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Synthesis and Evaluation of Estradiol Derivatives as Anti-Breast Cancer Agents

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Abstract

3-*N*-alkoxyestradiol derivatives were synthesized, characterized and tested for activity in MCF-7 human breast cancer cells. Among the compounds, the diisopropyl and piperidinyl derivatives were found to be more active than 4-hydroxytamoxifen (HO-Tam), the active metabolite of tamoxifen based upon IC₅₀ values. The IC₅₀s were correlated with structures using molecular modeling.

Keywords

Estradiol; Estrogen Receptors; SERMs; MCF-7 cells; 4-hydroxytamoxifen; Breast cancer

Introduction

Estrogens are important hormones in the female body. These steroids interact with multiple organ systems and have a major impact on the physiological events that occur through a woman's life.[1] Estrogen (Gr. Oistros, "mad desire" and gennan, "to produce") is the generic name that encompasses estradiol, estrone and estriol. This family of substances functions endogenously as hormones affecting the female reproductive function and/or anatomy. In premenopausal women 17- β -estradiol (2, Fig. (1)) biosynthesized in the ovaries, is the predominate estrogen with blood circulating levels ranging from 40 to 400 pg/ml depending on the point of time in the menstrual cycle.[2] 17 β -Estradiol is the most potent because of its high binding affinity for the estrogen receptors. Estrogen action is primarily mediated through binding to the estrogen receptor (ER), which dimerizes, binds to estrogen responsive elements (EREs) in the DNA and regulates transcription of estrogen responsive genes.

The link between estrogen and the breast dates back to over a hundred years ago when oophorectomy was first reported to have substantial therapeutic benefits in metastatic breast cancer patients.[3] It was this research that led to the development of endocrine therapy to treat patients with breast cancer. Endocrine therapy has been proven to be effective treatment of estrogen receptor positive breast cancer through disruption of estrogen action.[4] Selective estrogen receptor modulators (SERMs) are a group of compounds that bind to estrogen receptors and elicit an agonist or antagonist response based upon their chemical structure and

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target tissues. Tamoxifen is a well-known triphenylethylene SERM that is used as an adjuvant therapy in breast cancer. An estimated 60% of the patients with ER positive tumors respond to tamoxifen treatment.[5] It has also demonstrated efficient prevention of breast cancer. Nevertheless, its use has been associated with an increase risk of endometrial cancer. In addition, most patients with advance disease eventually develop resistance to tamoxifen treatment.[6] The active metabolite of tamoxifen is its 4-hydroxy analog (1, Fig. (1)), which has been found to be 100 times more active than the parent drug in cell proliferation studies using MCF-7 human breast cancer cell line.[7]

Although various chemically unrelated compounds have been found to demonstrate activities similar to that of SERMs through interaction with estrogen receptors[8]., We conceptualized by combining aminoalkyloxy with the rigid structure of estradiol, the naturally occurring ligand that these derivatives would have active equal to or better than 4-hydroxytamoxifen (HO-Tam). Therefore, in the present research we decided to combine a portion of the chemical feature of tamoxifen the aminoalkyloxy with the rigid structure of 17- β -estradiol (2, Fig. (1)) and evaluate these particular compounds in cell proliferation studies using MCF-7 cell line.

Results and Discussion

Synthesis

Estradiol was purchased from Steraloids, Inc (Newport, RI). All other chemicals were obtained from Sigma Aldrich Chemical Co. (Milwaukee, WI). These aminoalkyloxy derivatives were synthesized earlier as potential cholesterol lowering agents [9] and male fertility agents.[10] Using a similar published approach [9], we used the following procedure. Sodium metal (3 mole equivalent) was dissolved in 50 mL of ethanol then steroid (1 mole equivalent) was added. To the resulting solution, the alkylamine chloride (1.2 mole equivalent) was added and refluxed for 17 hours. The mixture was then filtered and the filtrate was concentrated to dryness under reduced pressure. The residue was re-dissolved in 150 mL of ethyl acetate and washed with 0.1 N aqueous hydrochloric acid (2×100 mL), then 2 N sodium hydroxide (2×100 mL) and water (100 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated under reduced pressure to obtain the residue. The residue was dissolved in 100 mL of diethyl ether and acidified with gaseous hydrochloric acid to reach a pH of 3. The acidified mixture was filtered, washed with ether and solid collected in respective yields (Scheme 1, Table 1).

