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Original article

Synthesis and biological evaluation of salpichrolide analogs as antiestrogenic agents

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ABSTRACT

The antiestrogenic activity of three natural salpichrolides A, G and B (**1**, **3** and **4**) and of five synthetic analogs containing an aromatic D ring and a simplified side chain (**5**–**9**), was evaluated on MCF-7 cells. The 2,3-ene-1-keto steroids **8** and **9** were obtained from 3β-acetoxy-17(13→18)-abeo-5αH-pregna-13,15,17-trien-20-one, the key step for these syntheses being a Wharton carbonyl rearrangement of a 1,2-epoxy-3-keto steroid to the allylic alcohol using hydrazine hydrate. The antiestrogenic activity was evaluated by performing dose–response experiments in ER(+) MCF-7 breast cancer cells. Dose-dependent proliferation was quantified via [^{3}H]-thymidine incorporation after 3 days treatment. Salpichrolides A, G and B and analogs **5**, **8** and **9** were active as antiestrogens with compound **9** being the most active of the synthetic analogs. Compounds **5** and **9** were also evaluated against the ER(−) cell line MDA-MB-231 and shown to be inactive.

1. Introduction

Breast cancer is the most frequent cancer among women with an estimated 1.67 million new cases in 2012 (25.2% of all malignancies), being the most common cause of cancer related deaths in women (over 500,000 in 2012) in both developed and developing regions [1]. Over 70% of breast tumors express estrogen receptor alpha (ERα) and most of them respond to antiestrogen therapies, at least at the beginning of the treatment [2]. In those cases, the endocrine therapy aims to inhibit estrogen signaling resulting in inhibition of cell proliferation or induction of cell death [3]. For this purpose the available antiestrogen agents are either Selective Estrogen Receptor Modulators (SERMs) or Selective Estrogen Receptor Down-regulators (SERDs). The latter (fulvestrant is the best known) bind to ERα and induce its proteasomal degradation [3]. However, most of these ER-positive breast tumors become hormone resistant and patients relapse within 5 years [2], giving rise to a need for novel antiestrogens with new potential properties. The withanolides are C-28 steroidal lactones and lactols isolated from several genera of the Solanaceae family [4], that exhibit a variety of biological activities including potential anticancer activity on breast cancer cells [5–8]. An evaluation of a series of withanolides against a panel of human breast cancer cell lines containing or lacking the estrogen receptor (ERα(+) and ERα(−) respectively), showed that while most of the compounds assayed exhibited antiproliferative activity on all cell lines, two withanolides were selective against hormone dependent ERα(+) cell lines [9]. A distinctive characteristic of the latter compounds isolated from the plant *Salpichroa origanifolia*, was a modified steroid nucleus with a six membered aromatic D ring [10], with salpichrolide A (**1**) being the most active (Fig. 1). The closely related salpichrolide D (**2**) with a five membered D ring was equally active against ERα(+) and ERα(−) cell lines, suggesting that the selectivity might be associated to the presence of the aromatic D ring. The authors proposed that the mode of action of the selective withanolides could involve the inhibition of the ER-dependent pathway, required for proliferation of the hormone-dependent ERα(+) breast cancer cell lines.

Those results prompted us to evaluate the antiestrogenic activity of salpichrolide A (**1**) and two structurally related compounds also isolated from *S. origanifolia* (**3**, **4**), by assaying their capacity to block the response to estradiol in ERα(+) breast cancer cells. We also speculated that if the modified steroid nucleus with an aromatic D ring was relevant to the observed selectivity, synthetic analogs with a simple side chain as **5**–**9** (Fig. 2) might retain this

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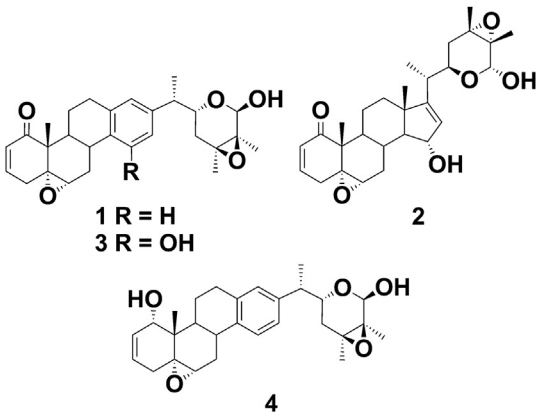

Fig. 1. Structures of the major natural salpichrolides.

property while being more amenable from a synthetic standpoint, to be used as leads for further development.

