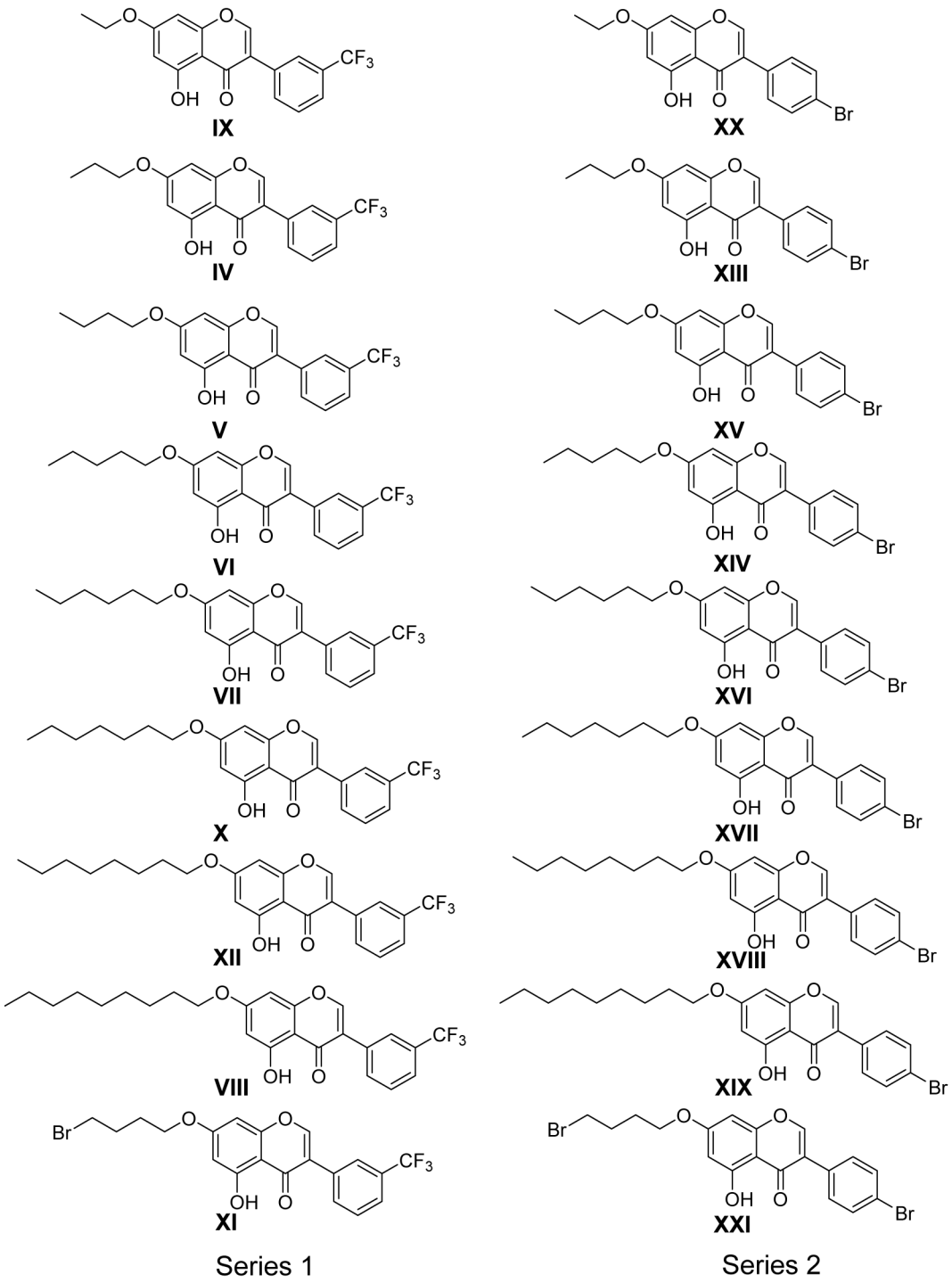

Fig. 2. Two series of genistein derivatives.

likely to be better anti-breast cancer agents. However, the cellular evaluation results were quite another story, in which compounds **VIII** and **XIX** with the longest alkyl side chains did not show any activity. The possible reason for this discrepancy is that the activity of compounds is dramatically affected by their hydrophilicity/lipophilicity balance.

