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# A STRATEGY TO TREAT THE BREAST CANCER THROUGH INHIBITING THE OVER EXPRESSION OF PROTEIN ESTROGEN *VIA* SCHIFF BASE FUSED COUMARIN: AN *IN-SILICO* BASED SYNTHETIC APPROACH

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**Keywords:**

Breast cancer, Estrogen, Coumarin fused Schiff base, *in-silico* study, *in-vitro* cytotoxicity

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**ABSTRACT:** Coumarin and its analogs have a wide range of attention in the treatment of hormone-dependent breast cancer by blocking the formation of estrogen through the inhibition of estrogen receptor. However, the Schiff bases are an important class of compounds with structural similarities of natural biological substances and also due to their presence of imine (-N=CH-), which have an impact on a biological system. Considering the above all facts, the present research work is aimed to design the series of building blocks of coumarin Schiff base moiety as target candidates for estrogen receptor. The designed molecules ensured their reliability through the *in-silico* drug designing model and subjected to a preliminary study by screening their violation of Lipinski rule of five, if any, predicted for their ADMET profile study by using online available tools. Later an attempt was made to synthesize the proposed compounds, and structural elucidation was done by IR, NMR, and Mass spectroscopy. Besides to this, an *in-vitro* cytotoxicity study by using human breast cancer cell lines also carried out to evaluate the anti-tumor potency of the synthesized coumarin conjugates as a promising candidate for treating the breast cancer. The results obtained from these tools show there is no Lipinski rule violation, and no toxicity is predicted for these synthesized compounds. Thus, the designed coumarin Schiff base derivatives might serve as the best leads for treating the breast cancer, which is additionally confirmed by performing their docking study via Accelrys discovery studio for inhibiting the production of estrogen.

**INTRODUCTION:** There is a cancer burden worldwide and have been raised to 18.1 million of new cases, and among them, 9.6 million deaths were recorded in the year of 2018.

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The increased case of cancer appearance is owing to several factors, together with population growth and aging, as well as the changing prevalence of certain causes of cancer linked to social and economic development, the presence of drug resistance, *etc*.

This leads to the search for a newer therapeutic agent with divergent and unique structure and with a mechanism of action possibly different from that of existing drugs that are urgently required to tackle the menace [1, 2].

However, when approaching breast cancer among the women community, it is the most frequent one, which impacts the 2.1 million women every year, and the reason for the greatest number of deaths. Upon the breast cancer survey done by WHO, in 2018, there were 627,000 women died from breast cancer. While breast cancer rates are higher among women in more developed regions, rates are increasing in nearly every region globally [3].

Breast, ovary, and gonads produce an abundance of estrogens *via* aromatase and sulphatase pathway. Estrogen stimulates the proliferation of normal and malignant cells in these organs through the estrogen receptor *via* the induction of nucleic acid synthesis and activation of growth regulatory genes[4]. Hormone therapy is a part of systemic therapy to treat various types of cancer. It works by either blocking the body's natural hormones or lowering the levels of those hormones, which can act to promote the growth of some cancers. Estrogen, a hormone produced by the ovaries in addition to other tissues, promotes the growth of HR+ breast cancers. Patients with these tumors can be given hormone therapy to lower the estrogen levels or block the effects of estrogen on the growth of breast cancer cells. The tamoxifen is a treatment that blocks the effects of estrogen in breast tissue but has estrogenic effects in other tissues, such as the liver, uterus, and bones. Tamoxifen can be used to treat both early- and advanced-HR+ breast cancer in both pre- and postmenopausal women [5]. Fulvestrant is another treatment used to treat metastatic breast cancer; moreover, it is an anti-estrogen drug given by intramuscular injection that reduces the number of estrogen receptors and blocks estrogen binding [6].

Based on the above fact, there is an urgent need to search for a novel potential compound that could fight breast cancer fruitfully. However, many research findings discuss cancer and its treatment. Currently, there is a hub who was actively participating in the anti-estrogen search and going to patent the drug molecule for the treatment of cancer, especially breast cancer [7]. These findings emerged us to go for synthesizing the novel coumarin fused Schiff base for prophylactic of breast cancer. Many research reports suggested that depending upon the structure of coumarins and their derivatives; they can act on various tumor cells by diverse mechanisms *viz*. DNA intercalating agents, DNA cross-linking agents, inhibition of the telomerase enzyme, topoisomerase, inhibition of protein kinases, reducing the hormones and down-regulation of oncogenes expression or induce the caspase-9- mediated apoptosis which suppress cancer cell proliferation by arresting cell cycle in G0/G1 phase, G2 /M phase and affecting the cancer cells [8]. Moreover, 7- methoxy- 8- isopentenyl coumarin, showed potential activity against lung cancer A549 cells and breast cancer cells line by arresting the cell cycle in G2 phase followed by inducing apoptosis through modulating PI3K/Akt pathway [9].

