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An *in-silico* Study of Novel Coumarin Analogues as Huamn Estrogen Receptor Inhibitors

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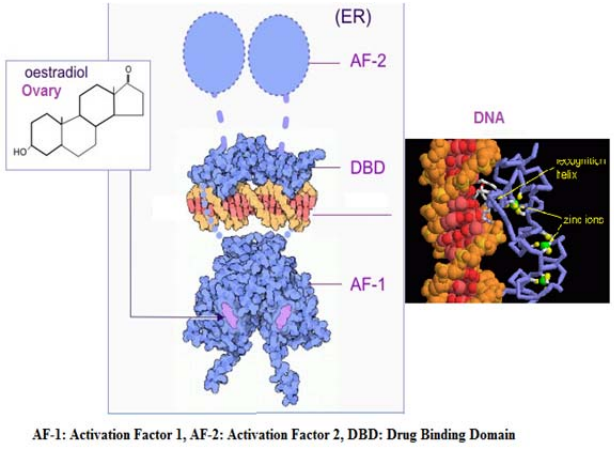
Abstract

The present study focuses a library of coumate analogues as ligands to the ligand-binding domain of the human estrogen receptor α (PDB ID 2IOG) and their binding affinities using GLIDE module of Schrodinger software after ascertaining their drug-likeness with QIKPROP. The compounds **COU 37**, **COU 36** and **COU 2** are the best hits based on their docking scores. Conclusively, *in silico* molecular docking studies have been very useful in predicting the pharmacokinetic profiles and the binding affinities of new hits before a detailed preclinical and clinical evaluation.

Keywords: Estrogen receptor, Schiff bass, Coumates, GLIDE.

INTRODUCTION

Breast cancer has become the most common malignancy and the leading cause for cancer specific death in women [1]. Breast, ovary and gonads produce an abundance of estrogens via aromatase and sulphatase pathway. Estrogen stimulates the proliferation of normal and malignant cell in these organs through estrogen receptor via the induction of nucleic acid synthesis and activation of growth regulatory genes [2]. Most of the post menopausal breast cancer patients have hormone dependent tumours involving the stimulation of estrogen receptor [3] **Figure 1**. A new approach for the successful treatment of post menopausal breast cancers (hormone dependent) involves the use of therapeutic agents that prevents the biosynthesis or physiological actions of estrogens in tumour cells [4]. Tamoxifen, a non steroidal triphenyl ethylene derivative and its analogues have been successfully used in treatment of hormone dependent breast cancer and has become the treatment of choice for this malignancy [5-6]. However they had shown to increase the risk of endometrial cancer [7-8]. Therefore, considerable interest by many research groups has been devoted to the search for more effective novel selective anti estrogens with better safety profiles.



AF-1: Activation Factor 1, AF-2: Activation Factor 2, DBD: Drug Binding Domain

Fig .1. Structure of ER.

The coumarin ring system, present in natural products display interesting pharmacological properties [9-10]. Many molecules based on the coumarin ring system have been synthesized by utilizing innovative synthetic techniques. The diversity oriented synthetic routes have led to the interesting derivatives of coumarin including the furanocoumarins and pyranocoumarins [11] which have been found to be useful in photochemotherapy, antitumor and anti-HIV therapy, central nervous system stimulants, antibacterial, anti-inflammatory, anti-coagulants, and dyes. Of particular interest in breast cancer chemotherapy, some coumarins and their active metabolite 7-hydroxycoumarin analogues have shown significant anti breast cancer activity through inhibition of sulfatase and aromatase enzymes. Coumarin based selective estrogen receptor modulators (SERMs) and coumarin-estrogen conjugates have also been described as potential anti-breast cancer agents [12-13]. A number of potential STS inhibitors are still in preclinical phase of development with only 667 COUMATE (**1**) set to enter clinical trials for the treatment of hormone-dependent breast cancer in postmenopausal women [14]. The structures of coumarin based sulphatase inhibitors are given in **(Fig.2)**.