As can be seen from Table 2, in series 1, compound **IX** with C_2H_5 side chain is more powerful than compounds (such as **IV**, **V**, and **VI**) with longer side chains. This is probably due to decreased hydrophilicity of compounds **IV**, **V**, and **VI** caused by their longer alkyl side chains. Compound **XI** with a longer C_4H_8Br chain exhibits the best activity,

which can be explained by its increased hydrophilicity related to a more stable O^- ion form. In series 2 (because of the lower lipophilicity of -Br group compared to -CF_3 group) compounds need relatively more lipophilic groups to get better activity. Accordingly, compounds **XIV** and **XV** showed the best potential activity in this series. These results indicate that the 7-O-alkylation of genistein is an effective approach to obtaining compounds with improved antiproliferative activity. Further pharmacodynamic and pharmacokinetic evaluation as well as toxicological study of these lead compounds is underway, in the hope of producing drug candidates for drug development.

3. EXPERIMENTAL CHEMICAL PART

3.1. Instrumentation and Chemicals

Melting points were measured on a Kofler apparatus, uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 400(400-MHz) spectrometer in $CDCl_3$ with Me_4Si as internal standard. All chemical shifts (δ) are expressed as parts per million (ppm) and coupling constants (*J*) are given in Hertz. Mass spectra were recorded on Waters Micromass GCT Premier and Thermo Fisher Scientific LTO FT Ultra using EI ionization at 70 eV and DART positive operation mode. All reagents were commercial materials used without further purification.

*3.2. General Procedures for Preparation of Compounds **IX – XXI***

A mixture of compound **I** (10 mmol, **Ia** 1.85 g, or **Ib** 1.96 g), phloroglucin (12 mmol, 1.52 g), $ZnCl_2$ (10 mmol, 1.36 g), and 80 mL of anhydrous diethyl ether were stirred on ice bath for 4 – 8 h under addition of dry HCl gas, and then the mixture was put into fridge for two days. Then, the addition of dry HCl gas was continued for another 4 – 8 h on ice bath, after which the mixture was placed in fridge for three days. The obtained solid was recrystallized in H_2O to give light yellow solid **II** (yield 54%, **IIa** 3.12 g, or **IIb** 3.22 g). Intermediate **II** (5 mmol, **IIa** 1.56 g, or **IIb** 1.62 g) was dissolved in 30 mL DMF, after which a mixture of boron trifluoride etherate (40 mmol, 5.68 g) and DMF (40 mmol, 3.1 mL) was added dropwise (on ice bath) and 20 – 30 min later the mixture was transferred onto 80°C oil bath, and a mixture of PCl_5 (15 mmol, 3.12 g) and DMF (15 mmol, 1.17 mL) was added dropwise. Then, the reaction mixture was refluxed for 3 – 5 h, ice water was added, and the mixture was filtered to get light yellow solid **III** (yield 85%, **IIIa** 1.37 g, or **IIIb** 1.41 g). A mixture of **III** (1 mmol, **IIIa** 0.32 g, or **IIIb** 0.33 g), K_2CO_3 (3 mmol, 0.41 g), KI (1mmol, 0.17 g), RBr (3 mmol), and 30 mL of acetone was stirred at 60 – 80°C with reflux for 2 – 5 h. Then, the mixture was concentrated and purified by flash column chromatography to obtain the desired product as white solid or pale yellow needles.

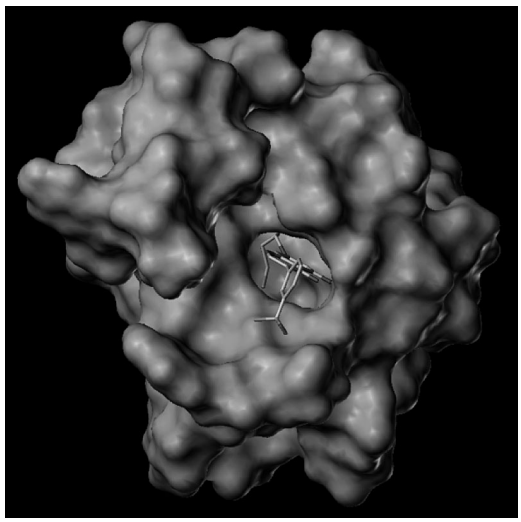

Fig. 3. The binding mode of a designed genistein derivative with estrogen receptor.