In recent years, the molecular hybridization strategy has emerged as a novel approach that involves the assembly of two or more pharmacophores in one molecular scaffold to develop hybrid multifunctional molecules. Such molecules have numerous biological activities with reduced undesired side effects due to the mixing of pharmacophores in one molecule and may be further modified to exhibit favorable pharmacokinetics and oral bioavailability. Using this approach, several researchers designed and synthesized the many hybrid scaffolds. Hybridization or coupling of different coumarin derivatives with varied bioactive molecules such as sulfonamides, pyrazoline, chalcone, triazoles, etc. has produced the novel hybrid molecules, which are endowed with vasorelaxant, platelet anti-aggregating, anticancer [10], monoamine oxidase-B (MAO-B) inhibitory, antimicrobial, antioxidant and anti-inflammatory properties. As a result, molecular hybridization approach is playing a vital role in the development of novel molecules for the treatment of numerous multifactorial diseases. Moreover, the hybridizing the coumarin nucleus with other heterocyclic moieties leads to new molecules with improved anticancer activity profile[11].

Recently, the 3- and 4-heteroarylcoumarins have been reported to exhibit significant biological activities such as anticancer, antimicrobial, antibacterial, anticancer (DNA cleavage), human monoamine oxidase inhibitory, antioxidant and anticholinesterase [12, 13]. On the other hand, azo-based heterocycles are interesting compounds due to their high chemotherapeutic potential. However,

diversely substituted hydrazine combined with coumarin system showed good cytotoxic and anti-proliferative activities toward a wide range of human tumor cell lines. For example, coumarin derivatives bearing 4,5-dihydro pyrazole moiety possess high antiproliferative activity [12, 13]. Creaven *et al*., [14] reported that the coumarin-derived Schiff bases and their copper (II) complexes, were cytotoxic to the breast cancer-derived MCF-7 mammalian cell line, at concentrations which were comparable to the cytotoxic activity of the commercially used drug, mitoxantrone. Apart from this, there are most of the Schiff base shows a crucial role in observing the conversion mechanism and racemization reaction in biological systems [15-21]. Many study reports state that there is a wide number of Schiff bases have been used for various essential biological activities, including antitumor, anti-HIV, antibacterial, antifertility activities, anti-mosquito larval, anti-inflammatory, and anti-cancer[22-29].

Keeping this point discussed in the above paragraph, based on the previously reported research work elsewhere [30, 31], and in view of our long-standing interest in the chemistry of the advantaged coumarin fused Schiff base scaffold, the present study encouraged us to explore the diverse coumarin motif as an active lead to target the cancerous cells. Hence, we have decided to combine the two pharmacophores Schiff base along with coumarin for testing their anti-tumor potency on human breast cancer cell line. There are many kinds of literature revealed that the Schiff bases are the key intermediates for the synthesis of numerous bioactive medicinal compounds from the primary amine [32]. In recent years, the chemistry of Schiff bases contains N-donor atom, which has been extensively studied and has acquired a great interest because of the azomethine RHC=N-R1 linkage, where R and R1 are alkyls, aryl, cycloalkyl, or heterocyclic groups which is essential for its biological activity [33, 34]. Also, now a day, the search for suitable cancer treatment has been going viral through computational biology, which is a trending tool to improve the domain of computer-aided drug designing. Before stepping into its synthesize and their biological evaluation on breast cancer cell lines, we have decided to go for their *in-silico* docking study for its affinity toward the receptor protein estrogen receptor.

To support this study further, the classical synthesis of these compounds involves the cyclization reaction of coumarin with assorted hydrazine such as hydrazines, phenyl-hydrazine, 2, 4 -dinitro phenylhydrazine, *etc*. to afford the diverse coumarin incorporated Schiff base [35].

## MATERIALS AND METHODS:

**Designing the Compound through *in-silico* Study:** The Schiff bases of 4-hydroxy coumarin with hydrazine and its derivatives were designed to evaluate the interaction with protein estrogen through the docking software Accelrys discovery studio.

**Ligand Preparation:** The chemical structures of the designed compounds to be docked were generated, corrected, verified, and optimized using chem sketch software and converted to 3D form. Then by using the "prepare ligand" protocol, the structures of the ligand were prepared for docking. All the tautomer's and isomers of the ligand were regarded, and an isomer or tautomer of the ligands possessing the lowest energy was approved for docking. All the ligands generated were followed the Lipinski's rule **Table 1**.

**TABLE 1: PREDICTION OF MOLECULAR PROPERTIES *VIA* LIPINSKI'S RULE**

| Compound Code | No. of H acceptors | No. of H donors | A log P | Molecular weight |
|---|---|---|---|---|
| D1 | 4 | 3 | 0.924 | 176 |
| D2 | 10 | 2 | 3.01 | 338 |
| D3 | 4 | 2 | 3.221 | 253 |

**Protein Preparation:** The crystal structure of protein estrogen receptor (PDB ID: 1HCQ) was downloaded from RCSB PDB and was prepared by eliminating the unessential water molecules, heteroatoms present, small ions, and alternate confirmations; completing the structure by modeling the missing loops; inserting the missing atoms standardizing atom names, protonating titratable residues using predicted pKa's. Checking the potential energy, van der Waals energy, Electrostatic energy, and RMS gradient of the complex before and after protein minimization and then finally merging the hydrogen receptors to the target receptor molecule using discovery studio 4.1 client and validating the protein generated through Ramachandran plot.

