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COMPUTATIONAL AND IN-VITRO APPROACHES FOR DESIGN, SYNTHESIS AND SCREENING OF ANILIDE BASED COMPOUNDS

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ABSTRACT

A novel series of anilide derivatives were formulated, produced, and assessed for their effectiveness in combating cancer. Within this investigation, three anilide derivatives were specifically designed and subjected to docking studies to determine their biological activity. A simple and highly effective method was developed for the synthesis of these intended compounds, and their structures were characterized through FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry analysis. Among the compounds examined, namely FAR1, FAR2, and FAR3, exhibited notable anticancer properties. The compound with the highest docking score (FAR1) was further evaluated for its cytotoxicity against the MCF7 cell line using the MTT assay. The results obtained revealed the potential anticancer activity of compound FAR1.

KEYWORDS: Anticancer activity, MCF7, Docking score, MTT assay, Anilide derivatives.

1. INTRODUCTION

Cancer is a multifactorial disease that affects millions of people every year worldwide and is characterised by uncontrolled cell growth.[1] Around 18 million new cases of cancer were reported in 2018; 9.5 million of these cases were in men and 8.5 million in women.[2] As per the WHO report, cancer represents the second leading cause of death and accounted for 9.6 million deaths in 2018.[3] Breast cancer is the most commonly diagnosed cancer and the main reason for cancer-related deaths in women. According to estimates, 1.67 million new cases of breast cancer are diagnosed each year.[4]

Breast cancer is an adenocarcinoma that mostly manifests as either ductal or lobular carcinoma, depending on whether the breast ducts or lobules are affected.[5] Tumour microenvironments, like stromal influences or macrophages, play crucial roles in the initiation and progression of breast cancer. Breast tumours typically begin as ductal hyperproliferation and then develop into benign tumours or even metastatic carcinomas after constant stimulation by various carcinogenic factors.[6] Different therapeutic approaches are available for the treatment of cancer, including chemotherapy and radiotherapy. However, the systemic toxicity of drugs and the development of drug-resistant tumours limit the success rates in the majority of cases.

Recently, the antiproliferative properties of numerous heterocyclic and non-heterocyclic scaffolds against various tumour cell lines have been studied.[7–15] Chalcones are one of those compounds showing promising anticancer activities.[16–17] These are chemically 1,3-diphenyl-2-propen-1-ones and have been shown to possess a variety of biological effects, such as antileishmanial, anti-inflammatory, anti-mitotic, and modification of P-glycoprotein-mediated multidrug resistance.[18–23] The chalcone moiety can be hybridised with other anticancer pharmacophores to create hybrids with the potential to overcome drug resistance and enhance therapeutic selectivity.[24]

In this study, three anilide derivatives were designed and docking studies were performed. A simple and highly effective method was developed for the synthesis of these intended compounds, and their structures were characterized through FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry analyses. The compound showing the highest binding affinity towards the target (1AS0) was evaluated for anticancer activity against MCF cell line using MTT assay.

2. MATERIAL AND METHODS

Materials

2.1. Chemicals used

Cinnamic acid, Aniline, O-toludine, Ethyl acetate, Ethanol, n-hexane, Thionyl chloride, Sodium hydroxide (Sigma Aldrich, Alchemy lab solutions, Edapally Kochi).

2.2. Instruments used

Bruker FT-IR (Shimadzu 8201 PC) spectrophotometers (Alshifa collage of pharmacy, Perithalmanna), Bruker Avance-500, FTNMR spectrometer (National Institute of Pharmaceutical Education And Research (NIPER), Mohali, Punjab), ESI-MS Q-ToF Micro Waters Mass Spectrometer instrument (National Institute of Pharmaceutical Education And Research (NIPER), Mohali, Punjab), Electronic weighing balance (Prince scale industries, Ahmedabad), Magnetic stirrer (Rotek; B & C industries, Kerala).

Methods

2.3 *In-silico* Study

All computational analysis was carried out on a Windows 10 Pro OS platform on a Desktop with an Intel(R) Pentium(R) CPU J3710 @ 1.60GHz and 4 GB RAM.

2.3.1 Physiochemical Properties

Molinspiration® Molecular Viewer allows the visualization of molecules which is encoded as SMILES or SD file for the calculation of important molecular description (Log P, polar surface area, number of hydrogen bond donors, number of hydrogen bond acceptors etc.) as well as prediction of bioactivity score of important drug targets.

2.3.2 Pharmacokinetic Study by SwissADME

SwissADME is a web tool giving free access into physiochemical properties (Molecular Weight, Molar refractivity, Polar surface area) pharmacokinetics (substrate or non-substrate of P-gp, CYP inhibition), drug-likeness of potent molecule. It also produces predictive models such as boiled egg allows the evaluation of Human Intestinal Absorption (HIA) and brain penetration (BBB) of drug molecule. The white region is for high probability of passive absorption by the GI tract and yolk region is for high probability of brain penetration.

2.3.3In-Silico Toxicity Prediction- OSIRIS

In silico toxicity prediction is done using OSIRIS® Property Explorer. It is a free software available for access in the Organic Chemistry Portal. Using this prediction tool, mutagenicity, tumerogenicity, skin irritation and reproductive effects can be calculated. The prediction properties rely on a precompiled set of structure fragment that gives rises to toxicity alerts in case they are encountered in the structure currently drawn. These fragment lists is created by rigorously shredding all compounds in the data base known to be active in a certain toxicity class. During the shredding any molecule is first cut at every rotatable bonds leading to a set of core fragments.