5-Hydroxy-7-propoxy-3-(3-(trifluoromethyl)phenyl)-4H-chromen-4-one (**IV**): white needles, 0.24 g, yield: 65.6%, m.p. 125 – 128°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.63 (s, 1H), 7.93 (s, 1H), 7.80 – 7.73 (m, 2H), 7.66 (d,

TABLE 1. Results Virtual Screening of the Synthesized Compounds

Compound	Side chain	B ring substituent	Total score
IX	C_2H_5	3′-CF_3	4.7837
IV	C_3H_7	3′-CF_3	6.2982
V	C_4H_9	3′-CF_3	7.9467
VI	C_5H_{11}	3′-CF_3	7.2333
VII	C_6H_{13}	3′-CF_3	7.8648
X	C_7H_{15}	3′-CF_3	8.4146
XII	C_8H_{17}	3′-CF_3	9.1928
VIII	C_9H_{19}	3′-CF_3	9.7859
XI	C_4H_8Br	3′-CF_3	8.3277
XX	C_2H_5	4′-Br	5.2892
XIII	C_3H_7	4′-Br	6.3325
XV	C_4H_9	4′-Br	6.7209
XIV	C_5H_{11}	4′-Br	6.9042
XVI	C_6H_{13}	4′-Br	7.1115
XVII	C_7H_{15}	4′-Br	8.2815
XVIII	C_8H_{17}	4′-Br	9.2914
XIX	C_9H_{19}	4′-Br	8.4162
XXI	C_4H_8Br	4′-Br	5.9875

J = 7.9 Hz, 1H), 7.58 (t, J = 7.8 Hz, 1H), 6.43 (d, J = 2.3 Hz, 1H), 6.40 (d, J = 2.3 Hz, 1H), 4.00 (t, J = 6.6 Hz, 2H), 1.90 – 1.80 (m, 2H), 1.06 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.0, 165.4, 162.6, 157.9, 153.5, 132.3(d, J = 1.0 Hz), 131.7, 131.0 (q, J = 32.4 Hz), 129.0, 125.6 (q, J = 3.9 Hz), 125.1 (q, J = 3.7 Hz), 128.09 – 119.79 (m), 122.8, 106.0, 98.9, 93.1, 70.2, 22.3, 10.4. MS (EI, 70 eV): 364.0923 ($C_{19}H_{15}O_4F_3$; calc. 364.0922).

7-Butoxy-5-hydroxy-3-(3-(trifluoromethyl)phenyl)-4H-chromen-4-one (**V**): white needles, 0.26 g, yield: 69.2%, m.p. 135 – 137°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.63 (s, 1H), 7.93 (s, 1H), 7.80 – 7.73 (m, 2H), 7.66 (d, J = 7.9 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 6.42 (d, J = 2.2 Hz, 1H), 6.40 (d, J = 2.3 Hz, 1H), 4.04 (t, J = 6.5 Hz, 2H), 1.81 (dt, J = 14.4, 6.5 Hz, 2H), 1.50 (dt, J = 14.8, 7.4 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.0, 165.4, 162.6, 157.9, 153.5, 132.3 (d, J = 1.0 Hz), 131.7, 131.0 (q, J = 32.4 Hz), 129.0, 125.6 (q, J = 3.8 Hz), 125.1 (q, J = 3.7 Hz), 128.05 – 119.79 (m), 122.8, 106.0, 98.9, 93.1, 68.5, 30.9, 19.1, 13.7. MS (EI, 70 eV): 378.1086 ($C_{20}H_{17}O_4F_3$; calc. 378.1079).

