Steroids 88 (2014) 44–52

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Discovery of novel steroidal pyran–oxindole hybrids as cytotoxic agents

Bin Yu [1], Ping-Ping Qi [1], Xiao-Jing Shi, Li-Hong Shan *, De-Quan Yu *, Hong-Min Liu *

School of Pharmaceutical Sciences and New Drug Research & Development Center, Zhengzhou University, Zhengzhou 450001, PR China

ARTICLE INFO

Article history:
Received 18 March 2014
Received in revised form 14 May 2014
Accepted 25 May 2014
Available online 11 June 2014

Keywords:
Pyran–oxindole hybrids
Cytotoxicity
Apoptosis
Cell cycle arrest

ABSTRACT

A series of novel steroidal pyran–oxindole hybrids were efficiently synthesized in a single operation through the vinylogous aldol reaction of vinyl malononitrile **3** with substituted isatins involving the construction of C–C and C–O bonds. Some compounds displayed moderate to good cytotoxicity against T24, SMMC-7721, MCF-7 and MGC-803 cells. Compounds **4f** and **4i** were more potent than 5-Fu against T24 and MGC-803 cells with the IC_{50} values of 4.43 and 8.45 μM, respectively. Further mechanism studies indicated that compound **4i** induced G2/M arrest and early apoptosis in a concentration- and time-dependent manner.

1. Introduction

Steroids are extensively available in nature and well known for their profound biological activities due to their high ability to penetrate cells and bind to nuclear and membrane receptors. Chemical modifications of the steroid ring system and side chain provide a way to alter the functional groups and numerous structure–activity relationships have been established by such synthetic alterations [1,2]. It is proved that a number of biologically important properties of modified steroids are dependent upon structural features of the steroid ring system and side chain [3–6]. In recent years, a number of steroidal heterocycles with interesting activities have been isolated or synthesized. For example, spironolactone, as the mineralocorticoid antagonist, is a clinically used drug for congestive heart failure (Fig. 1) [7]. A recent report from Puranik Purushottamachar *et al.* described that galeterone analogue had potential for development as new drugs for the treatment of all forms of prostate cancer with an IC_{50} value of 0.87 μM through degrading both full-length and truncated ARs in CWR22rv1 human prostate cancer cells [8]. Our group recently reported that [1,2,4] triazolo [1,5-a] pyrimidine-based phenyl-linked steroid dimer induced apoptosis through the mitochondrial pathway accompanied with the decrease of mitochondrial membrane potential, activations of caspase-9/-3, cleavage of MDM2 as well as upregulation of the expressions of p53 and Bax [9].

The oxindole nucleus is a privileged scaffold that is highly prevalent in natural and synthetic compounds of medicinal interest [10]. Molecules containing an oxindole nucleus display a diverse range of biological activities [11], such as inhibitors of the MDM2-p53 interaction [12], antimicrobial activities [13], cholinesterase inhibitors [14,15], anticancer properties [16], protein kinase activators [17] and so on. Particularly intriguing is the NITD609, which has been studied as an antimalarial drug candidate that acts through the distinct mechanism of action as existing antimalarial drugs have [18]. Besides, oxindoles can also serve as synthetic intermediates for alkaloids and many kinds of pharmaceuticals or drug precursors [19]. Fox example, coerulescine, the simplest spirooxindole–pyrrolidine hybrid found in nature, displays local anesthetic effect [10]. The spirotryprostatins have antimitotic properties and are of interest as anticancer lead compounds [20], and the recently discovered small-molecule MDM2 inhibitor MI-219 and its analogues are in preclinical development for cancer therapeutics (Fig. 2) [12,21]. Additionally, the six-membered pyran ring system, as an important structural motif, is also found in a wide range of biologically active compounds and is known to have wide applications in medicinal chemistry [22]. For example, pyrrolo [2, 3-h] chromenes (Fig. 2) showed excellent anticancer activity with an EC_{50} value of 13 nM against T47D cells [23,24].

On the basis of the above-mentioned observations regarding the potential of oxindoles and pyrans and our own previous observations regarding [25] the importance of the steroidal oxindoles in conferring cytotoxic activities, a series of steroidal pyran–oxindole hybrids were designed, incorporating both oxindole and

* Corresponding authors. Tel./fax: +86 371 67781739.
E-mail addresses: zzuyubin@hotmail.com (B. Yu), shlh@zzu.edu.cn (L.-H. Shan), dqyu@imm.ac.cn (D.-Q. Yu), liuhm@zzu.edu.cn (H.-M. Liu).
[1] These authors made equal contributions.

http://dx.doi.org/10.1016/j.steroids.2014.05.022
0039-128X/

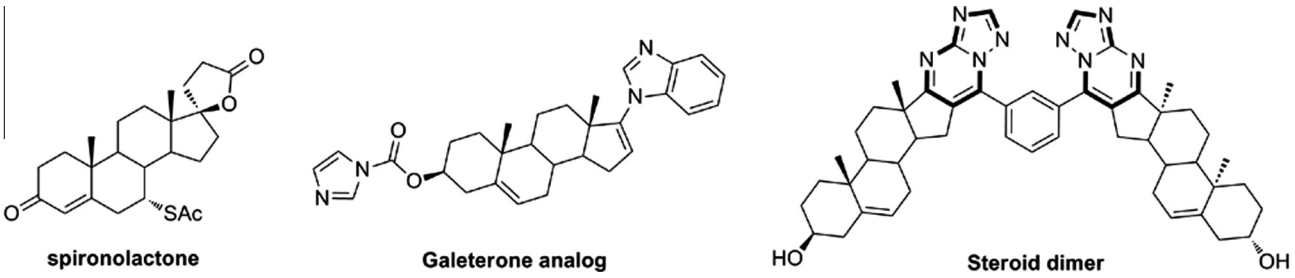

Fig. 1. Selective biologically active steroidal compounds.