2. Results and discussion

2.1. Chemistry

The synthetic analogs were selected so as to contain typical substituents present in rings A and B of the salpichrolides and related withanolides, i.e. the 5,6-epoxide (compounds **6** and **7**) or an oxygenated function at position 1 (compounds **8** and **9**). Compound **5** contains a 3β-hydroxyl, a common substituent present in many natural steroids including several withanolides. In all cases the simplified side chain was kept invariant. The synthesis of compounds **5**–**7** has been reported previously by us [11]. Analogs **8** and **9** were obtained from compound **10** as depicted in Scheme 1. Initial attempts to carry out this sequence with the ketone at C-20 were unsuccessful, thus compound **10** was reduced with $NaBH_4$ and the resulting 20-hydroxysteroid was protected as the *tert* butyldimethylsilyl ether to give **11** as a mixture of epimers at C-20. The transformation of the A ring into the enone intermediate **15** was accomplished by deacetylation and oxidation to the 3-ketosteroid **13**, followed by dehydrogenation with 2-iodoxybenzoic acid (IBX) in DMSO at 90 °C to give the Δ^1 steroid **15**. As the 20-silyl ether is cleaved by IBX, it was necessary to change the *tert*-butyldimethylsilyl ether group at position 20 (compound **13**) for an acetate group (compound **14**) prior to the dehydrogenation step. The enone **15** was epoxidized with hydrogen peroxide 30% in MeOH–KOH to give the α,β-epoxy ketone **16**. The key step of the synthesis was a Wharton rearrangement of the epoxyketone **16** to give the allylic alcohol **17**, upon reaction with hydrazine hydrate in EtOH [12,13]. The ^1H NMR spectrum of compound **17** exhibited two olefinic protons at δ 5.87 (H-2 and H-3), and a doublet at δ 3.84 ($J = 4.5$ Hz) corresponding to H-1. These data were consistent with the presence of a 1α-hydroxy group in ring A [13,14]. Compound **17** was treated with 5% KOH in MeOH to give the 20-hydroxy intermediate **18**, regioselective oxidation with MnO_2–Na_2CO_3 gave compound **8** (9% yield from **10**) while oxidation with PCC gave compound **9** (10% yield from **10**).

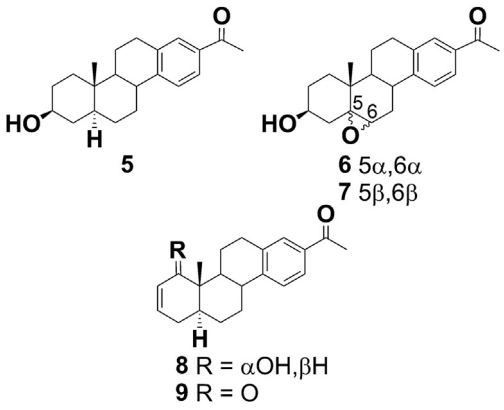

Fig. 2. Structures of the synthetic analogs.

2.2. Antiestrogenic activity

The antiestrogenic activity was assessed by incubating human breast cancer MCF-7 cells in the simultaneous presence of a stimulatory (but not saturating) concentration of estradiol and increasing concentrations of the compound to be tested. Besides salpichrolide A (**1**), two other natural salpichrolides with an aromatic D ring were selected for evaluation, salpichrolide G (**3**) with a phenolic group at position 15 that exhibited cytotoxicity to both ER(+) and ER(−) cell lines [9] and salpichrolide B (**4**) that is a minor component of *S. origanifolia* but can be easily obtained from **1** [14]. As shown in Fig. 3, all three natural salpichrolides significantly reversed the proliferative action of estradiol with an IC_{50} of 1.0×10^{-7} M, 1.4×10^{-7} M and 1.8×10^{-8} M for salpichrolides A (**1**) (panel a), G (**3**) (panel b) and B (**4**) (panel c) respectively. The effect of these compounds was compared to the classical antiestrogen and SERD fulvestrant (ICI 182,780). Fulvestrant at a 10 nM concentration significantly reversed the estrogen effect (IC_{50} 1.9×10^{-9} M) and inhibited cell proliferation below control values (Fig. 3 panel d). The latter effect may be due to the ability of fulvestrant of inducing apoptosis in MCF-7 cells [3]. None of the tested compounds was able to inhibit cell proliferation below control values, suggesting that their mechanism of action is different from the SERD fulvestrant.

The results obtained with the synthetic analogs are shown in Fig. 4. Compound **9** (IC_{50} 5.0×10^{-8} M), with an A ring that maintains the functionality of salpichrolide A (**1**), was the most effective of the synthetic compounds, with a significant antiestrogenic effect at a concentration of 100 nM (Fig. 4c). Compound **8**, that has the same functionality in ring A as compound **4**, showed antiestrogenic activity only at a concentration of 1 μM (Fig. 4b). Compound **6**, containing the 5α,6α-epoxide functionality present in most of the natural salpichrolides, lacks antiestrogenic activity (data not shown), while compound **7** with a 5β,6β-epoxide showed only incipient antiestrogenic activity at a concentration of 1 μM (data not shown). It is noteworthy that compound **5**, a very simple analog, that has neither the side chain nor any of the functionalities present in rings A and B of the salpichrolides, completely reversed estradiol effect at a concentration of 100 nM, with an IC_{50} of 3.3×10^{-7} M (Fig. 4a) while analogs of **5** with a normal steroid framework as pregnenolone, do not significantly bind to ERα [15]. This suggests that the aromatic D ring is a key structural motif for the observed antiestrogenic activity. However, the lack of activity of compounds **6** and **7** indicates that as expected, other structural factors are implied in the interaction with the ER.

In order to better assess if the synthetic analogs exerted their effect through the ER and thus maintained the selectivity for ER(+) cells, the breast cancer ERα(−) MDA-MB-231 cell line was incubated in the same conditions with the most active compounds **5** and **9**. As shown in Fig. 5, neither compound **5** nor compound **9** exerted any action on these cells, supporting the implication of the ERα in their action. This result also suggests that the action of these compounds on MCF-7 cells is not likely to be a toxic one.