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Letter

Synthesis and Antitumor Activity of Diosgenin Hydroxamic Acid and Quaternary Phosphonium Salt Derivatives

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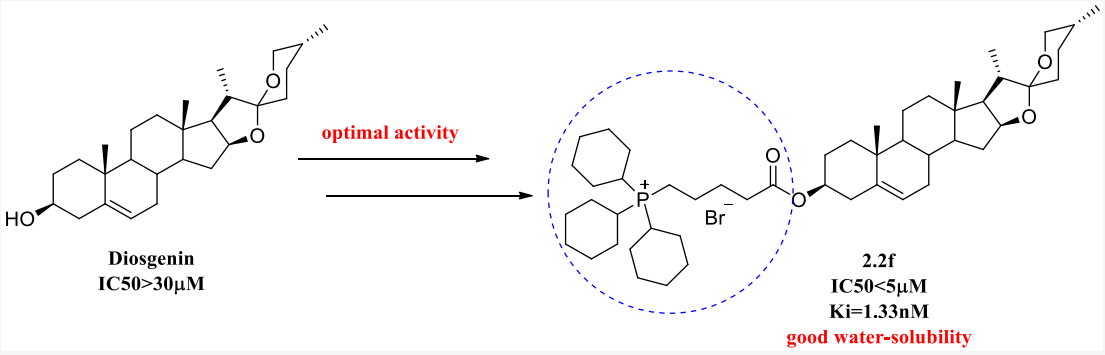

ABSTRACT: Diosgenin, a component separated from Dioscorea plants, is an important starting material for steroid hormone drugs and semisynthetic steroids. In the work, two series of diosgenin derivatives were designed, synthesized, and evaluated for their cellular anticancer activities. Most of the target compounds exhibited good inhibitory activities against four cell lines, Aspc-1 (human colon adenocarcinoma cells), H358 (human nonsmall cell lung cancer cells), HCT116 (human colorectal adenocarcinoma cells), and SW620 (human metastatic pancreatic cancer cells). Among them, the representative compound **2.2f** exhibited 7.9−341.7-fold antiproliferative activities against the above-mentioned four cell lines compared with the lead compound diosgenin.

KEYWORDS: *Diosgenin, antitumor, derivatives, quaternary phosphonium salt, hydroxamic acid*

Diosgenin (Figure 1) has the following properties: ability to regulate immunity,[1] antitumor,[2] antihyperlipidemia,[3]

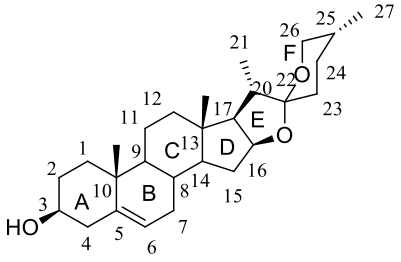

Figure 1. Structure of diosgenin.

anti-inflammatory,[4] relax blood vessels,[5] protects myocardium and cardiovascular system,[6,7] anti-AIDS,[8] anti-Alzheimer's disease,[9] etc. There are also important drugs for the treatment of lymphoid leukemia, cardiovascular diseases, meningitis, and demyelinating diseases and in treatments for patients.[10,11] In particular, it displays certain antitumor activity against a variety of cancer cells, making diosgenin a potential natural medicine.[12−14] However, diosgenin also has limitations, such as high cytotoxicity, poor solubility, and low bioavailability, which limits the application of the lead compound. Therefore, the development of diosgenin derivatives as drugs still has important theoretical significance and application value.

Based on the above properties of diosgenin, several research groups modified the C-3 hydroxyl group of the diosgenin A ring and C-26 of the diosgenin F ring.[15−20] In our previous studies, three series of nitrogen-containing derivatives were reported for antitumor activity.[21,22] So it was obvious that the antitumor activity of the A ring derivatives is generally better than that of the F ring derivatives. Nitrogen-containing heterocycles and quaternary ammonium salt derivatives have good activity, and it may be that nitrogen-containing heterocycles have unique biological activity and low biotoxicity, which play an important role in the structural modification of drug molecules.[23−25] Meta derivatives appropriately introduce hydrophilic groups to increase their

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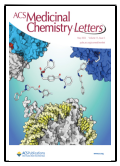

