SYNTHESIS AND MOLECULAR DOCKING STUDY OF NOVEL COMPOUND

Anurag Agrawal¹ and Richa Kothari*²

¹School of Pharmacy, ITM University, Gwalior, (M.P.) India-474001.
²Department of Chemistry, School of Sciences, ITM University, Gwalior, (M.P.) India-474001.

ABSTRACT
A novel synthesis of 3-{(2E)-2-[1-(4-chlorophenyl) ethyldene] hydrazinyl}-N-(4-methylphenyl)-3-oxopropanamide was carried out by the condensation of thiosemicarbazide hydrochloride with substituted carbohydrazone and well characterized by various physico-chemical techniques like elemental analysis, TLC, melting point, FT-IR, H¹NMR, and electronic spectral data. The molecular docking study was also carried out into the active site of estrogen receptors (ERα and ERβ) downloaded through RCSB (PDB ID: 1XP1 and 3OLS respectively). The compound exhibited good binding affinities on ERα and ERβ (~9.6 kcal/mol and ~9.1 kcal/mol respectively) and those were comparable with standard drug Tamoxifen. Molecular docking study suggests that particular compound may have significant anticancer activity.

KEYWORDS: Anticancer activity, Estrogen receptor, Molecular docking studies, Substituted carbohydrazone, Thiosemicarbazide.

INTRODUCTION
Breast cancer is the most common malignancy affecting women from western cultures. Approximately 1,80,000 women are diagnosed with breast cancer each year in united states alone and distressingly each year 44,000 women die of this disease. Breast cancer was already known to be associated with the steroid hormone estrogen more than a century ago. The discovery of estrogen receptor (ER) provided us not only with a powerful predictive and prognostic marker, but also an efficient target for the treatment of hormone- dependent breast cancer with anti-estrogens. The biological effects of estrogen are mediated by its binding to
Endocrine therapy using Tamoxifen, a selective estrogen receptor modulator and aromatase inhibitor, which ablate peripheral estrogen synthesis, has been shown to substantially improve disease-free survival. Endocrine therapy has also been shown to have a positive effect on the treatment of ER-positive breast cancer. Structure activity relationship of compound showing photocanthemotherapeutic activity is important to enhance the biological activity of the molecules by modifying various structural and/or functional parameters, viz. steric and electronic controls, lipophilicity and hydrophilicity and stability of molecules derived from the parent drug by suitable modifications. The macrocyclic pharmacophore has attracted more attention from medicinal chemists because of great importance in their biological as well as synthetic approach of medicinal chemistry. The macrocyclic pharmacophore are known to possess a range of biological activities including anticancer, anticonvulsant, antidepressant, antimicrobial, antiviral, anxiolytic, and anti-inflammatory activities. Keeping in view of biological importance of the compound, it has been felt worthwhile to study the condensation of thiosemicarbazide hydrochloride with substituted carbohydrazone as depicted in scheme 1.

2. EXPERIMENTAL

2.1 Material

All the chemicals used in the study are of analytical grade. p-toludene, dimethyl malonate, hydrazine hydrate, p-chloro benzaldehyde and thiosemicarbazide were purchased from Merck, India. Macroyclic ligand is synthesized using literature method.

2.2 Physical measurements

All glasswares were dried in an open flame before using in connection with an inert atmosphere. Solvents were evaporated under reduced pressure and evaporation was carried out at room temperature <50°C. TLC was performed using silica gel 60F254 plates with detecting agent iodine vapors spraying with 5% sulphuric acid in ethanol followed by heating at 100°C. Tetra methyl silane (0.0ppm) was used as an internal standard in 1H NMR. Infrared spectrum was taken with KBr on Perkin-Elmer RX-1. Melting Points were determined on a Buchi 535 digital melting point apparatus and were uncorrected. Microanalysis (Carbon, Hydrogen, Nitrogen) of compound were performed at SAIF, Lucknow India.

2.3 Synthesis of compound
A mixture of substituted carbohydrazone and thiosemicarbazide hydrochloride in absolute ethanol were added slowly with stirring in a round bottom flask. After the addition of both the reagents, the reaction was carried out for 8 hrs. under reflux. The solvent was evaporated under reduced pressure and the residue obtained was quenched with ethanol. The solid product was precipitated, filtered off, washed several times with cold ethanol and dried over fused CuCl$_2$ in desiccator. A good yield of product was obtained and the purity of the complex was confirmed by the TLC, elemental analysis and various spectroscopic techniques like IR, $^1$HNMR and UV-Visible spectra.

