Bioorganic & Medicinal Chemistry 21 (2013) 4120–4131

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and biological evaluation of novel tamoxifen analogues

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ARTICLE INFO

Article history:
Received 19 March 2013
Revised 2 May 2013
Accepted 8 May 2013
Available online 15 May 2013

Keywords:
Tamoxifen derivatives
Breast cancer (MCF-7)
Cervix adenocarcinoma (HeLa)
Biphasic mesothelioma (MSTO-211H)
Topoisomerase

ABSTRACT

A collection of compounds, structurally related to the anticancer drug tamoxifen, used in breast cancer therapy, were designed and synthesized as potential anticancer agents. McMurry coupling reaction was used as the key synthetic step in the preparation of these analogues and the structural assignment of *E*, *Z* isomers was determined on the basis of 2D-NOESY experiments. The compounds were evaluated for their antiproliferative activity on breast cancer (MCF-7), cervix adenocarcinoma (HeLa) and biphasic mesothelioma (MSTO-211H) human tumor cell lines. The estrogen like properties of the novel compounds were compared with those of the untreated controls using an estrogen responsive element-based (ERE) luciferase reporter assay and compared to 17β-estradiol (E2). Finally, with the aim to correlate the antiproliferative activity with an intracellular target(s), the effect on relaxation activity of DNA topoisomerases I and II was assayed.

1. Introduction

Tamoxifen (**1**, Fig. 1) is a triarylethylene compound that has been widely used in the treatment of breast cancer.[1,2] In addition to acute cancer therapy, tamoxifen is used as a preventative treatment[3] for high risk women as well as in long-term adjuvant therapy.[4] However, the use of tamoxifen has also been associated with increased risk of endometrial[5,6] and uterine[7] cancers as well as increased possibility of suffering a pulmonary embolism, stroke, or deep vein thrombosis.[8] Tamoxifen is converted to the active metabolite 4-hydroxytamoxifen (**2**, Fig. 1) which acts primarily through inhibition of the estrogen receptor transcriptional activity.[9] Furthermore, other mechanisms have been documented which may or may not be estrogen receptor dependent[10] and include induction of apoptosis,[11] interference with the insulin-like growth factor I receptor,[12] and suppression of telomerase activity by inhibition of protein kinase C.[13]

As a follow-up to our interest in the synthesis of anticancer compounds,[14] we describe in the present study, the synthesis of novel tamoxifen derivatives (Fig. 1) via McMurry reaction coupling.[15] The novel derivatives (Fig. 1), maintain the triarylethylene skeleton of tamoxifen, bearing OH groups in position 4 of the phenyl moieties of tamoxifen and substitute the side chain of tamoxifen with an amide side chain. The in vitro antiproliferative activity of these compounds was evaluated against three human tumor cell lines, MCF-7 (breast cancer), HeLa (cervix adenocarcinoma) and MSTO-211H (biphasic mesothelioma). Furthermore, we evaluated the estrogen like properties of the novel compounds using an ERE luciferase assay in HC11 cells (mouse mammary epithelial cells). The ERE luciferase reporter construct which is stably integrated into these cells harbors a transgene composed of 3 consensus ERE driving the expression of firefly luciferase protein, which allows the detection of estrogen activity by light produced by the luciferase enzyme. Finally, the effect on topoisomerases I and II was assayed to determine the potential intracellular target(s).

2. Results and discussion

2.1. Chemistry

The synthesis of the crucial keto-amides **6** is outlined in Scheme 1. Specifically, various *N*-(4-bromophenyl)amides (**4**), were reacted with 4-(benzyloxy)benzaldehyde (**3**) to produce the hydroxy-amides (**5**) from moderate to good yields (25–55%).

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http://dx.doi.org/10.1016/j.bmc.2013.05.012

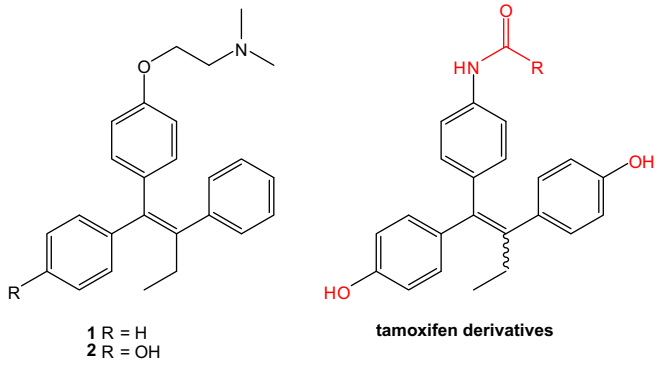


Figure 1. Structures of tamoxifen (**1**), 4-hydroxytamoxifen (**2**) and novel tamoxifen derivatives.

Oxidation of hydroxy-amides **5**, in the presence of Jones reagent, furnished the corresponding keto-amides **6**, in almost quantitative yields (90–95%).

Subsequently, keto-amides **6**, were treated under McMurry olefination conditions with 1-(4′-(benzyloxy)phenyl)propan-1-one (Scheme 2), to provide a mixture of *E*/*Z* isomers (**7** and **8**) in 1:1 ratio, that were separated by flash column chromatography in good yields (50–65%). Finally, deprotection of compounds **7** and **8** with BBr_3 at −78 °C (Scheme 2), afforded the target tamoxifen derivatives **9** and **10** in quantitative yields (90–95%).