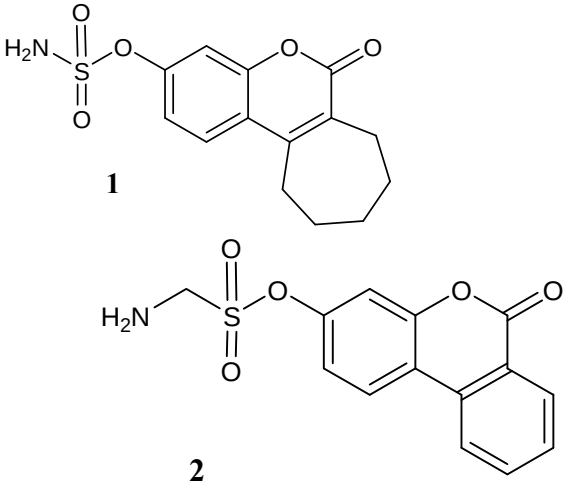


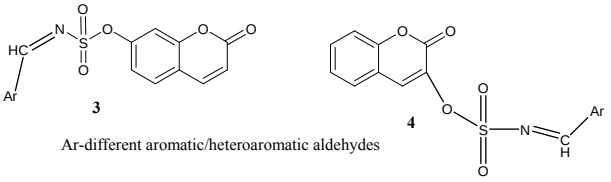
Fig .2. Coumarin based STS inhibitors

The Schiff bases are important class of compounds due to their flexibility, structural similarities with natural biological substances and also due to their presence of imine (-N=CH-) which imparts in elucidating the mechanism of transformation in biological system [15].
Keeping in mind the estrogenic receptor inhibitory properties of coumarin nucleus, it is proposed to design and develop novel pharmacophores containing coumates and Schiff bases and their *in-silico* docking studies towards the human alpha estrogen receptor and *in-silico* ADMET studies.

MATERIALS AND METHODS

In-silico docking study

The fourty compounds having different aromatic and heteroaromatic aldehydes substitution at 4^{th}, and 7^{th} positions of coumate nucleus were designed (**3-4**) and geometrically optimized with the help of Chem office Cambridge software 8.0. The 3D structure of ligands were prepared by using the builder panel in Maestro v11.3 and subsequently optimized using ligprep module (v4.1, schrodinger 2017-3). The energetically minimized analogues were used as input structures for processing in Ligprep 2017-3. An extensive set of conformations were generated using a liquid simulation (OPLS 3) force field in solvent water conditions. The starting coordinates of the human estrogen α receptor domain [PDB ID: 2IOG] were taken from the Protein Data Bank (www.rcsb.org). It was prepared by using the protein preparation wizard (Epik v4.1, schrodinger suite 2017-3). Before protein optimization, the water molecules were removed from the crystal structure, and missing side chain were added by using prime (v4.9 schrodinger 2017-3). The energy minimization of protein had undergone OPLS 3 force field with union of heavy atoms to root mean square deviation (RMSD) of $0.3A^0$. The docking study was performed as per the literature method by Glide integrated Maestro 11.3 [16]. The interaction and selectivity of the designed compounds were observed for alpha ligand-binding domain of ER. The docking procedure was validated by extracting ligand CM4 from the binding site and re-docking it to the ER (PDB: 2IOG).



ADMET studies

In silico, ADME properties of the compounds were calculated using QikProp (v5.3) module of Schrodinger [17]. It helps in predicting both the physically significant descriptors and pharmaceutically relevant properties. Different parameters such as predicted aqueous solubility (Log S), predicted apparent MDCK cell permeability (PMDCK), percent human oral absorption, octanol/water partition coefficient (QP log Po/w), brain/blood partition coefficient (QP log BB), and total solvent accessible surface area (SASA) were calculated. All compounds were neutralized before being used by QikProp. Compounds were also evaluated for acceptability of the inhibitors based on the Lipinski's rule.