5-Hydroxy-7-(pentyloxy)-3-(3-(trifluoromethyl)phenyl)-4H-chromen-4-one (**VI**: white needles, 0.25 g, yield: 63.3%, m.p. 110 – 112°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.62 (s, 1H), 7.92 (s, 1H), 7.80 – 7.72 (m, 2H), 7.65 (d, J = 7.9 Hz, 1H), 7.57 (t, J = 7.8 Hz, 1H), 6.41 (d, J = 2.2 Hz, 1H), 6.39 (d, J = 2.2 Hz, 1H), 4.02 (t, J = 6.6 Hz, 2H), 1.85 – 1.77 (m, 2H), 1.47 – 1.36 (m, 4H), 0.94 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.0, 165.4, 162.6, 157.8, 153.5, 132.3 (d, J = 0.9 Hz), 131.7, 131.0 (q, J = 32.4 Hz), 129.0, 125.6 (q, J = 3.8 Hz), 125.1 (q, J = 3.6 Hz), 128.04 – 119.83 (m), 122.7, 105.9, 98.9, 93.1, 68.8, 28.6, 28.0, 22.4, 14.0. MS (EI, 70 eV): 392.1236 ($C_{21}H_{19}O_4F_3$, calc. 392.1235).

7-(Hexyloxy)-5-hydroxy-3-(3-(trifluoromethyl)phenyl)-4H-chromen-4-one (**VII**): white needles, 0.23 g, yield: 57.7%, m.p. 97 – 99°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.63 (s, 1H), 7.93 (s, 1H), 7.79 – 7.74 (m, 2H), 7.66 (d, J = 7.8 Hz, 1H), 7.57 (t, J = 7.8 Hz, 1H), 6.42 (d, J = 2.2 Hz, 1H), 6.39 (d, J = 2.3 Hz, 1H), 4.03 (t, J = 6.6 Hz, 2H), 1.85 – 1.78 (m, 2H), 1.51 – 1.43 (m, 2H), 1.37 – 1.33 (m, 4H), 0.92 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ

TABLE 2. Anti-Breast Cancer Cell Activity of Synthesized Compounds

Compound	MCF-7		MBA-MD-231		MBA-MD-435	
	IC_{50}, μM (n = 5)	$^aE_{max}$, %	IC_{50}, μM (n = 5)	$^aE_{max}$, %	IC_{50}, μM (n = 5)	$^aE_{max}$, %
DDP	2.3 ± 0.1	96.8 ± 3.1	2.7 ± 0.3	95.3 ± 5.8	6.37 ± 0.4	92.1 ± 4.3
TAM	11.1 ± 1.9	94.4 ± 5.6	13.4 ± 1.5	84.8 ± 4.6	13.1 ± 2.1	90.5 ± 10.5
IV	>100	<50	>100	<50	>100	<50
V	>100	<50	>100	<50	>100	<50
VI	>100	<50	>100	<50	>100	<50
VII	>100	<50	>100	<50	>100	<50
VIII	>100	<50	>100	<50	>100	<50
IX	25.1 ± 3.4	85.5 ± 5.5	32.7 ± 2.8	84.0 ± 6.6	30.7 ± 2.9	77.5 ± 8.2
X	>100	<50	>100	<50	>100	<50
XI	14.6 ± 2.2	93.9 ± 8.4	18.6 ± 2.8	85.6 ± 9.4	22.3 ± 1.8	83.9 ± 4.2
XII	>100	<50	>100	<50	>100	<50
XIII	>100	<50	>100	<50	>100	<50
XIV	18.2 ± 2.8	89.7 ± 9.1	29.0 ± 4.4	78.3 ± 3.8	39.3 ± 6.9	72.3 ± 10.2
XV	25.9 ± 4.7	87.6 ± 7.8	46.8 ± 5.2	75.3 ± 6.0	52.0 ± 4.4	64.5 ± 2.9
XVI	>100	<50	>100	<50	>100	<50
XVII	>100	<50	>100	<50	>100	<50
XVIII	>100	<50	>100	<50	>100	<50
XIX	>100	<50	>100	<50	>100	<50
XX	>100	<50	>100	<50	>100	<50
XXI	>100	<50	>100	<50	>100	<50

[a] E_{max} is the maximum effect at high drug concentration.

180.0, 165.4, 162.6, 157.7, 153.5, 132.3 (d, J = 0.9 Hz), 131.7, 131.0 (q, J = 32.4 Hz), 129.0, 125.6 (q, J = 3.9 Hz), 125.1 (q, J = 3.7 Hz), 128.05 – 119.82 (m), 122.8, 106.0, 98.9, 93.1, 68.8, 31.5, 28.9, 25.6, 22.6, 14.0. MS (EI, 70 eV): 406.1389 ($C_{22}H_{21}O_4F_3$; calc. 406.1392).