2.3.4. Molecular Docking

Docking of small molecules and compounds into the binding site of receptor and estimating the binding affinity of complex is considered to the important part of structure based drug design. Molecular docking is achieved by Autodock Vina. The 3D crystallographic structure of proteins are uncovered from protein data bank (PDB ID-1AS0).

Autodock Vina is an open source program offering a complete molecular viewer and graphical support for all the steps inevitable for set up and docking analysis. PyMOL produce a high quality 3D image of protein as well as its visualization. PyRx is for docking analysis.

2.4 CHEMISTRY

2.4.1 General procedure for the Synthesis of cinnamoyl chloride

0.01M of Cinnamic acid (1.48g) is dissolved in 20ml chloroform in a round bottom flask. 10 drops of Dimethylformamide (DMF) is added slowly to reaction mixture with continuous stirring for 10min. 0.9ml of $SOCI_2$ (0.01M) was added drop wisely & continued stirring for 8hrs using magnetic stirrer. After cooling the reaction mixtures, the reflux condenser is replaced by a distillation unit and the excess of SOCl2 is removed by distillation under reduced pressure. A brownish solid remains as residue with melting point 30-33ºC.

2.4.2 General procedure for the synthesis of anilide derivatives

A mixture of 1.8g of cinnamoyl chloride (0.01M) and 0.01M of aniline derivatives in 10% NaOH was stirred at room temperature for 8 hrs using magnetic stirrer. Completion of reaction was monitored by TLC. Solid separated was filtered, washed with water until neutral and recrystallized from ethanol.

2.5 CHARACTERIZATION

2.5.1 Melting point

The melting point of the synthesized compound was determined by open capillary tube method. The temperature at which the compound starts losing its crystallinity and changes from solid to liquid form was found and recorded.

2.5.2 Thin layer chromatography

The reactants and products were dissolved in ethanol. It was spotted on the TLC plate. A single principal spot for the product and the absence of secondary spots for parent compounds and intermediates confirmed the purity of the product. Stationary phase: pre-coated silica gel GF using appropriate mobile phase was used. The spots were detected in a UV chamber.

2.5.3 IR spectrometry

Infrared spectroscopy is one of most commonly used spectroscopic technique for identification of functional groups in molecules. IR spectroscopy is an important tool in the structural elucidation of organic compounds. In IR spectroscopy finger print region is used to compare the two compounds. Infrared spectrum shows percentage transmittance versus frequency expressed as wave numbers.

2.5.3 NMR spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is an important analytical technique used in the structural elucidation of organic molecules. It involves the interaction of the electromagnetic radiation and the proton of a nucleus of an atom when placed under an externally applied static magnetic field. NMR spectra provide the detailed information about a molecule's structure. The chemical shift is used to predict the number of protons with refers to TMS as standard. The NMR spectra is recorded on 300 MHz BRUKER advance III NMR spectrometer. DMSO is used as a solvent.

2.5.4 LC-MS

LC-MS is a hyphenated technique, combining separation power of HPLC with the detection power of Mass Spectrometry. Mass spectra was recorded on Shimadzu LC-MS using Electron Spray Ionization Technique and was quantified using Lab Solutions Software 7.0.

2.6 Biological Evaluation

2.6.1 MTT assay

Principle

The MTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. The viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT to formazan (Mosmann et al., 1983). The insoluble formazan crystals are dissolved using a solubilizing solution (100% DMSO) and the resulting purple colored solution is quantified by measuring absorbance at 570 nm using an ELISA plate reader.

Procedure

The cells (2500 cells/well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions such as 37°C and 5% CO_2 environment in the incubator for 24 hrs. The test samples were prepared in DMEM media (100 mg/mL) and filter sterilized using 0.2µm Millipore syringe filter. The samples were further diluted in DMEM media and added to the wells containing cultured cells at final concentrations of 6.25, 12.5, 25, 50, 100 µg/mL respectively. Untreated wells were kept as control. All the experiments were done in triplicate and average values were taken in order to minimize errors. After treatment with the test samples the plates were further incubated for 24 hrs.

After incubation period, the media from the wells were aspirated and discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells. The plates were further incubated for 2 hrs for the development of formazan crystals. The supernatant was removed and 100 µL DMSO (100%) were added per well. The absorbance at 570 nm was measured with micro plate reader. Two wells per plate without cells served as blank.

The cell viability was expressed using the following formula:

$$\text{Percentage viability} = \frac{\text{Avg. absorbance of sample x 100}}{\text{Avg. absorbance of test}}$$

3. RESULT AND DISCUSSIONS

3.1. *Insilico* studies

Pharmacokinetic parameters of these derivatives were calculated using Molinspiration Online software. From all these parameters Table, the compounds obeying Lipinski's rule of five was selected for docking studies.

Table 1: Analysis of Lipinski rule of five using molinspiration.

