Synthesis and Pharmacological Evolution of Tetrahydroisoquinolines as Anti Breast Cancer Agents

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Abstract

Breast cancer is the second leading cause of cancer-related deaths in women and is the most common cancer among women, excluding non-melanoma skin cancers. An estimated 232,340 new cases of invasive breast cancer are expected among American women [1]. The nuclear receptors, Estrogen receptor (ER) and progesterone receptor (PR) and their associated steroid hormones are play an important role in the development, differentiation and function of normal breast and endometrial cells. There is strong evidence that estrogen plays an essential role in the growth of breast tumors and the status of these hormones is employed as diagnostic indicators for endocrine responsiveness and tumor recurrence [2]. The estrogen receptors (ERs) remain one of the attractive targets in the treatment of breast cancer till date. Till date, the two subtypes of human ERs identified, ER-α and ER-β have different tissue distribution pattern and transcriptional activity. One way of blocking the estrogen action on tumor cells is preventing the binding of estrogen (E₂) to estrogen receptor (ER) by designing novel inhibitors of ER capable of blocking E₂ without showing any estrogenic activity on their own [3-6]. E₂ and its derivatives are used by millions of women in Hormone Replacement Therapy (HRT) for the treatment of peri- and post-menopausal related symptoms [7-9]. Steroidal skeleton of E₂ was also used as the template for the attachment of various substituents for therapeutic applications in the treatment of hormon-dependent breast cancer [10-11]. In this regard, E₂ derivatives like ICI-182,780 Fulvestrant (3) and ICI-164,384 (4) proved to be effective anti breast cancer agents, whose activity is comparable to tamoxifen (TAM) [12,13]. However, steroid based anti-breast cancer agents have their own limitations and hence non-steroidal alternatives were sought. The search for non-steroidal, anti-estrogenic molecules commonly called as Selective estrogen receptor modulators (SERMs) as potential alternatives in the treatment of hormon-dependent ER(+) breast cancer is actively undertaken in recent years. In this regard, Tamoxifen (TAM) has been the leading drug to treat breast cancer for more than two decades and has proven to be an effective treatment for ER (+) breast cancer, particularly in the post-menopausal women [14-17]. However, it is not without adverse side effects. Tamoxifen behaves as ER antagonist in the breast tissue and as ER agonist in bone, and has prophyllactic use in breast cancer [18]. Its agonistic effect on the uterus is said to be associated with increased risk of developing endometrial cancer [19]. Thus, alternative chemical entities, preferably non-steroid estrogen receptor modulators are sought with no agonistic effects on uterus.

Tetrahydroisoquinoline derivatives were identified as subtype selective estrogen receptor antagonists/agonists hence, potential therapeutic agents for breast cancer [20,21]. Structure activity relationship studies (SAR) of ER-α selective tetrahydroisoquinolines were reported by Renaud et al. [22]. Tetrahydroisoquinolines incorporating conformationally restricted side chains as the replacement of the amonoethoxy residue of E₂, typical of SERMs were reported exhibiting binding affinity to ER-α and antagonistic properties [23]. More recently, new steroidomimetic tetrahydroquinolines were reported which act as microtubule disruptors [24].

However, in the development of anti-estrogenic drugs, it is critical that the compounds under study do not cause estrogenic stimulation of the uterus, which could lead to both increase in uterine bleeding

Keywords: Substituted Tetrahydroisoquinolines (THIQs); Antiproliferative activity; Breast cancer

Introduction

Breast cancer is leading cause of mortality among women, resulting in more than half a million deaths worldwide each year. Unfortunately, the recovery rate of advanced breast cancer by current available drug treatment is till unacceptably low. Chemotherapy is the main stay of cancer treatment and most of the drugs cause general toxicity to any non-proliferating cells, which can severely limit the therapeutic values of these drugs. Tetrahydroisoquinoline derivatives (THIQs) were identified as subtype selective estrogen receptor antagonists/agonists hence, potential therapeutic agents for breast cancer. Substituted THICs were synthesized and well characterized. Antiproliferative activity against human ER (+) MCF-7 (Breast), ER(-) MDA-MB-231 (breast) and Ishikawa (endometrial) cancer cell lines were studied after 72 hours drug exposure employing CellTiter-Glo assay at concentrations ranging from 0.01-100,000 nM. The activities of these compounds were compared with Tamoxifen (TAM). In-vitro results indicated that most of the compounds showed better activity than TAM. The most active compounds obtained in this study were 6a, 6b, 6d and 6j (IC<sub>50</sub>=0.63, 0.23; 0.93, 0.21; 043, 0.01; 0.7, 0.02 μg/ml) against MCF-7 and Ishikawa cell lines, in comparison to Tamoxifen activity (IC<sub>50</sub>=14. 4.55 μg/ml). The newly synthesized molecules were docked in the active sites of the ER-α (PDB: 3ERT) and ER-β (PDB: 3ERT) crystal structures and probable binding modes of this class of molecules were determined.