![Scheme: 1 Synthesis of compound.](image)

Yield: 75%. Analytical calculation for [C$_{38}$H$_{38}$N$_{12}$S$_2$Cl$_2$] (931.54gm$^{-1}$): C, 54.50; H, 6.32, N, 17.80; found C, 48.95; H, 4.07; N, 8.0. Selected IR (KBr) Cm$^{-1}$: 3249, 3088, 1604, 762. UV-Vis (ethanol, $\varepsilon$ M$^{-1}$Cm$^{-1}$, $\lambda_{max}$ = 365 nm) $^1$HNMR data (TMS, ppm) ($\delta$): 1.9(CH$_3$), 2.9-2.3 (-CH$_2$), 3.2-3.5 (H-NH), 6.7-6.8 (-C$_6$H$_6$), 10.4-10.5 (-HCN).

2.4 Molecular docking study

It is reported that estrogen can interact with estrogen receptor- alpha (ER$_\alpha$) and estrogen receptor- Beta (ER$_\beta$) therefore both types of receptors were downloaded from RCSB.org. (PDB ids for ER$_\alpha$ and ER$_\beta$ are 1XP1 and 3OLS respectively). Then these protein files were
optimized by software chimera version 1.10.2. The crystal structure of ligand was drawn and optimized by Chem 3D Ultra software and finally saved as pdb. format. Pyrx was used for virtual screening of ligand which utilizes Autodock vina function for docking purposes. Pymol software was used for graphical visualization, analyzing hydrogen bond interactions and producing quality images.

RESULTS AND DISCUSSION
Synthesized compound is non-hygroscopic in nature and air stable in solution and in the solid state at room temperature compound is completely soluble in DMF, DMSO and ethanol but partially soluble in water. The IR spectrum of the synthesized ligand was often used as the tool to establish the structure in the absence of the single crystal analysis.

MOLECULAR DOCKING STUDY
Synthesized compound maintained a square- pyramidal geometry with four nitrogens and two sulfur atoms of ligand in the same plane. Possible binding modes of ligand with estrogen receptors (ERα and ERβ) can be identified using docking analysis therefore molecular docking studies were carried out. The minimum binding energy indicated that both proteins (1XP1 and 3OLS) were docked successfully with ligand (table 1 & table 2). The study also reveals that ligand, in terms of binding energy, is more effective than tamoxifene figures 1-4.

Table 1: Binding Energy and Amino acids involved in the interactions

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Compound</th>
<th>Binding Energy (kcal/mol)</th>
<th>Mode</th>
<th>RMSD (lower bound)</th>
<th>RMSD (upper bound)</th>
<th>Amino acids Involved in H-bond interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1XP1_Ligand</td>
<td>-9.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Asn226, Glu74</td>
</tr>
<tr>
<td>2</td>
<td>3OLS_Ligand</td>
<td>-9.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Glu432</td>
</tr>
<tr>
<td>3</td>
<td>1XP1_Tamoxifen</td>
<td>-6.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Pro19</td>
</tr>
<tr>
<td>4</td>
<td>3OLS_Tamoxifen</td>
<td>-6.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Glu69</td>
</tr>
</tbody>
</table>

Figures1: (1XP1_Compound).
CONCLUSION

This paper describes the “Synthesis and molecular docking study of novel compound”. Compound was synthesized and characterized by using spectroscopic and elemental analysis etc. Molecular docking study revealed that ligand exhibit good binding affinities on ERα and ERβ (-9.6 kcal/mol and -9.1 kcal/mol respectively) and those were comparable with standard Tamoxifen drug. The molecular docking results highlight that the synthesized compound would be consider as possible hit as therapeutic agent.

Table: 2 Grid Size taken for docking between receptor and ligand.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Complex</th>
<th>Grid center X</th>
<th>Grid center Y</th>
<th>Grid center Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1XP1_Ligand</td>
<td>X = 20.7301586205</td>
<td>Y = 2.91052117237</td>
<td>Z = 22.1098</td>
</tr>
<tr>
<td>2</td>
<td>13OLS_Ligand</td>
<td>X = 11.6315841124</td>
<td>Y = -24.7463403588</td>
<td>Z = 0.7637</td>
</tr>
<tr>
<td>3</td>
<td>1XP1_Tamoxifen</td>
<td>X = 24.5359170961</td>
<td>Y = 3.40238797969</td>
<td>Z = 22.1098</td>
</tr>
<tr>
<td>4</td>
<td>3OLS_Tamoxifen</td>
<td>X = 11.1849104282</td>
<td>Y = -24.7361400709</td>
<td>Z = 0.7637</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENT
We are thankful to MPCST, Bhopal for financial support (scheme no. 4566/cst/ R&D/2010) and SAIF Chandigarh, Punjab University, Chandigarh for providing spectral data.

REFERENCES
1. Thomas C and Gustafsson JA., The different roles of ER Subtypes in cancer biology and therapy, Nature Reviews Cancer, 2011; 11(8): 597-608,