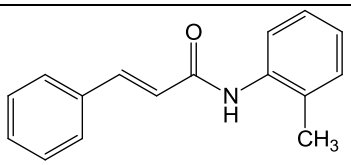
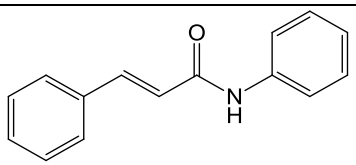
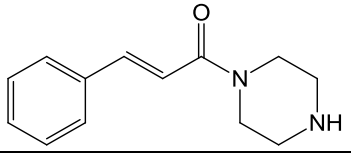
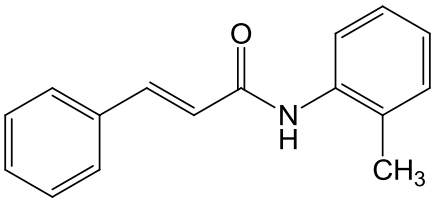
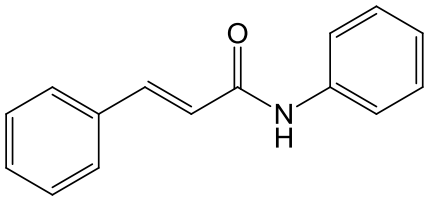
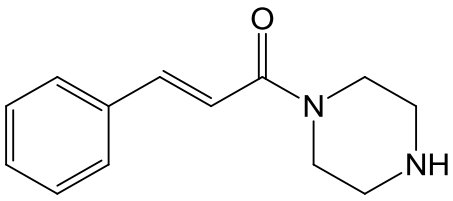
Compound id	Structure	MW	HA	HD	Log P	nrotb	Violation
FAR1		237.3	1	2	3.87	3	0
FAR2		223.28	1	2	3.47	3	0
FAR3		216..68	2	1	1.31	3	0

Table: Pharmacokinetic study by Swiss ADME.

Compound ID	GI Absorption (High/Low)	BBB Per meant (Yes/No)	P-GP substrate (Yes/No)	PAINS (alert)
FAR1	High	Yes	No	0
FAR2	High	Yes	No	0
FAR3	High	No	No	0

The designed molecules were docked against the selected GTP gamma S bond (1AS0). The best and stable pose was selected based on the docking score and the basis of multiple interactions. Comparing the binding structure of synthesized molecules, the aniline derivatives hold a key group for binding affinity in the binding pocket. Among the synthesized compound, FAR1 has the highest binding affinity towards the protein, and FAR3 has the least binding affinity. Where FAR3 have a piperazine ring instead of aniline which reduce the affinity and fitting of the drug in the binding pocket for the desired activity.

Sl. No	Compound	Structure	Docking score	Interacting residue
1	**FAR1**		10.2	SER:44, GLY:45, LYS:180, GLY:202, GLY:203, GLN:204, THR:181, ARG:178, LYS2:70, ASP:150, SER:151, THR:327, ALA:326, ASN:269,
2	**FAR2**		9.7	PHE:140, ASN:141, SER:143 ARG:144, LEU:148, ASN:149, ASP:150, ALA:152, ALA:153, ASP:231, LYS:280, SER:281
3	**FAR3**		8.4	ARG:90, GLY:89, LEU:91, MET:88, ALA:87, ILE:93, ASP:94