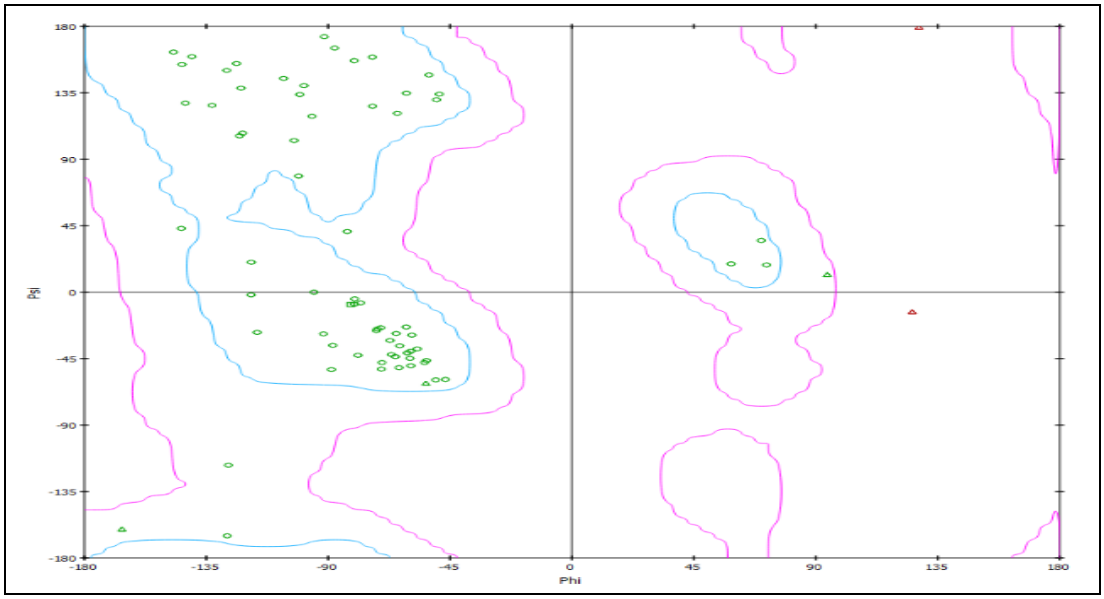



**FIG. 1: RAMACHANDRAN PLOT OF 1HCQ ESTROGEN RECEPTOR**

**Active Site Identification of Estrogen Receptor:** The estrogen receptor complex (1HCQ) was analyzed using discover studio version 4.1 and the active sites were identified out of which the eleven prominent sites were identified, and they were SER23, CYS24, GLU25, GLY26, CYS27, LYS28, ALA29, PHE30, PHE31, LYS32, ARG33 **Fig. 2**.

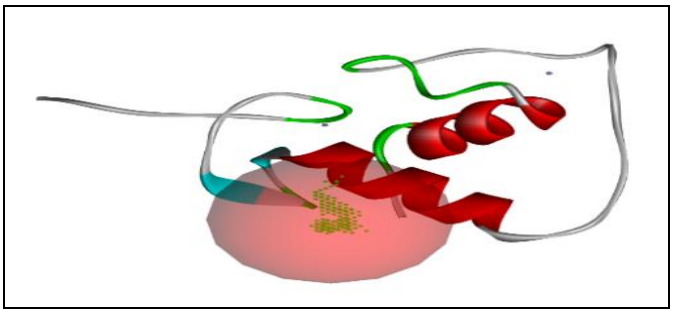


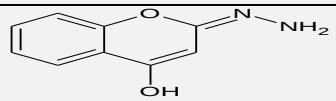
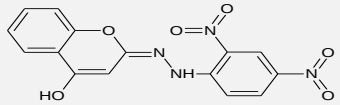
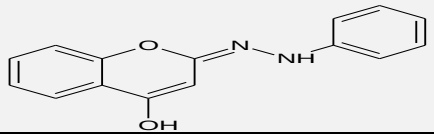
**FIG. 2: BINDING SITES OF 1HCQ ESTROGEN RECEPTOR**

**Molecular Docking Stimulation:**

**Cdocker studies:** The interaction of the many ligands with a single protein where the ligands stay flexible and protein is rigid was evaluated. The minimized structure of the designed compound was used as input ligand. Cdocker energies were calculated for each compound. The type of interaction has existed between the ligands, and protein was predicted using Cdocker protocol.