5-Hydroxy-7-(nonyloxy)-3-(3-(trifluoromethyl)phenyl)-4H-chromen-4-one (**VIII**): white needles, 0.23 g, yield: 52.4%, m.p. 85 – 88°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.61 (s, 1H), 7.90 (d, J = 2.3 Hz, 1H), 7.78 – 7.70 (m, 2H), 7.63 (d, J = 7.7 Hz, 1H), 7.55 (t, J = 7.7 Hz, 1H), 6.39 (t, J = 2.2 Hz, 1H), 6.36 (d, J = 2.3 Hz, 1H), 4.00 (td, J = 6.4, 1.5 Hz, 2H), 1.83 – 1.74 (m, 2H), 1.48 – 1.39 (m, 2H), 1.37 – 1.23 (m, 10H), 0.87 (t, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.0, 165.5, 162.7, 157.9, 153.5, 132.3 (d, J = 0.8 Hz), 131.7, 131.0 (q, J = 32.5 Hz), 129.0, 125.6 (q, J = 3.8 Hz), 125.1 (q, J = 3.7 Hz), 128.06 – 119.82 (m), 122.8, 106.0, 98.9, 93.1, 68.8, 31.9, 29.5, 29.3, 29.2, 28.9, 25.9, 22.7, 14.1. MS (EI, 70 eV): 448.1872 ($C_{25}H_{27}O_4F_3$; calc. 448.1861).

7-Ethoxy-5-hydroxy-3-(3-(trifluoromethyl)phenyl)-4H-chromen-4-one (**IX**): white needles, 0.26 g, yield: 75.2%, m.p. 180 – 182°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.66 (s, 1H), 7.95 (s, 1H), 7.82 – 7.76 (m, 2H), 7.69 (d, J = 7.8 Hz, 1H), 7.60 (t, J = 7.7 Hz, 1H), 6.44 (d, J = 2.2 Hz, 1H), 6.42 (d, J = 2.2 Hz, 1H), 4.14 (q, J = 7.0 Hz, 2H), 1.48 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.1, 165.2, 162.7, 157.9, 153.5, 132.3 (d, J = 1.0 Hz), 131.7, 131.0 (q, J = 32.4 Hz), 129.0, 125.6 (q, J = 3.8 Hz), 125.1 (q, J = 3.7 Hz), 128.12 – 119.77 (m), 122.8, 106.0, 98.9, 93.1, 64.3, 14.5. ESI-MS: 351.0837 ($C_{18}H_{14}O_4F_3^+$, (M+H($^+$).

7-(Heptyloxy)-5-hydroxy-3-(3-(trifluoromethyl)phenyl)-4H-chromen-4-one (**X**): white needles, 0.21 g, yield: 49.2%, m.p. 90 – 92°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.63 (s, 1H), 7.93 (s, 1H), 7.80 – 7.73 (m, 2H), 7.66 (d, J = 7.8 Hz, 1H), 7.57 (t, J = 7.8 Hz, 1H), 6.42 (d, J = 2.2 Hz, 1H), 6.39 (d, J = 2.2 Hz, 1H), 4.03 (t, J = 6.6 Hz, 2H), 1.86 – 1.77 (m, 2H), 1.51 – 1.42 (m, 2H), 1.39 – 1.29 (m, 6H), 0.90 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.1, 165., 162.74 (s), 157.97 (s), 153.59 (s), 132.42 (d, J = 1.0 Hz), 131.8, 131.14 (q, J = 32.6 Hz), 129.1, 125.7 (q, J = 3.8 Hz), 125.2 (q, J = 3.8 Hz), 128.23 – 119.85 (m), 122.9, 106.1, 99.0, 93.2, 68.9, 31.8, 29.1, 29.0, 26.0, 22.7, 14.2. ESI-MS: 421.1620 ($C_{23}H_{24}O_4F_3^+$, (M+H($^+$).