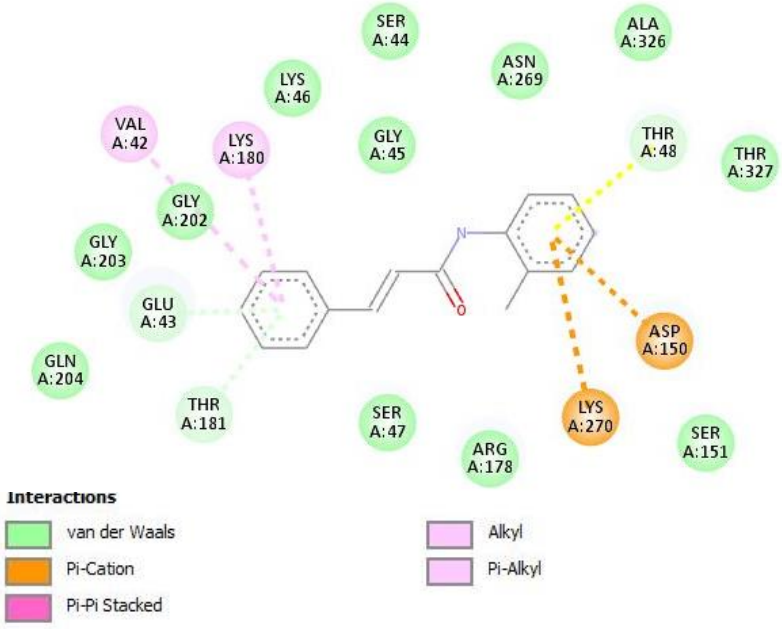


Figure: **binding interaction of FAR1.**

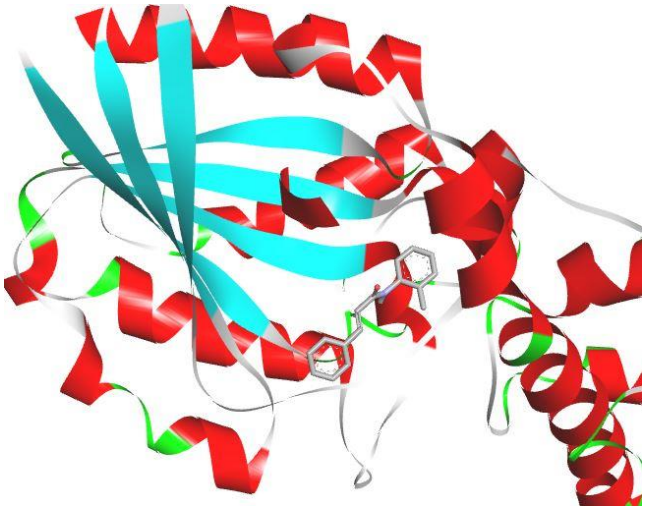


Figure: **Binding pattern of FAR1 in binding pocket.**

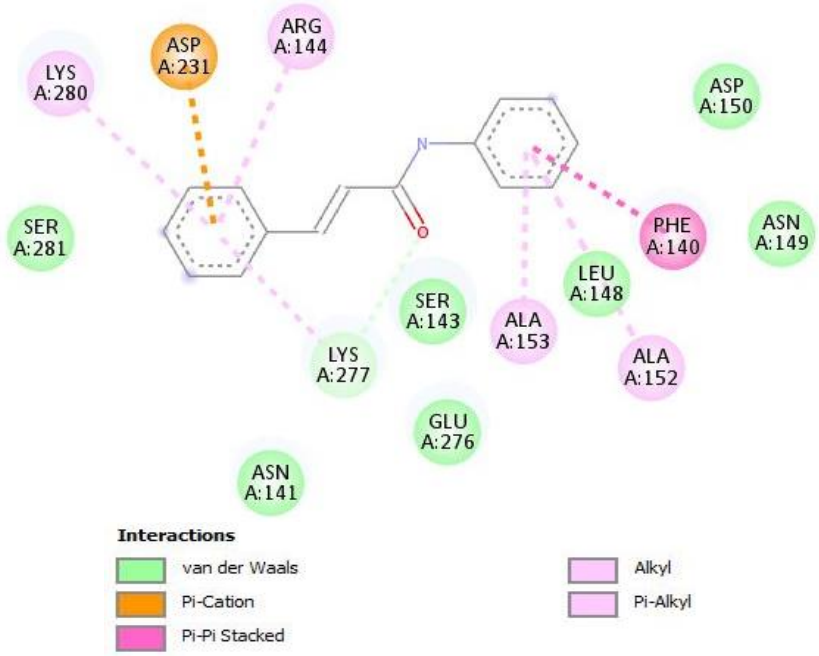


Figure: Binding interaction of FAR2.

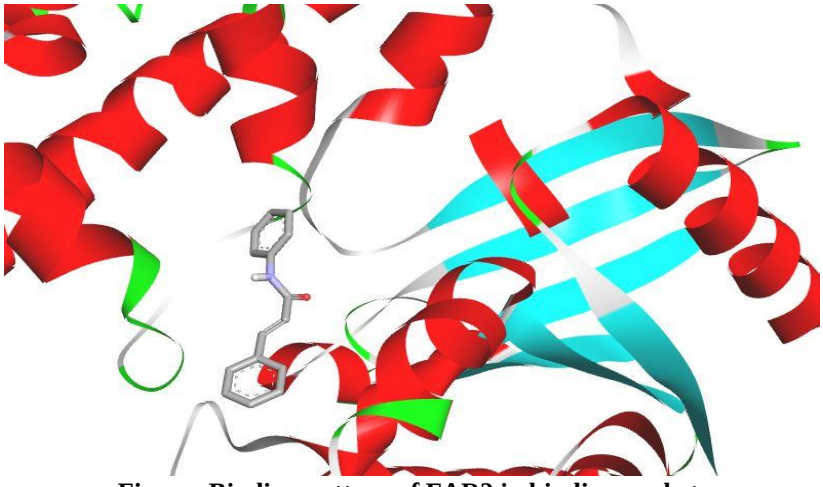


Figure: Binding pattern of FAR2 in binding pocket.

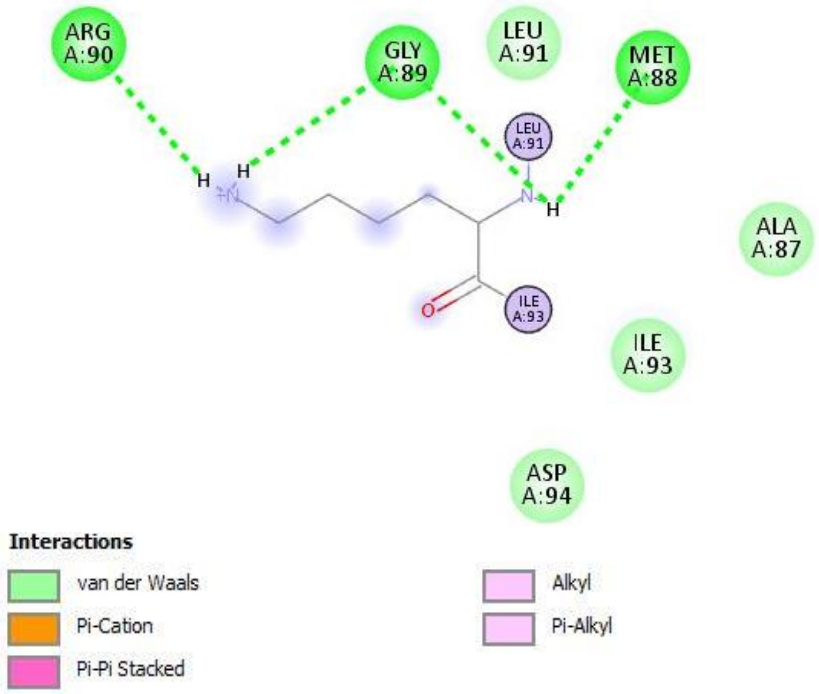


Figure: binding interaction of FAR3.

