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# Discovery of novel isatin–dehydroepiandrosterone conjugates as potential anticancer agents

Shaoyong Ke *,†, Liqiao Shi †, Ziwen Yang

*National Biopesticide Engineering Research Center, Hubei Academy of Agricultural Sciences, Wuhan 430064, People's Republic of China*

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ABSTRACT

A series of isatin–dehydroepiandrosterone hybrids were synthesised via a convenient condensation procedure, and which were evaluated for their potential anticancer activities. The preliminary assays indicated that some of the newly obtained compounds exhibited good antitumor activities against human hepatocellular liver carcinoma (HepG2), heptoma (Huh-7), melanoma (A875) and 5-fluorouracil-resistant human hepatocellular carcinoma (BEL-7402/5-FU) cell lines compared with 5-fluorouracil (5-FU), which might be considered as promising lead scaffold for further design and synthesis of highly potential anticancer agents.



With the ecological changes and environmental deterioration, cancer has gradually become a major threat to health of humans.[1] Among all current therapeutic methods, chemotherapy is still the most common options for cancer treatment.[2–4] However, with the rapid increasing multidrug-resistance, one of the strategies to overcome this problem is to discover novel molecules with highly potential anticancer activities.

It is well known that isatin is a natural product consisted in a number of plants, and which has also been found to be a common scaffold in various drugs, agrochemicals, and dyes.[5–7] Many isatin and its derivatives display diverse pharmacological activities mainly including antiviral, antitumor, anticonvulsants, antifungal etc.[8–12] Especially, some isatin derivatives have been developed as commercial anticancer drugs such as Sunitinib, Intedanib, Semaxanib (Fig. 1), which identify isatin moiety is an attractive pharmacophore in the discovery of new drugs. On the other hand, dehydroepiandrosterone (DHEA), one of the natural steroids, arouse many researchers' interest in recent years, and many DHEA-type derivatives have been evaluated as potential antibacterial, anticancer, antiviral agents and CYP17 inhibitors.[13–17] Recently, we have also synthesised a series of DHEA-dihydrazone derivatives[18], and some of the obtained compounds significantly inhibited the proliferation of human tumor cells in culture, suggesting that this natural four-ring scaffold might contribute to the cytotoxic activity.

In addition, pharmacophores hybridization and functionalization of natural products are the most widely used approaches for discovery of novel therapeutic agents in medicinal chemistry.[19] In view of these observations, as part of our medicinal program aimed at the discovery of novel biologically important heterocyclic compounds, we integrated the structural features of isatin and DHEA unit to design and synthesize a new class of isatin–DHEA conjugates as shown in Figure 2, wishing to identify novel functional molecules with potent antiproliferative effects on tumor cells. The results may provide useful information for design of novel chemotherapeutic drugs.

In the present study, a series of novel isatin–DHEA derivatives were prepared via a convenient condensation procedure. The general method for the preparation of these derivatives **2a–e** and **4a–e** is outlined in Scheme 1.

The easily available steroid DHEA **1** was selected as starting materials, which was linked to various isatins via a =N—N= bridge to the corresponding isatin–DHEA conjugates **4a–e**. To study the possible structure-activity relationships, five imine derivatives **2a–e** have also been prepared using a similar condensation method. All the newly prepared derivatives gave satisfactory chemical analyses.

Structures of target compounds **2a–e** and **4a–e** were confirmed by their $^1$H NMR, ESI-MS and elemental analyses, and all the spectra analyses were in good agreement with the proposed structures (Supplementary data). During the $^1$H NMR studies, assignments of

* Corresponding author. Tel.: +86 27 59101956.
*E-mail address:* keshaoyong@163.com (S. Ke).
† These authors contributed equally to this work.

