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Research paper

Facile and efficient access to Androsten-17-(1′,3′,4′)-pyrazoles and Androst-17β-(1′,3′,4′)-pyrazoles via Vilsmeier reagents, and their antiproliferative activity evaluation *in vitro*

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ABSTRACT

In this work, twenty-seven novel steroidal pyrazole derivatives were designed and effectively synthesized with two different commercially available staring material, Isopregnanolone **1** and 5,16-Pregnadienolone **7**, via the key intermediates, 17β-(4′-formyl)pyrazolylandrost-3β-yl formate and 17-(4′-formyl)pyrazolylandrost- 5,16-dienes-3β-yl formate, which were obtained from the cyclization of steroidal phenylhydrazone with Vilsmeier reagent catalyzed by phosphorous oxychloride followed by hydrolysis, then Borch reduction to afford the target derivatives under mild conditions. Structures of these compounds were identified by ^{1}H NMR, ^{13}C NMR and high resolution mass spectrometry. Based on our previous work, the cytotoxicity of these derivatives were evaluated by the SRB method against 293T cell lines and three cancer cell lines: A549, Hela and MCF-7. The results indicated that compounds **5b-d**, and **11a-e** exhibited moderate to high cytotoxic activities with IC_{50} values ranging from 0.62 to 7.51 μM. Among the eight hybrids, compound **11b**, with an ethyl amino and a dien-pregn moieties showed the highest potency, with an IC_{50} values of 0.87 μM and 0.53 μM for 293T cell lines and Hela cell lines, respectively. Some structure-activity relationships among the groups of the twenty-seven derivatives are discussed and identify several determinants important for the activity of these compounds. What's more, further molecular mechanism studies suggested that **11b** one of the most potent derivatives caused Hela cell lines apoptosis and arrested the cell cycle at S phase in a concentration dependent manner.

1. Introduction

Cancer, a chronic, uncontrolled, and pathological proliferation of abnormal cells, is the most burdensome disease known for the second death leading cause of death worldwide. About 12 million people are diagnosed with cancer and 7 million patients die of cancer annually. Furthermore, the global burden of cancer is increasing rapidly as the average age of the general population increases [1–3]. Despite advances in diagnosis and therapy, outcomes remain poor on account of the resistance against chemotherapy and targeted drugs which are as widespread as the use of these agents [4]. Another challenge for chemotherapy is lack of selectivity for general anticancer drugs destroy tumor cells as well as normal cells and often cause serious side-effects [5]. As a result, cures of cancer have to be versatile and sophisticated. Consequently, extensive efforts have been devoted to the development of novel anticancer medicines which will reduce limitations on cancer therapy.

In recent years, pyrazoles have attracted significant scientific attention due to their special skeleton and various bioactivities containing anti-inflammatory [6], antimicrobial [7,8], anticonvulsant [9], antidepressant [10], antimycobacterial [11], antioxidant [12], antiviral [13], insecticidal [14] and antitumor [15] activities. Presence of this nucleus in multiple pharmacological agents has made it an indispensible anchor for design and development of new pharmacological agents [16].

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Derivatives of steroids, compounds widely distributed in nature, can be either isolated from nature or synthesized. Steroidal derivatives possess diverse biological activities which play a pivotal role in the treatment of malignancies, and are treated as leading compounds in the discovery of novel anticancer drugs because they reduce systemic toxicity and improve specificity in cancer therapy [17–19]. Steroids bearing heterocycles (pyrazoles, pyrazolines, isoxazoles, isoxazolines, thiazoles) fused to the D-ring of the steroids backbone have been of pharmaceutical interest [20]. Synthetic of derivatives of steroidal pyrazoles have attracted a great deal of attention for the goals of developing leading compounds to withstand diseases [21,22].

Recently, our group [23] and the Eva Franck group [24] have demonstrated that the derivatives 17β-(1-phenyl-4-((ethylamino) methyl)-3-pyrazolyl)androst-5-ene -3β-ol and 17β-(1-phenyl-4-(hydroxymethyl)-3-pyrazolyl)androst-5,16-diene-3β-ol (Fig. 1) bearing a small hydrophilic group attached to 4 position of the pyrazole ring are the most potent compounds among the a series of derivatives. The former exhibited excellent cytotoxicity against A549 cell lines with an IC_{50} value of 0.91 μM. On the basis of our previous test results, we deduced that the hydrophilicity or configuration of the hybrids would dramatically affect their activities.

Base on the aforementioned findings and in the continuation of our ongoing research on 17-(4′-formyl)pyrazolylandrost derivatives related to their anticancer activities, herein we carried out the synthesis of two new series of steroidal pyrazole derivatives (Table 1) and evaluated their cytotoxicity on A549 cells (human alveolar adenocarcinoma cell lines), Hela cells (human cervical cancer cell lines), MCF-7 cells (human breast adenocarcinoma cell line), and 293T cells (human kidney cell line, transformed with large T antigen). Moreover, in order to gain a deeper insight into the mode of action of the title compounds onto the tumor cells some extra experiments, fluorescence microscopy, an annexin V/PI assay, and cell cycle evaluations, were carried out. For the most activate compound **11b**, the effect on the cell cycle and induction of apoptosis were performed against Hela cell lines to investigate its mechanism of action.