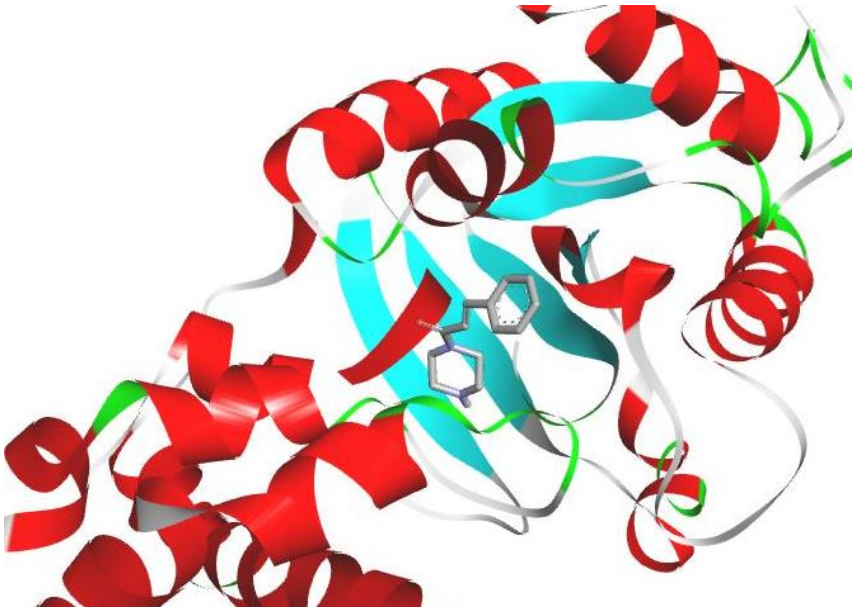


Figure: Binding pattern of FAR3 in binding pocket.

3.2. Chemistry

Three anilide derivatives were designed and synthesized by the reaction of equimolar amounts of the selected cinnamoyl chloride and different types of aniline derivatives (o-toludine, aniline, piperazine). The compound FAR1 and FAR2 took 8hrs, while FAR3 took 12hrs for the completion of the reaction compound. The progress of the reaction was monitored by TLC and melting point. The compounds' yield ranged from 60-75% with reasonable purity. All the synthesized derivatives were found to have a sharp melting point.

3.3. Characterization of the synthesized compounds

1. N-(o-toly) cinnamamide (FAR1): White powder (EtOH); MP = 148-156°C; Yield 67.5% w/w; IR (ZnSe) peaks: 3265cm^{-1} (NH stretching), 3028 cm^{-1} (Aromatic), 1651cm^{-1} (Amide stretching). ^{1}H NMR (500 MHz, DMSO D6) δppm= 9.47 (br, 1H, NH), 7.74 (d, J=16, 1H, CH), 7.62 - 7.1 (m, 10H, ArH), 6.8 (d, J=16, 1H, CH), 2.5 (d, 1H, CH_3); ^{13}C NMR (500MHz DMSO D6) δ 164.3,140, 138, 135, 131.1, 130.8, 130.1, 129.4, 128.1, 126.4, 125.4, 124.8, 122.2, 18.63; ESI MS: 238 [M+1].

2. N-phenyl cinnamamide (FAR2): White powder (EtOH); MP = 128-138°C; Yield 70.2 %w/w; IR (ZnSe)

peaks: 3236cm^{-1}(NH stretching), 3032cm^{-1}, 3126cm^{-1} (Aromatic C-H stretching), 1568cm^{-1} (amide stretching); ^{1}H NMR (500 MHz, $CDCl_3$) δppm= 7.7(d, 1H, CH), 7.117-7.65 (M, 10H, ArH), 6.645(d ,1H, CH); ^{13}CNMR (500 MHz $CDCl_3$) 164.34, 142.33, 138.1, 134.5, 129.9, 129.01, 128.8, 127.9, 124.4, 121.01, 120.01; ESI MS: 224 [M+1].