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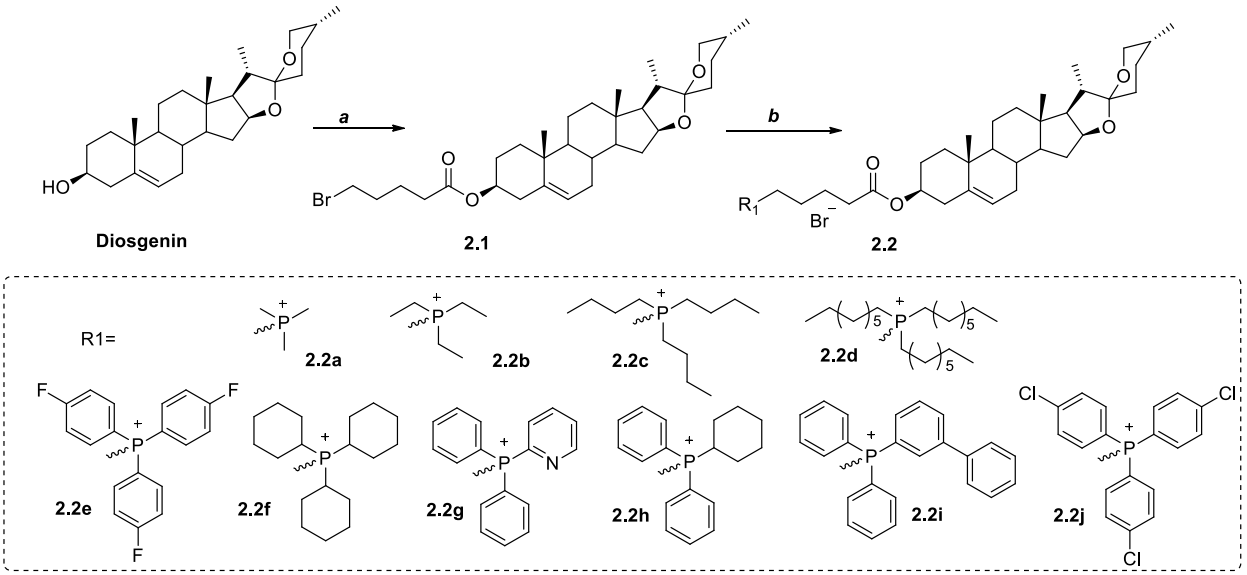
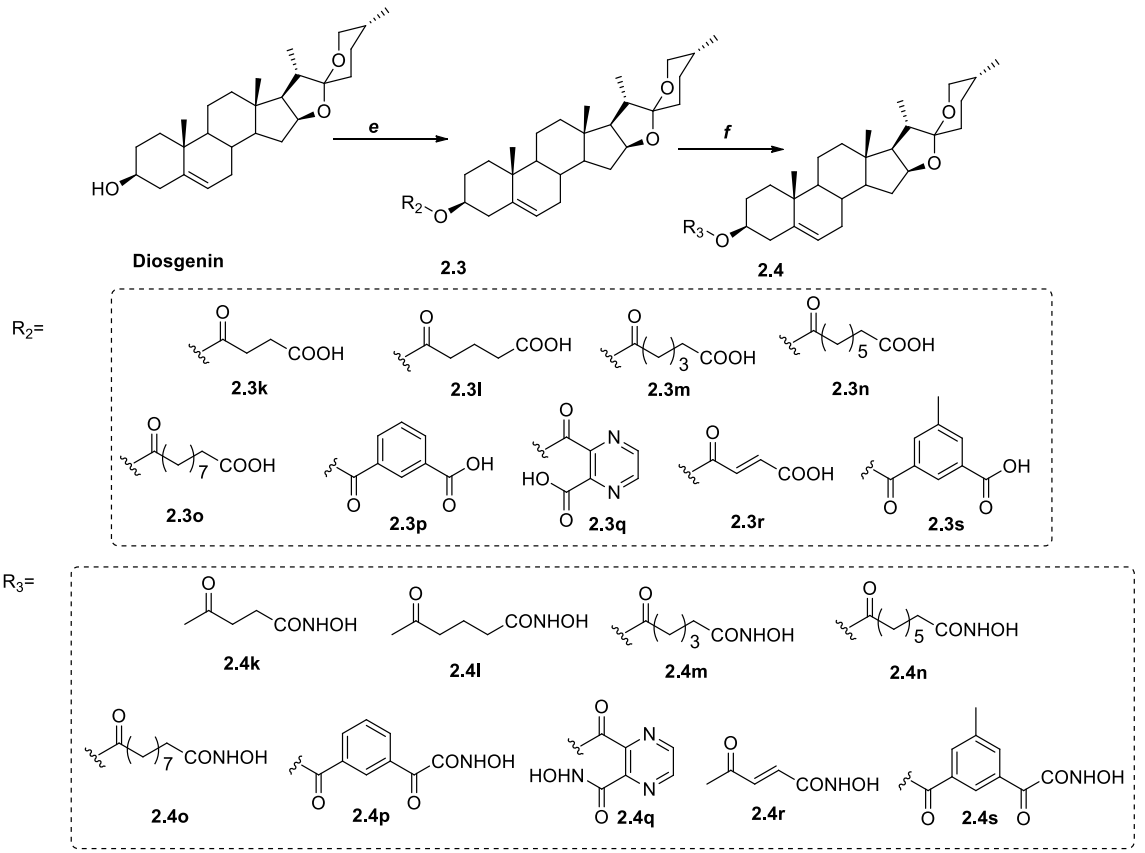
Scheme 1[a]