2. Results and discussion

2.1. Synthetic studies

The target 17-(4′-formyl)pyrazolylandrost-5,16-dienes-3β-ol and 17-(4′-formyl)pyrazolylandrost-3β-ol hybrids were synthesized from commercially available starting material (Isopregnanolone **1** and 5,16-Pregnadienolone **7**) as illustrated in Scheme 1a and b. The initial key intermediates **3** and **8** were prepared via the Vilsmeier reaction as per previously reported by our group with some modifications [23]. Compound **3**, bearing the formyl group at the 4-position of the pyrazole and formate moiety at the 3-position of the steroid was synthesized from compound **2** which could be easily prepared by condensation of Isopregnaolone **1** and phenylhydrazine in the present of acetic acid via cyclization on treatment with Vilsmeier reagents at 0 °C in 89% yield. Deformylation and reduction of compound 3 using K_2CO_3 or $NaBH_4$ gave 17-(4′-formyl)pyrazolylandrosta-3β-ol **4** and 17-(4′-hydroxymethyl)pyrazolylandrost-3β-ol **6**, respectively, in high yields. Further, the obtained products **4** underwent reaction with different amines in the present of $NaBH(AcO)_3$ in DCE to afford the desired derivatives **5a-5m**. However, following the above standard conditions did not result in the key intermediate **9** effectively, with a poor yield 9.5%. In this situation, we screened the effect of phosphorous oxychloride molar equivalents (3 equiv., 5 equiv.), temperature, and time (Table 2). Consequently, optimal conditions were as follows: 3 equiv. of phosphorus oxychloride, 40 °C, and 24 h; under these conditions, compound **9** could be synthesized efficiently in 91% yield. Subsequent reaction of product **9** through hydrolysis gave 17-(4′-formyl)pyrazolylandrost-5,16-dienes-3β-ol **10** quantitatively. Finally, the aldehyde **10** was subjected to reductive amination to afford the target hybrids **11a-11m**.

Spectra (^1H NMR, ^{13}C NMR and high resolution mass) of the newly synthesized compounds were in full agreement with the proposed structure. In the ^1H NMR spectra of compounds **3** and **9**, the formation of the heterocyclic unit was confirmed by the signal around $\delta = 7.81$ ppm or $\delta = 8.38$ ppm corresponding to the 5′–H on the pyrazole ring. Moreover, the signals of the aldehyde group and the formoxyl group were detected around $\delta = 9.98$ ppm and $\delta = 8.02$ ppm, respectively, and the signals of the phenyl hydrogen atoms appeared in the aromatic region. In addition, the signals of the vinyl hydrogens of pregndiene moiety were observed at $\delta = 6.37$ ppm and $\delta = 5.45$ ppm corresponding to the 6-H and 15-H respectively, and the signals of 3-H, CH_3-18 and CH_3-19 of pregn moiety showed a multiplet at around $\delta = 4.78$ ppm, and two singlets ranging from $\delta = 1.16$ ppm to 0.61 ppm. The above chemical shifts appeared for compounds **3** and **9** indicating the formation of the steroidal pyrazoles.

When Borch reduction was carried out to afford the products ***5a-m***, ***11a-m***, the representative multiplets signals from $\delta = 4.12$ ppm to $\delta = 4.26$ ppm of $-CH_2-$ and the broad peak ranging from $\delta = 3.56$ ppm to $\delta = 4.22$ ppm of $-NH-$ in the ^1H NMR spectra were the vital characteristic peaks of these derivatives. In the ^{13}C NMR of hybrids **3** and **9,** the characteristic peak of the aldehyde group and formoxyl group appeared at about $\delta = 185$ ppm and $\delta = 160$ ppm, respectively. Further, the chemical shift of the aromatic carbons ranging from $\delta = 155$ ppm to $\delta = 119$ ppm showed

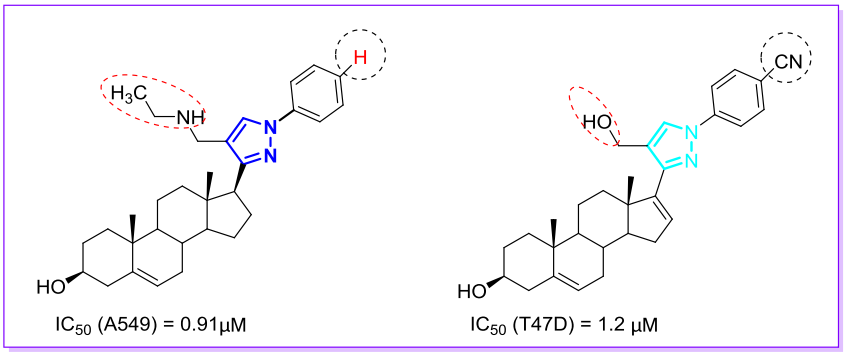

Fig. 1. Structures of previous reported steroidal pyrazole derivatives [23,24].