**ADMET Studies:** ADME properties of the compounds were calculated using QikPro (v5.3) module of Schrodinger. Solubility of the drugs in water at 25 °C, human intestinal adsorption level after oral administration, metabolism of the administered drug by the inhibition enzyme Cytochrome P450 2D6 (CYP2D6) using 2D input, hepatoxicity of the drug, Plasma protein binding extent, 95% and 99% confidence ellipses in the ADMET_PSA_2D, ADMET_AlogP98 plane were calculated. Based on the results obtained from the *in-silico* study, we have planned to synthesize the three diverse compounds, and those were presented in **Table 2**.

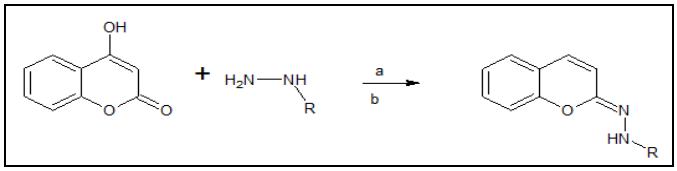
**TABLE 2: LIST OF COMPOUNDS TO BE SYNTHESIZED**

| Compound code | Structure | Molecular formula | IUPAC name |
|---|---|---|---|
| D1 |  | $C_9H_8O_2N_2$ | (2E)-2-hydrazinylidene-2H-chromen-4-ol |
| D2 |  | $C_{15}H_{10}O_6N_4$ | (2E)-2-[2-(2,4-dinitro phenyl)-Hydrazinylidene]-2H-chromen-4-ol |
| D3 |  | $C_{15}H_{13}O_2N_2$ | (2E)-2-(2-phenyl Hydrazinylidene )-2H-chromen-4-ol |

**Synthesis of Schiff Bases of Coumarin:**

**Experimental Section:** All the chemicals in this synthesis were of AR and LR grade and were obtained from Merck, Hi-Media, and Sigma-Aldrich, SD Fine chem., Mumbai. The melting points were determined in open capillaries on a Thomas Hoover apparatus and were uncorrected. The synthesized compounds were characterized by the following methods IR spectra of synthesized compounds were recorded on Shimadzu (8300, Kyoto, Japan) Fourier transform infrared spectrophotometer in the range of 4000 $cm^{-1}$ – 400 $cm^{-1}$ using KBr pellet technique.

**General Procedure for the Synthesis of Coumarin Schiff Base Analogs (D1-D3):** A mixture of 4- hydroxycoumarin (1g), hydrazine/ hydrazine derivative (600 mg) is taken in a round-bottomed flask in the molar ratio 1:1.2:1.2. To this 10 ml of ethanol was added, and it was refluxed for 2 h at 78 °C and stirred using magnetic stirrer with a hot plate. The residue obtained was subjected to TLC to determine the completion of the reaction and then poured into crushed ice with continuous stirring, and the precipitate obtained was filtered washed with cold water and air-dried. The product was recrystallized from absolute ethanol. The reaction sequences for synthesized compounds leading to the formation of new compounds are outlined in **Scheme 1** [36].




**SCHEME 1: SYNTHESIS OF COUMARIN SCHIFF BASE ANALOGS**

**Reagents and Conditions:** a) 20 ml of ethanol, b) 2 h reflux.

**Substitution Pattern for R:** D1- 2,4- dinitro phenylhydrazine; D2- Phenylhydrazine; D3- Hydrazine.

***In-vitro* Cytotoxicity Activity:**

**MTT Assay:** The cell line used for this study was MCF-7 (Breast carcinoma, Human). The monolayer cell culture was trypsinized, and the cell count was adjusted to 1.0 × 105 cells/ml using DMEM medium containing 10% fetal bovine serum (FBS). To each well of 96 well microtitre plates, the 100µl of diluted cell suspension was added. The supernatant was brushed upon the formation of the partial monolayer at after 24 h, the monolayer was cleansed once again with medium, and 100 µl of different test sample concentrations (250-15.625 µg/ml) prepared in maintenance media was added per well to the partial monolayer in microtitre plates. Then the plates were incubated at 37 °C for 48 h in a 5% $CO_2$ atmosphere, and the microscopic examination was carried out and recorded every 24 h. After 48 h, the sample solutions in the wells were discarded, and 20µl of MTT in MEM-PR (MEM with phenol red) was added to each well. The plates were gently shaken and incubated for 3 h at 37 °C in 5% $CO_2$ atmosphere. The supernatant was removed, and 50µl of isopropanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage of growth inhibition was calculated using the following formula, and the concentration of drug samples needed to inhibit the cell growth by 50% was found from the dose-response curves [7].

Cell viability = Mean OD of individual test group × 100 / Mean OD of the control group

## RESULTS AND DISCUSSION:

**Cdocker Results:** The spatial arrangement of the synthesized compounds in the estrogen receptor was proposed by the *in-silico* docking study. The crystalline structures of the estrogen receptor (PDB ID: 1HCQ) [37] were used for the identification of possible binding modes of the synthesized coumarin derivative. The ligand was docked into the predefined cyclooxygenase active site of both estrogen receptor. The detailed results were demonstrated in **Table 3**.

