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Molecular Mapping and Docking Analysis for Selectivity of Anti-fertility Potency of Estrogen Analogs

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Abstract— **Considering the long-term benefit-to-risk ratio of estrogen analogs as oral contraceptives, the present study has been focussed to deduce the active pharmacophore features required to differentiate the anti-fertility potency from estrogenic activity of steroidal motif. Conformational rigidity and presence of hydrogen bond acceptors have been deduced to be the distinguishing features of estrogenic and anti-fertility activities as revealed from the molecular field and similarity analyses and receptor-dependent docking studies.**

Keywords-Estrogen analogs, estrogenic and anti-fertility potencies, molecular field and similarity analyses, molecular docking

I. INTRODUCTION

The action of estrogen, a prime reproductive hormone, is mediated through estrogen receptor (ER), belonging to the class of nuclear receptors [1], acting via a series of cell signaling pathway. Three major naturally occurring estrogens – estradiol (E2), estriol (E3) and estrone are orally active, but are degraded rapidly [2]; however synthetic estrogens are orally effective and used for hormone replacement therapy (HRT), treatment of breast and prostate cancer, menstrual disorder, endometrial carcinoma, thromboembolic diseases, etc and as contraceptives at high doses, but are associated with untoward effects including thrombic disorder, hypertension and certain types of malignancy [3].

The scope of molecular modeling has allowed the search for novel contraceptive with improved toxicity profiles. Attempts range from the use of simple 2D graph theoretical approaches to highly sophisticated 3D techniques [4-9], including docking studies. Considering the long-term benefit-to-risk ratio of steroidal estrogen analogs as oral contraceptives, a number of potential contraceptive agents with significant anti-fertility activity accompanied by reduction of other estrogenic activities have been developed. SAR studies show that alkyl or allyl substituent on silicon side chain produces potent oral anti-fertility activity with reduced oral estrogenic activity [10]. Van der Waals volumes of estrogen analogs show useful correlation with the biological activity [3]. Classical QSAR models and receptor-independent pharmacophore hypotheses have been explored previously [8]. Consequently, the present work is taken up to explore 3D pharmacophore contour maps to deduce the selectivity requirement for differentiating the anti-fertility potency from the estrogenic activity considering the steroidal scaffold as small ligand [10-12] based on molecular fields and similarity studies. The analyses are further validated with the receptor-ligand interaction study at the active site cavity of ER.

II. MATERIALS AND METHODS

In the present work, 53 compounds belonging to the silicon-substituted analogs of ethynyl estradiol [10], 31 compounds to the analogs of {(triethyl) ethynyl}estradiol [11] and 43 compounds to 17-deoxy estrogen analogs [12] have been considered, and are divided into training (Tr, n_E = 98 for estrogenic (E) potency, n_A = 60 for anti-fertility (A) potency) and test (Ts, n_E = 24 and n_A = 20) sets for 3D QSAR models development. The E and A potencies of these compounds are considered as biological activities and implemented as logarithmic function, pE [$\log_{10}$ (1000/E)] and pA [$\log_{10}$ (A)] respectively for modeling purpose. The general structure of estrogen analogs is depicted in Fig. 1.

The 3D contour maps for anti-fertility and estrogenic potencies are derived from the molecular field [13] and similarity [14] studies. These models are analysed through statistical parameters using PLS approach, such as R^2 (correlation coefficient of PLS analysis without validation), *see* (standard error of estimate), R^2_{CV} (crossvalidated correlation coefficient by LOO method), R^2_{bs} (bootstrapped correlation coefficient) and s_{bs} (Standard deviation for bootstrap). The models are further validated with the test set compounds, estimating R^2_{pred} and *se* (standard error of prediction) [15]. These 3D-QSAR models permit the understanding of steric (s), electrostatic (e), lipophilic (p), and hydrogen bond (HB) acceptor (a) and donor (d) parameters requirement/ arrangement for ligand binding. As a consequence, the structural variations in the compounds that give rise to variation in the molecular fields at particular regions of the space are correlated to the biological properties. The best conformers are considered from pharmacophore hypothesis developed with hypogen [16] and used to generate molecular fields and similarity indices. Partial atomic charges are calculated after energy minimization, using the Tripos force field [17] method, and subsequently used for CoMFA and CoMSIA studies.