7-(4-Bromobutoxy)-5-hydroxy-3-(3-(trifluoromethyl)-phenyl)-4H-chromen-4-one (**XI**): white needles, 0.24 g, yield: 53.4%, m.p. 113 – 116°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.64 (s, 1H), 7.94 (s, 1H), 7.81 – 7.73 (m, 2H), 7.66 (d, J = 7.8 Hz, 1H), 7.58 (t, J = 7.8 Hz, 1H), 6.42 (d, J = 2.0 Hz, 1H), 6.39 (d, J = 2.0 Hz, 1H), 4.08 (t, J = 5.7 Hz, 2H), 3.50 (t, J = 6.4 Hz, 2H), 2.07 (dd, J = 14.4, 7.6 Hz, 2H), 2.00 (dd, J = 13.0, 6.3 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.1, 165.0, 162.7, 157.8, 153.5, 132.3 (d, J = 0.9 Hz), 131.6, 130.9 (d, J = 1.6 Hz), 129.0, 125.6 (q, J = 3.9 Hz), 125.2 (dd, J = 7.4, 3.7 Hz), 127.42 – 120.05 (m), 122.8, 106.1, 98.8, 93.1, 67.6, 33.1, 29.2, 27.6. ESI-MS: 457.0254 ($C_{20}H_{17}O_4BrF_3^+$, (M+H($^+$).

5-Hydroxy-7-(octyloxy)-3-(3-(trifluoromethyl)phenyl)-4H-chromen-4-one (**XII**): white needles, 0.21 g, yield: 48.5%, m.p. 90 – 92°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.65 (s, 1H), 7.95 (s, 1H), 7.82 – 7.75 (m, 2H), 7.68 (d, J = 7.8 Hz, 1H), 7.60 (t, J = 7.8 Hz, 1H), 6.44 (d, J = 2.2 Hz, 1H), 6.41 (d, J = 2.2 Hz, 1H), 4.05 (t, J = 6.6 Hz, 2H), 1.88 – 1.79 (m, 2H), 1.48 (dd, J = 15.0, 7.0 Hz, 2H), 1.41 – 1.29 (m, 8H), 0.92 (t, J = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.0, 165.4, 162.6, 157.9, 153.5, 132.3 (d, J = 1.0 Hz), 131.7, 131.0(q, J = 32.4 Hz), 129.0, 125.6 (q, J = 3.8 Hz), 125.1 (q, J = 3.7 Hz), 128.09 – 119.74 (m), 122.8, 106.0, 98.9, 93.1, 68.8, 31.8, 29.3, 29.2, 28.9, 25.9, 22.6, 14.1. ESI-MS: 435.1776 ($C_{24}H_{26}O_4F_3^+$, (M+H($^+$).

3-(4-Bromophenyl)-5-hydroxy-7-propoxy-4H-chromen-4-one (**XIII**): white needles, 0.27 g, yield: 72.0%, m.p. 140 – 142°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.69 (s, 1H), 7.87 (s, 1H), 7.57 (d, J = 8.3 Hz,2H), 7.41 (d, J = 8.3 Hz, 2H), 6.39 (d, J = 7.2 Hz, 2H), 3.98 (t, J = 6.5 Hz, 2H), 1.89 – 1.78 (m, 2H), 1.05 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.2, 165.3, 162.6, 157.8, 153.1, 131.7, 130.4, 129.7, 122.9, 122.6, 106.0, 98.8, 93.0, 70.2, 22.3, 10.4. ESI-MS: 375.0226 ($C_{18}H_{16}O_4Br^+$, (M+H($^+$).

3-(4-Bromophenyl)-5-hydroxy-7-(pentyloxy)-4H-chromen-4-one (**XIV**): white needles, 0.27 g, yield: 67.4%, m.p. 117 – 119°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.66 (s, 1H), 7.85 (s, 1H), 7.54 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.3 Hz, 2H), 6.37 (d, J = 2.0 Hz, 1H), 6.35 (s, 1H), 3.99 (t, J = 6.5 Hz, 2H), 1.83 – 1.74 (m, 2H), 1.40 (qd, J = 13.8, 6.7 Hz, 4H), 0.92 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.2, 165.3, 162.6, 157.8, 153.1, 131.7, 130.4, 129.7, 122.9, 122.6, 106.0, 98.8, 93.0, 68.7, 28.6, 28.0, 22.4, 14.0. ESI-MS: 403.0539 ($C_{20}H_{20}O_4Br^+$, (M+H($^+$).