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and an increased risk of developing uterine cancer. Ishikawa cell line, a well-differentiated human endometrial adenocarcinoma cell line expresses functional estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) isoforms. Therefore testing the new potential anti-proliferative moieties on Ishikawa cell line in the initial stages of the design is a good idea to understand the risk associated in developing endometrial cancer and its treatment. Also the compounds which act as antiproliferative agents against endometrial cancer can be used in combination with Tamoxifen (TAM) in the treatment of breast cancer, particularly in post-menopausal women.

Here in we report the synthesis and in-vitro anti-proliferative activity of new tetrahydroisoquinolines (THIQs) against MCF-7, MDA-MB-231 human breast cancer cell lines and Ishikawa human endometrial adenocarcinoma cell lines. These cell lines are widely accepted in vitro models for assessing potent anti-proliferative and anti-estrogenic compounds including SERMs. Tamoxifen (TAM) was used as a standard for comparison of activities in all these studies. An in-silico docking analysis of these compounds in the active sites of the ER-α and ER-β crystal structures, ER-α-4-OHT complex (3ERT) and probable binding modes of the ERα-RAL complex (1QKN) and probable binding modes of the molecules in their active sites were determined.

Materials and Methods

Experimental section

General: Melting points were determined on a Mel-Temp 3.0 melting point apparatus and are uncorrected. The structures of the products described were confirmed by IR, 1H NMR and elemental analysis data. 1H NMR spectra were recorded on Varian Gemini HX 300 MHz spectrometer and are reported in ppm. Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, GA, and are within ± 0.4% of theoretical values unless otherwise noted. Flash chromatography was performed on Combi-Flash (Teledyne Isco) using RediSep columns. All Chemicals and solvents were purchased from Sigma-Aldrich and were used without further purification.

General procedure for the synthesis of 2-Aminoisoquinolinium Iodide (3): A solution of hydroxylamine-O-sulfonic acid 2 (2 g, 1 equiv), water (10 mL) and Isoquinolin-2-ium-2-yl(4-methoxybenzoyl)amide (5c): Yield 58%; mp 169.5-171.2°C; 1HNMR (CDCl3) δ (ppm): 3.85 (s, 3H, OCH3), 7.16 (d, 2H, 2H, J=2.1, 6.9 Hz, C9-H), 8.28 (dd, 1H, J=2.1, 7.5 Hz, C1-H).

Yield 58%; mp 174.3-175.8°C; 1HNMR (CDCl3) δ (ppm): 2.38 (s,3H, CH3 group), 7.28 (2H, J=8.1 Hz, C1, C5-H). 1HNMR (CDCl3) δ (ppm): 0.89 (t, 3H, J=7.2 Hz, CH3), 7.25 (d, 2H, J=6.0 Hz, C3', C5'-H), 7.79-7.85 (m, 1H, C7-H), 7.93-8.03 (m, 2H, C3, C5-H), 8.11-8.15 (m, 3H, C4, C2', C6'-H), 8.45 (dd, 1H, J=1.5, 5.7 Hz, C-CH3), 9.87 (s, 1H, C1-H).

Yield: 58%; mp 164.2-165.7°C; 1HNMR (CDCl3) δ (ppm): 0.94 (t, 3H, J=7.5 Hz, -CH2-CH3), 2.66-2.73 (q, 2H, J=7.2 Hz, -CH2-CH3), 2.79 (2H, J=1.8, 6.3 Hz, C4, C5-H), 7.37-7.85 (m, 1H, C7-H), 7.93-8.03 (m, 4H, C4, C5, C6, C8-H), 8.12-8.17 (m, 3H, C3, C2', C6'-H), 8.46 (dd, 1H, J=1.2, 5.7 Hz, C5-H), 9.87 (s, 1H, C1-H).

Yield: 55%; mp 154.7-156°C; 1HNMR (CDCl3) δ (ppm): 0.93 (t, 3H, J=6.9 Hz, -CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-), 1.36 (q, 2H, J=7.5 Hz, -CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-), 2.66 (2H, J=7.8, -CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-CH2-), 7.25 (2H, J=6.0 Hz, C3', C5'-H), 7.79-8.15 (m, 1H, C7-H), 7.91-7.94 (m, 2H, C2', C6'-H), 8.11-8.15 (m, 3H, C3, C2', C6'-H), 8.45 (dd, 1H, J=1.5, 5.7 Hz, C-CH3), 9.87 (s, 1H, C1-H).