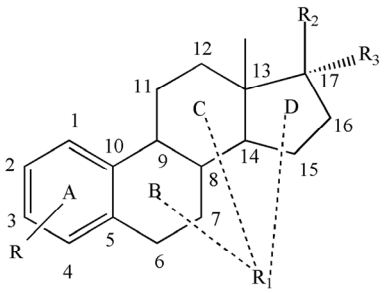


Figure 1. General structure of estrogen analogs.

Receptor-dependent molecular docking study highlights the binding interaction at the active site residues [18]. Crystal structure of estrogen receptor (PDB code: 3OS9) [19] of its LBD and reference protein coordinates used for docking has been obtained from RCSB Protein Data Bank (http://www.rcsb.org/pdb/). The docking study has been performed in Discovery Studio 1.7 [20] by using LigandFit of 'Receptor-ligand interactions' protocol. Pre-treatment process for both the active ligand and the receptor are performed with ligand preparation and binding site definition. Constraint parameters used for ligand preparation are ionization change, tautomer and isomer generation; Lipinski filter and 3D generator albeit all the duplicate structures are removed. Finally the docked receptor-ligand complex is analyzed to investigate the type of interactions and compare dock score.

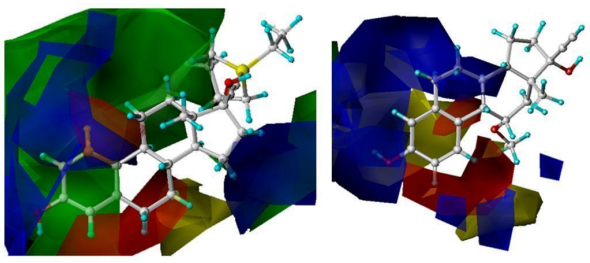
III. Results and Discussion

A. 3D-QSAR study

Molecular field and similarity studies are explored to derive selective pharmacophores for differentiating estrogenic and anti-fertility potencies and permit to develop 3D QSAR models. The constructed models are robust with cross-validated $R^2_{CV} > 0.6$ (0.671, 0.705 for estrogenic and 0.842, 0.775 for anti-fertility potency in molecular fields and similarity studies respectively), which indicate good predictive ability of both the models. The non-cross validated PLS analyses resulted correlation coefficient, $R^2 > 0.8$ and *see* < 0.4 (0.885, 0.372 and 0.936, 0.279 for estrogenic and 0.991, 0.079 and 0.971, 0.142 for anti-fertility potency of fields and similarity analyses respectively). High R^2 value shows the self consistency of the models. The high R^2_{bs} value > 0.9 and low $s_{bs} < 0.3$ on bootstrapping analyses also support the significance of the developed models. The contributions of steric (s) and electrostatic (e) fields for CoMFA models are 50.2% and 49.8% for estrogenic and 51.8% and 48.2% for anti-fertility potencies (Fig. 2A and B) respectively, which indicate that both the fields are predominant for interactions with the enzyme. In CoMSIA study, the contribution of physicochemical parameters are 17.3% (s), 27.0% (d), 27.0% (a) and 28.7% (p) for estrogenic and 19.4% (s), 31.0% (e), 21.8% (a) and 27.7% (p) for anti-fertility properties (Fig. 3A and B). To verify the predictive ability of the models, test compounds (n_E=24 and n_A =20) are used for estimating the predictive correlation. $R^2_{pred} > 0.65$ and *se* < 0.3 indicate good predictive ability of both the models generated in field and similarity analyses. The observed vs predicted estrogenic and anti-fertility activities of the compounds as per CoMFA and CoMSIA models are plotted in Fig. 4.

In case of field analyses, electrostatic fields, blue (favorable) and red (unfavorable) contours represent 80% and 20% level of contributions for both estrogenic and anti-fertility activities (Fig. 2A and B) respectively. The region around the blue contour indicates that increased positive charges will favor the activity, but negative charges near the red contours may be favorable for the activity. Both the models indicate that negative charged substituents around C_{11} favour both the activities, whereas positive charged substituent on C_2 increases antifertility potency; presence of electron-withdrawing group at C_2 increases hydrophobicity of the molecule, in turn increases estrogenicity. High electron density at C_5 or electron withdrawing groups in the neighbouring positions help to improve both the activities respectively. However presence of red contour around ring A suggests that presence of electrophilic substituents in ring A is essentially required for anti-fertility potency. The regions of green contours (steric favourable) suggest that bulky substituents in those positions may improve the biological activity, while the yellow region (steric unfavourable) indicates that an increased steric bulk is unfavorable for both the activities (Fig. 2A and B). The maps illustrate some astonishing difference between the activities that indicate the increase of steric influences around ring A as well as the whole molecule except at C_7 position is essential for estrogenic activity, on the contrary, steric influence is unfavorable for contraceptive property.