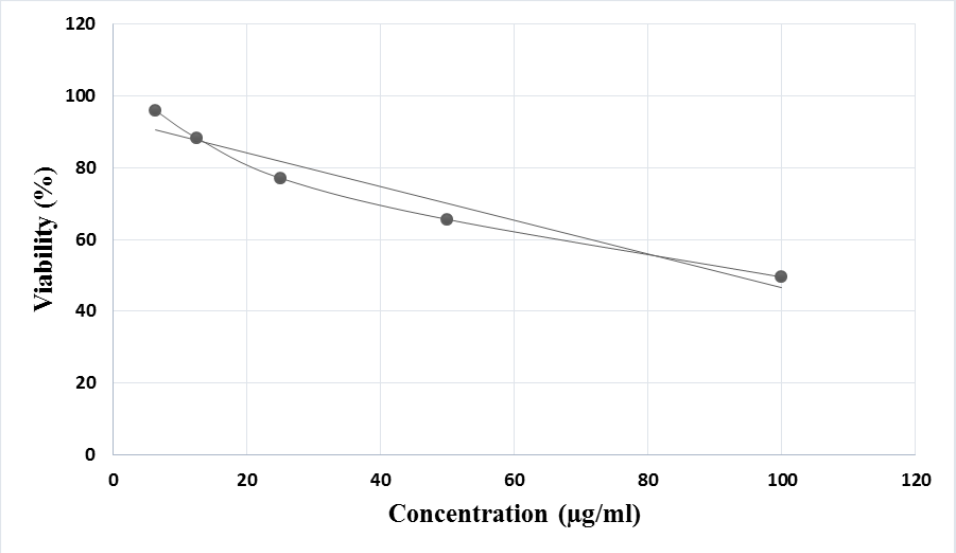
3. (E)-3-phenyl-1-(piperazin-1-yl) prop-2-en-1-one (FAR3): White powder (EtOH); MP = 250-260° C; Yield 70.2 %w/w; IR(ZnSe) peaks : 3354 cm^{-1} (NH stretching), 3034 cm^{-1} (Aromatic CH stretching), 2864cm^{-1} (C-H stretching), 1499cm^{-1} (Aromatic C-H stretching), 1644cm^{-1} (Amide stretching) ,1037 cm^{-1} (CN stretching); ^{1}H NMR (500 MHz, $CDCl_3$) δppm= 7.7(d , J=15.5,1H,CH), 7.7-7.3(m, 5H, ArH), 6.8 (d, J=15.5, 1H, CH), 3.8(d, 4H, CH_2)' 1.65(s, 3H, CH_2); ^{13}C NMR (500 MHz, $CDCl_3$) 165, 148.7, 134.9, 129.9, 128.8, 127.8, 116.3, 45.6, 42.1; ESI MS: 218[M+2]

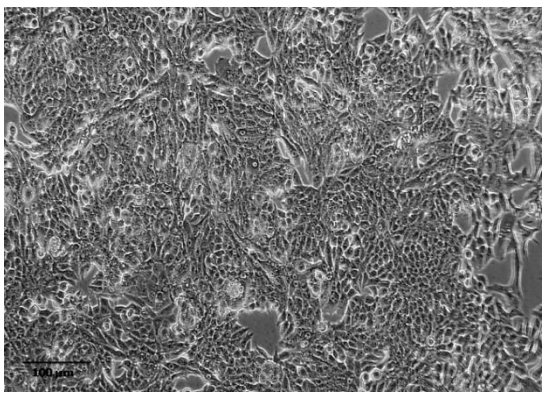
3.4. *In vitro* studies

The compound with the highest docking score (FAR1) was selected for in-vitro studies. To assess its cytotoxic activity against the MCF7 breast cancer cell line, the MTT assay was employed. The reduction in cell viability dependent on the administered dose was observed in MCF-7 cancer cells when exposed to various concentrations of the sample. The highest cytotoxicity was observed at a concentration of 100µg/ml. The calculated IC_{50} value for the sample was determined to be 92.69µg/ml. These findings provide a preliminary indication of the anticancer potential of the tested sample.

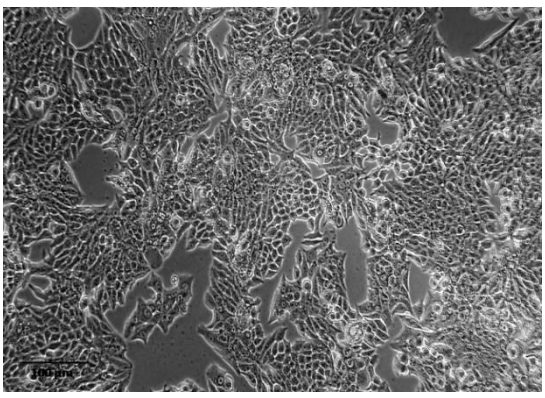
Furthermore, the biological activity assessed through enzymatic assay aligned with the results obtained from molecular modeling studies. FAR1, identified as a potent molecule during insilico evaluation, demonstrated consistent biological activity. Thus, it can be concluded that computational tools are highly effective in identifying potent molecules even before their synthesis.

