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Design, synthesis and biological evaluation of novel steroidal spiro-oxindoles as potent antiproliferative agents

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ABSTRACT

Two series of novel steroidal spiro-pyrrolidinyl oxindoles **3a**–**t** and **6a**–**c** were designed and synthesized from dehydroepiandrosterone using the 1,3-dipolar cycloaddition as the key step and further evaluated for their antiproliferative activities for four human cancer cell lines (MGC-803, EC109, SMMC-7721 and MCF-7). This protocol achieved the formation of two C—C bonds, one C—N bond and the creation of one new five-membered pyrrolidine ring and three contiguous stereocenters in a single operation. Biological evaluation showed that these synthesized steroidal spiro-pyrrolidinyl oxindoles possessed moderate to good antiproliferative activities against the tested cell lines and some of them were more potent than 5-Fu. Particularly, compound **3g** showed good antiproliferative activity against SMMC-7721 (IC_{50} = 0.71 μM). Steroid dimer **6b** showed improved antiproliferative activities against SMMC-7721 and MCF-7 with the IC_{50} values of 4.30 and 2.06 μM, respectively. Flow cytometry analysis demonstrated that compound **3n** caused the cellular early apoptosis and cell cycle arrest at G2/M phase in a concentration- and time-independent manner.

1. Introduction

Steroids are a family of molecules that play a crucial role in a wide range of biological processes and in human physiology. Their hormonal action *via* binding to a specific receptor is well known and has led to the development of antagonists to treat certain hormone-dependent diseases [1]. It is proved that a number of biologically important properties of modified steroids depend upon structural features of the steroid ring system [2] and side chain [3]. Chemical modifications of the steroid ring system and side chain provide a way to alter the functional groups, and numerous structure activity relationships (SARs) have been established by such synthetic alterations [4]. Among them, dimeric steroids are a special group of compounds, which have recently received significant attention [5,6]. There is evidence that dimerization of steroid skeleton offers some unique characteristics that are applicable in different areas, especially in pharmacology [7–9].

The specificity of biological activities found in natural compounds is generally in connection with the characteristic structural complexity and well-defined stereo-architecture [10,11]. The biological activity and structural complexity found in nature has stimulated generations of synthetic chemists to design strategies for assembling challenging structures found in natural products [12,13]. Particularly intriguing are the spirocyclic oxindole scaffolds, which feature in a large number of natural and unnatural compounds with important biological activities [14–18] and can also serve as key intermediates for the synthesis of alkaloids and many kinds of pharmaceuticals or drug precursors [19]. Among them, the spiro-pyrrolidinyl oxindoles possess a myriad of biological activities. For example, coerulescine [13], the simplest spirooxindole-pyrrolidine hybrid found in nature, displays local anesthetic effect. Pteropodine modulates the function of muscarinic serotonin receptors [20]. The spirotryprostatins have antibiotic properties and are of interest as anticancer lead compounds [21], and the recently discovered small-molecule MDM2 inhibitor MI-219 and its analogs are in advanced preclinical development for cancer therapeutics [14,16] (Fig. 1).

Recently, we have achieved the synthesis and antiproliferative evaluation of steroidal dienamides from dehydroepiandrosterone [22,23]. Inspired by the varied and significant biological activities of spiro-oxindole skeletons [24–29] and being involved in finding new biologically active modified steroids [30,31], we are interested in the design, synthesis and biological evaluation of novel steroidal spiro-pyrrolidinyl oxindoles. In this paper, we report the efficient and catalyst-free construction of steroidal spiro-oxindoles with spirotricyclic skeleton *via* a one-pot three-component protocol and evaluate their antiproliferative activities against human cancer cell lines *in vitro* and the effects toward the cell cycle and apoptosis.

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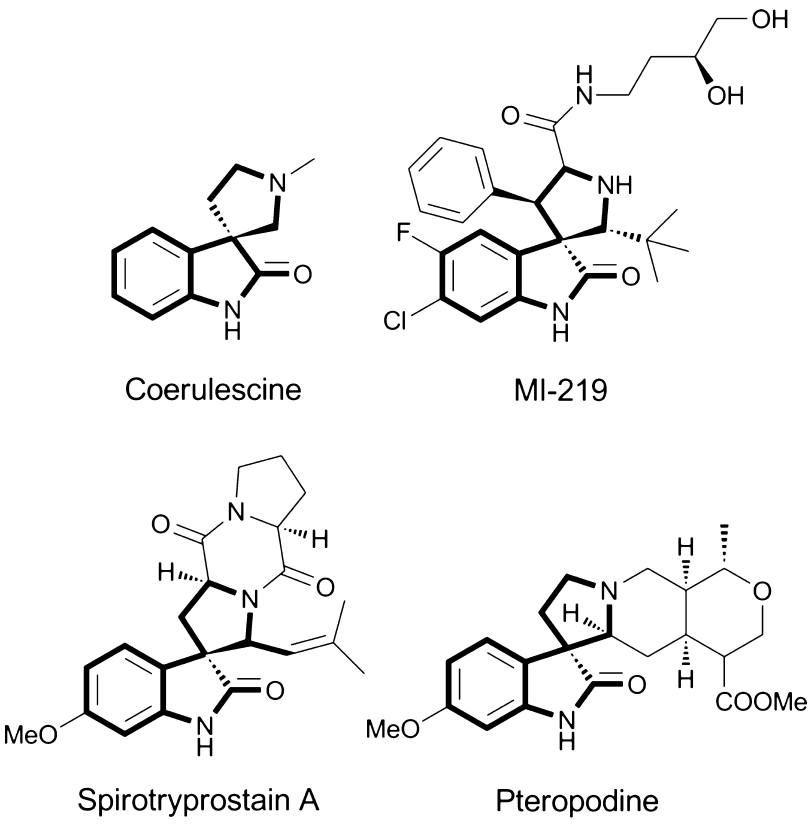

Fig. 1. Some naturally occurring and synthetic spiro-pyrrolidinyl oxindoles.