Yield: 55%; mp 172.2-173.5°C; 1HNMR (CDCl3) δ (ppm): 1.36 (s, 3H, t-butyl group), 7.45 (dd, 2H, J=2.1, 8.7 Hz, C1, C5-H), 7.77-7.83 (m, 1H, C7-H), 8.15 (m, 3H, C4, C5, C6-H), 8.45 (dd, 1H, J=1.5, 5.7 Hz, C-CH3), 9.91 (s, 1H, C1-H).

Yield: 49.5%; mp 183.1-184.8°C; 1HNMR (CDCl3) δ (ppm): 7.35-7.45 (m, 2H, C1-H), 7.75 (d, 2H, J=1.5, 6.6 Hz, C3', C5'-H), 7.83-7.89 (m, 1H, C6-H), 7.97-8.17 (m, 4H, C4, C5, C6, C8-H), 8.47 (dd, 1H, J=6.3 Hz, C2-H), 8.56 (dd, 1H, J=6.3 Hz, C5-H), 9.74 (s, 1H, C1-H).

(4-Chlorobenzoyl)(isoquinolin-2-ium-2-yl)amide (5i): Yield 64%; mp 195.2-195.6 °C; 1HNMR (CDCl₃) δ (ppm): 7.49 (d, 1H, J=8.4 Hz, C₃-H), 7.83 (d, 2H, J=2.1, 8.1 Hz, C₂',C₆'-H), 9.19 (s, 1H, C₁-H).

(6-Chloro-2-carbonyl)(isoquinolin-2-ium-2-yl)amide (5s): Yield 65.8%; mp 147.5-148.9 °C; 1HNMR (CDCl₃) δ (ppm): 6.51 (d, 1H, J=1.5 Hz, C₄-H), 7.02 (d, 1H, J=6.3 Hz, C₃-H), 7.17-7.20 (m, 3H, C₃, C₅, C₇-H), 7.43 (s, 1H, C₄-H), 7.64 (s, 1H, C₂-H), 8.01 (d, 1H, J=8.4 Hz, C₃'-H), 8.10 (d, 1H, J=5.7 Hz, C₅'-H), 9.21 (s, 1H, C₁-H).

(4-Bromobenzoyl)(isoquinolin-2-ium-2-yl)amide (5j): Yield 58.6%; mp 189.2-190.1 °C; 1HNMR (CDCl₃) δ (ppm): 7.38-7.47 (m, 2H, C₃, C₅-H), 7.59-7.67 (m, 2H, C₄, C₆'-H), 7.94-8.03 (m, 3H, C₅, C₆, C₈-H), 8.08 (d, 1H, J=1.5 Hz, C₃'-H), 8.45 (dd, 1H, J=1.5, 5.7 Hz, C₅'-H), 9.84 (s, 1H, C₁-H).

(4-Trimethylsilylphenyl)sulfonyl)(isoquinolin-2-ium-2-yl)amide (5o): Yield 51.5%; mp 192.3-204.6 °C; 1HNMR (CDCl₃) δ (ppm): 7.50-7.56 (m, 4H, C₆, C₇, C₈, C₉-H), 7.65 (d, 2H, J=1.8, 5.1 Hz, C₃-H), 9.21 (s, 1H, C₁-H).

(2-Chloro-6-methylisoquinolin-2-ium-2-yl)amide (5u): Yield 57.8%; mp 196.7-197.8 °C; 1HNMR (CDCl₃) δ (ppm): 2.55 (s, 3H, CH₃ group), 7.03 (d, 1H, J=6.3 Hz, C₇-H), 7.16-7.20 (m, 3H, C₃, C₅, C₇-H), 7.44 (d, 2H, J=7.8 Hz, C₃'-H), 8.09 (d, 1H, J=8.1 Hz, C₅'-H), 8.45 (dd, 1H, J=1.5, 5.7 Hz, C₅'-H), 9.84 (s, 1H, C₁-H).

(Benzyl(2-thiazole-2-carbonyl)isoquinolin-2-ium-2-yl)amide (5v): Yield 55.4%; mp 225.9-226.6 °C; 1HNMR (CDCl₃) δ (ppm): 7.32-7.34 (m, 1H, C₇-H), 7.47-7.52 (m, 1H, C₈-H), 8.01-8.09 (m, 2H, C₃, C₅-H), 8.10-8.15 (m, 2H, C₂', C₆'-H), 8.20 (dd, 1H, J=1.5, 5.7 Hz, C₅'-H), 8.24 (d, 1H, J=6.2 Hz, C₃'-H), 8.47 (dd, 1H, J=1.5, 5.7 Hz, C₅'-H), 9.87 (s, 1H, C₁-H).