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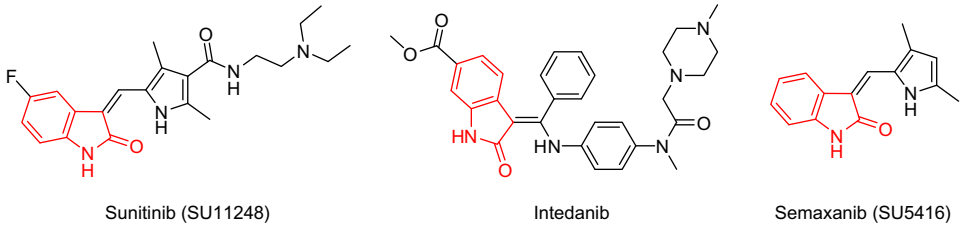



**Figure 1.** Structures for some anticancer drugs derived from isatin.

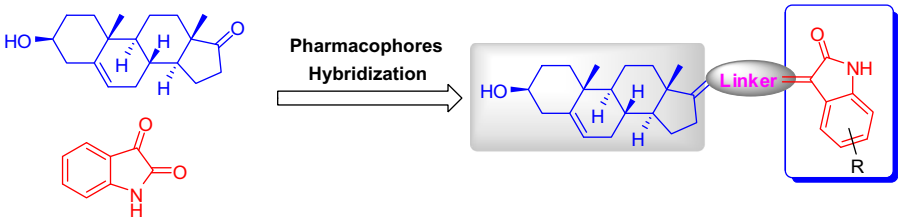
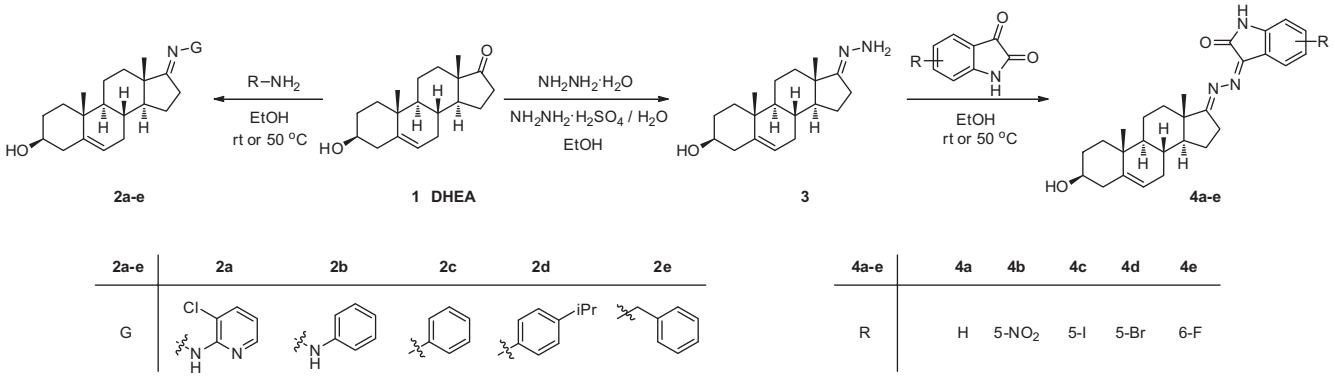



**Figure 2.** Design strategy of novel isatin–dehydroepiandrosterone conjugates.




**Scheme 1.** General scheme for the preparation of novel dehydroepiandrosterone derivatives.

the signals are based on the chemical shifts and intensity patterns. For all isatin–DHEA conjugates **4a**–**e**, the signals at lower fields in the range of 8.65–6.70 ppm were assigned to the aromatic protons in isatin moiety, and the signals at 11.49–10.06 ppm were attributed to NH protons as shown in general structures in Scheme 1. All $^1$H NMR spectra of compounds **2a**–**e** and **4a**–**e** indicated distinctive signals for protons of alkene bond in the part of DHEA were resonated as a singlet between $\delta$ 5.30–5.37 ppm, and the signals of methine proton attached to hydroxyl group were presented at about 4.00 ppm. The several set of signals that appeared in their $^1$H NMR spectra at higher fields were attributed to the other protons of DHEA unit. All the characteristic peaks observed within the $^1$H NMR spectra for title compounds are given in Supplementary data.

