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Original article

Synthesis of 2-alkoxy and 2-benzyloxy analogues of estradiol as anti-breast cancer agents through microtubule stabilization

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ABSTRACT

2-Methoxyestradiol (2ME2) is an investigational anticancer drug. In the present study, 2-alkoxyesters/acid and 2-benzyloxy analogues of estradiol have been synthesized as analogues of 2ME2. Three of the derivatives exhibited significant anticancer activity against human breast cancer cell lines. The best analogue of the series i.e. **24** showed stabilization of tubulin polymerisation process. It was substantiated by confocal microscopy and molecular docking studies where **24** occupied 'paclitaxel binding pocket' to stabilize the polymerisation process. Compound **24** significantly inhibited MDA-MB-231 cells (IC_{50}: 7 μM) and induced arrest of cell cycle and apoptosis in MDA-MB-231 cells. In acute oral toxicity, **24** was found to be non-toxic and well tolerated in Swiss albino mice up to 1000 mg/kg dose.

1. Introduction

Breast cancer is a leading cause of women mortality worldwide. It is one of the most common malignant cancers for women causing 1.7 million patients and about 0.52 million deaths in 2012 [1]. Many factors are believed to contribute to this burden of breast cancer including genetic, environmental, life style and biological etc. The mechanism involved in cell proliferation, invasion and metastasis of breast cancer are not fully understood. Breast cancer is a heterogeneous disease consisting of multiple molecular subtypes [2a–c]. Based on responsiveness, it is mainly subdivided in to hormone responsive (ER/PR/Her-2) and hormone non-responsive. Various approaches have been developed to tackle these. For ER positive breast cancers selective estrogen receptor modulators (SERMs) and aromatase inhibitors have been developed while cytotoxic drugs like paclitaxel, doxorubicin, cyclophosphamide etc. are used to combat ER-negative cancers. However, there is a general consensus that breast cancer treatment needs multimodality approach to eradicate residual cancer cells and prevent recurrence of the disease. Several anti-breast cancer drugs have been developed, but the morbidity and mortality of the disease is so high that it is still a challenge to scientific fraternity.

Microtubules have been considered as an ideal target for anticancer drug development due to their essential role in mitosis forming the dynamic spindle apparatus. Microtubules play crucial role in the maintenance of cell structure, chromosomal segregation, protein trafficking and mitosis [3]. Both, microtubule stabilisers like paclitaxel, epothilones, discodermolide etc. and microtubule inhibitors like colchicine, podophyllotoxin, combretastatins, vinca alkaloids etc. induce cell cycle arrest and apoptosis and thus act as anticancer agents.

In the recent past, 2-methoxyestradiol (2ME2, **1**) a metabolite of endogenous estrogen 17β-estradiol has emerged as a potent anticancer molecule. It is an investigational drug. 2ME2 is an anti-angiogenic drug which induces G2/M arrest and apoptosis [4]. It selectively targets endothelial cell adhesion and migration. It also inhibits microtubule assembly after binding to tubulin near the colchicine binding site [5–7]. Two of its water soluble prodrugs 3-mono sodium phosphate and 3,17-diphosphate salts have also been prepared for better bioavailability [8]. Being an important investigational drug, several routes of its total synthesis have also been

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done [9–11]. 2ME2 completed its phase II clinical trials against ovarian cancer in 2007 and against prostate cancer in 2010 [12]. There is an increasing interest on synthesis of 2ME2 analogues for better activity and bioavailability. Two such analogues ENMD-1198 (**2**) and STX-140 (**3**) have shown better potency than the parent molecule, 2ME2. Both the analogues have oral bioavailability. ENMD-1198 has completed phase-I clinical trial for advanced cancers. Few more potent analogues of 2ME2 depicted in Fig. 1 are 2-ethoxyestradiol (**4**), 16α-methyl-2ME2 (**5**), 18-ethyl-2ME2, 17(20) tetra-ene, 3-ol (**6**), 2-ethoxyestra 1,3,5(10),17(20) tetra-ene, 3-ol (**7**) [13a–g] possessing better or comparable activity.

In the present communication, we have synthesized several 2-alkoxyesters/acids and 2-benzyloxy analogues of 2ME2 (Fig. 2). The best analogue was further evaluated for its effect on cell cycle phases of MDA-MB-231 cells, *in-vitro* tubulin polymerisation activity, confocal microscopy of cellular cytoskeleton network, *in vivo* estrogenicity/antiestrogenicity, and *in-vivo* acute oral toxicity against Swiss albino mice at various doses. Docking experiments have also been conducted to validate the findings.

2. Results and discussion

2.1. Chemical synthesis

The synthetic plan of 2-benzyloxy and 2-akyloxy analogues of 2-methoxyestradiol has been shown in Scheme 1. Estrone was transformed to 2-formyl-3-hydroxyestra-1,3,5 (10)-17-acetate (**13**) as reported earlier by our group [11,14]. Two different approaches were done to get 2-benzyloxy (**16**–**27**) and 2-alkyloxy (**31**–**40**) derivatives [15]. Compound **13** was acetylated with acetic anhydride in pyridine at room temperature to afford 2-formylestradiol-3,17-diacetate (**14**). On Baeyer–Villiger oxidation aldehyde **14** was transformed to 2-hydroxyestradiol-3,17-diacetate (**15**). Various benzyl bromides were hooked-up at 2-hydroxyl of **15** using anhydrous potassium carbonate in acetone to afford 2-benzyloxy derivatives of 2ME2 (**16**–**20**). The respective benzyl bromides were in turn synthesized from suitable benzaldehyde on reduction ($NaBH_4$/MeOH) followed by bromination of alcohols (PBr_3). In the last step, all these diacetates were saponified with 5% KOH in 90% methanol to get final 2-benzyloxyestradiols (**22**–**26**).