General procedure for reduction (6a-x): A solution of Ylide (5 mmol) in 20 ml of absolute ethanol was added drop-wise to a solution of sodium borohydride (50 mmol) in 25 ml of absolute ethanol pre-cooled to 0 °C. The reaction was allowed to proceed for 5-7 h at 0°C with stirring. Water (35 ml) was added, and allowed to warm up to room temperature. Extraction with dichloromethane (3×50 ml), drying over anhydrous sodium sulfate, and removal of the solvent in vacuo gave the crude product, which was purified on Combiflash using ethyl acetate: dichloromethane (2:3 v/v) as an eluent to afford a pure compounds 6a-w in fair to good yields.

N-(1,2,3,4-tetrahydroisoquinolin-2-yl)benzamide (6a): Yield 60%; mp 197.4-198.1 °C; 1HNMR (CDCl₃) δ (ppm): 3.06 (t, 2H, J=5.7 Hz, C₃-H), 3.34 (t, 2H, J=6.0 Hz, C₂'-H), 4.21 (s, 2H, C₇-H), 7.01 (d, 1H, J=3.0 Hz, C₃-H), 7.08 (s, 1H, NH, D₂O exchange), 7.14-7.18 (m, 3H, C₃, C₅, C₇-H), 8.31 (d, 1H, J=6.9 Hz, C₆'-H), 7.39-7.50 (m, 3H, C₃, C₇-H), 7.75 (d, 2H, J=7.5 Hz, C₃'-H). Anal. Calcd. for C₂₅H₂₆N₂O·C₂H₅OH: C, 76.16; H, 6.39; N, 11.10. Found: C, 75.97; H, 6.28; N, 11.05.

4-Methyl-N-(1,2,3,4-tetrahydroisoquinolin-2-yl)benzamide (6b): Yield 65%; mp 195.9-196.4 °C; 1HNMR (CDCl₃) δ (ppm): 2.39 (s, 3H, CH₃ group), 3.06 (t, 2H, J=5.7 Hz, C₃-H), 3.35 (t, 2H, J=6.0 Hz, C₂'-H), 4.26 (s, 2H, C₇-H), 6.98 (d, 2H, J=6.0 Hz, C₂'-H), 7.01 (d, 1H, J=3.0 Hz, C₃-H), 7.04 (s, 1H, NH, D₂O exchange), 7.14-7.20 (m, 4H, C₅, C₇-H).
Yield 58%; mp 159.4-160.4°C; 1HNMR (CDCl3) δ (ppm): 3.30 (2H, p-OCH3 group), 3.95 (s, 3H, OCH3 group), 4.19 (s, 1H, C1-H), 4.23 (s, 1H, C1'-H), 7.08 (d, 1H, J=8.1 Hz, C2'-H), 7.74 (d, 2H, J=8.1 Hz, C3'-H), 7.91 (m, 3H, C3, C6, C8-H). Anal. Calcd. for C18H18F2N2O: C, 69.07; H, 4.83; N, 9.03. Found: C, 68.99; H, 4.79; N, 9.09.

4-Methoxy-N-(1,2,3,4-tetrahydroisoquinolin-2-yl)benzamide (6i): Yield 60%; mp 191.6-192.7°C; 1HNMR (CDCl3) δ (ppm): 2.19 (3H, CH3 group), 2.75 (t, 2H, J=5.3 Hz, C4-H), 3.38 (2H, C1-H), 3.69 (t, 2H, J=5.7 Hz, C3-H), 4.04 (s, 2H, C1-H), 4.33 (s, 2H, C1'-H), 7.05 (d, 1H, J=8.1 Hz, C2'-H), 7.78 (d, 1H, J=8.1 Hz, C3'-H), 7.90 (m, 3H, C3, C6, C8-H). Anal. Calcd. for C18H17F2N2O: C, 70.40; H, 4.78; N, 9.05. Found: C, 70.36; H, 4.72; N, 9.04.

4-Methyl-N-(1,2,3,4-tetrahydroisoquinolin-2-yl)benzamide (6j): Yield 54%; mp 181.5-183.5°C; 1HNMR (CDCl3) δ (ppm): 1.19 (6H, -CH2-CH2-CH3), 3.08 (2H, C3-H), 3.32 (2H, C1-H), 3.56 (s, 2H, C1'-H), 3.87 (s, 3H, OCH3 group), 4.21 (s, 2H, C1-H), 7.06 (d, 2H, J=8.1 Hz, C3',C5'-H), 7.80 (d, 2H, J=8.1 Hz, C6',C8'-H). Anal. Calcd. for C18H18F2N2O: C, 71.66; H, 5.64; N, 9.12. Found: C, 71.58; H, 5.61; N, 9.13.
4-Ethyl-N-(1,2,3,4-tetrahydroisoquinolin-2-yl)benzenesulfonamide (6g): Yield 49.5%; mp 137.1-138.3°C; 1H NMR (CDCl3) δ (ppm): 1.27 (t, 3H, J=7.5 Hz, -CH2-CH3), 2.66-2.75 (q, 2H, J=7.5 Hz, -CH2-CH3), 2.84 (t, 2H, J=5.7 Hz, C3-H), 2.94 (t, 2H, J=5.1 Hz, C2-H), 3.83 (s, 2H, C2-H), 5.54 (s, 1H, -NH, D-O exchange), 6.89 (dd, 1H, J=1.8, 6.6 Hz, C1-H), 7.05-7.15 (m, 3H, C6-C6-C6-H), 7.32 (d, 2H, J=6.0 Hz, C6-C6-C6-H), 7.88 (d, 2H, J=6.3 Hz, C3-C3-H). Anal. Calcld. for C16H16ClN3O: C, 63.68; H, 4.34; N, 12.09. Found: C, 63.42; H, 6.33; N, 8.81.