Molecular docking

The human estrogen receptor catalytic site prediction was carried out using the cast p program [18]. The program calculated the area and volume of each pocket analytically. The best ligand binding site was observed to be at pocket number 36 of volume 1178.9 Aº3 and area of 901.1 Aº2. This pocket contains 36 amino acid residues, such as Met343, Leu346, Thr347, Leu349, Ala350, Asp351, Glu353, Leu354, Trp383, Leu384, Leu387, Met388, Leu391, Arg394, Phe404, Val418, Glu419, Gly420, Met421, Ileu424, Phe425, Leu428, Gly521, His524, Leu525, Tyr526, Met528, Lys529, Cys530, Lys531, Asn532, Val533, Val534, Pro535, Leu536, Leu539. The same pocket with above amino acid residues have been observed in our study.
To validate the docking protocol, the root mean square deviation (RMSD) between the co-crystallized native ligand and the redocked native ligand should be within 2 angstrom as can be visibly appreciated. The redocked native ligand and co-crystal structure are superimposed and the RMSD was found to be 0.8205. Their superimposition was also correctly reproduced (**Figure 3**) within the binding domain of the target receptor. The Ramachandran plot was generated which revealed that most of the residues (97.8%) were in most favored regions (**Figure 4**).

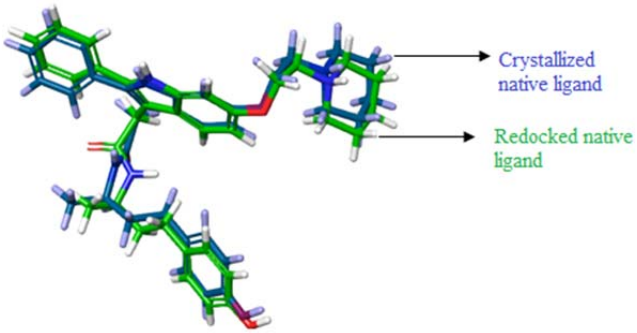


Figure 3. LBD of hERα superimposed with co-crystallized native ligand.

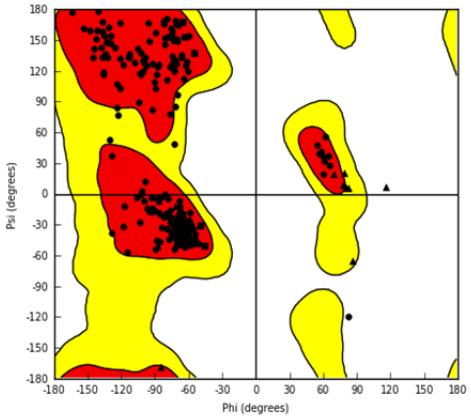


Figure 4. Ramachandran plot

RESULTS AND DISCUSSION

Molecular docking was performed to elucidate the binding mode competence of human estrogen receptor and 40 coumarin analogues. The designed molecules were docked along with the native ligand and a reference standard, Tamoxifen. G- score was used to access the binding affinities of the studied ligands to the target receptor. It was well known that the G- scores greater than −7 kcal/mol is considered as promising, and that it can go as high as −13 or even more [19]. The G-scores of our designed compounds range from −14.2 to -7, indicated good binding affinities to the target receptor and the results are depicted in **Table 1**. The coumarin analogue with di-hydroxy phenyl and mono hydroxy phenyl substitution **COU 37** and **COU 36** were ranked as top scorers.

The roles of certain crucial amino acids in the ligand-binding domain of the human estrogen receptor α (hERα), was also established. Major non-covalent interactions between the studied ligands and the ligand-binding domain of the hERα was investigated. These amino acids have been repeatedly implicated during ligand interaction with the hERα and also play important role in the inhibition of the ligand-binding domain of hERα [20]. These non-covalent interactions Van der Waals, columbic interaction, π-π interaction and hydrogen interaction are shown in **Table 1** and **Figure 4a–d**

The designed compound had appropriate log P (octanol/water) value for biological efficacy with zero to one Lipinski violation. They also had satisfying pharmacological properties of 95% available drugs with high to medium predicted oral absorption availability without any toxic functionality. Molecular weight of each ligand was within the range of 500. Log S values within the acceptable range of 95% of existing drugs. **Table 2**.