In order to achieve 2-alkoxy esters and acids of 2-ME2, the 3-hydroxyl of compound **13** was protected through benzylation with benzyl bromide to get 3-benzylated estradiol derivative (**28**) in quantitative yield. **28** on Baeyer–Villiger oxidation with *m*-CPBA yielded estra-2,3,17-triol-2-yl formate (**29**). Formate ester **29** was hydrolysed to 3-benzylestra-2,3,17-triol (**30**). Various ethyl bromoalkanoates were hooked up at 2-hydroxy of **30** to get various 2-alkoxy esters of 3-benzyl estra-1,3,5(10)-17-ol (**31**–**33**). These

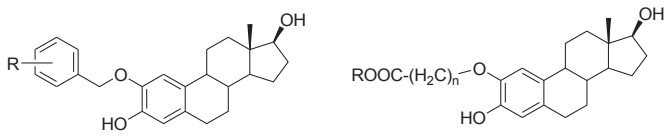

Fig. 2. Proposed pharmacophores.

esters on debenzylation (hydrogenolysis) by Pd–C/H_2 in dry THF yielded 2-alkoxy esters of estradiol (**34**–**37**). Finally, on saponification 2-alkoxyacids derivatives of estradiol (**38**–**40**) were achieved.

Additionally, compound **21** was prepared on treatment of **15** with ethyl bromocrotonate to yield 2-alkoxy derivatives. The diacetoxyester **21** was hydrolysed to **27** to get 2-alkoxy acid at 2-position of estradiol. All the compounds were confirmed by spectroscopy [Supporting information].

2.2. Biological evaluation

2.2.1. Cytotoxicity

Only six compounds of this series exhibited significant cytotoxicity against human breast cancer cell lines, MCF-7 (ER+ ve) and MDA-MB-231 (ER− ve) and rest were inactive (Table 1). Compounds **23** and **24** showed potent activity against ER− ve MDA-MB-231 cells. Previously, most of the 2ME2 derivatives have exhibited anticancer activity against ER− ve breast cancer cell lines [13f,g].

However, both the compounds were inactive against MCF-7 cells. MCF-7 is an estrogen-dependent breast cancer cell line and contains functional estrogen receptor (ER). 2ME2 derivatives show poor affinity to ERs and it might be possible that **23** and **24** both do not have good affinity to ER, hence inactive against MCF-7. It is further confirmed by *in-vivo* estrogenicity/antiestrogenicity results of **23** and **24**. From estrogenicity and antiestrogenicity experiments compound **23** was found to have low level of antiestrogenicity, while **24** was devoid of it. Also both the compounds have shown synergistic increase in uterine weight in presence of E2. Hence, both may be considered to act through non-ER pathway. This also indicates that compounds **23** and **24** possibly induce cytotoxicity in MDA-MB-231 cells through a non-ER dependent mechanism.

Both the active compounds were also evaluated against HEK-293 cells to see the toxicity against normal cells. Both the compounds in comparison to MDA-MB-231 cells, showed less toxicity to HEK-293 cells, having selectivity index >2.5 for both the compounds **23** and **24**. Since, compound **24** has better activity against MDA-MB-231 cells in comparison to compound **23**, it was selected as a lead molecule for further studies.

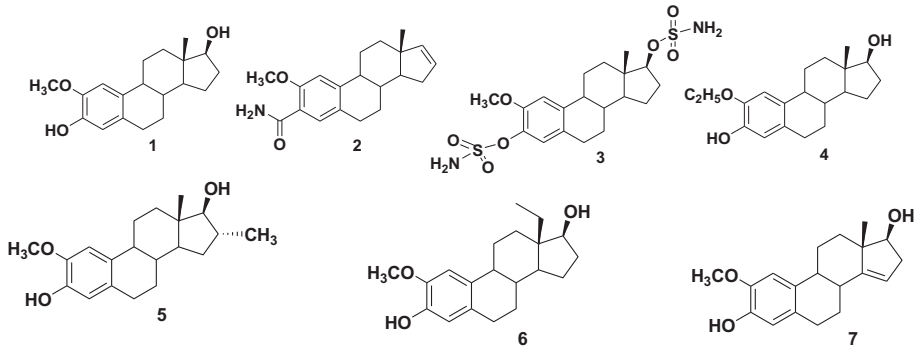

Fig. 1. 2-Methoxyestradiol and some of the notable leads; **1**: 2-methoxyestradiol, **2**: ENMD-1198, **3**: STX-140, **4**: 2-ethoxyestradiol, **5**: 16α-methyl-2ME2, **6**: 18-ethyl-2ME2, 17(20) tetra-ene, 3-ol, **7**: 2-ethoxyestra 1,3,5(10),17(20) tetra-ene, 3-ol.