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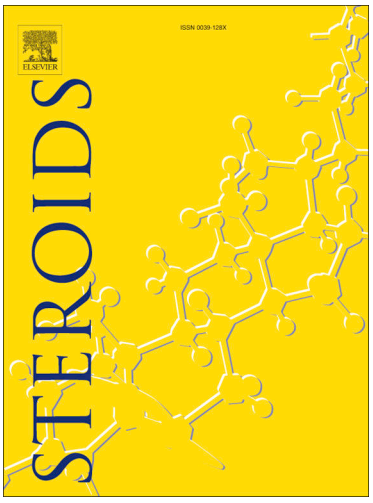

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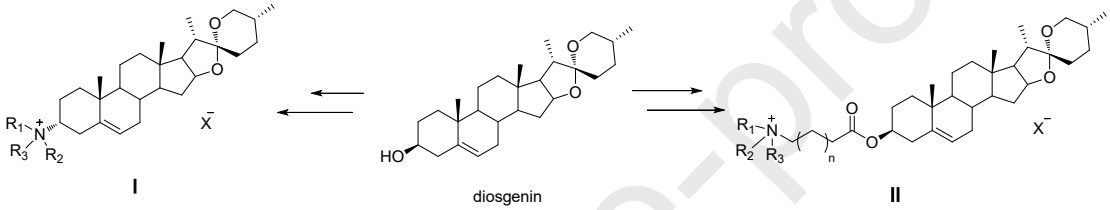
Synthesis of Diosgenyl Quaternary Ammonium Derivatives and their antitumor activity

Xi Xia[1], Yu Chen[1], Lin Wang[1], Zhi-Gang Yang[1], Xiao-Dong Ma[2], Zhi-Gang Zhao[1], Hong-Jun Yang*[,1],

[1] Key Laboratory of Basic Chemistry of the State Ethnic Affairs Commission, School of Chemistry and Environment, Southwest Minzu University, Chengdu 610041, P. R. of China

[2] College of Pharmacy, Dalian Medical University, Dalian 116044

Graphical Abstract



Abstract

Giosgenin is a naturally steroidal saponin exhibiting a variety of biological activities including antitumor ones. A series of novel diosgenyl quaternary ammonium derivatives were designed and synthesized to develop potential anti-tumor agents in our research. All novel derivatives were characterized by ^{1}H NMR, ^{13}C NMR and HR-MS, and evaluated for their in vitro anti-proliferative activities using MTT assay. The human cancer cell lines were A549 (Human lung cancer cell), H1975 (Human lung adenocarcinoma cell), A431 (Human skin squamous cell carcinoma), HCT-116 (Human colorectal adenocarcinoma cell), Aspc-1 (Human metastatic pancreatic cancer cell), Ramos (Human B lymphoma cell), HBE (Human bronchial epithelioid cell) and LO2 (Human normal hepatocyte).

Key words: Diosgenin; Antitumor activities; Derivatives; Quaternary ammonium derivatives

* Corresponding author at: Key Laboratory of Basic Chemistry of the State Ethnic Affairs Commission, School of Chemistry and Environment, Southwest Minzu University, Chengdu, China
E-mail address: yanghj@swun.edu.cn (Hong-Jun Yang).

1. Introduction

Malignant tumor, known as cancer, is one of the most dangerous diseases endangering human health [1]. Now, the discover of the antineoplastic drugs form natural plants has been accredited with a great amount of attention over the years by medicinal chemists. Besides, restudy on the efficacious components form plants and structural modification play an important role in field of anti-tumor drugs.

Giosgenin is a naturally steroidal saponin, it can be found in several plants species including dioscorea, yams, smilax, costus, fenugreek, et al [2, 3]. Giosgenin is widely used as a precursor to synthetic steroids [4], such as prednisone, norethindrone, dexamethasone and so on. A large number of studies have shown that diosgenin has a wide range of anti-tumor activities on a variety of tumor cells [2, 5-9], and it can effectively plays its role by inhibiting cell proliferation and inducing apoptosis [10], and blocking cell cycle [11, 12]. In addition, diosgenin exhibits anti-inflammatory [13, 14], anti-photoaging [3], anti-cardiovascular disease [15, 16] and the like [17]. However, the use of these compounds was always limited by the high toxicity, poor water-soluble and low bioavailability. A number of studies were attempted by structural modification to improve utilization of diosgenin, and many successful examples have been reported [5, 18-22]. Therefore, the design of novel families of giosgenin derivatives to ameliorate their severe side effect is a pivotal task.