4-Butyl-N-(1,2,3,4-tetrahydroisoquinolin-2-yl)benzenesulfonamide (6r): Yield 60%; mp 197.3-199.1°C; 1H NMR (CDCl3) δ (ppm): 0.93 (t, 3H, J=7.5 Hz, -CH2-CH2-CH2-CH3) 1.36 (q, 2H, J=7.2 Hz, -CH2-CH2-CH2-CH3) 1.57-1.67 (m, 2H, -CH2-CH2-CH2-CH3), 2.69 (t, 2H, J=7.5 Hz, -CH2-CH2-CH2-CH3) 2.84 (t, 2H, J=5.7 Hz, C3-H), 2.93 (t, 2H, J=5.4 Hz, C3-H), 3.54 (s, 1H, -NH, D-O exchange), 6.88 (dd, 1H, J=2.1, 6.3 Hz, C1-H), 7.07-7.15 (m, 3H, C6-C6-C6-H), 7.32 (d, 2H, J=6.0 Hz, C6-C6-C6-H), 7.87 (d, 2H, J=6.3 Hz, C6-C6-C6-H). Anal. Calcld. for C17H20N2O2S: C, 66.12; H, 7.02; N, 8.81. Found: C, 66.34; H, 7.31; N, 8.76.

N-(1,2,3,4-tetrahydroisoquinolin-2-yl)-2-furan-2-carboxamide (6s): Yield 68%; mp 153.8-154.5°C; 1H NMR (CDCl3) δ (ppm): 3.08 (t, 2H, J=6.0 Hz, C3-H), 3.37 (t, 2H, J=6.0 Hz, C3-H), 4.24 (s, 2H, C2-H), 6.51 (d, 2H, J=1.5 Hz, C1-H), 7.02 (d, 1H, J=6.3 Hz, C1-H), 7.15 (s, 1H, -NH, D-O exchange), 7.17-7.20 (m, 2H, C4-C4-C4-H), 7.43 (s, 2H, C2-H), 7.64 (s, 1H, C6-H). Anal. Calcld. for C14H14N2O2: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.68; H, 5.76; N, 11.54.

6-Chloro-N-(1,2,3,4-tetrahydroisoquinolin-2-yl)-nicotinamide (6t): Yield 60%; mp 197.3-199.1°C; 1H NMR (CDCl3) δ (ppm): 3.10 (t, 2H, J=6.0 Hz, C3-H), 3.42 (s, 2H, C2-H), 4.29 (s, 2H, C1-H), 5.12 (d, 2H, J=5.7 Hz, C1-H), 7.14 (s, 1H, -NH, D-O exchange), 7.16-7.22 (m, 3H, C6-C6-C6-H), 7.38 (d, 1H, J=8.1 Hz, C1-H), 8.13 (d, 1H, J=8.1 Hz, C1-H). Anal. Calcld. for C14H13ClN3O: C, 62.61; H, 4.90; N, 14.46. Found: C, 62.43; H, 4.92; N, 14.46.

(2-Chloro-6-methyl)-N-(1,2,3,4-tetrahydroisoquinolin-2-yl)-isonicotinamide (6u): Yield 55%; mp 172.6-174.3°C; 1H NMR (CDCl3) δ (ppm): 3.10 (t, 2H, J=6.0 Hz, C3-H), 3.43 (t, 2H, J=6.0 Hz, C3-H), 4.29 (s, 2H, C2-H), 7.03 (d, 1H, J=6.9 Hz, C1-H), 7.12 (s, 1H, -NH, D-O exchange), 7.14-7.18 (m, 3H, C6-C6-C6-H), 7.97-8.03 (m, 1H, C1-H), 8.05-8.08 (m, 1H, C6-H). Anal. Calcld. for C14H14ClN3O: C, 62.72; H, 4.16; N, 12.76. Found: C, 62.63; H, 4.19; N, 12.79.

