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Multicomponent assembly of novel antiproliferative steroidal dihydropyridinyl spirooxindoles

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ABSTRACT

Multicomponent assembly of steroidal dihydropyridinyl spirooxindoles from pregnenolone, isatins, malononitrile, and ammonium acetate is described, which involves the formation of two C–C bonds, two C–N bonds, and an all-carbon quaternary stereogenic center in a single operation. MTT assays showed that some of these compounds had moderate to excellent cytotoxicity against the tested cancer cell lines and were more potent than 5-FU. Particularly, compound **5o** represented excellent inhibitory effect toward EC-109 (IC_{50} = 0.3 μM), being about 33-fold more potent than 5-FU.

1. Introduction

Steroids, as an important class of polycyclic molecules, have played pivotal roles in regulating normal physiological processes and targeting disease-related biological sites. Diverse biological functions have been observed and some of steroidal derivatives are currently used in clinic or have advanced into clinical trials for the treatment of diseases. Two representative examples are galeterone [1,2] and abiraterone [3,4] for the treatment of advanced prostate cancer. Galeterone possesses a unique dual mechanism of action by selectively inhibiting the C17, 20-lyase activity of CYP17 and by acting as an androgen receptor antagonist, and abiraterone is an androgen synthesis inhibitor by blocking the action of CYP17 enzyme, which is involved in the androgen biosynthesis.

Galeterone and abiraterone share the similar structure features, namely bearing an *N*-heterocycle at the D-ring. Modifications on steroid nucleus have been highly pursued in last decades with an aim of identifying potent steroidal derivatives for disease treatment [5–9]. Among them, incorporating heterocycles into steroid nucleus has been recognized as a promising strategy in discovering new steroids with biological potential.

Spirooxindoles have drawn extensive attention among scientific community as a consequence of their diverse biological activities, intriguing 3D structural features, and prevalence in natural products. Considerable efforts have been devoted to constructing such scaffolds and exploring their biological activities [10–14]. Significant progress has been observed in new drug discovery. For example, SAR405838 [15] and CFI-400945 [16–19], as the MDM2–p53 interaction and PLK4 inhibitors, respectively, are currently undergoing clinical assessment for cancer therapy, while NITD609 developed by the Novartis Institute for Tropical Diseases can inhibit growth of *Plasmodium falciparum* by targeting protein synthesis and has advanced into clinical trials for the treatment of malaria as well (Fig. 1) [20]. Recently, three types of structurally novel steroidal spirooxindoles have been disclosed by our group, biological evaluation indicated that these compounds can inhibit growth of human cancer cells potently and induce cell cycle arrest and apoptosis in a time-/concentration-dependent manner [21–23] (Fig. 1). Additionally, several dihydropyridines have been conventionally used for the treatment of hypertension in clinic as *L*-type calcium channel blockers [24] and have been reported to be able to inhibit growth of cancerous cells as well [25–27]. These findings, coupled with our previous observations regarding the anticancer potential of steroids [28–32], promoted us to explore the anticancer potential of steroidal dihydropyridinyl spirooxindoles, which were efficiently accessed from pregnenolone (PREG), isatins, malononitrile and ammonium acetate (Fig. 1). To the best of our knowledge, this is the first description concerning the multicomponent assembly and cytotoxic evaluation of steroidal dihydropyridinyl spirooxindoles.

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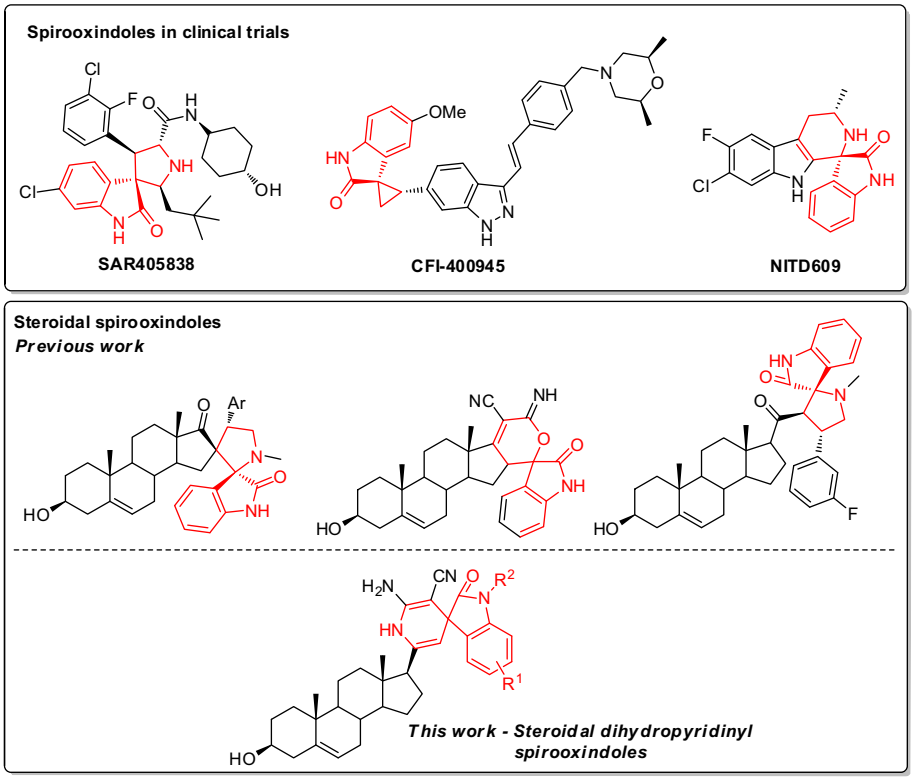

Fig. 1. Spirooxindoles in clinical trials and related molecules previously synthesized in our group.