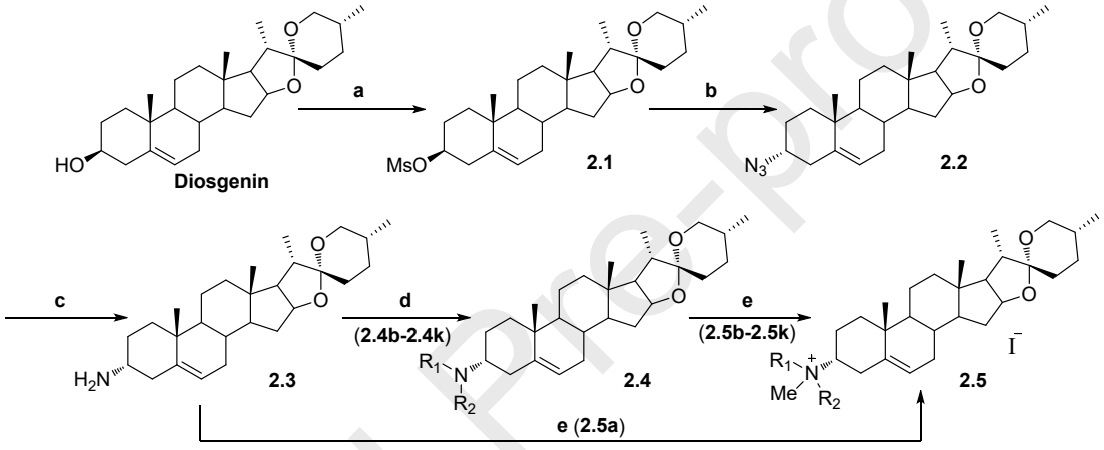
It is found that the introduction of nitrogen atoms into the structure of drugs or modification of existing nitrogen atoms to make them into quaternary ammonium derivatives can improve its water-soluble and enhance the biological activity [23-27]. In the reported derivatives of diosgenin, the derivatives modified with triazolium salt or diazolium salt in C-3 position have good antitumor activity [28-31]. Therefore, in order to improve the water-soluble of diosgenin and increase its antitumor activity, two series of quaternary ammonium derivatives were designed and synthesized with diosgenin as the lead compound, and their antitumor activity was tested in vitro.

2. Results and Discussion

2.1 Synthesis

2.1.1 Synthesis of quaternary ammonium series I derivatives of diosgenin [32, 33]

Series I of quaternary ammonium derivatives **2.5a**-**2.5k** were prepared using the sequences shown in Schemes 1. First, giosgenin as the lead compound was sulfonated with methylsulfonyl chloride in the presence of trimethylamine to afford compound **2.1**, which was reacted with sodium azide to provide compound **2.2**. Then, the azidine of compound **2.2** was reduced to amino by triphenylphosphine to obtain compound **2.3**. Finally, the compound **2.3** obtained through the 3-step reaction was used as the common intermediate of the C3 derivatives of diosgenin, and substituted with different halogenated hydrocarbons to afford the corresponding series of quaternary ammonium derivatives **2.5a**-**2.5k**.