All newly prepared compounds **2a**–**e** and **4a**–**e** were screened for their *in vitro* cytotoxic effects against HepG2 (human hepatocellular liver carcinoma), Huh-7 (human heptoma), A875 (human melanoma) and BEL-7402/5-FU (5-fluorouracil-resistant human hepatocellular carcinoma) cell lines by the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay using 5-FU (5-fluorouracil) as a positive control.[20,21] The preliminary antitumor results were summarized in Figure 3.

Generally, as shown in Figure 3, most of the synthesised steroidal analogues **2a**–**e** and **4a**–**e** displayed moderate to good antitumor activities against four human cancer cell lines compared with the control. Notably, the isatin–DHEA conjugates **4a**–**e** exhibited significant better inhibitory activities against all tested cell lines at 20 μg/mL concentration than that of the positive control 5-FU. However, the other DHEA derivatives containing imine unit **2a**–**e** indicated moderate to lower activities compared with the control. The preliminary results demonstrated that the strategy combined isatin and DHEA was right and effective, and these novel isatin–DHEA conjugates might be used as a high potential active scaffold for optimization of anticancer agents.

Subsequently, in order to further investigate the highly potential activities, the $IC_{50}$ values for all compounds were also evaluated. The cytotoxic activities expressed as $IC_{50}$ values for tested compounds are described in Table 1, which testified that the synthesised isatin–DHEA conjugates **4a**–**e** exhibited obviously higher inhibition activities than the commercial 5-FU. As indicated in Table 1, compounds **4a**, **4d**, and **4e** have higher cytotoxicity activities (entries 6, 9, and 10) against all tested cancer cell lines than that of the control 5-FU. However, compounds **2a**–**e** indicated poor activities at the same tested conditions. Especially, all isatin–DHEA conjugates **4a**–**e** displayed significant cytotoxic activities against BEL-7402/5-FU cell lines that resistant to 5-FU, and the $IC_{50}$ value for compound **4d** bearing a bromo group is up to 5.97 ± 2.67 μM. In addition, compound **4b** bearing a nitro group exhibited selective inhibitory effect on HepG2, Huh-7 and BEL-7402/5-FU cell lines.

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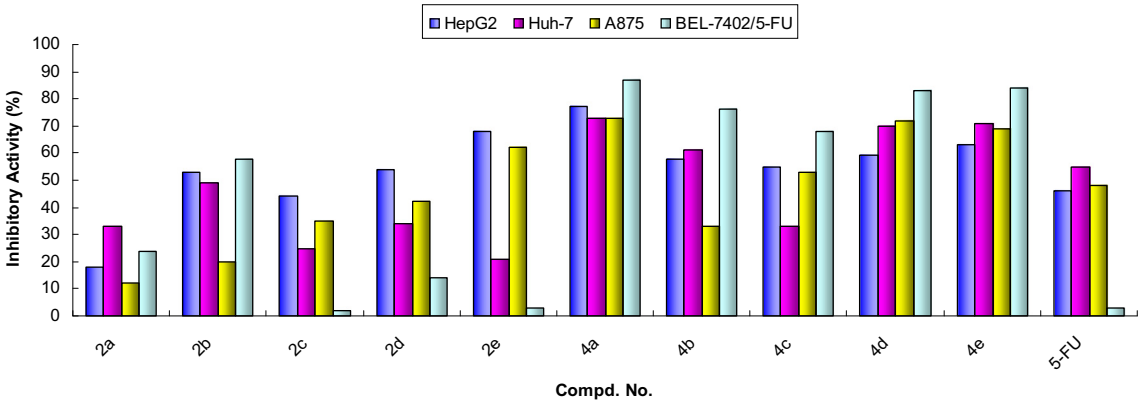


**Figure 3.** Antitumor activities of compounds **2a–e** and **4a–e** at 20 μg/mL. Abbreviations: HepG2—human hepatocellular liver carcinoma cell line; Huh-7—human hepatoma cell line; A875—human melanoma cell line; BEL-7402/5-FU—5-fluorouracil-resistant human hepatocellular carcinoma cell line; 5-FU—5-fluorouracil, used as a positive control.

