Comparative Cytotoxic Activities of the Propanamide and Spiro-γ-lactone Derivatives of Δ^{14}-17α- and 17β-Estradiol Stereoisomers against Human Cancer Cell Lines

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Biological activities were evaluated for the 17-spiro-γ-lactone and 17-propanamide derivatives of Δ^{14}-17α- and 17β-estradiols diastereoisomeric at C-17, which are the possible candidates of a 17β-hydroxysteroid dehydrogenase (17β-HSD) inhibitor. Cytotoxic activities of these compounds were assessed against seven variants of human cancer cell lines (BT-474, MCF-7, HCC-1806, HCC-1937, A-549, HCT-116, and HT-29). Of the four substrates examined, the best IC_{50} values were observed for 3,17β-dihydroxy-19-nor-17α-pregna-1,3,5(10),14-tetraene-21,17-lactone (2a) against HCC-1806, HCT-116 and HT-29 cells and the cytotoxic activities of this novel estradiol derivative were nearly identical to those of cisplatin.

Keywords: Estradiol, Estrone, Spiro-γ-lactone, Propanamide, 17β-HSD, Inhibitor, Cytotoxic activity

Introduction

Based on the three dimensional structure for the active center of 17β-hydroxysteroid dehydrogenase (17β-HSD), a wide variety of potential anti-estrogenic inhibitors with reduced estrogenic activity as antitumor agents have been proposed by structure-based drug design. Their chemical syntheses and bioassays have also been attained by many groups of workers. In particular, Poirer et al.’s work have developed the 17β-HSD inhibitors of human placenta cytosolic enzyme as well as inhibitors of human placenta microsomal one. Nonetheless, the therapeutic use of these inhibitor candidates has been limited because of their estrogenic activity and/or cytotoxicity.

We sought to design a more electron-enriched estradiol derivative to inhibit the enzyme as well as to improve estrogenic activity and/or cytotoxicity. Our laboratory has currently attained the chemical synthesis of the four possible candidates of 17β-HSD inhibitors, the enzyme involved in the biosynthesis of the potent mitogenic estrogen, estradiol, from estrone. Those compounds include 3-(3',17β-dihydroxy-1',3',5'(10'),14'-estratetraene-17α-yl) propanamide (Δ^{14}-3,17β-estradiol 17α-propanamide; 1a) and 3,17β-dihydroxy-19-nor-17α-pregna-1,3,5(10),14-tetraene-21-carboxylic acid-γ-lactone (Δ^{14}-C-17β-O-/C-21-spiro-γ-lactone; 2a) and their C-17 epimers, Δ^{14}-3,17α-estradiol 17β-propanamide (1b) and Δ^{14}-C-17α-O-/C-21-spiro-γ-lactone (2b), as shown in Figure 1.

In continuation of our studies on new and scarce bioactive steroids, we describe herein bioassay of the new estradiol stereoisomers. Since a target in question in this study is estrogen-dependent breast cancer, the cytotoxic activities of these compounds were assessed against seven variants of human cancer cell lines, i.e., BT-474, MCF-7, HCC-1806, HCC-1937, A-549, HCT-116, and HT-29.

Materials and Methods

Compounds and reagents

Cisplatin [cis-diaminedichloroplatinum (II)] was pur-
chased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals and solvents were of analytical reagent grade.

Cancer cell lines
All human cancer cells (BT-474, MCF-7, HCC-1806, HCC-1937, A-549, HCT-116, and HT-29) used in this study were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and subcultured in Roswell Park Memorial Institute (RPMI) 1640 medium or Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), in 5% CO₂ humidified air at 37 °C.

Evaluation of cytotoxic activities
Test compounds were dissolved in DMSO at a concentration of 10 mg/mL and diluted with RPMI 1640 medium prior to use. Thousand cells of each cell line were suspended in 50 μL of medium and incubated in a 96 well plate for overnight. After the incubation, 50 μL of the substrate solution was added to the well with the final concentrations of 0.01 - 100 μg/mL. After additional 96 h incubation, viability of the cells was determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay using CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega Corporation, Madison, WI, USA), and the values of LD₅₀ were estimated.

Results and Discussion
As described in detail by a previous paper, a 17β-O-spirolactone moiety in estradiol derivatives has been established as an essential factor for the appearance of 17β-HSD inhibition. A tert-amide function in the steroidal D-ring was also effective, because it provides antiestrogenic property. Thus, estradiol derivatives possessing these functionalities in the steroidal D-ring are well recognized as an important pharmacophore for the inhibition of 17β-HSD, though the potent estrogenic activity and cytotoxicity of some of these estradiol derivatives have been reported. Hence, we supposed that introduction of an additional electron-enriched functionality (as Δ₁₄-bond) is more preferable for 17β-HSD inactivation.³ ⁶

Figure 2 shows the relationship between substrate concentration [Log (g/mL)] and cell viability, shown as % of control. Structure-activity relationships were expressed in terms of the LD₅₀ values. Table 1 summarizes cytotoxic activities of the substrates 1a, 1b, 2a, and 2b against seven

![Fig. 1 Structures of the stereoisomeric spiro-γ-lactone and propanamide derivatives of estradiol.](image-url)
human cancer cell lines. The tumor cell lines comprised 2 estrogen-receptor positive breast cancer cell lines (MCF-7 and BT-474), 2 estrogen-receptor negative breast cancer cell lines (HCC-1806 and HCC-1937), 2 colon cancer cell lines (HCT-116 and HT-29) and a lung cancer cell line (A-549). Cisplatin was also tested to serve as a positive control.