3-(4-Bromophenyl)-7-butoxy-5-hydroxy-4H-chromen-4-one (**XV**): white needles, 0.28 g, yield: 71.3%, m.p. 123 – 126°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.69 (s, 1H), 7.88 (s, 1H), 7.57 (d, J = 8.3 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 6.40 (d, J = 2.2 Hz, 1H), 6.38 (d, J = 2.1 Hz, 1H), 4.03 (t, J = 6.5 Hz, 2H), 1.84 – 1.75 (m, 2H), 1.49 (dt, J = 14.7, 7.4 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.2, 165.3, 162.6, 157.8, 153.1, 131.7, 130.4, 129.7, 122.9, 122.6, 106.0, 98.8, 93.0, 68.4, 30.9, 19.1, 13.8. ESI-MS: 389.0384 ($C_{19}H_{18}O_4Br^+$, (M+H($^+$).

3-(4-Bromophenyl)-7-(hexyloxy)-5-hydroxy-4H-chromen-4-one (**XVI**): white needles, 0.25 g, yield: 60.4%, m.p. 122 – 124°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.69 (s, 1H), 7.88 (s, 1H), 7.58 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 6.40 (d, J = 2.2 Hz, 1H), 6.38 (d, J = 2.2 Hz, 1H), 4.02 (t, J = 6.6 Hz, 2H), 1.86 – 1.75 (m, 2H), 1.47 (dt, J = 14.4, 7.0 Hz, 2H), 1.39 – 1.31 (m, 4H), 0.92 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.2, 165.3, 162.6, 157.8, 153.1, 131.7, 130.4, 129.7, 122.9, 122.7, 106.0, 98.8, 93.0, 68.8, 31.5, 28.9, 25.6, 22.6, 14.0. ESI-MS: 417.0694 ($C_{21}H_{22}O_4Br^+$, (M+H($^+$).

3-(4-Bromophenyl)-7-(heptyloxy)-5-hydroxy-4H-chromen-4-one (**XVII**): white needles, 0.24 g, yield: 56.8%, m.p. 166 – 169°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.69 (s, 1H), 7.88 (s, 1H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 6.40 (d, *J* = 2.1 Hz, 1H), 6.37 (d, *J* = 2.1 Hz, 1H), 4.02 (t, *J* = 6.6 Hz, 2H), 1.85 – 1.76 (m, 2H), 1.50 – 1.41 (m, 2H), 1.36 (dd, *J* = 14.4, 11.1 Hz, 6H), 0.90 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.2, 165.3, 162.6, 157.9, 153.1, 131.7, 130.4, 129.7, 122.9, 122.7, 106.0, 98.8, 93.0, 68.8, 31.7, 29.0, 28.9, 25.9, 22.6, 14.1. ESI-MS: 431.0852 ($C_{22}H_{24}O_4Br^+$, (M+H($^+$).

3-(4-Bromophenyl)-5-hydroxy-7-(octyloxy)-4H-chromen-4-one (**XVIII**): white needles, 0.22 g, yield: 48.8%, m.p. 92 – 93°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.69 (s, 1H), 7.88 (s, 1H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 6.40 (d, *J* = 2.2 Hz, 1H), 6.38 (d, *J* = 2.1 Hz, 1H), 4.02 (t, *J* = 6.5 Hz, 2H), 1.85 – 1.76 (m, 2H), 1.46 (dt, *J* = 15.0, 7.7 Hz, 2H), 1.32 (dd, *J* = 14.7, 7.6 Hz, 8H), 0.89 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.2, 165.3, 162.6, 157.9, 153.1, 131.7, 130.4, 129.7, 122.9, 122.7, 106.0, 98.8, 93.0, 68.8, 31.8, 29.3, 29.2, 28.9, 25.9, 22.6, 14.1. ESI-MS: 445.1006 ($C_{23}H_{26}O_4Br^+$, (M+H($^+$).