**Table 1**
Cytotoxic activity of the compounds against different human liver cells

| Entry | Compd No. | In vitro cytotoxicity $IC_{50}$[a] (μM) | | | |
|---|---|---|---|---|---|
| | | HepG2[b] | Huh-7[b] | A875[b] | BEL-7402/5-FU[b] |
| 1 | **2a** | >90 | >90 | >90 | NT[c] |
| 2 | **2b** | 43.14 ± 8.99 | 55.35 ± 4.73 | >100 | 29.87 ± 7.53 |
| 3 | **2c** | 63.45 ± 8.20 | >100 | 82.74 ± 9.22 | >100 |
| 4 | **2d** | 44.31 ± 4.91 | >90 | 61.53 ± 2.07 | >90 |
| 5 | **2e** | 32.60 ± 4.39 | >100 | 42.48 ± 3.71 | >100 |
| 6 | **4a** | 13.33 ± 3.78 | 28.19 ± 3.59 | 25.23 ± 1.32 | 21.79 ± 4.87 |
| 7 | **4b** | 27.43 ± 4.77 | 30.55 ± 3.91 | 69.00 ± 4.18 | 10.10 ± 3.04 |
| 8 | **4c** | 22.54 ± 4.45 | >70 | 43.72 ± 7.86 | 14.11 ± 3.59 |
| 9 | **4d** | 16.22 ± 4.65 | 13.90 ± 3.91 | 14.83 ± 1.47 | 5.97 ± 2.67 |
| 10 | **4e** | 19.72 ± 5.97 | 22.26 ± 6.21 | 29.59 ± 2.76 | 12.85 ± 4.88 |
| 11 | 5-FU[d] | >150 | >100 | >150 | >700 |

[a] $IC_{50}$—compound concentration required to inhibit tumor cell proliferation by 50%.
[b] Abbreviations: HepG2—human hepatocellular liver carcinoma cell line; Huh-7—human hepatoma cell line; A875—human melanoma cell line; BEL-7402/5-FU—5-fluorouracil-resistant human hepatocellular carcinoma cell line.
[c] NT—no test.
[d] 5-FU, used as a positive control.

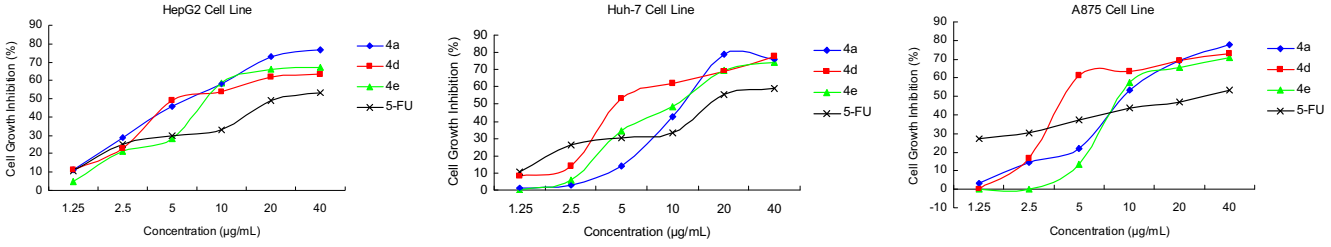


**Figure 4.** Dose-response analysis of cell growth inhibition activity for representative compounds **4a**, **4d**, **4e** and **5-FU** (positive control) against HepG2 (left), Huh-7 (middle) and A875 (right) cell lines.