2,6-Dichloro-5-fluoro-N-(1,2,3,4-tetrahydroisoquinolin-2-yl)-nicotinamide (6x): Yield 63%; mp 169.5-171.0°C; 1H NMR (CDCl3) δ (ppm): 3.09 (t, 2H, J=6.3 Hz, C3-H), 3.42 (t, 2H, J=6.0 Hz, C3-H), 4.26 (s, 2H, C2-H), 7.03 (d, 1H, J=6.9 Hz, C1-H), 7.15 (s, 1H, -NH, D-O exchange), 7.16-7.20 (m, 3H, C6-C6-C6-H), 7.92 (d, 1H, J=6.0 Hz, C1-H). Anal. Calcld. for C16H14N3OF3: C, 52.96; H, 3.56; N, 12.35. Found: C, 52.78; H, 3.60; N, 12.09.

Antiproliferative Activity Studies

The antiproliferative activity of compound 6a-x was evaluated at the Southern Research Institute (SRI, Birmingham, Alabama, USA) according to procedure [25]. The compounds were screened against human ER (+) MCF-7 (breast), ER (-) MDA-MB-231 (breast), and Ishikawa (endometrial) cancer cell lines in comparison to tamoxifen (TAM).

Material

Human MCF-7 and MDA-MB-231 breast cancer cell lines were purchased from the NCI. The human Ishikawa endometrial cancer cell line was purchased from Sigma Aldrich. All three cell lines were cultured in phenol red-free RPMI-1640 (HyClone) (500 mL) supplemented with L-glutamine-dipeptide (HyClone) (5 mL), and 10% fetal bovine serum (Atlanta Biologicals) (50 mL).

Method

The cell lines were cultured and treated with compounds under study including the standard TAM ranging from 0.01-100.0 nM concentration in the presence of 10 nM estradiol using the previously reported method [26]. The results expressed as IC50 (inhibitory concentration) of 50%) were the averages of three data points for each concentration and were calculated using GraphPad Prism 4.0.

Molecular modeling studies

Docking method: The crystal structures of Era-4-OHT complex (PDB: 3ERT) and ERβ-RAL complex (PDB:1QKN) respectively, HYBRID v3.0.1 of OEDocking [30,31] was chosen as the appropriate docking method for our studies. Briefly, HYBRID docks molecules by an exhaustive search algorithm that systematically searches rotations and translations of each ligand conformation within the active site. In the process, unrealistic poses are filtered out, and those that survive are scored. The specified number of top scoring poses (in this study, 10), are subject to systematic solid body optimization. The best scoring pose is then used to rank the ligand against other ligands in the database. The protein and the conformers are held rigid during the docking process. Ligand flexibility is implicitly included by docking a conformer ensemble of each molecule and the bound cocrystal ligand in the protein active site is used as a guide for docking.

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the external ligand and scoring. The default parameters were set for HYBRID and OMEGA in this study. The scoring function used in this process to evaluate the poses in HYBRID is HYBRID-Chemgauss4 [32]. It uses Gaussian-smoothed potentials to measure the complementary nature of ligand poses within the active site.

Docking studies: The X-ray structure of Ligand Binding Domain of estrogen receptors has provided a way to better understand the ER binding site. The validation of the docking poses of the bound ligands (antagonists) in the crystal structures of ERα-4-OHT complex (3ERT) and ERβ-RAL complex (1QKN) was done using OEdocking application, HYBRID [30,31]. The re-docking of the co-crystallized ligands has been undertaken to make sure that the bound conformations of the ligands (OHT, RAL) are reproduced by the selected docking method. All the best 10 poses retrieved through re-docking using HYBRID method were identical with the original poses of the cognate ligands in the crystal structures with root mean square deviation (rmsd) values between them being <2 Å, a criterion often used for the correct bound structure prediction and validation. This led us to believe that the OEdocking method, HYBRID can be used reliably as a docking tool in our modeling studies.

Results and Discussion

Chemistry

Substituted tetrahydroisoquinoline derivatives 6a-x were prepared according to the procedure depicted in Scheme 1. The amino salt, 2-aminoisoquinolinium Iodide (3) used in the present study was prepared by the reaction of isoquinoline and hydroxylamine-O-sulfonic acid and water, which was refluxed at 90°C for 2h as previously reported procedure [33]. Reaction of (3) with corresponding substituted acylating agents like acylchlorides or sulfonyl chlorides, followed by treatment with a base afforded N-ylides 5 stable crystalline solids. Sodium borohydride reduction of 5a-x in absolute ethanol furnished the title compounds 6a-x in fair to good yields. It should be noted that some of the compounds synthesis have been reported [34-36]. However, none of these compounds has been examined for their antiproliferative activity against human Ishikawa endometrial cell line, MCF-7 (ER positive breast cancer cell line) and Ishikawa (endometrial) cancer cell lines at concentration ranging from 0.01-100,000 nM in the presence of 10nM estradiol (E2) using CellTiter-Glo assay (E2 was used for competitive growth inhibitory studies). As shown in Table 1, compounds 6a-f, 6i-k, 6o (IC50=0.4-3.5 μg/mL) demonstrated significant antiproliferative activity against human Er (+) MCF-7 breast cancer cell line in comparison to TAM (IC50=5.14 μg/mL, Table 1). These results indicate that compounds 6d may lower the risk of developing uterine cancer based upon the IC50 value in comparison with TAM [26] (Figure 2).