2.5a $R_1=R_2=$ Me

2.4b, 2.5b $R_1=R_2=$

2.4c, 2.5c $R_1=R_2=$

2.4d, 2.5d $R_1=R_2=$

2.4e, 2.5e $R_1=R_2=$

2.4f, 2.5f $R_1=R_2=$

2.4g, 2.5g $R_1=R_2=$ O

2.4h, 2.5h $R_1=R_2=$ OH

2.4i, 2.5i $R_1=R_2=$ N O O

2.4j, 2.5j $R_1=R_2=$

2.4k $R_1=$ N $R_2=$ H

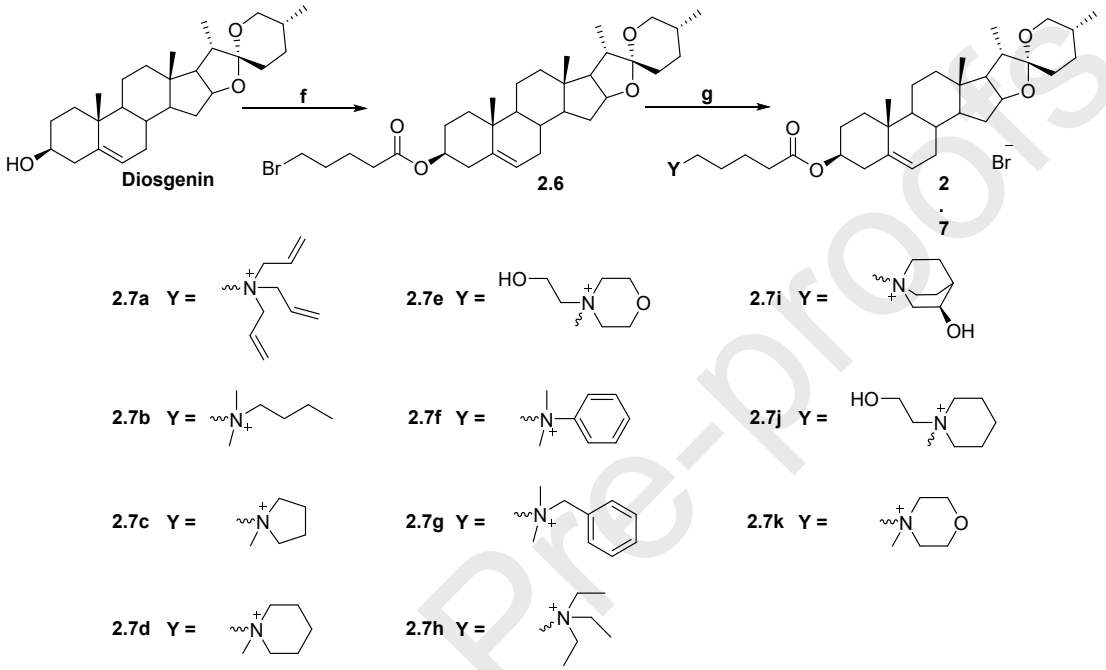
2.5k $R_1=$ N $R_2=$ Me

Scheme 1. Reagents and conditions: (a) MsCl, Et_3N, THF, 0 °C, 1 h, yield 93%. (b) NaN_3, DMF, 90 °C, 7 h, yield 51%. (c) Ph_3P, THF/H_2O, 60 °C, 24 h, yield 60%. (d) halohydrocarbon, K_2CO_3, CH_3CN or CH_3CN/CH_2Cl_2 (v/v=1:1), 40-80 °C, yield 63-93%. (e) CH_3I, (K_2CO_3), CH_3CN/CH_2Cl_2 (v/v=1:1), reflux, yield 41-96%.

2.1.2 Synthesis of quaternary ammonium series II derivatives of diosgenin [34, 35]

Series II of quaternary ammonium derivatives **2.7a**-**2.7k** were synthesized as

shown in Scheme 2. We designed to derive diosgenin into a steroidal halogenated hydrocarbon and then react with different tertiary amines to obtain quaternary ammonium derivatives. First, diosgenin, the lead compound, was condensed with 5-bromo- pentanoic acid to provide intermediate **2.6**. The compounds **2.7a**-**2.7k** were synthesized through **2.6** reaction with different tertiary amines.



Scheme 2. Reagents and conditions: (f) DMAP, EDC·HCl, 5-Bromovaleric acid, CH_2Cl_2, r.t., 3 h, yield 79%. (g) Tertiary amine or Triphenylphosphine, CH_3CN, reflux, yield 35-90%.

2.2 Water solubility

Water solubility of all derivatives is shown in table 1.

The water solubility of the two series of diosgenin quaternary ammonium salt derivatives **2.5a**-**2.5k**, **2.7a**-**2.7l** is superior to the lead diosgenin. In the quaternary ammonium salt series I (**2.5a**-**2.5k**), the water solubility of **2.5h** and **2.5k** is the best, about 300 times higher than the water solubility of the lead. In the quaternary ammonium salt derivative series II (**2.7a**-**2.7l**), the water solubility of **2.7c** is superior to all synthetic derivatives and is about 3000 times higher than the lead.

Table 1 Water solubility of diosgenyl quaternary ammonium derivatives

Compound	Water-soluble*(mg/mL)	Compound	Water-soluble

			(mg/mL)
Diosgenin	0.0001	**2.7a**	0.0782
2.5a	0.0021	**2.7b**	0.0999
2.5b	0.0116	**2.7c**	0.2938
2.5c	0.0235	**2.7d**	0.0613
2.5d	0.0115	**2.7e**	0.0758
2.5e	0.0170	**2.7f**	0.1004
2.5f	0.0113	**2.7g**	0.0269
2.5g	0.0108	**2.7h**	0.1992
2.5h	0.0351	**2.7i**	0.1987
2.5i	0.0074	**2.7j**	0.0399
2.5j	0.0019	**2.7k**	0.1276
2.5k	0.0351	**2.7l**	0.0046

* The above results are relative values of water solubility of each derivative.

2.3 Inhibitory activity

All diosgenyl quaternary ammonium derivatives were tested with MTT assay on five kinds of tumor cells and two kinds of normal human cells, and the results were shown in table 2 and table 3.

Antiproliferation activity of quaternary ammonium series I derivatives of diosgenin is showed in table 2.

For tumor cells H1975, Aspc-1, HTC-116 and A549, the antitumor activity of derivative **2.5a**-**2.5k** was better than the lead compound. Among them, the inhibitory activity of **2.5g** on H1975 (IC_{50} = 5.09 μM) was comparable to that of Adriamycin (IC_{50} < 5 μM). Compounds of **2.5b**, **2.5d**, **2.5f**, **2.5g**, **2.5i** and **2.5j** (IC_{50} was 4.06, 4.07, < 5, 5.03, 4.78, < 5 μM, respectively) had comparable inhibitory activity to that of Adriamycin against Aspc-1 cells (IC_{50} < 5 μM). The inhibitory activity of **2.5d**, **2.5f** and **2.5j** on HTC-116 (IC_{50} = 1.12, 1.03, 0.34 μM, respectively) was better than that of Adriamycin (IC_{50} > 1.25 μM). The inhibitory activity of **2.5b**, **2.5d**, **2.5f**, **2.5g** and **2.5h** on A549 (IC_{50} = 6.64, 2.69, 2.42, 6.33, 5.35 μM, respectively) was better than