**TABLE 3: LIGAND PROTEIN INTERACTION DOCKING SCORE**

| Compound code | Cdocker Interaction Energy |
|---|---|
| D1 | 13.7681 |
| D2 | 21.6917 |
| D3 | 18.4268 |
| Fulvestrant | 32.5392 |
| Tamoxifen | 20.3513 |

In compound D1, the hydrogen bond interaction was seen with LYS28, the hydrophobic bond interaction was seen with ALA29, LYS32, and electrostatic bond interaction were noticed with

LYS28, LYS32. Whereas in compound D2, the hydrogen bond interaction was seen with LYS28, a hydrophobic bond interaction was seen with TYR19, ALA29, LYS32, and an electrostatic bond interaction were seen with LYS28, LYS32. However, the compound D3 exhibited a hydrogen bond with LYS28, hydrophobic bond with TYR19, LYS32, and the electrostatic bond with LYS32. The interactions results were shown in **Table 4**. The 2D & 3D diagram of the binding mode was represented in **Fig. 3a-3c**.

**TABLE 4: LIGAND PROTEIN INTERACTION ENERGIES FOR COMPOUNDS D1, D2 & D3**

| Compound | Hydrogen bond | | Hydrophobic bond | | Electrostatic bond | |
|---|---|---|---|---|---|---|
| | From | To | From | To | From | To |
| D1 | A:LYS28:HE2 | Div1:O9 | Div1 | A:ALA29 | A:LYS28:NZ | Div1 |
| | | | Div1 | A:LYS32 | A:LYS28:NZ | Div1 |
| | | | | | A:LYS32:NZ | Div1 |
| | | | | | A:LYS32:NZ | Div1 |
| D2 | Div2:H32 | Div2:O22 | A:TYR19 | Div2 | A:LYS28:NZ | Div2:O22 |
| | A:LYS28:HE1 | Div2:O22 | Div2 | | A:LYS28:NZ | Div2 |
| | A:LYS28:HE2 | Div2:O9 | Div2 | A:ALA29 | A:LYS32:NZ | Div2 |
| | A:LYS28:HE2 | Div2:O22 | | A:LYS32 | A:LYS32:NZ | Div2 |
| D3 | A:LYS28:HE1 | Div3:O9 | A:TYR19 | Div3 | A:LYS32:NZ | Div3 |
| | A:LYS28:HE2 | Div3:N12 | A:TYR19 | Div3 | | |
| | | | Div3 | A:LYS32 | | |