2-imino-3-cyano pyran key scaffolds, with an aim to obtain potent and selective cytotoxic agents. Besides, we also explored the effects toward the cell cycle and possible mechanism of inducing apoptosis.

2. Experimental section

2.1. General

Reagents and solvents were purchased from commercial sources and were used without further purification. Isatins used were purchased from Aladdin Company (www.aladdin-e.com). 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 $CDCl_3$. 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 a Waters Micromass Q-T of Micromass spectrometer by electrospray ionization (ESI).

2.2. Synthesis of steroidal α, α-dicyanoalkene (**3**)

The steroidal α, α-dicyanoalkene (**3**) was efficiently prepared via Aldol condensation of 3β-acetyl dehydroepiandrosterone **2** with malononitrile in ethanol catalyzed by ammonium acetate according to our previously reported method [4,6].

2.3. General procedure for the synthesis of steroidal pyran–oxindole hybrids ***4a–j***

To a solution of compound **3** (1.0 mmol) and substituted isatin (1.0 mmol) in ethanol, 1, 8-diazabicyclo [5.4.0] undec-7-ene (DBU) (2.0 mmol) was added. The reaction mixture was stirred at room temperature for about 1 h. The solvent was removed and CH_2Cl_2 was added, the organic phase was washed with water and brine, dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel chromatography using acetone/petroleum ether (1/2) as the eluent to give the corresponding steroidal pyran–oxindole hybrids.

2.3.1. Compound ***4a***

White solid, yield: 75%, m. p. 230.7–232.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.10 (d, *J* = 7.9 Hz, 1H), 7.06 (d, *J* = 5.5 Hz, 1H), 6.56 (d, *J* = 7.6 Hz, 1H), 5.29 (s, 1H), 4.69–4.51 (m, 1H), 3.96 (s, 1H), 2.04 (s, 3H), 1.56 (s, 3H), 1.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.66, 175.50, 170.57, 169.41, 143.96, 139.93, 131.45, 127.39, 124.40, 121.25, 119.99, 112.20, 110.23, 104.38, 73.49, 72.66, 57.16, 56.05, 49.28, 47.70, 37.97, 36.74, 36.64, 34.60, 32.27, 30.45, 29.28, 27.60, 21.40, 19.98, 19.29, 15.57. HRMS (ESI): *m/z* calcd for $C_{32}H_{34}BrN_3NaO_4$ $(M + Na)^+$, 626.1630; found, 626.1654.

2.3.2. Compound ***4b***

Yellow solid, yield: 78%, m. p. 232.1–233.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.51 (s, 1H), 7.19 (s, 1H), 7.18–7.10 (m, 1H),

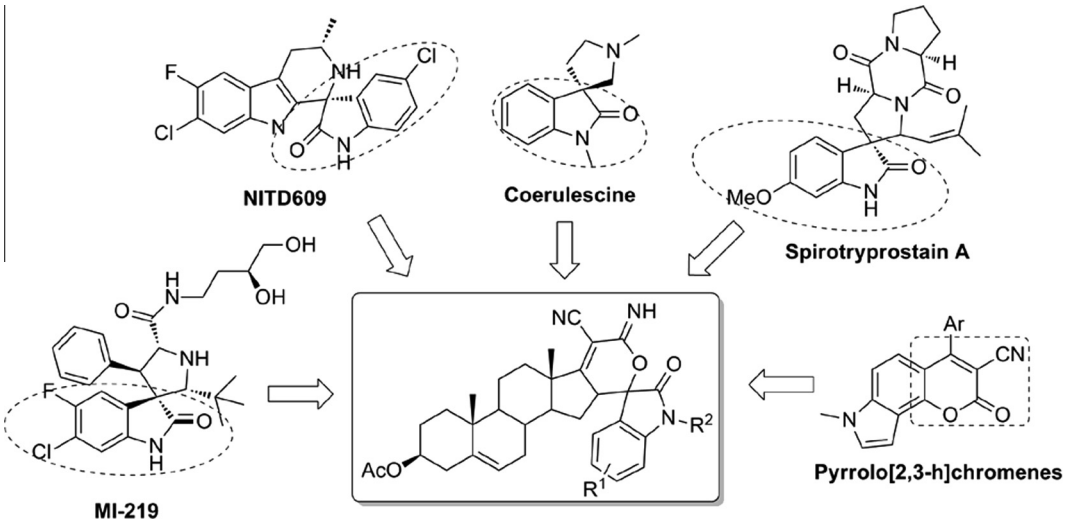

Fig. 2. Designed strategy of steroidal pyran–oxindole hybrids. The dashed boxes indicate the key core structure present in previously reported molecules.