that of the control group Tae226 (IC_{50} = 9.67 μM). For tumor cell Ramos, except **2.5f** and **2.5h**, other derivatives showed better levels of inhibitory effects on the anti-tumor than lead compound. For tumor cells A431, we observed that most derivatives exhibit better anti-tumor activity than the lead compound, among which, the inhibitory activity of **2.5j** (IC_{50} < 2.5 μM) was similar to that of the control group Adriamycin (IC_{50} < 2.5 μM).

Results from biological activity studies of compounds **2.5a**-**2.5k** indicate that all derivatives are more toxic than their lead compounds. However, compared with control group, the cytotoxicity of all the derivatives except compound **2.5j** (IC_{50} < 5 μM) was less than Adriamycin (IC_{50} < 5 μM) on HBE. For the LO2, all the derivatives except compound **2.5g**、**2.5j** (IC_{50} = 7.44, 2.58 μM) exhibit weaker toxic than Adriamycin (IC_{50} = 7.88 μM), even compound **2.5c**、**2.5f**、**2.5k** (IC_{50} = 18.88, 21.00, 26.77 μM) showed frailer toxic than Tae226 (IC_{50} = 18.14 μM).

Comparison of the observed IC_{50} values suggest that derivatives **2.5a**-**2.5k** had stronger anti-tumor activity against tumor cells H1975, Aspc-1 and HCT-116 than Ramos on the whole, and stronger against HCT-116 than H1975. Overall, **2.5h** and **2.5k** showed less antitumor activity than the rest of the derivatives. While, derivative **2.5d** demonstrated excellent anti-tumor activity against tumor cells H1975, Aspc-1, HCT-116, Ramos and A549 (IC_{50} = 5.32, 4.07, 1.12, 7.52, 2.69 μM, respectively). Compound **2.5j** had better antitumor activity against tumor cells H1975, Aspc-1, HCT-116 and A431 (IC_{50} was 5.72, <5, 0.34, <2.5 μM), especially against HCT-116 and A431. The anti-tumor activity of diosgenin can be improved by improving water solubility and introducing hydrophobic groups, which may be one of the reasons why compound **2.5j** has better anti-tumor activity.

Table 2 Antiproliferation activity of quaternary ammonium series I derivatives of diosgenin

	Antiproliferation activity (IC_{50}, μM)							
Compound	**A549**	**A431**	**H1975**	**HTC-116**	**Aspc-1**	**Ramos**	**HBE**	**LO2**
2.5a	15.23	6.38	18.96	9.67	9.44	39.06	28.67	14.43

2.5b	6.64	4.01	5.96	<5	4.06	7.18	7.17	10.99
2.5c	12.67	14.53	5.92	4.71	4.41	9.56	8.99	18.44
2.5d	2.69	4.71	5.32	1.12	4.07	7.52	6.84	8.07
2.5e	10.48	10.36	11.17	3.94	5.54	22.96	13.00	12.54
2.5f	2.42	3.87	6.39	1.03	<5	>40	10.11	21.00
2.5g	6.33	2.85	5.09	>1,<5	5.03	32.57	9.67	7.44
2.5h	5.35	3.26	21.70	11.45	17.77	>40	19.73	11.89
2.5i	10.72	4.33	9.17	3.23	4.78	11.05	9.94	8.70
2.5j	9.86	<2.5	5.73	0.34	<5	15.47	<5	2.85
2.5k	27.53	8.25	20.59	9.74	19.32	38.35	18.89	26.77
Diosgenin	71.23	4.75	50.59	31.41	63.11	>40	46.56	>40
Adriamycin	0.78	<2.5	<5	>1.25	<5	<2.5	<5	7.88
Tae226	9.67	\	\	\	\	\	\	18.14

Note: The symbol "\ " indicates that the test was not performed.

Anti-proliferation activity of quaternary ammonium series II derivatives of diosgenin is presented as table 3.

The anti-proliferative activity of all the derivatives were superior to the lead compound diosgenin for tumor cells A549, H1975 and aspc-1. And for A549, all the derivatives had better effect on anti-proliferative than Tae226. For H1975, all the derivatives were more excellent than Adriamycin (IC_{50} < 5) except **2.7d** and **2.7k** (IC_{50} =5.08, 5.45 μM). Compounds **2.7a-d**, **2.7f** and **2.7i** (IC_{50} =1.23, 2.27, 2.15, 2.83, 4.18, 4.46 μM) showed better anti-proliferative than Adriamycin (IC_{50} < 5 μM) on Aspc-1. All the derivatives had better effect than diosgenin (IC_{50} =4.75 μM) on anti-proliferative for A431 except compounds **2.7e**, **2.7i**, and **2.7k** (IC_{50} = 6.21, 6.12, 5.88 μM). Compound **2.7f** (IC_{50} = 2.80 μM) had comparable anti-proliferative activity to that of Adriamycin (IC_{50} < 2.5 μM) on Ramos, and the rest of derivatives was similar levels effect on the anti-proliferative activity to diosgenin against Ramos.