Concentration of FAR1 (µg/ml)	Optical density	Percentage viability
control	0.643	-
6.25	0.617	96.01
12.5	0.568	88.28
25	0.496	77.09
50	0.422	65.58
100	0.318	49.51
		IC_{50} = 92.69

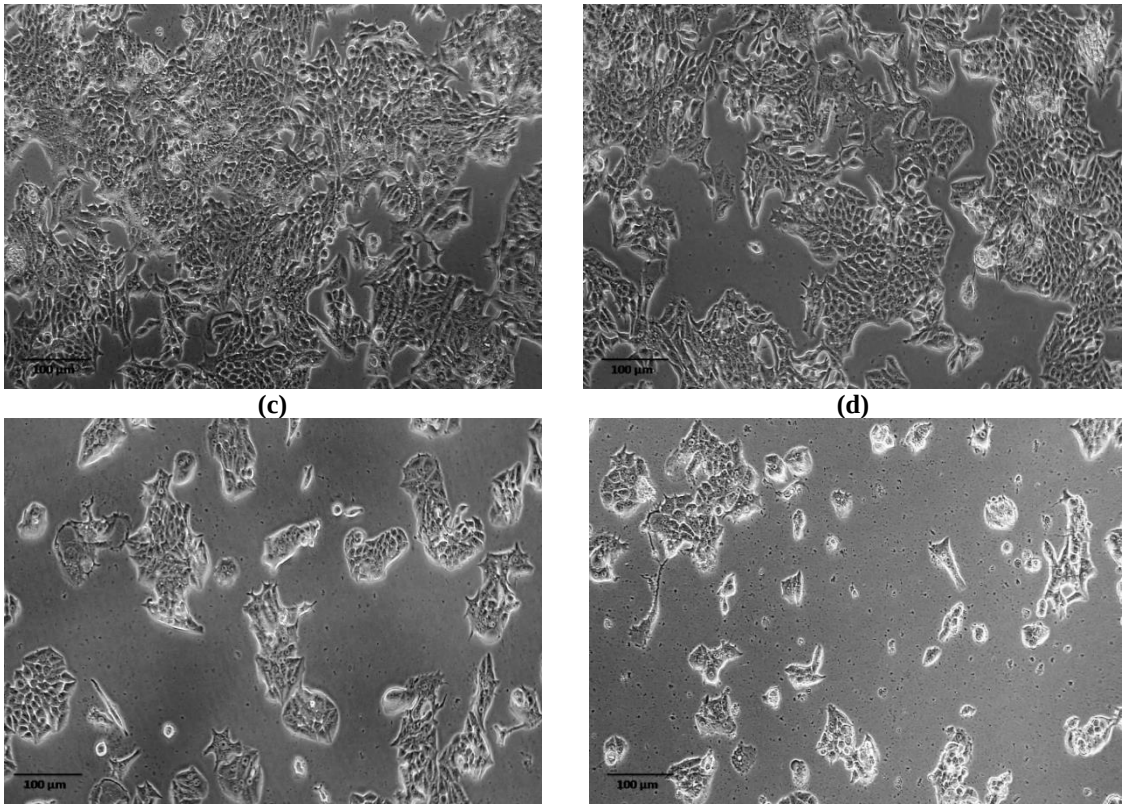




(a)



(b)



(c) **(d)**

(e) **(f)**

Figure: **Microscopic image of MCF7 cell line treated with different concentration of FAR1 (a) control, (b) 6.25, (c) 12.5, (d) 25, (e) 50 and (f) 100 µg/mL.**

4. CONCLUSION

From this study we concluded that, all the developed compounds were able to successfully interact with the target enzyme within a favorable range. The results of molecular docking indicated that FAR1, FAR2, and FAR3 displayed efficient interactions with the active site, surpassing the interactions observed with the co-crystallized ligand. Notably, N-(o-toly) cinnamamide (FAR1), which possesses a secondary amine and two aromatic rings, exhibited specific interactions with LEU A 295, PRO A 203, ILE A 182, PRO A 192, LYS A 185, and ALA A 184. Furthermore, FAR1 demonstrated the highest activity among the developed compounds against the MCF7 cell line.

A simple and efficient method for synthesizing the designed compounds, identified through molecular modeling studies, has been successfully developed. The synthesis of all the compounds involved magnetic stirring at room temperature for a duration of 3 to 4.5 hours. The progress of the reactions was monitored using TLC (thin-layer chromatography) and the determination of melting points. The yields of the compounds ranged from 60% to 75%, and they exhibited reasonable purity. To ensure higher purity required for spectral studies, all the synthesized compounds underwent two rounds of recrystallization in ethanol.

The formation of anilide derivatives was confirmed by conducting spectral studies, including IR (infrared spectroscopy), ^{1}H NMR (proton nuclear magnetic resonance), and ^{13}C NMR (carbon-13 nuclear magnetic resonance). These spectroscopic analyses provided evidence for the structural characteristics of the synthesized compounds. Additionally, mass spectrometry was employed to determine the molecular mass of the compounds, further confirming the identification and structure of the anilide derivatives.

The compound FAR1, which exhibited the highest docking score among the synthesized compounds, was selected for in-vitro analysis. Through MTT assay, it was discovered that FAR1 demonstrated significant activity against the MCF7 cell line. The compound FAR1 exhibited an IC_{50} value of 92.69 µg/ml.

To determine the efficacy of the synthesized molecules, it is necessary to conduct in vivo evaluations using small animals. This step is crucial for establishing the effectiveness of the compounds. Our study aims to offer valuable insights to researchers involved in the development of anti-cancer drugs.