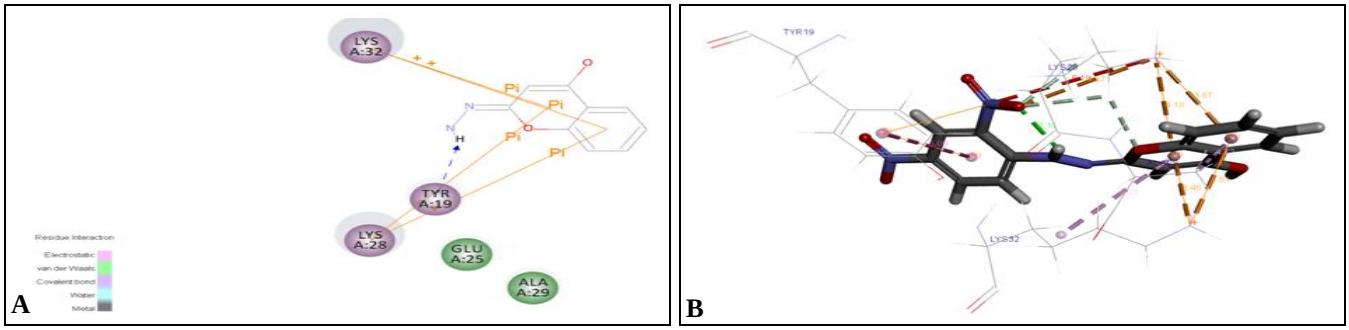



**FIG. 3A: LIGAND PROTEIN INTERACTION ENERGIES FOR COMPOUND D1**

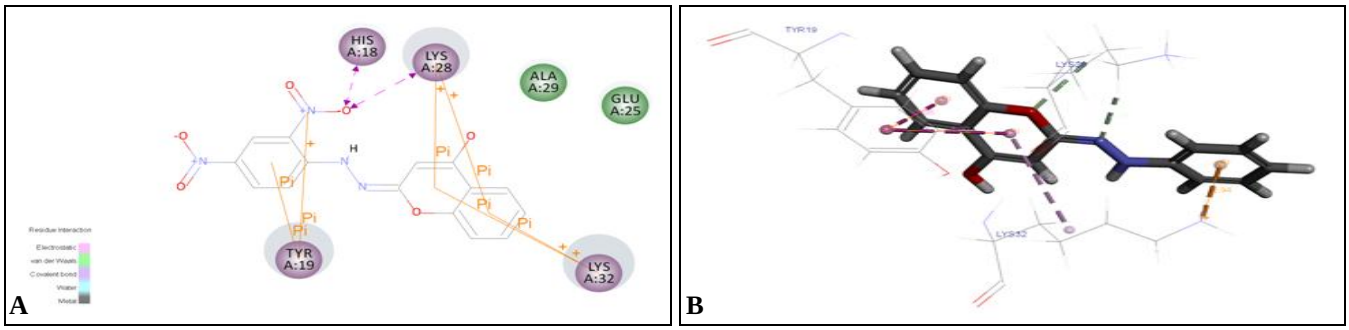



**FIG. 3B: LIGAND PROTEIN INTERACTION ENERGIES FOR COMPOUND D2**

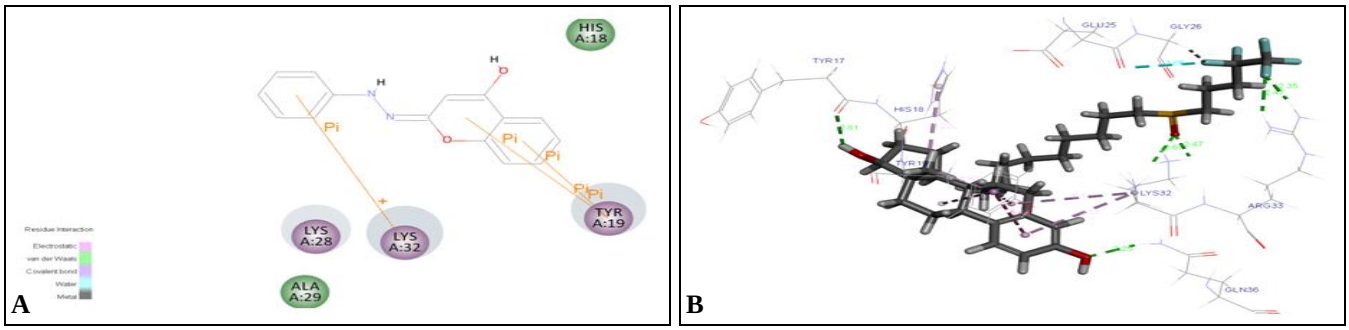



**FIG. 3C: LIGAND-PROTEIN INTERACTION ENERGIES FOR COMPOUND D3**

Foot Note: A: 2D diagram for the binding mode of D1, D2 & D3 with estrogen receptor. Residues were annotated with their 3 letter amino acid code. The distance of hydrogen bonds and electrostatic interactions are presented in $A^0$. B: 3D diagram for the binding site of D1, D2 & D3 with estrogen receptor.

**ADMET Studies:** The pharmacokinetic and pharmacodynamic properties of the synthesized drugs were predicted through the ADMET study. The results were also gathered from the ADMET plot. The studies help in predicting and excluding compounds that may be toxic or may be unable to cross the membrane. The predictions made through the studies were given in the following tables with the plot **Table 5** and **Fig. 4**.

The solubility of each compound was predicted; the ADMET solubility level ranges from 2 to 4 for the three synthesized compounds; thus, the three synthesized compounds were soluble in water at 25°C. AlogP computes the lipophilicity of a molecule and represents the partition co-efficient values (logarithm) of the compounds in octanol/water system. It is an important parameter and affects bio-availability, membrane permeability, and distribution and clearance route of drugs. This parameter also plays an essential role in the pharmacological and toxic properties of drugs. AlogP values for all the synthesized compounds were within the required limit below five. The polar surface area of the compound is in an inverse relationship with human intestinal absorption, and all three compounds exhibited values less than 150 and thus predicted to have high absorption. However, the blood barrier level of compound D2 was found to be undefined and that of D1 and D3 to be medium and low, respectively.

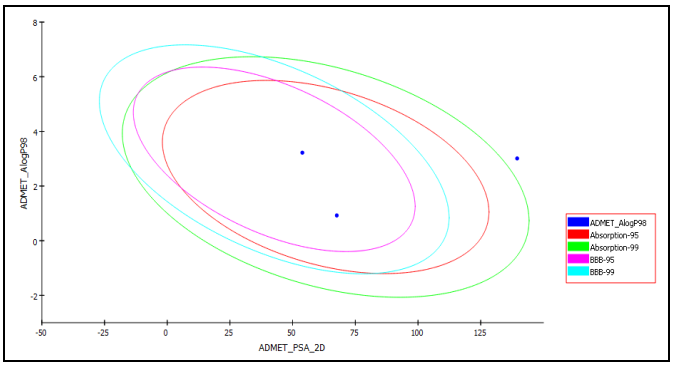



**FIG. 4: ADMET PLOT OF THE SYNTHESISED COMPOUNDS.** The above plot highlights four BBB penetration levels for the three derivatives synthesized: Very high penetrants (blue; log BB ≥ 0.7); High penetrants (green; 0 ≤ log BB< 0.7); Medium penetrants (cyan; -0.52 <log BB< 0); Low penetrants (orange; log BB ≤ -0.52). Here D2 is in the undefined region.