The contour maps of CoMSIA models are depicted in Fig. 3A and B for estrogenic and anti-fertility activities respectively. Steric fields are favourable near C_3, C_4 and C_{17} and in ring C, suggesting the conformational rigidity of the ligand for estrogenic activity, except for C_7, C_8 and C_9 positions showing negative impact for substitutions with bulky group at these positions. However for anti-fertility activity, the contour shows steric unfavourable feature implying flexibility of the ligand to be important for contraception purpose. Importance of hydrophobicity (Fig. 3A and B) is indicated by purple (80% favorable) and orange (20% unfavorable) colors suggesting hydrophobic nature of the ligand to be important for estrogenic activity. The models further indicate magenta (80% favorable) and cyan (20% unfavorable) for acceptor.



A B

Figure 2. Mapped features for most active compounds in CoMFA study. (A) Estrogenic activity and (B) anti-fertility activity.

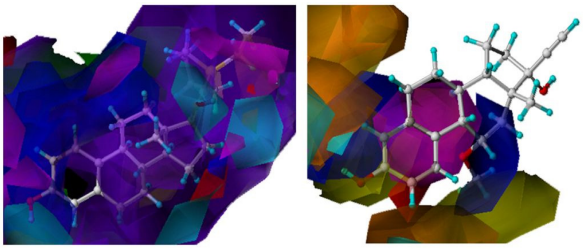
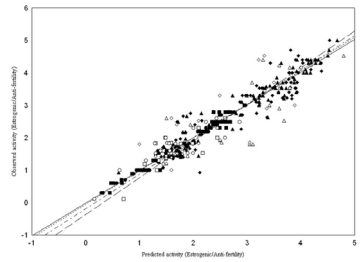


Figure 3. Mapped features for most active compounds in CoMSIA study. (A) Estrogenic activity and (B) anti-fertility activity.

Presence of HB 'a' at C_{11}, and substituent at C_{17} and C_3 positions enhance estrogenic activity, whereas presence of acceptors at rings A and B favour anti-fertility potency, but acceptor at C_2-substituent will increase charge functionality at atom C_2, that does not favour activity. Donor is represented by blue (80% favorable) and red (20% unfavorable) colours. Substitution with donor group increases electronegativity at C_{16} and presence of donors near rings A and C are required for estrogenicity. The electrostatic fields are represented by blue (80% favorable) and red (20% unfavorable). Negative charged substituents decrease charge functionality at C_2, increase of positive charges in the neighbourhood of C_5 enhance electron density at that position and positive charged substituents can form HB with C_{11} substituent. All of these features are essential for anti-fertility activity. The results are quite correlated with QSAR study and pharmacophore mapping previously performed [19], illustrating the importance of conformational rigidity for estrogenic and flexibility for anti- fertility activities, and importance of HB acceptor at ring A to be determining features for anti-fertility activity from estrogenicity.