[a]Reagents and conditions: (a) DMAP, EDC·HCl, 5-bromovaleric acid, CH_2Cl_2, rt, 3 h, yield 81%. (b) Tertiary phosphine, CH_2Cl_2 (**2.2a**), and CH_3CN (**2.2b**−**2.2j**), reflux, yield 41-87%.

Scheme 2[a]



[a]Reagents and conditions: (e) DMAP, EDC·HCl, dicarboxylic acid, CH_2Cl_2 (**2.3k**−**2.3o**) or DMF (**2.3p**−**2.3s**), rt, 10−24 h, yield 56−95%. (f) CDI, NH_2OH(50%), CH_2Cl_2 (**2.4k**−**2.4p**), rt, 4−6 h, or EDC·HCl, HOBt, NH_2OTHP, P-TsOH, CH_2Cl_2 (**2.4q**−**2.4s**), rt, 12−18 h, yield 40−87%.

water solubility, so as to obtain better pharmacological activities.[26]

At the moment, for cancer, histone deacetylases (HDACs) are closely related to the pathogenesis of tumors and are one of the main targets. It can be found from many previous studies that the design of multitarget drugs with other activities based on HDAC inhibition is an effective way to treat tumors.[27] These molecules can not only overcome the resistance of

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single drug but also effectively avoid the problems caused by drug combination.[28] The pharmacodynamic structure of HDAC inhibitors consists of three parts: (1) Zinc binding group (ZBG) which chelates zinc ions at the bottom of the pocket of HDAC; (2) the surface recognition region (cap group) acting on the entrance edge of the active pocket of HDAC; (3) linker region acting on the hydrophobic channel of the active site and connecting Cap and ZBG.[29,30] However, the "Cap" group can accept a wider range of structural changes; the design of multitarget molecules for HDAC usually starts from this structure and combines or fuses with ZBG groups (mostly isohydroxamic acid and benzoimide groups) by introducing other active molecular fragments.[31] Considering the effect of water solubility on the activity of diosgenin derivatives, the hydroxamic acid group with better activity was introduced in diosgenin to obtain optimal inhibitory activity.

We used diosgenin as a starting material to synthesize a series of quaternary ammonium salts and conducted in vitro antiproliferative activity experiments. The results showed that the water solubility of the derivatives was higher than that of diosgenin and that the derivatives had antitumor activity on most cells. The antitumor activity was also better than that of diosgenin, indicating that increasing water solubility can effectively improve the antitumor activity of the diosgenin derivatives.[22] At the same time, the intracellular transport rate of phosphine cations is 10^7-10^8 times higher than that of hydrophilic sodium ions[32] and quaternary phosphonium salts also have antitumor effects.[33] The hydrogen bond in the structure of hydroxamic acid can interact with protein molecules, combined to exert its antitumor effect but also increase its water solubility. Therefore, we have designed and synthesized diosgenin hydroxamic acid by modifying its structure at the C-3 position of the diosgenin A ring to increase the hydrophilicity of the derivative to obtain better antitumor activity diosgenin derivatives.[18,20,34]

During these studies, we have synthesized diosgenin hydroxamic acid and diosgenin quaternary phosphonium salts. The synthetic route of diosgenin quaternary phosphonium salt derivatives is shown in Scheme 2. Diosgenin introduces 5-bromovaleric acid at the C-3 position to obtain compound **2.1** (Scheme 1). Compound **2.1** is reacted with different tertiary phosphine compounds to obtain the corresponding target compound diosgenin quaternary phosphonium salt derivatives **2.2a−2.2j**[35,36] (Scheme 1, Supporting Information (SI), page S2). Next, we focused on the addition of hydroxamic acid. Diosgenin introduces different dicarboxylic acids at position C-3 to obtain compounds **2.3k−2.3s**. Next, compounds **2.3k−2.3o** are reacted with carbonyl diimidazole (CDI) at 25 °C for 2 h, and 50% NH_2OH aqueous solution is added to obtain compounds **2.4k−2.4o** (Scheme 2, SI page S7). Compounds **2.3p−2.3s** react with *o*-(tetrahydro-2-hydropyran-2-yl) (NH_2OTHP), and then TsOH is added for deprotection to obtain the target compounds **2.4p−2.4s** (Scheme 2, SI page S8).