Pharmacological Evaluations in cell culture

MCF-7 cells, purchased from ATCC (Rockville, MD), were grown in RPMI medium 1640 with 10% FBS (GIBCO, Grand Island, NY) and 1% Antibiotic-Antimycotic (GIBCO, Grand Island, NY) in an incubator (NUAIRE NU-1500, Plymouth, MN) with 5% CO₂ and 95% air at 37 °C. Cells were grown as monolayer, doubling time was about 22 hours. The stock solution of the test compound was prepared at 1mg/ml drug concentration. The stock solution was diluted serially to obtain the desired drug concentrations, e.g. 20, 100 and 200 μ g/ml. Plates with twelve wells 5 ml capacity/well) were employed for all the experiments. In a typical experiment, each well contained 1×10^5 initial cells and 100 μ l drug solution in a total volume of 2 ml media. The final concentrations of compounds were 0, 10, 50 and 100 μ g/ml in triplicate sample wells. The plates were incubated at 5% CO₂ and 37 °C for 72 hours under humid conditions. After incubation, cells were washed with PBS 1 $\times$, treated by 0.5 ml trypsin-EDTA for 10 minutes and the cells were suspended by adding 1.5 ml medium. Cells were counted by using a hemacytometer under a Nikon TMS 104 microscope. The data was converted to micromolar concentration using the various molecular weights and represent the mean values with standard error mean of less than ± 0.6 .

Molecular modeling studies

To evaluate structure activity relationship (SAR), we carried out molecular modeling studies looking at docking calculations using the FlexX [11] and Consensus Score (Cscore) [12-17] programs of the Sybyl 6.9 software [18]. Modeling was performed on SGI O2 workstations. The co-crystal structure of 4-hydroxytamoxifen-estrogen receptor was down loaded from the protein data bank (PDB) file coded 3ERT [19] as the reference structure, and 30 ranked conformations for each of the compounds were generated from their minimized structures. Minimizations were carried out using Tripos® force field. The docking calculations were performed with an active-site encompassing a sphere of 6.5 Å around the reference structure position in the PDB file. Due to the rigid structure of the estradiol derivatives studied in comparison with the more flexible reference compound, 4-hydroxytamoxifen, the FlexX scores were poor. This was expected due to the fact that FlexX scores are in part determined from structural matching which is based upon the similarity between components.

The overall FlexX results, which included all 30 conformers, showed very little correlation with bioactivity data not shown. The results of the two highest CScores docking outcomes are reported in Table 2. Certain Cscore values displayed strong correlation with bioactivity as shown Fig. (2), (3), and (4).

In Fig. (2), ChemScore, a module of Cscore, is a four term function consisting of lipophilic interactions between ligand and receptor, metal-ligand binding (not necessary in this study), hydrogen bonding and rotational terms. There appears to be strong correlation between the ChemScores and the bioactivity ($R^2=0.89$), excluding the morpholinyl derivative. Another module of Cscore is PMF score which dealt with Helmholtz free energy interactions between protein-ligand atom pairs shown Fig (3). The D score module of CScore involves scoring based upon electrostatic and hydrophobic interactions between ligand and receptor shown in Fig. (4).

In addition, there appears to be fairly strong correlation between PMF scores, and D Scores with bioactivity with R^2 values of 0.72 and 0.78, respectively shown in Fig. (3) and (4). Which is a good indication of binding affinity since ChemScores, PMF scores and D scores are estimations of the free energy of interaction of the ligand and active-site.

Conclusion

By alkylating the 3-hydroxyl group with an alkylamino substituent, we were able to inhibit the growth of MCF-7 human breast cancer cells. Based upon IC_{50} values, our compounds activities were very much similar to that of 4-hydroxytamoxifen, the active metabolite of tamoxifen, with the compounds **7** and **8** displaying IC_{50} values of 9.0, 4.1 and 5.6, respectively (shown in Table 1). Further studies are being carried out to determine if cell growth inhibition is due to receptor antagonism through binding studies. In addition, studies are being carried out to determine if these compounds are selective modulators of other tissues such as bone and uterus.