Table 1.G- Score from the glide XP docking run of novel compounds in the active site of 2IOG

Comp.Code	G-score	LipophilicEvdW	Hbond	LowMW	RotPenal	Penalties	HB Penal
Tamoxifen	-13.6	-8.355	-0.9	-0.258	0.418	0	0
COU37	-10.499	-6.062	-1.227	-0.289	0.125	0	0
COU36	-10.195	-5.973	-0.747	-0.342	0.135	0	0
COU02	-10.104	-6.567	0	-0.234	0.173	0	0
COU06	-10.075	-5.28	-0.8	-0.295	0.176	0	0
COU08	-10.009	-6.418	0	-0.086	0.142	0	0
COU13	-9.875	-6.571	0	-0.287	0.124	0	0
COU18	-9.839	-6.568	0	-0.139	0.101	0	0
COU16	-9.806	-5.746	-0.526	-0.349	0.143	0	0
COU39	-9.763	-6.276	-0.304	-0.252	0.118	0	0
COU05	-9.647	-6.162	0	-0.199	0.219	0	0
COU32	-9.674	-6.319	0	-0.281	0.123	0	0
COU26	-9.612	-5.768	-0.478	-0.349	0.143	0	0
COU07	-9.702	-5.338	-0.785	-0.242	0.163	0	0
COU28	-9.54	-6.396	0	-0.139	0.101	0	0
COU12	-9.534	-6.153	0	-0.287	0.124	0	0
COU22	-9.504	-6.51	0	-0.287	0.124	0	0
COU14	-9.454	-6.471	0	-0.252	0.177	0	0
COU23	-9.435	-6.294	0	-0.287	0.124	0	0
COU31	-9.457	-6.115	0	-0.281	0.123	0	0
COU17	-9.415	-5.639	-0.687	-0.296	0.132	0	0
COU04	-9.275	-5.849	0	-0.199	0.219	0	0
COU11	-9.261	-6.333	0	-0.287	0.124	0	0
COU20	-9.25	-6.541	0	-0.259	0.179	0	0
COU34	-9.29	-6.068	0	-0.246	0.117	0	0
COU30	-9.224	-6.858	0	-0.202	0.165	0	0
COU33	-9.264	-6.032	0	-0.281	0.123	0	0
COU35	-9.267	-6.044	0	-0.246	0.117	0	0
COU01	-9.218	-5.885	0	-0.234	0.173	0	0
COU25	-9.204	-6.501	0	-0.252	0.177	0	0
COU29	-9.196	-6.449	0	-0.259	0.179	0	0
COU21	-9.168	-6.261	0	-0.287	0.124	0	0
COU40	-9.153	-6.479	0	-0.195	0.109	0	0
COU15	-9.054	-6.409	0	-0.252	0.177	0	0
COU10	-9.043	-5.759	0	-0.149	0.205	0	0
COU19	-8.943	-6.637	0	-0.202	0.165	0	0
COU03	-8.927	-5.533	0	-0.234	0.173	0	0
COU27	-9.048	-5.68	-0.499	-0.296	0.132	0	0
COU24	-8.901	-5.941	0	-0.252	0.177	0	0
COU09	-7.193	-5.13	0	-0.205	0.221	0	0