Molecular modeling studies

The top scoring conformations of the THIQs (6a-6k, 6o, 6r and 6v-6x) collected by docking the conformer ensemble (generated by OMEGA [28,29]) on ERα and ERβ receptors (Figures 4 and 5) clearly show the preference for ERα as the ligands fit better in the bigger ligand binding pocket of ERα. These studies also give us an idea of the probable bioactive conformations and binding mode of the newly synthesized THIQs which would assist in further optimization studies. Around their mechanism of action. The results showed that compounds 6a-c, 6j-k and 6o (IC50=0.3-4.85 μg/mL) were more potent than TAM (IC50=5.64 μg/mL) shown in Table 1. Furthermore compound 6d (IC50=0.37 μg/mL) possessing ethyl group on phenyl ring, the most active of the series in this cell line. This results suggests that compound 6d may also inhibit cell proliferation via ER-independent mechanism in comparison to TAM (Figure 2).

In the Present investigation, evaluation of the antiproliferative activity of these compounds against human Ishikawa endometrial cell line were revealed that compounds 6a, 6b, 6d and 6j (IC50=0.23, 0.21, 0.01 and 0.02 μg/mL respectively) were more potent than TAM (IC50=4.55 μg/mL, Table 1). These results indicate that compounds 6d may lower the risk of developing uterine cancer based upon the IC50 value in comparison with TAM [26] (Figure 3).

Antiproliferative activity

In vitro antiproliferative activity of compounds 6a-x were evaluated against human MDA-MB-231 (ER negative breast carcinoma cell line), MCF-7 (ER positive breast cancer cell line) and Ishikawa (endometrial) cancer cell lines at concentration ranging from 0.01-100,000 nM in the presence of 10nM estradiol (E2) using CellTiter-Glo assay (E2 was used for competitive growth inhibitory studies). As shown in Table 1, compounds 6a-f, 6i-k, 6o (IC50=0.4-3.5 μg/mL) demonstrated significant antiproliferative activity against human Er (+) MCF-7 breast cancer cell line in comparison to TAM (IC50=5.14 μg/mL, Table 1). (Note: IC50 is the concentration of test drug where a 50% reduction is observed in cell growth compared to the untreated control after a 72 h period of exposure to test drug). Compounds bearing phenyl (6a) and ethyl (6d) groups, the most active of the series in this cell line, exhibit higher antiproliferative activity against MCF-7 cell line based on IC50 value (Figure 1).

The ER (-) MDA-MB-231 breast cancer cell line constitutes an original model for identifying the ER-independent mechanisms of TAM antiproliferative effects [37,38]. Thus, in the present study the antiproliferative activity of compounds 6a-x against human ER (-) MDA-MB-231 breast cancer cell lines were also investigated to know

Figure 1: In vitro antiproliferative activity of compounds 6a-x against ER (+) MCF-7 cell line.
ten tetrahydroisoquinolines (THIQs) synthesized were more potent than Tamoxifen on all the cell lines understudy. The best being the THIQ, 6d as shown in Table 1, the high potency of these compounds on Ishikawa cell lines as compared with Tamoxifen, indicate that these compounds may lower the risk of developing uterine cancer. Tetrahydroisoquinolines were reported as subtype selective estrogen agonists/antagonists as they are structurally similar to Lasofoxifene. The selectivity towards ERα and ERβ is crucial, as they have their own tissue distribution patterns and transcriptional properties. ERα is mainly involved in reproduction events in uterus and mammary glands and ERβ is more generally expressed and not dominant in uterus and breast tissues.

Discussion

The antiproliferative activity of twenty four substituted tetrahydroisoquinoline analogs (6a-x) were evaluated in two human breast cancer cell lines (MCF-7, MDA-MB-231) and human endometrial cancer cell lines (Ishikawa) in order to understand their underlying mechanism of action of their antiproliferative activity. This study also helps us to evaluate the effect of various substitutions on the phenyl ring of the THIQs (Scheme 1) towards antiproliferative activity. In this regard, high-quality biological testing results were collected for the newly synthesized compounds along with Tamoxifen (Table 1). The most active compounds are 6a, 6b, 6c, 6d, 6i, 6j, 6k and 6p. These results strongly indicate that the antiproliferative activity of most of our compounds on Ishikawa cell lines were far better than the standard Tamoxifen, indicating that the compounds in the present study have low associated risk in developing endometrial cancer and its treatment. The active compounds mentioned above showed nearly similar activity on MCF-7 and MDA-MB-231 cell lines, except for 6j where the compound is more active on MCF-7 cell lines than MDA-MB-231. These results indicate that except for compound 6j, all other active compounds in the present study may also inhibit cell proliferation.