A cytochrome enzyme catalysis the metabolization of the drug through computation it was found that all the synthesized compounds have values less than 0.161 and thus was predicted to be metabolized by the liver. The hepatotoxicity value, which determines the extent of toxicity caused by the compound on the organs, is greater than -4.145 and holds true for all the synthesized compounds. Plasma protein binding affects the distribution of drugs and can be categorized as restrictive binding and permissive binding. It is also meant to measure the efficiency of a drug. All the synthesized compounds showed a Bayesian score higher than –2.226 (true), suggesting that they are likely to bound (≥90%) in blood with a carrier protein.

**TABLE 5: ADMET PROPERTIES OF THE SYNTHESIZED COMPOUNDS D1, D2, AND D3**

| ADMET | Compound Code | | |
|---|---|---|---|
| | D1 | D2 | D3 |
| **Predicted profile regression** | | | |
| Solubility | -1.649 | -4.669 | -3.874 |
| Solubility level (drug-likeness) | 4 | 2 | 3 |
| Alogp98 | 0.924 | 3.01 | 3.221 |
| bbb | -0.938 | - | -0.011 |
| bbb level | 3 | 4 | 2 |
| Adsorption level | 0 | 2 | 0 |
| psa 2d | 67.608 | 139.55 | 53.879 |
| **Metabolism SAR prediction** | | | |
| Ext cyp2d6 | -6.16294 | -5.86012 | -0.884875 |
| Ext cyp2d6 prediction | False | False | False |
| Ext cyp2d6 applicability | 13.4214 | 13.6559 | 10.9153 |
| **Hepatotoxicity prediction** | | | |
| Ext hepatotoxicity | 1.17173 | 4.17277 | 0.166502 |
| Ext hepatotoxicity prediction | True | True | True |
| Ext hepatotoxicity applicability | 13.1198 | 14.3857 | 14.2733 |
| **QP log PPB** | | | |
| Ext ppb | -1.93927 | 3.21709 | 6.42693 |
| Ext ppb prediction | True | True | True |
| Ext ppb applicability | 13.4782 | 14.4642 | 13.6421 |

**Topkat Values:** After the ADMET studies, the compounds were screened further for the prediction of toxicity using the TOPCAT tool in DS to check their safety. The drug's approval and rejection by regulatory are decided after screening for mutagenicity, which is performed *in-vitro* by Ames test. Ames test assesses the genotoxicity of the drugs, which is related to direct (mutation inclusions involved in carcinogenesis) and indirect effects (surrogate events) in DNA, thereby leading to its mutations **Table 6**.

**TABLE 6: TOPKAT VALUES**

| Code | Mouse F NTP | Mouse M NTP | Rat F FDA | WOE | Rat Oral $LD_{50}$ | Rat Inhalational $LC_{50}$ | Fathead Minnow $LC_{50}$ | Daphnia $EC_{50}$ | Carcinogenic potency $TD_{50}$ Mouse | Chronic LOAEL |
|---|---|---|---|---|---|---|---|---|---|---|
| D1 | NC | C | NC | NC | 0.22986 | 543.71 | 0.0406689 | 9.91068 | 125.969 | 0.201714 |
| D2 | NC | NC | NC | NC | 0.145952 | 281.16 | 0.000334748 | 0.726665 | 179.379 | 0.096828 |
| D3 | NC | C | NC | C | 0.111642 | 1,289.38 | 0.00213395 | 1.82376 | 65.3993 | 0.0829428 |

The rat oral level of $LD_{50}$ for the synthesized compounds was to more than 0.100 g/kg and thus predicted to be less toxic. $TD_{50}$ tumorigenic dose 50% showing carcinogenic potency was low for all the synthesized compounds indicating low carcinogenic effect. LOAEL, which represents the lowest observed adverse effect level, was also low. The *in-silico* assessment of toxicity in Daphina Magna was performed to check for the acute, and reproductive toxicity of the compound using an effective concentration of 50%, and the values for all the compounds were indicating toxicity at production was lowered for lower doses.

**Result on Synthesis:** The compounds are synthesized through Schiff's reaction between 4-hydroxy coumarin and hydrazine/hydrazine derivatives refluxing for about 2 h at 78 °C with continuous stirring. The synthesized compounds are purified through re-crystallization with a suitable solution. The purity of the compound is determined through Thin Layer Chromatography and by identifying the melting point of the compounds through the open capillary method **Table 7**. The final synthesized compounds were interpreted with IR spectral analysis report.