A comparison of the cytotoxic activity of the Δ^{14}-17β-hydroxy-17α-propanamide 1a and its C-17 epimer 1b revealed that 1a always showed much smaller LD_{50} values than 1b. Essentially identical relationship was also observed between the two stereoisomeric Δ^{14}-17ξ-O/17ξ-C-spiro-γ-lactones, 2a and 2b. Above the finding strongly suggests that the presence of a 17β-O-moiety is more effective and essential factors as an inhibitor. In addition, the IC_{50} values for the Δ^{14}-spiro-γ-lactone derivatives, 2a and 2b, were found to be always smaller than those observed for the corresponding propanamide derivatives (1a and 1b), thus indicating that the presence of a tert-amide moiety in the side chain at C-17 is preferable to a non-alkylated amide linkage in 1a and 1b, probably owing to decreased electron density of the nitrogen atom.

Of particular noteworthy was that the Δ^{14}-spiro-γ-lactone 2a with 17β-O/17α-C stereochemistry was found to be the most potent and significant cytotoxicity (IC_{50} value of less than 5 μg/mL) with a unique profile against the three strains of human cancer cell lines, HCC-1806, HCT-116 and HT-29. The observed IC_{50} values of 2a against HCC-1806 (1.6), HCT-116 (3.3), and HT-29 (3.0) cancer cells were similar to those observed for cisplatin (1.0, 3.1 and 3.1, respectively), an agent widely used in cancer therapy. The crucial importance of the 17β-orientation of the oxygen atom was also confirmed, since the 17α-O/17β-C compound 2b was approximately 10 to 30 fold less cytotoxic activity than the

Fig. 2 Cytotoxic activities of the compounds of 1a, 1b, 2a, and 2b. The responses of the human cancer cell lines against the test compounds and cisplatin (CDDP) were determined using an MTT assay after 96 hours of exposing the cells with the compounds.
corresponding 17β- O/17α - C epimer 2a. Therefore, 2a might be a novel tool for the investigation of proliferation of cancer cell lines.

One possible explanation for the biological activity of 2a may be its acting as a competitive inhibitor for an estrogen-receptor. In this study, estrogen-receptor positive and negative cancer cell lines were exposed to our synthesized compounds in the culture media that is known to contain phenol red and fetal bovine serum (FBS). It has been known that phenol red possesses a hormonal-like activity and that FBS contains steroids. Our preliminary experiments revealed that the growth of both estrogen-receptor positive cell lines, MCF-7 and BT-474, was drastically reduced by culture media, which was preparing without phenol red and with charcoal-treated FBS replacing untreated FBS (data not shown). Therefore, the estrogen-receptor positive cells were likely to have had a stimulated estrogen-receptor pathway during exposure to the synthesized compounds. However, 2a is likely to be a weak competitive inhibitor of the estrogen receptor, as its IC₅₀ value (38.0 µg/mL) is far higher than that of tamoxifen (0.04 µg/mL), a clinically available inhibitor of an estrogen-receptor. The activities of 2a against BT-474 (IC₅₀=5.7) and MCF-7 (IC₅₀=38.0) were different from each other, though both cells are estrogen-receptor positive. Thus, the activity of 2a against BT-474 was found to be 6 fold higher than that against MCF-7, whereas BT-474 was more sensitive to 2a than cisplatin. Although the mechanism of action of 2a remains obscure, the combination of a Δ¹⁴ - bond and a 17β- O - /17α - C - spiro-γ- lactone moieties present in the D-ring of the C₁₈ steroid nucleus appears to be important and essential factors to the efficient inhibition of enzyme activity, but the precise role of these functionalities is not fully understood. Therefore, the IC₅₀ values of the 2a analogue without the Δ¹⁴- bond (a known compound)¹⁰ should be measured in order to reveal the evidence of the importance of the olefinic functionality. Additional bioassay data, such as 17β - HSD enzyme - inhibitory effect and receptor binding data, may provide further support for the design rationale and for the putative mechanism of action. These are now undergoing in my laboratory.

Acknowledgements

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Table 1 Cytotoxic activities (LD₅₀ values, µg/mL) observed for substrates (1a, 1b, 2a, and 2b) tested against human cancer cell lines.

<table>
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<th>Cell</th>
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<th>1a</th>
<th>1b</th>
<th>2a</th>
<th>2b</th>
<th>cisplatin</th>
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<td>1.6</td>
<td>33.0</td>
<td>1.0</td>
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<td>49.9</td>
<td>6.6</td>
<td>35.7</td>
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<tr>
<td>HT-29</td>
<td>colon cancer</td>
<td>36.4</td>
<td>&gt;100</td>
<td>3.0</td>
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References and Notes