2. Experimental

2.1. General

Reagents and solvents were purchased from commercial sources and were used without further purification. Thin-layer chromatography (TLC) was carried out on glass plates coated with silica gel (Qingdao Haiyang Chemical Co., G60F-254) and visualized by UV light (254 nm). The products were purified by column chromatography over silica gel (Qingdao Haiyang Chemical Co., 200–300 mesh). Melting points were determined on a X-5 micromelting apparatus and are uncorrected. All the NMR spectra were recorded with a Bruker DPX 400 MHz spectrometer with TMS as internal standard in $CDCl_3$ or DMSO-d_6. Chemical shifts are given as δ ppm values relative to TMS. High-resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-T of Micromass spectrometer by electrospray ionization (ESI).

2.2. General procedure for the synthesis of 16-arylidene-17-ketosteroids (**2**)

A mixture of dehydroepiandrosterone (DHEA, **1**) (2.0 mmol), aromatic aldehydes (2.1 mmol) and KF/Al_2O_3 (2.0 mmol) in EtOH (20 mL) was heated under reflux for about 1 h. After completion of the reaction as evident from TLC, the slurry was filtered and the residue was washed thoroughly with CH_2Cl_2. The filtrate was condensed under reduced pressure, and the solid obtained was crystallized from EtOH or MeOH to yield the corresponding 16-arylidene-17-ketosteroids **2**. All the intermediates **2** were synthesized following the procedure previously reported by our group [30]. All the intermediates were reported before by our group [30] and Kumar [41], so spectral data for a representative compound **2a** was given below. White solid, yield 93%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 8.3 Hz, 2H, ArH), 7.71 (d, J = 8.4 Hz, 2H, ArH), 7.46 (s, 1H, Ar-C<u>H</u>=), 5.45–5.36 (m, 1H, 6-H), 3.60–3.50 (m, 1H, 3α-H), 3.09 (s, 3H, Ar-$SO_2C\underline{H}_3$), 2.94–2.82 (m, 1H), 2.50 (ddd, J = 16.0, 12.8, 3.0 Hz, 1H), 2.40–2.32 (m, 1H), 2.32–2.26 (m, 1H), 2.26–2.15 (m, 1H), 2.01 (dd, J = 9.6, 3.1 Hz, 1H), 1.86 (ddd, J = 15.8, 12.0, 4.3 Hz, 2H), 1.80–1.63 (m, 4H), 1.57 (ddd, J = 14.5, 11.9, 3.3 Hz, 2H), 1.47–1.33 (m, 2H), 1.19–1.11 (m, 1H), 1.09 (s, 3H, 19-CH_3), 1.01 (s, 3H, 18-CH_3). HRMS (ESI): m/z calcd for $C_{27}H_{34}O_4SNa$ $(M+Na)^+$, 477.2075; found, 477.2075.

2.3. General procedure for the synthesis of steroidal spiro-pyrrolidinyl oxindoles (**3a**–**t**)

A mixture of 16-arylidene-17-ketosteroids (**2a**–**t**, 1 mmol) (substituted) isatin (1.5 mmol) and sarcosine (2.0 mmol) in methanol/1,4-dioxane mixture (v/v = 1/1, 20 mL) was refluxed for about 5 h. After completion of the reaction as evident from TLC (petroleum ether/ethyl acetate = 2/1), the mixture was evaporated and purified by column chromatography on silica gel using petroleum ether/ethyl acetate = 2/1(v/v) as the eluent to obtain the pure products **3a**–**t**.

2.3.1. Spiro [2′.16]-1′-methyl-2′-(indolin-2-one)-4′-(4″-methylsulfonyl phenyl)-tetrahydro-1H-pyrrolo-dehydroandrosterone (**3a**)

White solid, yield: 89%, m.p. 262.0–264.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H, NH), 7.92 (d, J = 8.2 Hz, 2H, ArH), 7.70 (d, J = 6.7 Hz, 2H, ArH), 7.28–7.15 (m, 1H, ArH), 7.23 (t, J = 5.9 Hz, 1H, ArH), 7.07–6.96 (m, 1H, ArH), 6.85 (d, J = 7.7 Hz, 1H, ArH), 5.10 (d, J = 4.0 Hz, 1H, 6-H), 4.11 (t, J = 8.7 Hz, 1H, 12′-H), 3.92 (t, J = 9.4 Hz, 1H, 11′-H), 3.54 (t, J = 8.1 Hz, 1H, 11′-H), 3.51–3.39 (m, 1H, 3α-H), 3.09