**TABLE 7: PHYSICAL CHARACTERIZATION DATA OF THE SYNTHESIZED COMPOUNDS**

| Code | Molecular weight | Melting point | % yield | Solubility | Colour | $R_f$ value |
|---|---|---|---|---|---|---|
| $D_1$ | 176 | 205 °C - 210 °C | 60.7% | Hot water, ethanol, ethyl acetate, DMSO, chloroform, acetone, acetone nitrile | Pale yellow | 0.7647 |
| $D_2$ | 338 | 235 °C-240 °C | 46.9% | Acetone, ethyl acetate, DMSO, chloroform, ethanol | Rosy red | 0.6857 |
| $D_3$ | 253 | 218 °C-220 °C | 37.9% | Hot water, ethanol, DMSO, chloroform, acetone, ethyl acetate | Pale orange | 0.6829 |

**D1:** (2E)-2-hydrazinylidene-2H-chromen-4-ol, IR ($cm^{-1}$): 2471.29 (-N-); 1620.76 (C=N); 1341.96 (C-OH); 1205.08 (C-O-C); 829.01 (aromatic ring).

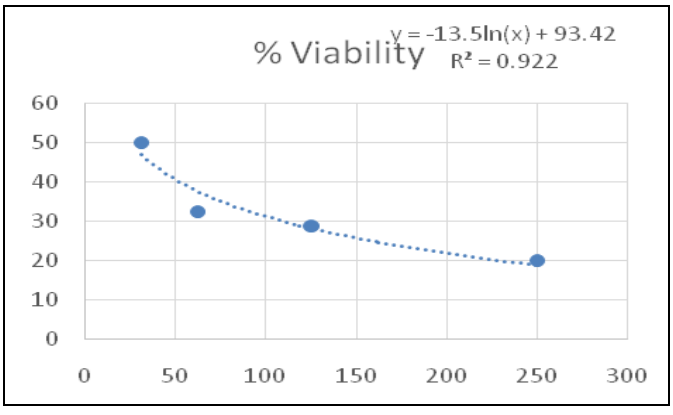
**D2:** (2E)-2-[2-(2, 4-dinitro phenyl)-Hydra-zinylidene]-2H-chromen-4-ol, IR ($cm^{-1}$): 1647.69 (C=N); 1274.28 (NH aryl); 1598.87 ($NO_2$); 1038.37 (C-O-C); 1103.51 (C-OH); 831.59 (aromatic ring).

**D3:** (2E)-2-(2-phenyl Hydrazinylidene)-2H-chromen-4-ol, IR ($cm^{-1}$); 1273.60 (NH aryl); 1649.15 (C=N); 1028.51 (C-O-C); 1243.42 (C-OH); 830.49 (Aromatic ring).

**Cytotoxicity Studies:** All three compounds were screened by the MTT assay method on human breast cancer cell lines (MCF-7), and the results were depicted in **Table 8**. All the synthesized compounds showed significant cytotoxicity effect of below 100 µg/ml; however, the compound DI exhibited significant effects on suppressing of MCF-7 cells in the concentration of 24 µg/ml. The Coumarin Schiff base D1 significantly down-regulated the viability of the MCF-7 cells in a dose-dependent manner. The data here suggested that coumarin Schiff base D1 possessed an anti-tumor role in breast cancer cell proliferation without causing cytotoxicity in normal cells.

**TABLE 8: $IC_{50}$ VALUES OF SYNTHESIZED COMPOUNDS**

| S. no. | Sample code | MCF-7 ($IC_{50}$µg/ml) |
|---|---|---|
| 1 | D1 | 24.7584 |
| 4 | D2 | 46.46148 |
| 5 | D3 | 73.39237 |



**CONCLUSION:** The lack of selective binding is the major drawback of existing drugs used for estrogen-positive breast cancer therapy. Coumarin and its analogs have an inhibitory effect against the estrogen receptor. To increase the selective binding of estrogen receptor alpha, it was proposed to synthesize and screen for *in-vitro* cytotoxicity of Schiff bases of coumarin. Based on the literature review, a three compound library of novel coumarin analogs with Schiff bases were designed, and structural based drug design was performed with human estrogen receptor (PDB ID: 1HCQ). Based on the *in-silico* outcomes, the three coumarin Schiff bases were synthesized. The compounds were obtained in good yield. The novelty had been ascertained by SciFinder software. The synthesized molecules were consistent with their assigned IR spectral data, which confirmed their formation. The *in-vitro* cytotoxicity study was performed by MTT assay for all the synthesized compounds. Most of the compounds have good $IC_{50}$ values of below 200 µg/ml; however, the compounds D1 has shown significant $IC_{50}$ values such as 24 µg/ml and suppressed the proliferation of estrogen receptor overexpressed MCF-7 cells. Further, it was confirmed by its noteworthy docking score against the estrogen receptor. Thus, the designed coumarin Schiff base derivatives might serve as the best drug lead for treating breast cancer, which is additionally confirmed by performing their docking study *via* Accelrys discovery studio for inhibiting the production of estrogen.

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