As is evident from data, **2.7a-2.7k** are more toxic than their lead compounds. Compounds **2.7a**, **2.7c**, **2.7d**, **2.7h** and **2.7j** (IC_{50} = 5.61, 7.65, 6.06, 6.42, 7.10 μM) had

less toxic than Adriamycin (IC_{50} < 5) on HBE. All the derivatives had more toxic than Tae226 (IC_{50} = 18.14 μM) on LO2, while compound **2.7e** (IC_{50} =8.233 μM) showed less toxic than Adriamycin (IC_{50} = 7.88 μM).

In general, **2.7a**-**2.7k** had stronger anti-proliferative activity against tumor cells H1975, Aspc-1 and HCT-116 than Ramos overall. Compounds **2.7b**-**c**, **2.7f**-**g**, **2.7j** had excellent anti-proliferative activity on the tumor cells tested. Compound **2.7k** exerted less anti-proliferative activity compared to the impact of rest of the investigated compounds.

Table 3 Anti-proliferation activity of quaternary ammonium series II derivatives of diosgenin

Compound	Anti-proliferation activity(IC_{50}, μM)[b]						
	A549	**A431**	**H1975**	**Aspc-1**	**Ramos**	**HBE**	**LO2**
2.6	>40.0	>10.0	>40.0	>40.0	>40.0	>40.0	>40.0
2.7a	1.84	2.55	0.788	1.23	>40.0	5.61	4.57
2.7b	2.81	3.11	3.68	2.27	36.5	4.29	5.54
2.7c	4.47	4.05	3.44	2.15	31.9	7.65	6.26
2.7d	5.83	3.03	5.08	2.83	>40.0	6.06	6.30
2.7e	12.6	6.21	3.36	11.1	~ 39.1	3.39	8.233
2.7f	6.63	3.52	4.09	4.18	2.80	4.32	5.79
2.7g	5.30	1.85	1.51	7.12	35.4	4.19	7.12
2.7h	2.52	3.46	3.23	6.56	>40.0	6.42	5.72
2.7i	4.07	6.12	2.88	4.46	33.6	3.88	6.70
2.7j	4.68	4.14	1.68	6.07	37.0	7.10	5.75
2.7k	5.83	5.88	5.45	8.48	>40.0	4.26	7.21
Diosgenin	71.23	4.75	50.59	63.11	>40	46.56	>40
Adriamycin	0.78	<2.5	<5	<5	<2.5	<5	7.88
Tae226	9.67	\	\	\	\	\	18.14

Note: The symbol "\ " indicates that the test was not performed.

In the investigated two series of compounds, series II had better anti-proliferative activity than series I on A549, A431, H1975 and Aspc-1. However, series II was also more toxic. Besides, the anti-proliferation activity of the two series derivatives against Ramos was worse than that of other tumor cells tested, but the anti-proliferation activity of series I was better than that of series II.

Analyzing water solubility and anti-tumor activity of two series of diosgenyl quaternary ammonium derivatives, we observed that the water-soluble of two series of

derivatives was better than diosgenin and the water-soluble and anti-proliferation activity of series II were preferable to series I. Therefore, we deduced that increasing water-soluble could improve the anti-tumor of diosgenyl quaternary ammonium derivatives. Particularly, compounds **2.7c** and **2.7f** had fantastic water-soluble, both of which showed excellent anti-proliferation activities against tumor cells A549, A431, H1975, Aspc-1 and Ramos. Besides, we also found an interesting phenomenon that compounds **2.5h**, **2.5k**, **2.7h** and **2.7k**, which were more water-soluble, had weaker antitumor activity than other derivatives, while **2.5d**, **2.5j**, **2.7a**, **2.7g** and **2.7j** showed stronger anti-proliferation activity in most tumor cells with relatively small water solubility. This research indicates that not only to improve the water solubility, but also to further study the structure-activity relationship of the drug, so as to design the diosgenin derivative with better antitumor activity.