To evaluate the inhibitory activity, all derivatives **2.2a−2.2j** and **2.4k−2.4s** are tested using the CCK8 method. SW620 (human colon adenocarcinoma cells), H358 (human nonsmall cell lung cancer cells), HCT-116 (human colorectal adenocarcinoma cells) and Aspc-1 (human metastatic pancreatic cancer cells) cell lines were tested for inhibitory activity in vitro (Table 1, see the SI page S10). The results of the biological tests show the IC_{50} values of diosgenin derivatives **2.2a−2.2j** and **2.4k−2.4s** for tumor cells Aspc-1, H358, HCT-116, and SW620, and all derivatives except **2.4n** for HCT116 cells and **2.4q** for SW620 cells. The antitumor activity of the derivatives are superior to that of diosgenin. First, **2.2b**, **2.2c**, **2.2e−2.2j**, **2.4l**, **2.4m**, and **2.4p** have inhibitory activities against Aspc-1 (IC_{50} are 2.265, 1.092, respectively, 0.7281, 0.1847, 0.6483, 0.3131, 0.2905, 0.6523, 2.139, 2.307, 4.961 μM) that are equivalent to that of doxorubicin in the control group (IC_{50} < 5 μM); for **2.2b**, **2.2c**, **2.2e−2.2j**, and **2.4l** versus H358, the inhibitory activities (IC_{50} of 3.153, 2.18, 3.823, 4.038, 3.538, 3.515, 3.656, 1.707, 4.316 μM respectively) are equivalent to that of the control doxorubicin (IC_{50} < 5 μM); the inhibition rate of all derivatives of HCT116 are all not superior to the control group doxorubicin; **2.2b**, **2.2c**, **2.2e−2.2i**, **2.4l**, and **2.4m** inhibit SW620 activity (IC_{50} are 4.388, 1.173, 1.145, 0.4483, 0.778, 0.8726, 1.159, 2.431, respectively, 1.92 μM) that is equivalent to that of doxorubicin in the control group (IC_{50} < 5 μM). On the whole, the IC_{50} value can be analyzed; all derivatives are stronger than diosgenin for the H358, HCT-116, and SW620 cell lines, and the antiproliferative activity of **2.2a−2.2j** quaternary phosphonium salt derivatives is generally better than that of **2.4**. The **2.4k−2.4s** hydroxamic acid series derivatives are better. The antiproliferative activity of **2.2e−2.2j** is better for the four kinds of tumor cell lines, and the antiproliferative activity of **2.4n**, **2.4q**, and **2.4r** is weaker than those of the other derivatives.

Table 1. Antiproliferative Activities of Diosgenin Derivatives

	IC_{50} (μM)			
compds	Aspc-1	H358	HCT116	SW620
2.2a	7.435	13.57	10.06	9.594
2.2b	2.625	3.513	5.001	4.388
2.2c	1.092	2.18	3.994	1.173
2.2d	7.627	27.11	31.4	29.02
2.2e	0.7281	3.823	4.164	1.415
2.2f	0.1847	4.038	4.001	0.4483
2.2 g	0.6483	3.538	4.25	0.778
2.2h	0.3131	3.515	4.148	0.8726
2.2i	0.2905	3.657	4.224	1.159
2.2j	0.6523	1.707	4.318	6.805
2.4k	7.994	7.473	31.81	5.217
2.4l	2.139	4.316	32.51	2.431
2.4m	2.307	12.6	38.77	1.92
2.4n	22.86	23.11	>40	11.07
2.4o	15	10.37	33.41	23.37
2.4p	4.961	9.541	34.41	6.8
2.4q	9.291	35.51	35.79	>40
2.4r	11.36	36.84	39.02	16.85
2.4s	7.799	15.22	32.8	10.74
diosgenin	63.11	>40	31.41	>40
adriamycin	<5	<5	>1.25	<5

To understand which structural element of diosgenin derivatives can be responsible for binding to certain sites of the enzyme active site, we performed molecular docking and attempted a structure− activity relationship analysis. According to literature review, the EGFR enzyme (epidermal growth factor receptor) is widely distributed on the surface of mammalian epithelial cells, fibroblasts, glial cells, keratinocytes, etc., which inhibits proliferation, angiogenesis, tumor invasion, metastasis, and apoptosis of EGFR and tumor cells.[37,38] Mutations in the EGFR kinase domain are a common cause of