Figure 2: In vitro antiproliferative activity of compounds 6a-x against ER (-) MDA-MB-231 cell line.

Figure 3: In vitro antiproliferative activity of compounds 6a-x against Ishikawa cell line.

Figure 4: Top scoring binding poses of the active THIQs (6a-6k, 6o, 6r, 6t and 6v-6x) at the active site of ERα-4-OHT complex (3ERT).

Figure 5: Top scoring binding poses of the active THIQs (6a-6k, 6o, 6r, 6t and 6v-6x) at the active site of ERβ-RAL complex (1QKN).
The IC50 values were determined from the graphs (GraphPad Prism) using mean values of data points at various concentrations. The data represent the average of triplicate determinations at various concentrations.

In vitro results indicated that some of the compounds in the study determined and compared to that of standard antiestrogen drug TAM. (-) MDA-MB-231 (breast) and Ishikawa (endometrial) cell lines were antiproliferative activities against human ER(+) MC-7 (breast), ER(-) MDA-MB-231 lines over MDA-MB-231 cell lines indicating a possible ER-dependent antiproliferative action. Compound 6d with an ethyl substitution on the 4-th position of the phenyl ring was the best compound in this series for future studies with IC50 values on MCF-7 and MDA-MB-231 lines for future studies with IC50 values on MCF-7 and MDA-MB-231 lines.

via ER-independent mechanism in comparison to TAM. As far as the structure activity relationship is concerned, ethyl group with the right steric bulk, halogens, OCH3 and CH3 groups at the -para position (4th position) of the aromatic ring is the cause of activity. Replacing the phenyl right with other heterocycles and additional ring substitutions on the phenyl ring lead to loss of activity. Similarly, hetero atoms incorporated inside the aromatic ring as in led to loss in activity.

Conclusion

The newly synthesized substituted tetrahydroisoquinolines (THIQs) were characterized thoroughly by 1H NMR and elemental analysis to understand how these compounds interact with ER. and antiuterotropic activities on immature rats in order to further investigate how these compounds interact with ER.

Table 1: IC50 values (µg/mL) for compounds 6a-x tested against MCF-7, MDA-MB231 and Ishikawa Cancer Cells.

<table>
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<th>CODE</th>
<th>R</th>
<th>X</th>
<th>IC50 µg/mL</th>
<th>MCF-7</th>
<th>ISHIKAWA</th>
<th>MDA-MB-231</th>
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<td>9.95</td>
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THIQ

TAMOXIFEN

Table 1: IC50 values (µg/mL) for compounds 6a-x tested against MCF-7, MDA-MB231 and Ishikawa Cancer Cells.

Conclusions

The synthesized substituted tetrahydroisoquinolines (THIQs) were characterized thoroughly by 1H NMR and elemental analysis to make sure the purity of these compounds for in vitro testing. Their antiproliferative activities against human ER(+) MC-7 (breast), ER(-) MDA-MB-231 (breast) and Ishikawa (endometrial) cell lines were determined and compared to that of standard antiestrogen drug TAM. In vitro results indicated that some of the compounds in the study 6a, 6b, 6c, 6d, 6i, 6j, 6k, and 6p showed better activity than TAM on the above cell lines. Compound 6j showed better selectivity on MCF-7 cell lines over MDA-MB-231 cells lines indicating a possible ER-dependent antiproliferative action. Compound 6d with an ethyl substitution on the 4-th position of the THIQ phenyl ring was the best compound in this series for future studies with IC50 values on MCF-7 and MDA-MB-231 being 0.43 and 0.37 µg/mL respectively. Pose prediction of the active compounds were undertaken by docking these compounds on the published crystal structures of ER-a and ER-β receptors. These in silico preliminary studies indicate a preference for these compounds to the relatively larger ER-a binding site. The future studies in this direction involve exploring the activity profile of this class of compounds by substitutions on the THIQ ring keeping the ethyl group intact on the 4-th position of the phenyl ring, ER binding studies, uterotropic and antitumor activities on immature rats in order to further understand how these compounds interact with ER.

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References


27. SYBYL-X 1.3, Tripos International: 1699 S. Hanley Rd., St. Louis, MO, 63144, USA.


30. McGann M, OEDOCKING 3.0.1. : OpenEye Scientific Software, Santa Fe, NM.