Table 2. Predictions of ADMET for compounds by QIKPROP 5.3

Compound code	QPlogPw	QPlogPo/w	QPlogS	QPlogBB	QPlogKp	IP	HOA	PSA	Rule Of Five
COU37	17.953	-0.165	-2.912	-2.557	-5.674	0	2	157.366	0
COU36	16.01	0.391	-3.271	-2.274	-5.052	0	2	137.369	0
COU02	11.938	1.348	-2.647	-1.21	-3.166	0	3	97.265	0
COU06	14.286	0.657	-2.946	-2.01	-4.147	0	3	120.017	0
COU08	11.838	1.455	-2.543	-1.094	-3.166	0	3	97.493	0
COU13	11.783	1.357	-2.23	-0.997	-2.638	0	3	94.355	0
COU18	11.795	1.434	-2.353	-0.995	-2.645	0	3	94.227	0
COU16	14.38	0.841	-2.824	-1.753	-3.17	0	3	118.316	0
COU39	14.353	1.438	-4.112	-1.893	-4.231	0	3	118.111	0
COU05	13.335	0.165	-2.174	-2.502	-4.978	0	2	142.967	0
COU32	13.736	1.466	-3.579	-1.517	-4.046	0	3	114.775	0
COU26	14.336	0.682	-2.746	-1.904	-3.576	0	3	119.953	0
COU07	16.542	0.323	-3.23	-2.496	-4.634	0	2	142.159	0
COU28	11.971	1.475	-2.717	-1.138	-2.725	0	3	95.799	0
COU12	12.023	1.538	-2.509	-0.955	-2.227	0	3	96.34	0
COU22	12.026	1.361	-2.545	-1.169	-2.696	0	3	98.038	0
COU14	13.086	0.157	-1.286	-1.99	-4.128	0	2	138.234	0
COU23	11.962	1.396	-2.597	-1.144	-2.723	0	3	95.806	0
COU31	13.672	1.536	-3.744	-1.538	-4.16	0	3	114.834	0
COU38	13.679	1.607	-4.142	-1.537	-4.169	0	3	114.868	0
COU17	16.251	0.199	-2.48	-2.134	-4.065	0	2	136.729	0
COU04	13.505	0.425	-2.444	-2.376	-4.458	0	2	142.603	0
COU11	11.857	1.476	-2.39	-0.912	-2.328	0	3	93.386	0
COU20	12.443	1.301	-2.248	-1.25	-2.551	0	3	97.208	0
COU34	14.93	0.522	-3.347	-2.393	-5.318	0	2	157.889	0
COU30	12.534	0.911	-1.868	-1.438	-2.788	0	3	111.486	0
COU33	13.677	1.541	-3.756	-1.539	-4.158	0	3	114.869	0
COU35	15.036	0.369	-3.562	-2.756	-5.897	0	2	159.859	0
COU01	11.95	1.402	-2.847	-1.253	-3.249	0	3	97.452	0
COU25	13.376	0.188	-1.983	-2.396	-4.423	0	2	142.422	0
COU29	12.507	1.17	-2.319	-1.43	-2.868	0	3	100.15	0
COU21	11.798	1.279	-2.243	-1.071	-2.771	0	3	96.073	0
COU40	14.392	1.261	-3.842	-1.906	-4.185	0	3	130.534	0
COU15	13.415	0.351	-2.062	-2.24	-4.014	0	3	140.774	0
COU10	12.826	1.236	-2.78	-1.554	-2.95	0	3	114.279	0
COU19	12.596	1.092	-2.024	-1.283	-2.366	0	3	109.007	0
COU03	11.827	1.383	-2.418	-1.098	-3.172	0	3	97.498	0
COU27	16.404	0.234	-2.749	-2.299	-4.156	0	2	139.634	0
COU24	13.08	0.168	-1.261	-1.954	-4.04	0	2	141.201	0
COU09	12.785	1.511	-3.189	-1.505	-2.958	0	3	102.576	0
Standard Values	*4- 45*	*2-6.5*	*-6.5-0.5*	*-3-1.2*	*-8 to -1*	*-7.9-10.5*	*-1.5to+1.5*	*7-200*	*0-4*

QP log Pw Predicted water/gas partition co-efficient HOA predicted human oral absorption
QP log Po/w Predicted octanol/water partition co-efficient PSA percent human oral absorption
QP log S Predicted aqueous solubility
QP log BB Predicted brain/blood partition coefficient
QP log Kp Predicted skin permeability IP PM3—calculated ionization potentia
HOA Predicted human oral absorption
PSA Percent human oral absorption
Rule of 5 Number of violations of Lipinski's rule of five