The stereochemical assignments of the *E*, *Z* isomers were determined on the basis of 2D-NOESY experiments. For example the stereochemistry of compound **7d** (Scheme 2) was determined on its 2D-NOESY spectrum (Supplementary data), obtained at 400 MHz. The CH_2CH_3 protons at 2.50 ppm demonstrate NOE cross peaks with the proton at 7.07 ppm (H-2‴′) and the CH_2CH_3 protons at 0.96 ppm with the proton at 7.17 ppm (H-2‴). These NOE data establish the stereochemical assignment of compound **7d** to be in the *E* configuration. Similarly, the stereochemistry of compound **8d** was determined to be in the *Z* configuration based on its 2D-NOESY spectrum (Supplementary data), obtained at 400 MHz. The CH_2CH_3 protons at 0.94 ppm demonstrate NOE cross peaks with the proton at 7.17 ppm (H-3′) and the CH_2CH_3 protons at 2.48 ppm with the proton at 7.06 ppm (H-2‴′). A similar strategy using 2D-NOESY data was employed for the stereochemical assignment of compounds **7** and **8**.

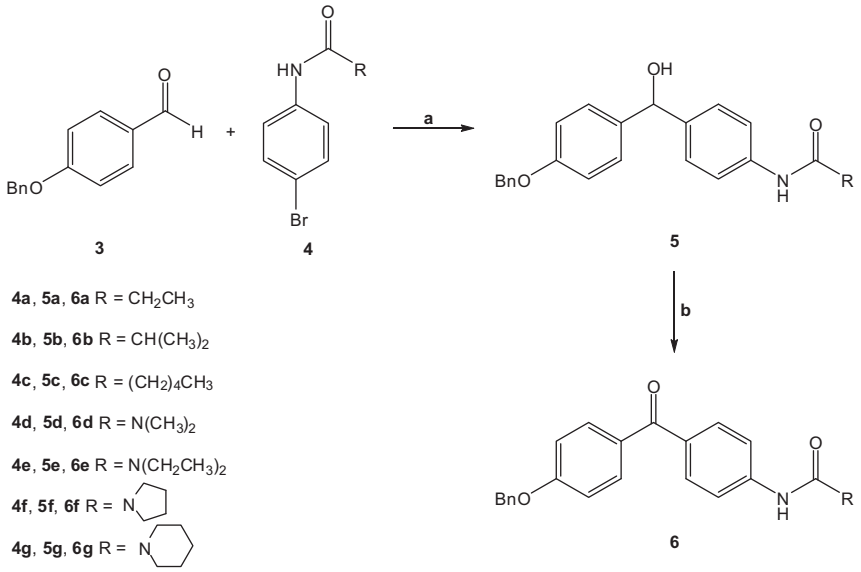
2.2. Antiproliferative activity

The solubility of compounds **9** and **10** has been evaluated theoretically by *c*log*P*[16] and have been compared to tamoxifen and 4-hydroxytamoxifen (Table 1). The theoretical calculations for the new compounds, showed values that are generally lower than that of tamoxifen, which indicates that compounds **9** and **10** present a reduced lipophilicity.

The novel compounds were evaluated by an in vitro assay performed on three human cancer cell lines comprised of breast cancer (MCF-7), which is known to overexpress the estrogen receptor,[17] cervix adenocarcinoma (HeLa) and biphasic mesothelioma (MSTO-211H), using tamoxifen and 4-hydroxytamoxifen as reference compounds. The results, expressed as GI_{50} values, are shown in Table 1.

As regard the estrogen sensitive MCF-7 cells, the obtained results highlighted for the compounds carrying in R a branched group (**b**, **d** and **e** series) or the piperidine (**9g** and **10g**) an antiproliferative activity comparable or even higher with respect to that of tamoxifen (Scheme 2). In particular, the most active compound is **10b** that shows an antiproliferative activity about 4 times higher with respect to the reference drug. Nevertheless, the comparison with 4-hydroxytamoxifen underlined for all new derivatives a significantly lower cytotoxicity. In this connection, it seems that the addition of a further 4-hydroxyl group in the triarylethylene moiety could play a crucial role in the occurrence of cytotoxic effect. As regard the compounds characterized in R by a linear substituent (**a** and **c** series) or by a pyrrolidine (**9f** and **10f**), they are practically ineffective. Moreover, taking into consideration the stereochemistry, *Z* isomers seem generally slightly more active toward MCF-7 cells, showing GI_{50} values from 1.8 (compounds **d**) to 3.6 times (compounds **g**) lower with respect to *E* isomers.

The investigation of the antiproliferative effect on two estrogen-independent human tumor cell lines, HeLa and MSTO-211H, shows, as expected, a significant decrease of the sensitivity toward tamoxifen. In contrast, for the novel derivatives the GI_{50} values obtained for HeLa and MSTO-211H remain practically unchanged with respect to those obtained for MCF-7, with the exception of **10b**. Indeed, interestingly, for this latter compound a decrease in



Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −50 °C, 2 h; (b) Jones reagent, acetone, 0 °C, 30 min.