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Table 2. Scoring Results of Molecular Docking

ligand	binding energy (kcal/mol)	intermolecular energy (kcal/mol)	electrostatic energy (kcal/mol)	internal energy (kcal/mol)	predicted K_i
gefitinib	−8.84	−11.22	−0.09	−0.69	333.47 nM
2.2f	−12.11	−15.09	0.02	−4.02	1.33 nM

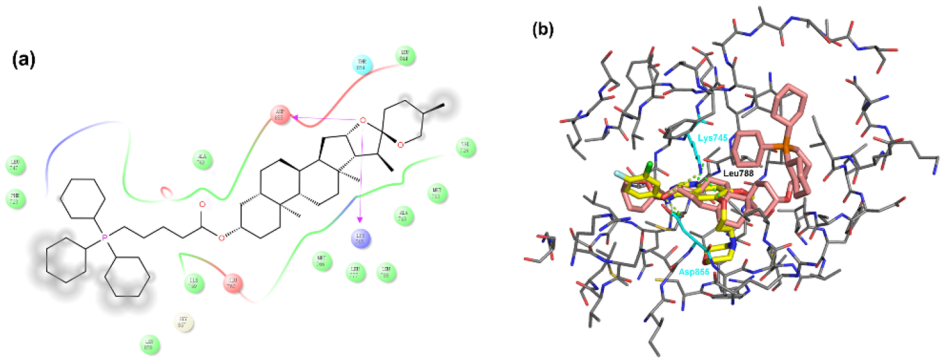

Figure 2. (a) Two-dimensional binding pattern of compound **2.2f** with EGFR. (b) Docking model of compound **2.2f** (pink), gefitinib (yellow), and EGFR.

non-small cell lung cancer (NSCLC), and NSCLC is the main subtype of lung cancer. Gifitinib is an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) and the first NSCLC targeted drug with the most mature research. In our previous studies, we tested the enzyme activity of derivatives with better inhibitory activity and lower biotoxicity.[21] The EGFR enzyme was used to investigate the survival rate of cells at different concentrations, and the corresponding IC_{50} value was calculated to further verify the good activity of the derivatives. Therefore, the enzyme activity test was not repeated. We used Autodock molecular docking software to establish a molecular docking model to compare the binding of compound **2.2f**, gifitinib, and EGFR (SI, page S11). As shown in Table 2, we can find from the predicted binding energy that the binding energy between compound **2.2f** and EGFR is stronger than that of gefitinib. The further interaction mode analysis results are shown in Figure 2. From the point of view of the binding site, the binding region and occupancy site of compound **2.2f** and EGFR are partly the same as the binding site of gifitinib, Ala743, Lys745, Cys775, Arg776, Leu777, Leu788, Met790, Thr854, and Asp855, and other binding sites are basically the same. The hydrogen bond between **2.2f** and EGFR is expected to reduce a group of gefitinib, but the hydrophobic interaction between **2.2f** and the pocket is significantly stronger than that of gefitinib, which further enhances the affinity between compound **2.2f** and EGFR. It may be the main reason for its enhanced binding energy with EGFR.

In summary, diosgenin hydroxamic acids and diosgenin quaternary phosphonium salts were designed and synthesized for the purpose of improving the structure of diosgenin to increase the hydrophilicity of its derivatives and obtaining better antitumor activity. For two novel series of diosgenin derivatives, in vitro antitumor activity experiments were carried out to investigate the cell activities of SW620, H358, HCT-116, and Aspc-1 cell lines. The experimental results show that the antitumor activity of most of the diosgenin derivatives is better than that of the lead compound, the antitumor activity of some derivatives is equivalent to that of adriamycin, and the antitumor activity of the quaternary phosphonium salt derivatives is overall better than that of hydroxyl oxamic acid derivatives. Close attention to the introduction of hydrophilic groups in the C-3 position of the A ring, in particular the hydrophilic groups (such as nitrogenous group, amide group, salt group, hydroxamic acid, etc.), led to compounds with better pharmacological activity. Moreover, the limitations of in vivo bioactivity, pharmacokinetics, and potential toxicity will also be the focus of our future studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00581.

Synthesis of compounds **2.1**, **2.2a**−**2.2j**, and **2.4k**−**2.4s**, antiproliferative activity method, molecular modeling methods, and NMR spectra (PDF)

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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