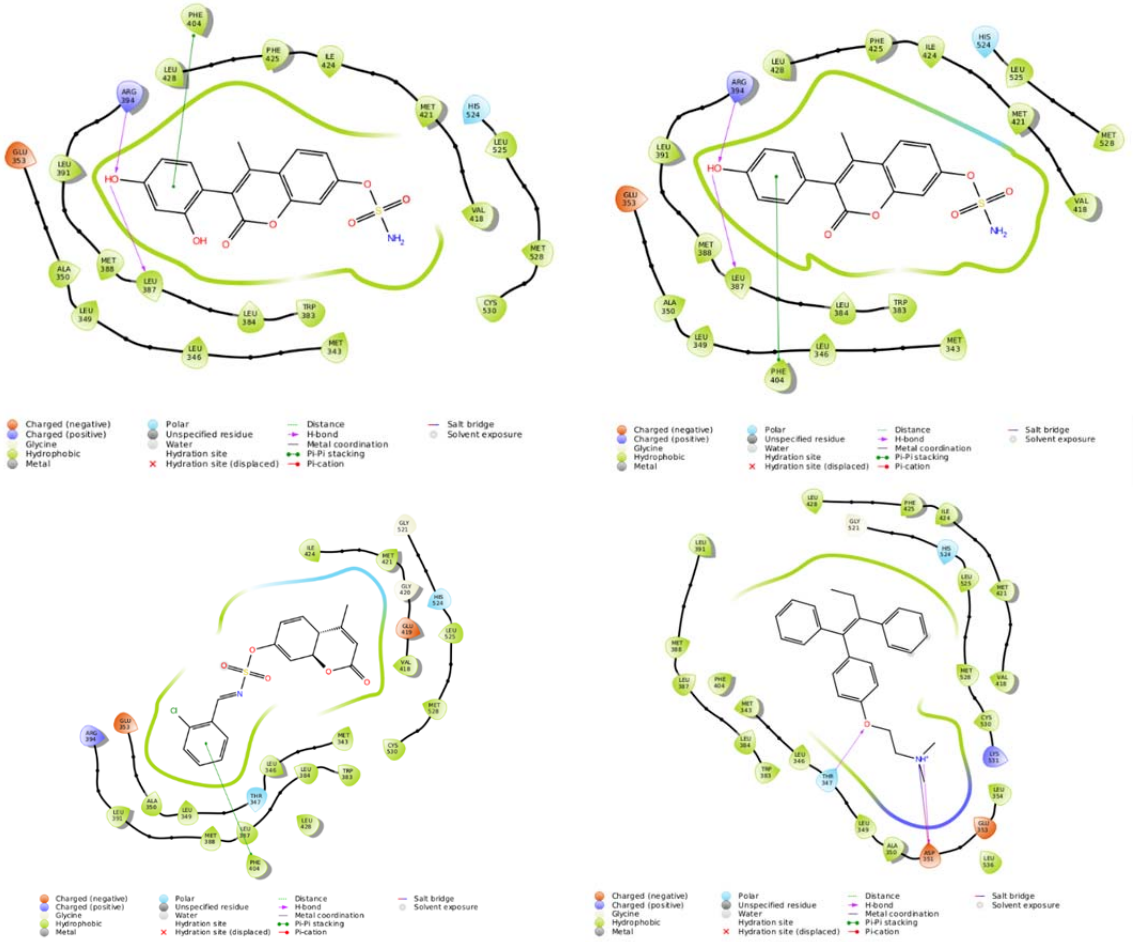


Figure 4a-4d Molecular interaction studies ligands with amino acid at the ligand binding domain of ERα. a. COU37 8, b. COU36, c. COU2 2, d.Tamoxifen.

CONCLUSION

This study has revealed potent estrogen receptor inhibitors with good predicted pharmacokinetic profiles that may be further investigated for their *in vitro* as well as *in vivo* activity towards estrogen receptor positive breast cancer. Structure-activity relationship studies of the "locking effect" of the lactone ring structure of the COUMATE has confirmed that the conformational restriction of the conjugated C=C bond plays an important role in the potency inhibitory activity displayed by coumarin based compounds in addition to the overall size of the inhibitors.

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