We carried out other experiments on the best anti-proliferative activity of **2.7a** in the two series of compounds to further verify the drug activity, including cell scratch test, natural light imaging, dual AO/EB flurorescence staining and DAPI staining. In these four groups, Gefitinib and Osimertinib (AZD9291) were used as the control group to carry out in vitro anti-proliferative activity experiments on H1975 with a concentration of 0, 100, 500, and 2500 nM, respectively. In the scratch test (Fig. **1**), the repair ability of cancer cells decreased with the increase of **2.7a** concentration, indicating that the higher the concentration, the stronger the anti-proliferation activity. Following 72h of treatment with different concentration, as the concentration increases, the number of cancer cells is significantly reduced in natural light imaging (Fig. **2**). In the AO/EB test (Fig. **3**), we can observe that the normal cancer cells represented by green decreased significantly with the increase of drug concentration, and the anti-proliferation activity of **2.7a** compound was similar to that of Gefitinib and Osimertinib. In the DAPI test (Fig. **4**), blue indicates normal cancer cells, as shown in the figure, the number of cancer cells decreases as the concentration of **2.7a** increases. It can be concluded that **2.7a** shows good anti-proliferation activity, and the anti-proliferation activity increases with the increase of concentration.

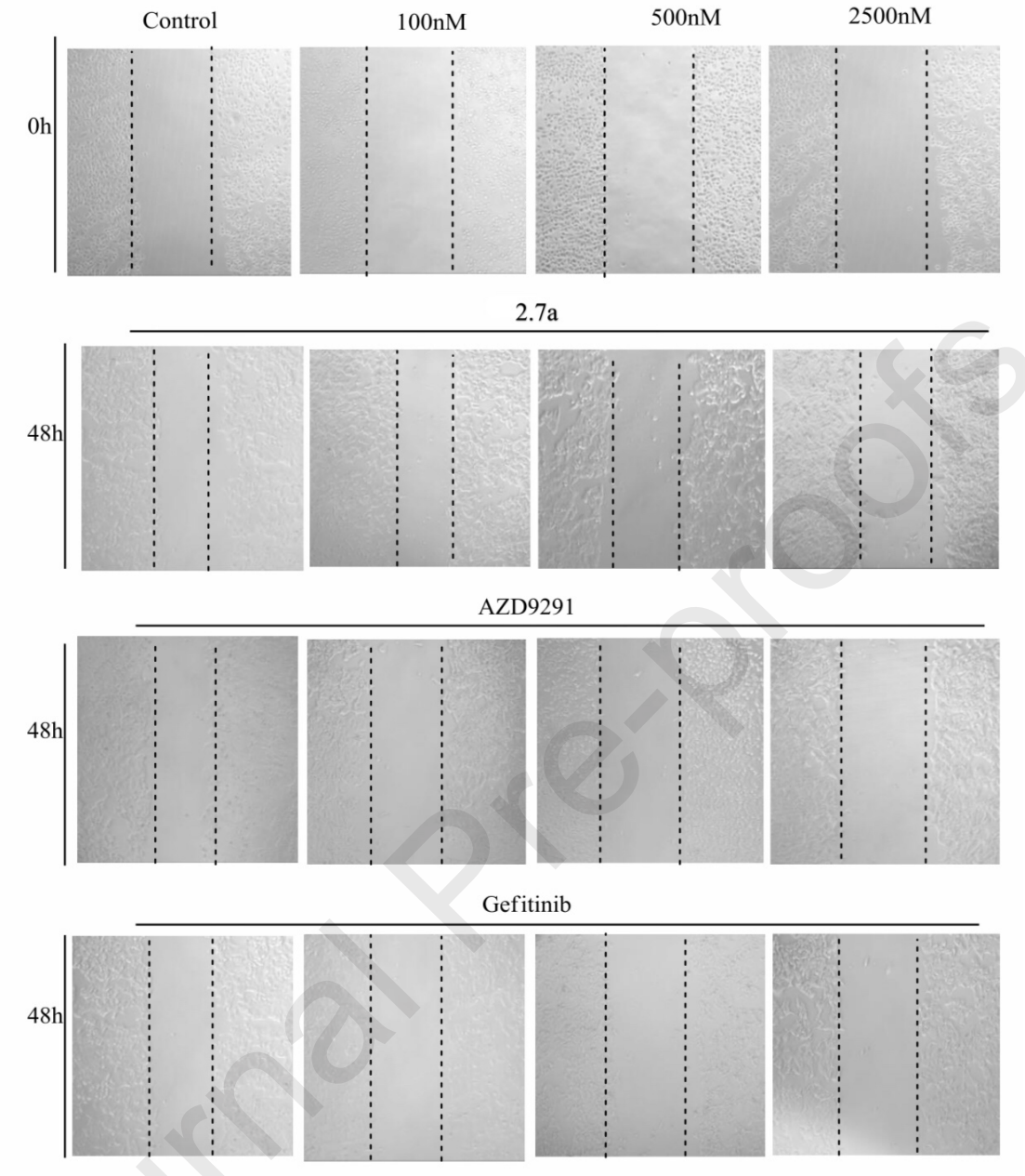


Fig. **1** The scratch test of H1975

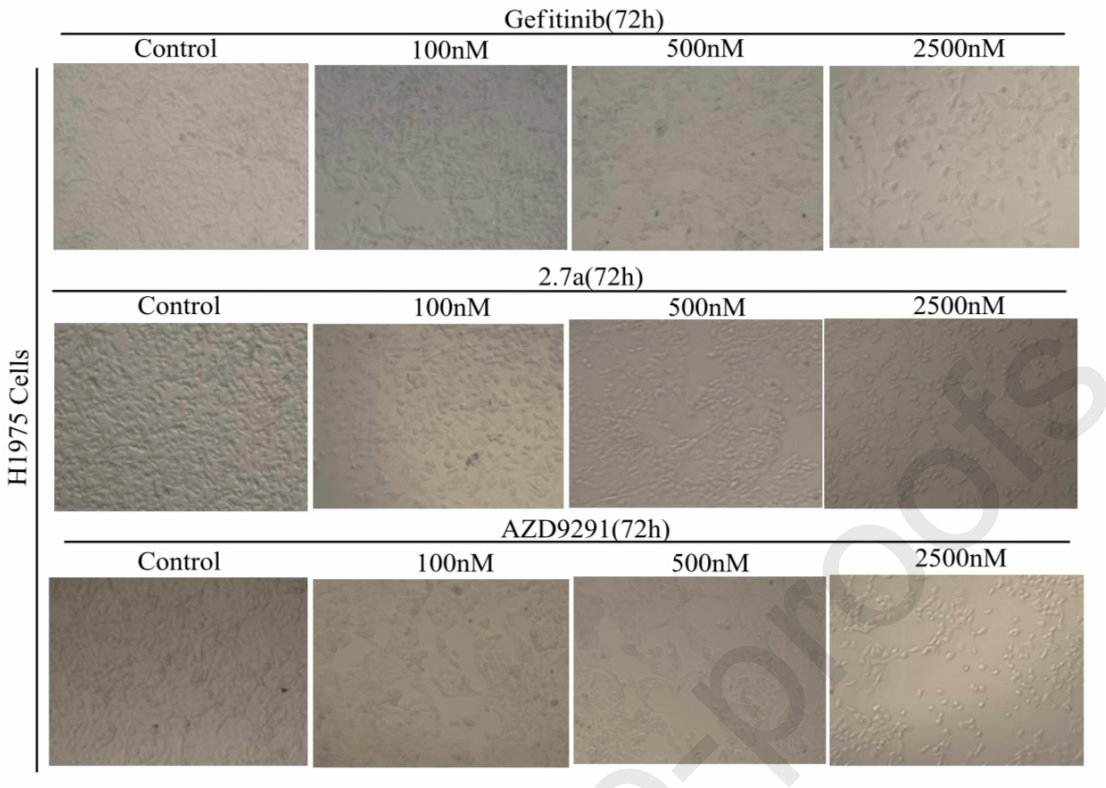


Fig. **2** Natural light imaging of H1975

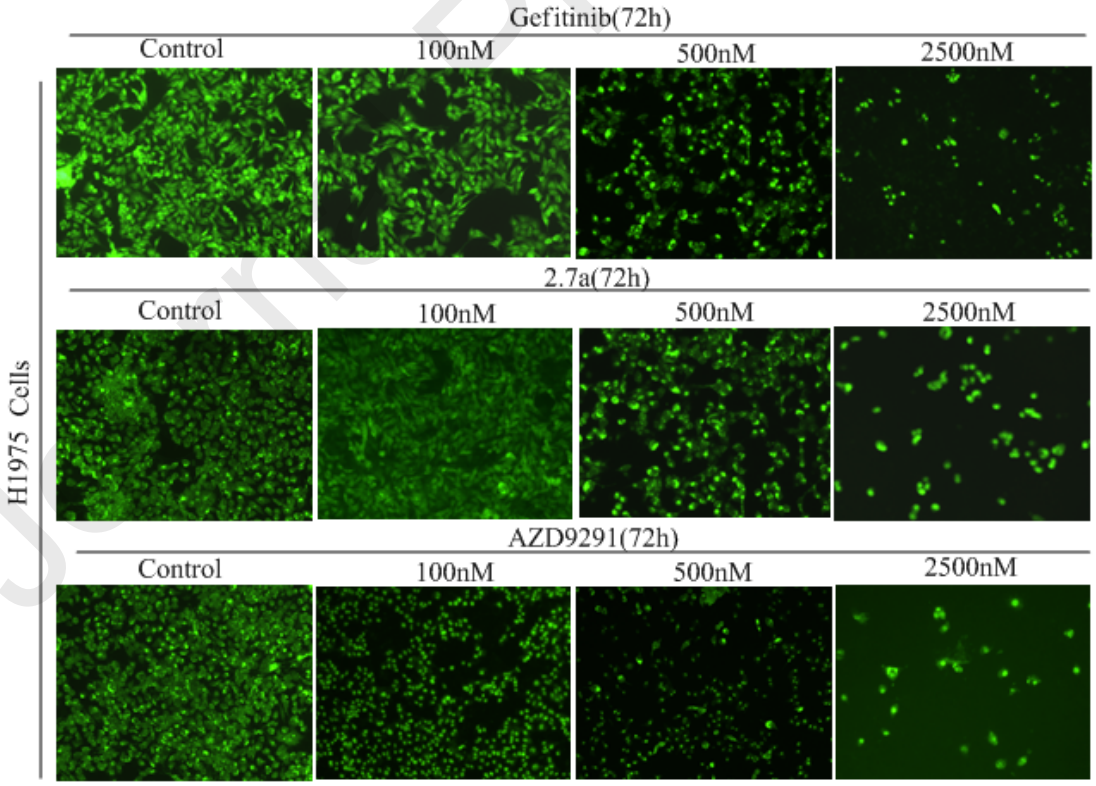


Fig. **3** The AO/EB test of H1975

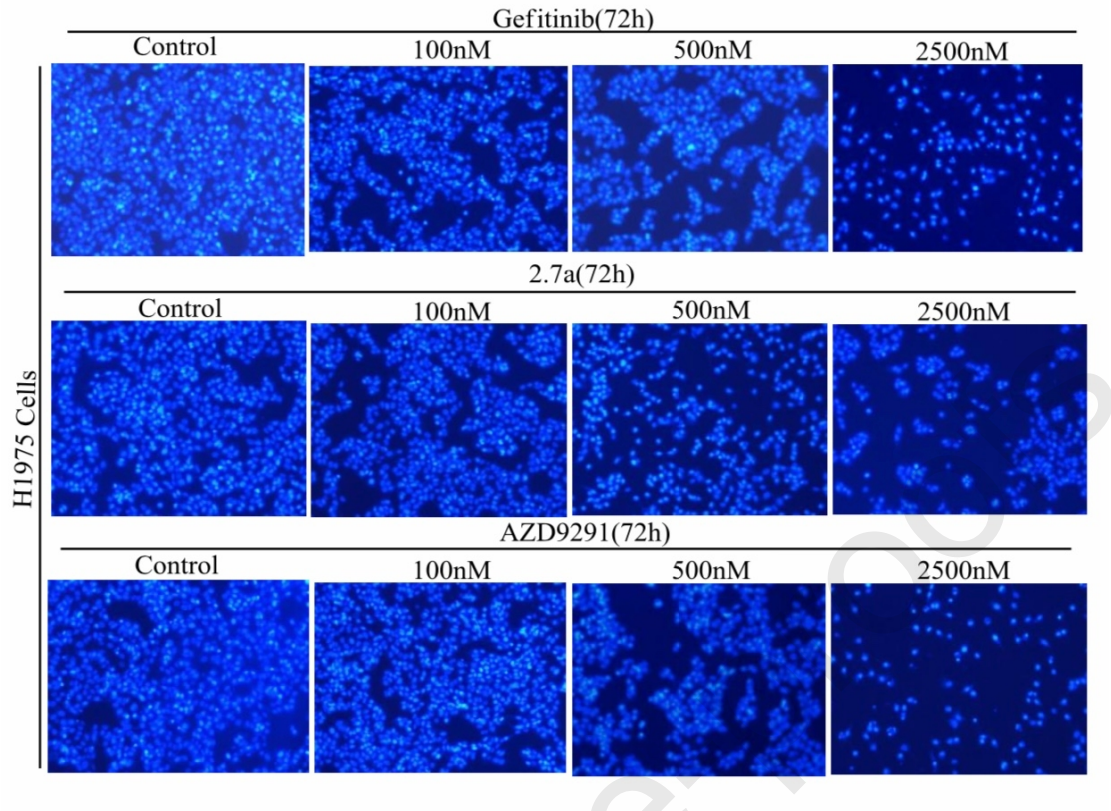


Fig. 4 The DAPI test of H1975

3 Conclusion

22 new diosgenyl quaternary ammonium derivatives were synthesized from 11 new diosgenin amines and ester derivatives with diosgenin as a precursor. The water solubility of diosgenyl quaternary ammonium derivatives and their anti-proliferation activity against 8 kinds of human cells were investigated. The results showed that the water solubility of all diosgenyl quaternary ammonium derivatives was higher than that of the precursors. The anti-tumor activity of most derivatives was better than that of diosgenin, and the anti-tumor activity of some derivatives was similar to that of Adriamycin and Tae226. In summary, improving the water solubility of diosgenyl derivatives is beneficial to improve its antitumor activity. The results of this study have certain theoretical significance and potential application value for the structural repair of diosgenin.

Financial & interests disclosure

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Acknowledgements

Supplementary data

Reference and notes

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Highlights:

1.Two series of novel diosgenyl quaternary ammonium derivatives were designed and synthesized.

2.The structures of all novel derivatives were confirmed by ^{1}H NMR, ^{13}C NMR and HR-MS, and evaluated for their *in vitro* anti-proliferative activities using MTT assay.

3. The experimental results show that improving the water solubility of diosgenyl derivatives is beneficial to improve its antitumor activity.