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Synthesis, characterization and antitumor activities of some steroidal derivatives with side chain of 17-hydrazone aromatic heterocycle

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ABSTRACT

Here a series of dehydroepiandrosterone-17-hydrazone and estrone-17-hydrazone derivatives possessing various aromatic heterocycle structures in 17-side chain of their steroidal nucleus were synthesized and their structures were evaluated. The antiproliferative activity of synthesized compounds against some cancer cells was investigated. The results have demonstrated that some dehydroepiandrosterone-17-hydrazone derivatives show distinct antiproliferative activity against some cancer cells through inducing cancer cell apoptosis, and compound **8** with a quinoline structure in 17-side chain displays excellent antiproliferative activity in vitro against SGC 7901 cancer cell (human gastric carcinoma) with an IC_{50} value of 1 μM. In addition, estrone-17-hydrazone derivatives having a key feature of indole group in the structure showed a special obvious cytotoxicity against HeLa cells, but almost inactive against other cells. The information obtained from the studies is valuable for the design of novel steroidal chemotherapeutic drugs.

1. Introduction

Heterocycles are ubiquitous in drug molecules because they possess hydrogen bond donors and acceptors in a rigid framework, and they can therefore effectively interact with target enzymes and receptors via hydrogen bond interactions. They can enhance binding affinity and improve in vitro potency. Heterocycles can modulate lipophilicity of the drug molecules or improve aqueous solubility of the compounds, thus providing desired pharmacokinetic properties and pharmaceutical properties [1]. The compounds containing heterocycles had been widely applied to pharmaceutical and pesticide field for its good biological activity. The versatility of heterocycles in modern anticancer drug discovery is amply demonstrated in various anticancer new drugs, such as axitinib [2], ponalinib [3], imatinib [4], gefitinib [5], lapatinib [6], etc.

It is well known that steroids play an important biological role in life. They can regulate a variety of biological processes and have been widely used in medicine as essentials of anti-inflammatory, anabolic, anticancer and contraceptive drugs. Scientists have found that introducing some heterocycles into the steroids [7–14], changing the steroidal side chain or substitution of the steroidal skeleton, introducing heteroatom or replacing one or more carbon atoms in steroidal molecule with heteroatom may result in change of its biological activities [15–19]. So far, the steroids containing heterocycles had been widely researched and reported [20–22]. Literatures suggested that such compounds displayed distinct cytotoxicity against cancer cell lines [23–27].

In our previous studies, we synthesized some novel steroidal oximes, hydrazones, lactone and lactams, and investigated their cytotoxic activity against different types of cancer cells [28–34]. The results showed that some steroidal oximes, hydrazones, A-homo or B-homo steroidal lactams possessing a cholesteric side chain and the 3-, 6- or 7-hydroxyl or hydroximino group displayed distinct cytotoxic activity against some cancer cells. In order to obtain biologically potent compounds with diverse structures, therefore in this paper, a series of steroidal compounds containing heterocycle attached to the steroidal 17-sidechain by a hydrazone structure were designed and synthesized using dehydroepiandrosterone and estrone as staring materials. Furthermore, the antiproliferative activity of the compounds against some cancer cells was evaluated in vitro.

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2. Result and discussion

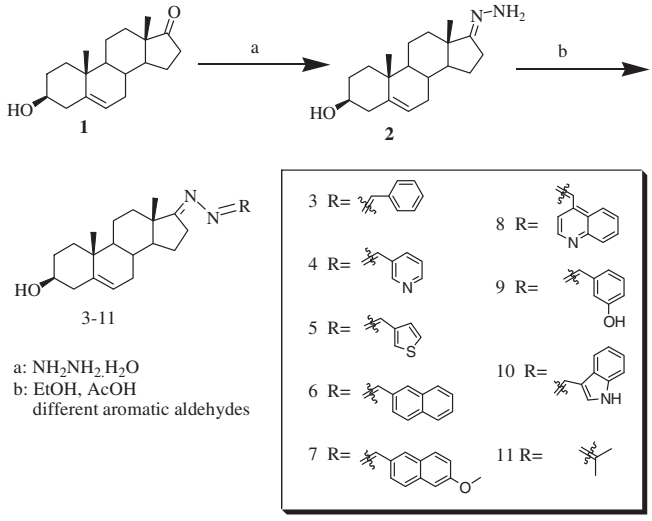
2.1. Chemistry

2.1.1. Synthesis of dehydroepiandrosterone-17-hydrazone aromatic heterocycle derivatives

Scheme 1 outlines the synthetic procedures of compounds **3–11**. First, the dehydroepiandrosterone was converted to the corresponding dehydroepiandrosterone-17-hydrazone (**2**) via reaction with hydrazine hydrate in anhydrous ethanol. After crystallized, the reaction of pure steroidal hydrazone with appropriate aromatic aldehydes gave steroidal hydrazone derivatives **3–11**. Their structures were confirmed by IR, NMR and HRMS spectrum. In the ^{1}H NMR spectrum the resonances showing of C22-H at 8.310 ppm (s) and 22-C at 151.2 ppm demonstrate a formation of 17=N—N=CAr bond in **4.** The downfield chemical shifts of Ar—H at 7.350, 8.115, 8.630, 8.883 ppm and Ar—C at 123.7, 130.5, 134.5, 149.8, 154.3 ppm show the presence of pyridine ring in compound **4**.