The results in Table 1 can further demonstrate the cytotoxic effect of isatin–DHEA conjugates are highly potential scaffold to cancer cell lines.

Furthermore, the dose-response curve of cell growth inhibition activities for high potential compounds **4a**, **4d**, **4e** and 5-FU have been displayed in Figure 4, which demonstrated that the target compounds exhibited obviously cytotoxic effects on HepG2, Huh-7, and A875 cell lines in a dose-dependent manner. Especially, compound **4d** displayed the highest potent growth inhibitory activities against all tested cell lines with the $IC_{50}$ values of 16.22 ± 4.65, 13.90 ± 3.91, and 14.83 ± 1.47 μM (Entry 9), respectively, which were obviously better than that of the control 5-FU.

During the experiments, significant morphological changes of cells have also been observed by inverted microscope. So we present the selective morphological image of the cells treated with compounds **4a**, and **4d** at 10 μg/mL for 48 h in following pictures (Fig. 5). Contrast with the images in Figure 5, we found that the quantities of the cells treated with compounds **4a**, and **4d** were obviously decreased greatly for the cancer cell lines, and there were significant volume shrink have also been observed. However, such morphological changes were not apparent in the control groups (A and D).

In summary, a novel series of isatin–DHEA conjugates were synthesised, characterized and evaluated for their *in vitro* cytotoxicity

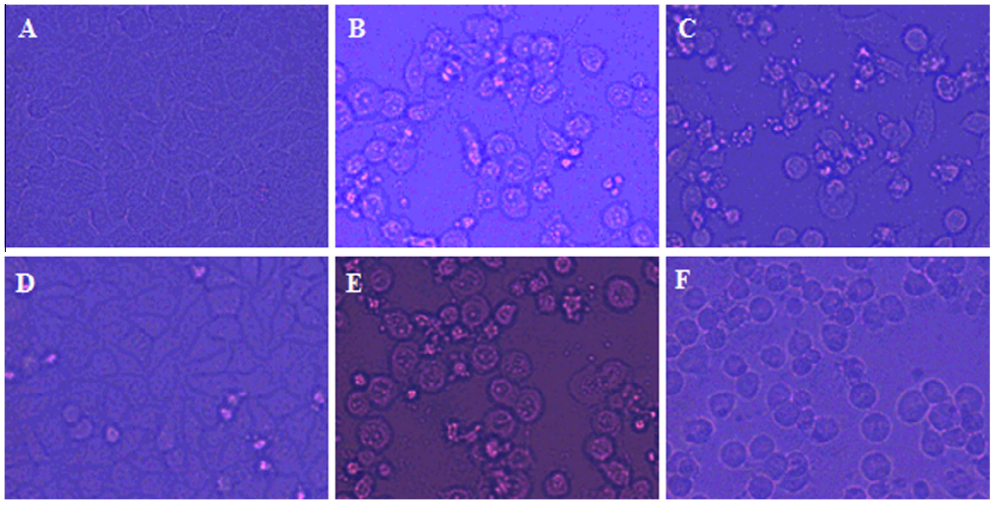


**Figure 5.** Morphology image of HepG-2 (A–C) and A875 (D–F) cells treated with the compounds **4a**, or **4d** for 48 h (400×). A and D is the control, the cells treated with DMSO 0.1% (v/v) as a vehicle control. B and E, C and F are treated with the compounds **4a**, or **4d** at concentration of 10 mg/mL, respectively.

activities against various cell lines. From the present data, we can find that the target compounds displayed obviously good antitumor activities against HepG2, Huh-7, A875 and BEL-7402/5-FU cell lines compared to 5-FU. The highly potential compound **4d** exhibited significant inhibition activities ($IC_{50}$: 5.97 ± 2.67 μM) against BEL-7402/5-FU cell lines that resistant to 5-FU, which might be developed as novel lead scaffold for anticancer agents.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.08.041.

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