Estrogenic: CoMFA, training set, n_E=98, R^2=0.885, Q^2=0.671, *see*=0.372

test set, n_E=24, R^2_{pred}=0.696, *se*=0.319

CoMSIA, training set, n_E=98, R^2=0.936, Q^2=0.706, *see*=0.279

test set, n_E=24, R^2_{pred}=0.666, *se*=0.313

Anti-fertility: CoMFA, training set, n_A=60, R^2=0.991, Q^2=0.86, *see*=0.079

test set, n_A=20, R^2_{pred}=0.688, *se*=0.302

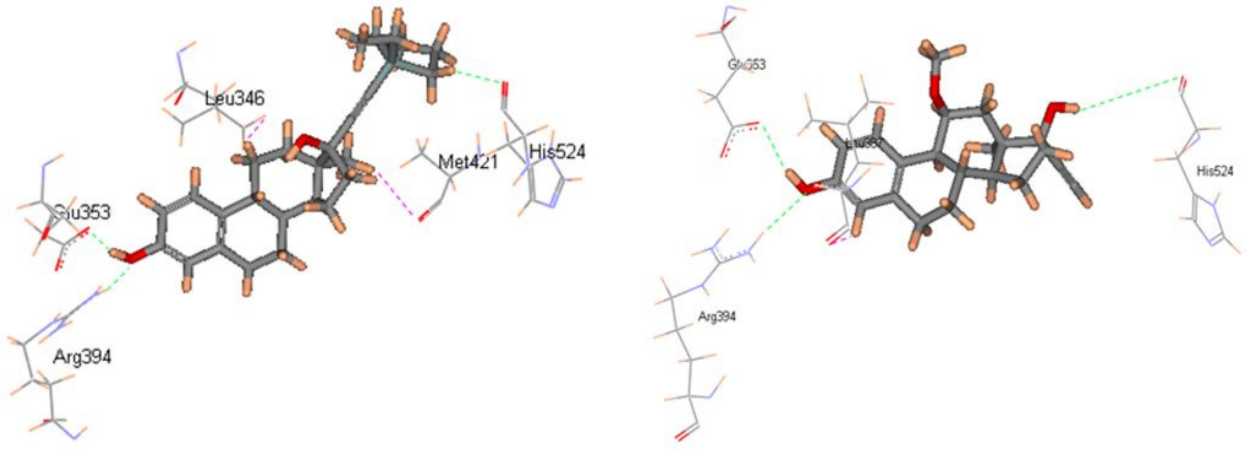
CoMSIA, training set, n_A=60, R^2=0.971, Q^2=0.795, *see*=0.142

test set, n_A=20, R^2_{pred}=0.731, *se*=0.318

Figure 4. Observed vs predicted activity of CoMFA and CoMSIA models

B. Docking study

The most active compounds for both the activities are considered for docking at the catalytic cleft of the receptor (PDB code: 3OS9) [19] in order to explore the binding modes. The essential amino acids in the active site cavity (within 4Å) required for binding interactions are Arg394, His524, Glu353 (polar amino acids) and Leu346, Leu384, Leu387, Met421 (non polar amino acids). The interaction mechanisms revealed from docking study indicate that both type of ligands anchor to the binding pocket by means of HB interactions of electronegative (oxygen) atom and amide backbone of Glu353 and Arg394 respectively with the hydroxyl group located at the phenolic site (C_3) of the



A B

Figure 5. Active ligands estrogenic activity (A) and anti-fertility (B) activities at the binding site of 3OS9 [19]. The catalytic residues have been labeled.

compounds. The bond lengths are 1.917, 2.038 and 3.930, 2.492Å respectively for estrogenic and anti-fertility potencies. Third HB interaction is observed at C_{17} chain, at a distance of 2.562 and 3.680Å with His524 for compounds showing estrogenic and anti-fertility activities respectively. These kind of hydrophobic interactions of the ligands with hydrophobic core of catalytic cleft of receptor are required for both kind of activities, suggesting the importance of presence of hydroxyl groups at C_3 and C_{17} positions. Again estrogenic compound forms two van der Waals interactions with Leu346 and Met421 at distances of 1.73 and 3.41Å respectively, demonstrating influence of conformational rigidity and hydrophobicity of the molecule to be quintessential for estrogenicity. The contraceptive compound shows steric clash of oxygen atom of Leu387 with C_4 atom of ring A of the compound at a distance of 3.123Å. This interaction modulates compound with anti-fertility activity towards improved selectivity. The active ligands along with vital amino acid residues and binding interactions are depicted in Fig. 5A (estrogenic) and B (anti-fertility). The binding interactions at the active site cavity of the receptor are fairly compliant with the molecular field and similarity studies as well as the QSAR and pharmacophore mapping studies.

IV. Conclusion

In view of these observations, the present study accounts for some of important pharmacophoric features of steroidal derivatives for differentiating anti-fertility potency from estrogenic activity. Steric influence on the molecule results in enhanced estrogenic activity, whereas conformational flexibilty may be important feature of anti-fertility activity. Presence of additional HB acceptor on ring A may be crucial to develop novel compounds with improved receptor binding.

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