2.1.2. Synthesis of estrone-17-hydrazone aromatic heterocycle derivatives

To determine the effect of A-ring's structure in steroidal nucleus to the cytotoxicity, we synthesized compounds **14–18** (Scheme 2). Compounds **14–18** were prepared similarly as the procedures for the synthesis of compounds **3–11** and their structures had been confirmed by IR, NMR and HRMS spectra.



Scheme 1. Synthesis of compounds **3–11**.

2.2. Biological results and discussion

2.2.1. Cell culture and assay for cell viability

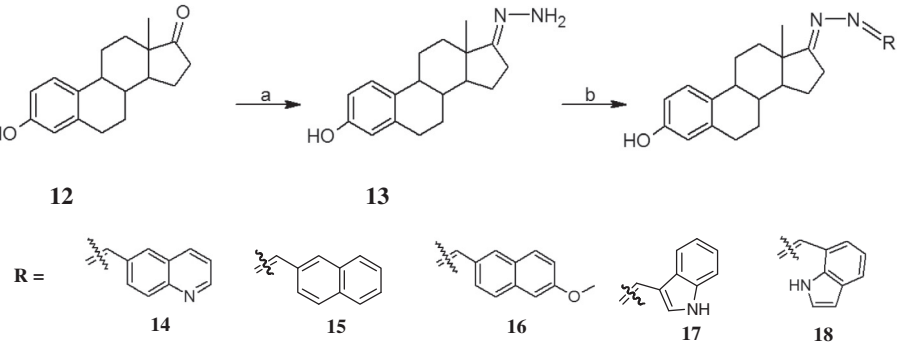
To evaluate the antiproliferative activity of the compounds, we determined their IC_{50} values on HeLa (human cervical carcinoma), HT-29 (human colon carcinoma), Bel 7404 (human liver carcinoma) and SGC 7901 (human gastric carcinoma) cancer cells using a MTT assay. MTT is a compound that can be taken up by viable cells and reduced by a mitochondrial dehydrogenase forming a formazan product in living cells. The absorbance of the formazan product at 492 nm is in linear proportion to cell numbers. The results were summarized as IC_{50} values in μmol/L in Table 1.

As showed in Table 1, dehydroepiandrosterone-17-hydrazone aromatic heterocycle compounds exhibit distinct antiproliferative activity, but compound **11** with a structure of propan-2-ylidenehydrazone is almost inactive against these cancer cells. The compounds **8–10** with 17-quinoline-4′-methanylidenehydrazone, 17-(3′-hydroxy)benzylidenehydrazone and 17-indol-3′-methanylidenehydrazone showed better cytotoxicity. Thereinto, compound **8** with the structure of a quinoline was found to be the most potent compound as anticancer agent. It displayed a better antiproliferative activity than cisplatin did (a positive control) against the tested cancer cells and owns an IC_{50} value of 1.0 μM on SGC-7901 cell. However, after the A-ring in the steroidal nucleus of compounds **6–8** was transformed into a benzene ring, compounds **14–16** obtained resulted in a dramatic decrease of the cytotoxicity. This showed the importance of the structure of steroidal nucleus in steroids.

Interestingly, compounds **17** and **18** having a key feature of indole group in their structures showed a special cytotoxicity against HeLa cells with an IC_{50} value of 5.0 μM and was almost inactive against other cells. It shows that compounds **17** and **18** are better potent compounds to the proliferate inhibition of HeLa tumor cell line, therefore, the further structural modification and antitumor activity study in vivo would be in progress.

2.2.2. Annexin V assay

To further disclose the antiproliferative mechanism of compounds, the HeLa and Bel-7404 cells were treated with compound **8**, and Annexin V assay was performed. The translocation of membrane phospholipid phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane is an early event of cell apoptosis. Annexin V is a 35–36 kD Ca^{2+} dependent, phospholipid-binding protein that has a high affinity for PS. Therefore, FITC-conjugated Annexin V is commonly used to determine apoptotic cells at an early stage. As shown in Fig. 1, treatment with different concentration of compound **8** resulted in different amounts of PI/Annexin V double-labeled apoptotic cells (control: 0.0%) after 24 h incubation (the lower right quadrant and the upper right quadrant which contains early and late apoptotic cells, respectively), suggesting compound **8** is a potent apoptotic inducer in these carcinoma cells.



Scheme 2. Synthesis of compounds **14–18**.