It is clear based upon the top two C Scores for each module that compound **9** docking results showed little correlation between with activity. This may suggest that compound **9** does not act by the same mechanism as the other compounds despite their molecular similarities. Also, conformational searches not discussed in this work show that several of the low-lying conformations of these derivative are similar in shape. The results show a lesser correlation with activity between those docking functions (Dscore and PMF scores) that do not possess both the hydrogen bonding and lipophilic contact terms in comparison to the ChemScore results that do. The later hypothesis coincides with evidence of hydrogen bonding found in the crystal structure, 3ERT, of 4-hydroxtamoxifen. [19]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Canez MS, Lee KJ, Olive DL. Progestogens and estrogens. *Infertil Reprod Med Clin North Am* 1992;3:59–77.
2. North American Menopause Society. Menopause core curriculum study guide. Cleveland, OH: North American Menopause Society; 2000.
3. Kaufmann MA. Review of Endocrine Options for the Treatment of Advanced Breast Cancer. *Oncology* 1997;54(Suppl 2):2–5. [PubMed: 9394852]
4. Buzdar AU. Endocrine therapy in the treatment of metastatic breast cancer. *Semin Oncol* 2001;28:291–304. [PubMed: 11402439]
5. Osborne CK, Yochmowitz MG, Knight WA III, McGuire WL. The value of estrogen and progesterone receptors in the treatment of breast cancer. *Cancer* 1980;46:2884–2888. [PubMed: 7448733]
6. Mouridsen H, Palshof T, Patterson J, Battersby L. Tamoxifen in advanced breast cancer. *Cancer Treat Rev* 1978;5:131–141. [PubMed: 365330]
7. Katzenellenbogen BS, Norman MJ, Eckert RL, Peltz SW, Mangel WF. Bioactivities, estrogen receptor interactions, and plasminogen activator-inducing activities of tamoxifen and hydroxy-tamoxifen isomers in MCF-7 human breast cancer cells. *Cancer Res* 1984;44:112–119. [PubMed: 6537799]
8. Chen HY, Dykstra KD, Birzin ET, Frisch K, Chan W, Yang YT, Mosley RT, DiNinno F, Rohrer SP, Schaeffer JM, Hammond ML. Estrogen receptor ligands. Part 1: The discovery of flavanoids with subtype selectivity. *Bioorg Med Chem Lett* 2004;14:1417. [PubMed: 15006374]
9. Allais, A.; Paturet, MUS. Patent 3,212,971. 1965.
10. Bonfils, A.; Philbert, D. Roussel Uclaf France. Method of controlling male infertility. US Patent 5,739,124. Jan 17. 1997
11. Rarey M, Kramer B, Lengauer T, Klebe G. A fast flexible docking method using an incremental construction algorithm. *J Mol Biol* 1996;261:470–489. [PubMed: 8780787]
12. Jones G, Willett P, Glen R, Leach AR, Taylor R. Development and validation of a genetic algorithm for flexible docking. *J Mol Biol* 1997;267:727–748. [PubMed: 9126849]
13. Muegge I, Martin YC. A General and Fast Scoring Function for Protein-Ligand Interactions: A Simplified Potential Approach. *J Med Chem* 1999;42:791–804. [PubMed: 10072678]
14. Kuntz D, Blaney JM, Oatley SJ, Langridge R, Ferrin TE. A Geometric Approach to Macromolecule-Ligand Interactions. *J Mol Biol* 1982;161:269–288. [PubMed: 7154081]
15. Eldridge MD, Murray CW, Auton TR, Paolini GV, Mee RP I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes. *J Comp-Aided Molec Des* 1997;11:425–445.
16. Böhm HJ. The development of a simple empirical scoring function to estimate the binding constant for a protein-ligand complex of known three-dimensional structure. *J Comp-Aided Molec Des* 1994;8:243–356.
17. Clark RD, Strizhev A, Leonard JM, Blake JF, Matthew JB. Consensus Scoring for Ligand/Protein Interactions. *J Mol Graph Mod* 2002;20:281–295.
18. Sybyl® 6.9.2 Tripos Inc. 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA
19. Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* 1998;95:927–937. [PubMed: 9875847]

Figure 1.
The structures of 4-hydroxytamoxifen, estradiol and N-alkyloxy derivatives of 17-β- estradiol.

Fig. (2).

The ChemScore includes lipophilicity, H-bonding, and rotational terms energies.

Fig. (3).
The PMF scores are Helmholtz free energy interactions between protein-ligand atom pairs.

Fig. (4).

D score values are the energies derived from charge van der Waals interactions.

Scheme 1. Reagents

i. alkylamino, sodium ethoxide stirred at reflux; ii. sat. HCl etherate

Table 1

Structures of synthesized compounds (**4-9**), reaction yields and IC₅₀ values (50% growth inhibitory concentration) against MCF-7 human breast cancer cells.

Compd. no.	R	%Yield	Inhibition IC ₅₀ , μM^a
4	CH ₂ CH ₃	83	8.7
5	CH ₃	74	16.2
6	Pyrrolidine ring	66	10.8
7	Piperidine ring	39	4.1
8	(CH ₃) ₂ CH	73	5.6
9	Morpholine ring	63	19.8
HO-TAM			9.0

^aValues are means of three experiments with standard error mean less than 0.6 $\pm$. IC₅₀ is the concentration (μM , micromolar) at which 50% cell growth inhibition occurs.

Table 2

The PMF score, Dscore, ChemScore and Bioactivity values of the two highest ranked conformations based on the consensus F Score in FlexX and Cscore programs.

Compd. no.	PMF Score (kcal/mol)	D Score (kcal/mole)	ChemScore (kcal/mol)
4	-13.6	-121.96	-33.4
4	-13.9	-119.84	-33.19
5	-11.67	-105.7	-29.63
5	-3.81	-107.05	-29.64
6	-18.99	-114.4	-33.28
6	-16.56	-110.37	-33.17
7	-20.53	-121.82	-35.1
7	-22.91	-118.01	-35.36
8	-24.88	-123.75	-36.86
8	-25.04	-122.01	-36.45
9	-24.12	-116.06	-33.07
9	-22.99	-115.77	-33.18