3-(4-Bromophenyl)-5-hydroxy-7-(nonyloxy)-4H-chromen-4-one (**XIX**): white needles, 0.20 g, yield: 45.2%, m.p. 108 – 110°C, ^{1}H NMR (400 MHz, $CDCl_3$ä 12.69 (s, 1H), 7.88 (s, 1H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 6.40 (d, *J* = 2.1 Hz, 1H), 6.38 (d, *J* = 1.9 Hz, 1H), 4.02 (t, *J* = 6.6 Hz, 2H), 1.85 – 1.76 (m, 2H), 1.46 (dt, *J* = 14.4, 7.1 Hz, 2H), 1.32 (dt, *J* = 26.5, 10.8 Hz, 10H), 0.89 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.2, 165.4, 162.7, 157.9, 153.1, 131.8, 130.5, 129.7, 123.0, 122.7, 106.0, 98.8, 93.0, 68.8, 31.9, 29.5, 29.3, 29.2, 28.9, 25.9, 22.7, 14.1. ESI-MS: 459.1169 ($C_{24}H_{28}O_4Br^+$, (M+H($^+$).

3-(4-Bromophenyl)-7-ethoxy-5-hydroxy-4H-chromen-4-one (**XX**): white needles, 0.24 g, yield: 67.3%, m.p. 161 – 163°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.69 (s, 1H), 7.87 (s, 1H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.40 (d, *J* = 8.6 Hz, 2H), 6.39 (d, *J* = 2.2 Hz, 1H), 6.37 (d, *J* = 2.2 Hz, 1H), 4.10 (q, *J* = 7.0 Hz, 2H), 1.45 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.2, 165.1, 162.6, 157.8, 153.1, 131.7, 130.4, 129.7, 122.9, 122.7, 106.0, 98.7, 93.0, 64.3, 14.5. ESI-MS: 361.0070 ($C_{17}H_{13}O_4Br^+$, (M+H($^+$).

7-(4-Bromobutoxy)-3-(4-bromophenyl)-5-hydroxy-4H-chromen-4-one (**XXI**): pale yellow needles, 0.25 g, yield: 54.0%, m.p. 116 – 118°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.70 (s, 1H), 7.89 (s, 1H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.41 (d, *J* = 8.5 Hz, 2H), 6.40 (d, *J* = 2.1 Hz, 1H), 6.37 (d, *J* = 2.1 Hz, 1H), 4.06 (dd, *J* = 10.5, 5.8 Hz, 2H), 3.50 (t, *J* = 6.4 Hz, 1H), 3.27 (t, *J* = 6.7 Hz, 1H), 2.12 – 1.89 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.2, 165.0, 162.7, 157.8, 153.1, 131.8, 130.4, 129.6, 123.0, 122.7, 106.2, 98.7, 93.0, 67.6, 33.1, 29.2, 27.6. ESI-MS: 466.9487 ($C_{19}H_{17}O_4Br_2^+$, (M+H($^+$).

4. EXPERIMENTAL BIOLOGICAL PART

Setting cisplatin (DDP) and tamoxifen as the positive control drugs, all synthesized compounds were tested for their antiproliferative effect on three cancer cell lines: MCF-7, MDA-MB-231 and MDA-MB-435 (all purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences). The cancer cell lines were cultured in high glucose DMEM medium (from HyClone) supplemented with 10% fetal bovine serum (from ScienCell) and permitted in a humidified incubator at 37°C under 5% CO_2 atmosphere.

MTT assay. The MCF-7, MDA-MB-231 and MDA-MB-435 cells were seeded in a 96-well plate (at density of 2×10^3, 3×10^3 and 5×10^3 per well, respectively) and allowed to grow to the desired confluence. The cells were treated with various concentrations of genistein derivatives for 72 h (genistein derivatives were dissolved in DMSO to final concentrations of 100, 30, 10, 3, 1, 0.3 μM, and the DMSO content never exceeded 0.05%). After 72 h exposure, 20 μL MTT solution (5 mg·mL^{-1} in DMSO) was added to each well and then incubated for 4 h. Control cells were incubated with a media containing an equivalent solvent amount with DDP or TAM instead of genistein derivatives. The supernatant was removed carefully by pipetting from wells without disturbing the attached cells, and formazan crystals were solubilized by adding 200 μL of DMSO to each well and shaking for 15 min. The absorbance at 490 nm was measured with a microplate reader, and the IC_{50} value was defined as the concentration at which 50% survival of cells was observed.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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