5. ACKNOWLEDGEMENT

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6. REFERENCES

1. Hussain, S.; Singh, A.; Nazir, S.U.; Tulsyan, S.; Khan, A.; Kumar, R.; Bashir, N.; Tanwar, P.; Mehrotra, R. Cancer drug resistance: A fleet to conquer. J. Cell. Biochem, 2019; 120: 14213–14225.
2. Forouzanfar MH, Afshin A, Alexander LT, Anderson HR, Bhutta ZA, Biryukov S and Charlson FJ: Global, regional, and national comparative risk assessment of 79 behavioral, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet, 2016; 388(10053): 1659-24.
3. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer, 2015; 136: E359-E386.
4. https://www.britannica.com/science/anticancer-drug.
5. https://www.researchgate.net/publication/5396094_Overview_of_breast_cancer_staging_and_surgical_treatment_options.
6. Dumars C, Ngyuen JM, Gaultier A, et al. Dysregulation of macrophage polarization is associated with the metastatic process in osteosarcoma.Oncotarget. 2016; 7: 78343-78354.
7. Bandgar, B.P.; Gawande, S.S.; Bodade, R.G.; Totre, J.V.; Khobragade, C.N. Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, anti-inflammatory and antioxidant agents. Bioorg. Med. Chem., 2010; 18: 1364-1370.
8. Martirosyan, A.; Rahim-Bata, R.; Freeman, A.; Clarke, C.; How-ard, R.; Strobl, J. Differentiation-inducing quinolines as experimental breast cancer agents in the MCF-7 human cancer cell model. Biochem. Pharmacol., 2004; 68: 1729-1738.
9. Mól, W.; Matyja, M.; Filip, B.; Wietrzyk, S. Synthesis and antiproliferative activity in vitro of novel (2-buthynyl)thioquinolines. Bioorg. Med. Chem., 2008; 16: 8136-8141.
10. Hu, L.; Jiang, J-D.; Qu, J.; Li, Y.; Jin, J.; Li, Z-R.; Boykin, D.W. Novel potent antimitotic heterocyclic ketones: Synthesis, antiproliferative activity, and structure activity relationships. Bioorg. Med. Chem. Lett., 2007; 17: 3613-3617.
11. Issa, S.; Walchshofer, N.; Kassab, I.; Termoss, H.; Chamat, S.; Geahchan, A.; Bouaziz, Z. Synthesis and antiproliferative activity of oxazinocarbazole and N,N-bis(carbazolylmethyl)amine derivatives. Eur. J. Med. Chem., 2010; 45: 2567-2577.
12. Hadjikakou, S.K.; Hadjiliadis, N. Antiproliferative and anti-tumor activity of organotin compounds. Coord. Chem. Rev., 2009; 253: 235-249.
13. Solomon, V.R.; Hu, C.; Lee, H. Design and synthesis of chloroquine analogs with anti-breast cancer property. Eur. J. Med. Chem., 2010; 45: 3916-3923.
14. Solomon, V.R.; Hu, C.; Lee, H. Hybrid pharmacophore design and synthesis of isatin-benzothiazole analogs for their anti-breast cancer activity. Bioorg. Med. Chem., 2009, 17: 7585-7592.
15. Solomon, V.R.; Hu, C.; Lee, H. Design and synthesis of anti-breast cancer agents from 4-piperazinylquinoline: a hybrid pharmacophore approach. Bioorg. Med. Chem., 2010; 18: 1563-1572.
16. Agarwala, P.K. Carbon-13 NMR of flavonoids. Elsevier Science Publishers B. V: The Netherlands,, 1989.
17. Begum, N.A.; Roy, N.; Laskar, R.A.; Roy, K. Mosquito larvicidal studies of some chalcone analogues and their derived products: structure–activity relationship analysis, Med. Chem. Res., 2010, (on-line).
18. Go, M.L.; Wu, X.; Liu, X.L. Chalcones: an update on cytotoxic and hemoprotective properties. Curr. Med. Chem., 2005; 12: 481-499.
19. Chimenti, F.; Fioravanti, R.; Bolasco, A.; Chimenti, P.; Secci, D.; Rossi, R.; Ya´n˜ez, M.; Orallo, F.; Ortuso, F.; Alcaro, S. Chalco-nes: A valid scaffold for monoamine oxidases inhibitors. J. Med. Chem., 2009; 52: 2818-2824.
20. Liu, M.; Wilairat, P.; Go, M-L. Antimalarial alkoxylated and hydroxylated chalones: Structure-activity relationship analysis. J. Med. Chem., 2001; 44: 4443-4452.
21. Ducki, S.; Forrest, R.; Hadfield, J.A.; Kendall, A.; Lawrence, N.J.; McGown, A.T.; Rennison, D. Potent antimitotic and cell growth inhibitory properties of substituted chalcones. Bioorg. Med. Chem. Lett., 1998; 8: 1051-1056.
22. Bois, F.; Boumendjel, A.; Mariotte, A.; Conseil, G.; Di Petro, A. Synthesis and biological activity of 4-alkoxychalcones: potential hydrophobic modulators of P-glycoprotein-mediated multidrug re-sistance. Bioorg. Med. Chem., 1999; 7: 2691-2695.
23. Dimmock, J.R.; Elias, D.W.; Beazely, M.A.; Kandepu, N.M. Bio-activities of chalcones. Curr. Med. Chem., 1999, 6: 1125-1149.
24. Zi X, Simoneau AR. Flavokawain A. A novel chalcone from kava extract, induces apoptosis in bladder cancer cells by involvement of Bax protein-dependent and mitochondria-dependent apoptotic pathway and suppresses tumor growth in mice. Cancer Res., 2005; 65: 3479–86.
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