2. Experimental

2.1. General remarks

Most of reagents and solvents were used directly without special treatment. Substituted isatins were prepared following our previously reported methods [23]. Thin layer chromatography (TLC) was carried out on glass plates coated with silica gel and visualized by UV light (254 nm). The products were purified by column chromatography over silica gel. Melting points were determined on a Beijing Keyi XT4A apparatus and are uncorrected. All NMR spectra were recorded with a Bruker DPX 400 MHz spectrometer with TMS as an internal standard in DMSO-*d*6. Chemical shifts are given as δ ppm values relative to TMS (Most of the peaks due to the steroidal skeleton are merged and could not be differentiated. Thus δ values of only those peaks that distinguish the product and could easily be differentiated are reported). High-resolution mass spectra (HRMS) were recorded on Esquire3000 mass spectrometer by electrospray ionization (ESI).

2.2. General procedure for the synthesis of steroidal dihydropyridinyl spirooxindoles derivatives ***5a–o***

Pregnenolone (PREG) **1** (1 mmol), substituted isatins **2** (1 mmol) and malononitrile **3** (1 mmol) ammonium acetate **4** (1.2 mmol) were dissolved in EtOH (5 ml), the resulting solution was kept under reflux for 18–24 h. Upon completion, EtOH was removed and the residue was purified by column chromatography (acetone/40–60 petroleum ether = 3/4) to give the corresponding steroidal derivatives in good yields.

2.2.1. Compound ***5a***

White solid, yield 82%, m.p. 280.6–282.2 °C. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.15 (s, 1H), 7.44 (s, 1H), 7.03–6.73 (m, 3H), 5.78 (s, 2H), 5.27 (d, J = 5.5 Hz, 1H), 4.61 (d, J = 4.8 Hz, 1H), 4.05 (s, 1H), 3.30–3.21 (m, 1H), 0.96 (s, 3H), 0.62 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 180.8, 160.0, 157.7, 153.6, 141.8, 139.7, 139.6, 139.1, 139.0, 137.2, 137.1, 136.4, 120.7, 115.4 (J = 70 Hz), 112.8, 110.5, 96.9, 70.4, 55.7, 53.9, 52.1, 50.4, 43.9, 42.7, 37.9, 37.4, 36.6, 31.9, 31.7, 24.4, 23.5, 23.2, 20.9, 19.7, 13.3. HRMS (ESI): m/z calcd for $C_{32}H_{37}FN_4O_2$ $(M-H)^-$, 527.2823; found, 527.2824.

2.2.2. Compound ***5b***

White solid, yield 80%, m.p. 244.1–245.6 °C. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.11 (s, 1H), 7.39 (s, 1H), 7.15 (t, J = 7.7 Hz, 1H), 7.09 (d, J = 7.3 Hz, 1H), 6.98 (t, J = 7.2 Hz, 1H), 6.77 (d, J = 7.8 Hz, 1H), 5.71 (s, 2H), 5.27 (d, J = 5.6 Hz, 1H), 4.61 (d, J = 5.0 Hz, 1H), 4.02 (s, 1H), 3.30–3.22 (m, 1H), 0.96 (s, 3H), 0.63 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 180.8, 153.5, 141.8, 141.0, 138.0, 135.9, 128.5, 125.2, 122.5, 121.5, 120.7, 109.7, 97.7, 70.4, 55.7, 53.9, 53.0, 51.5, 50.4, 43.9, 42.7, 37.9, 37.4, 36.6, 32.1, 31.9, 31.7, 24.4, 23.5, 20.9, 19.7, 13.3. HRMS (ESI): m/z calcd for $C_{32}H_{38}N_4O_2$ $(M-H)^-$, 509.2917; found, 509.2918.

2.2.3. Compound ***5c***

White solid, yield 83%, m.p. 267.0–268.1 °C. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.26 (s, 1H), 7.45 (s, 1H), 7.21 (d, J = 8.5 Hz, 1H), 7.04 (s, 1H), 6.80 (d, J = 8.3 Hz, 1H), 5.80 (s, 2H), 5.27 (d, J = 5.5 Hz, 1H), 4.60 (d, J = 4.8 Hz, 1H), 4.06 (s, 1H), 3.29–3.19 (m, 1H), 0.96 (s, 3H), 0.62 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 180.5, 153.7, 141.8, 140.0, 139.9, 136.6, 128.5, 126.3, 125.1, 121.3, 120.7, 111.3, 100.0, 96.7, 70.4, 55.8, 53.9, 52.4, 51.9, 50.3, 43.9, 43.7, 42.7, 38.0, 37.4, 36.6, 32.1, 31.9, 31.7, 24.3, 23.6, 20.9, 19.7, 13.2. HRMS (ESI): m/z calcd for $C_{32}H_{37}ClN_4O_2$ $(M-H)^-$, 543.2527; found, 543.2529.

2.2.4. Compound ***5d***

White solid, yield 78%, m.p. 193.1–194.3 °C. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.28 (s, 1H), 7.44 (s, 1H), 7.34 (d, J = 9.3 Hz, 1H), 7.15 (s, 1H), 6.76 (d, J = 9.0 Hz, 1H), 5.81 (s, 2H), 5.26 (s, 1H), 4.61 (s, 1H),