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Computational design synthesis of chalcone scaffolds as potent antioxidant agent

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ABSTRACT
Chalcone derivatives possess various activities such as, antibacterial, anti- inflammatory, analgesic, anticancer, anticholinergic, antiulcer, antiplatelet, antioxidant, antimalarial, and antidiabetic activities etc. In this study, we have designed a chalcone derivativethrough structure-based drug design utilizing Human aldose reductase protein (PDB ID:1Z3N) and the drug with highest docking score was synthesized. A convenient Claisen-Schmidt condensation method for the synthesis of chalcone is reported in this study. The reaction progress is monitored by thin layer chromatography, the physical properties such as solubility, melting pointwas found and the synthesized compound was characterized using IR Spectroscopy. Antioxidant activity of the synthesized compound was estimated by DPPH radical scavenging assay by UV-Visible spectroscopy the result showed a remarkable antioxidant activity for the synthesized compound.

Keywords: Chalcone, Claisen-Schmidt condensation reaction, Antioxidant activity, CADD, DPPH

I. INTRODUCTION

Computer-aided drug design (CADD) is a multidisciplinary field that combines computational techniques and molecular modeling to discover, design, and optimize new pharmaceutical compounds, particularly drugs. This method helps to reduce time and resources, CADD helps as to create three-dimensional models of biological molecules, such as proteins, enzymes, and receptors, to understand their structures and functions. Virtual screening is an application of CADD, helps to screen large databases of chemical compounds and to identify potential drug candidates that could bind to a specific target molecule.[1,2]Docking simulations helps to predict, how small molecule (a potential drug) interacts with target protein or receptor at atomic level and also identify potential binding sites. CADD can predict the absorption, distribution, metabolism, excretion (ADME), and toxicity properties of potential drugs, and helps to filter out compounds with unfavorable properties. Recent advancements in machine learning and AI have enhanced CADD by improving predictive models and speeding up the drug discovery process. CADD is an integral part of modern drug discovery, as it helps researchers narrow down the pool of potential drug candidates and prioritize those with the highest likelihood of success.[3, 4, 5, 6]

The family of flavonoids includes a group of organic substances known as chalcones. They are the secondary metabolites of edible or medicinal plants. Chalcones are abundant in nature and mostly found in plants including fruits, vegetables, and spices.

Chemically, chalcones are 1,3-diphenyl-2-propene-1-one derivative with two aromatic rings that are linked through a three carbon α, β-unsaturated system, serve as a define characteristic for them.[7]Chalcones are utilized to create a variety of heterocyclic compounds as well as to undertake several chemical processes, by condensing aromatic aldehydes and aryl ketones with the proper quantity of condensing agents, chalcones can be synthesized.

Antioxidants are compounds that help to protect the body against oxidative stress and the harmful effects of free radicals. Free radicals are unstable molecules that can damage cells, proteins, and DNA in the body, potentially leading to various diseases and aging processes. Antioxidants neutralize free radicals by donating an electron to stabilize them, thereby reducing their potential harm. Vitamins C and E are well-known antioxidant. Antioxidants helps to reduce the risk

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of chronic diseases such as heart disease, cancer, and neurodegenerative conditions.

Chalcones have been proven in studies to have strong antioxidant activity, scavenging free radicals and reducing oxidative stress. They can aid in the protection of cells and tissues against reactive oxygen species (ROS) and other damaging chemicals.[8] Chalcones may offer numerous possible healthadvantages via lowering oxidative stress**.**Chalcones' capacity to donate hydrogen atoms or electrons, which efficiently quenches and stabilizes free radicals, is thought to be the basis for their antioxidant action. Chalcones are able to prevent the negative effects of oxidative stress and stop oxidative damage chain processes by doing this.[9]

II. METHADOLOGY

In-silico study

All computational analysis was carried out on windows 10 platform on an Acer desktop with i7 8 core 8 processors, NVIDIA Quadro K2200 4GB graphics card and 16GB RAM.

Selection and preparation of protein

The X-ray crystal structure of Human aldose reductase in complex with NADP+ and the inhibitor lidorestat at 1.04 angstrom (PDB ID: 1Z3N) was retrieved from Protein Data Bank.[10] Protein structure was processed by protein preparation wizard. Crystal structure was subjected to protein preparation wizard for filling missing loops and side chains (using Prime), ionization, H-bond optimization, heterogeneous state generation, protonation and overall minimization. All other ligands, water and ions were removed except ATP molecule.

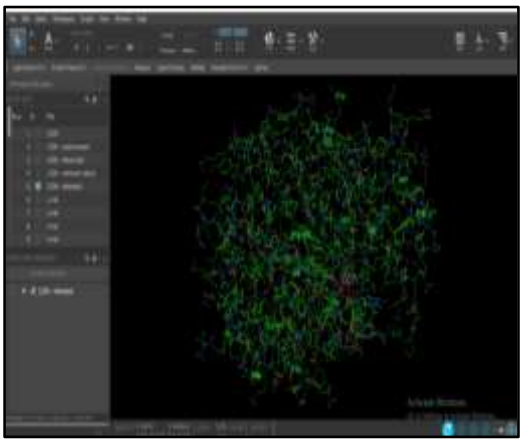

Figure 1: Protein preparation wizard

Receptor grid generation

Generation of grid around active sites was done by receptor grid generation panel of Glide version 6.9. The grid points in the x-, y-, z- axes (2.02 6.72 21.05) and grid size was kept default at 20 Å.

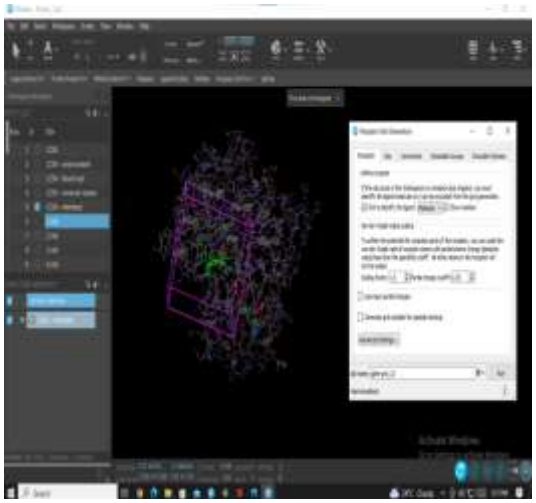

Figure 2: Receptor grid generation

Selection and preparation of ligand

The ligands were sketched using ChemDraw version.19.1 and saved in mol format. Minimization of ligands was carried out by Ligprep version 3.6 using OPLS4 Force Field.

Ligand based docking

All the docking and scoring calculations were executed by the Glide version 6.9. All ligands were docked with the protein grid (PDB ID: 1Z3N) using ligand docking application of Glide version 6.9 in Schrodinger Maestro version 12.8.Glide Extra Precision (XP) flexible liganddocking was carried out in Glide of Schrödinger Maestro v12.8.[11]

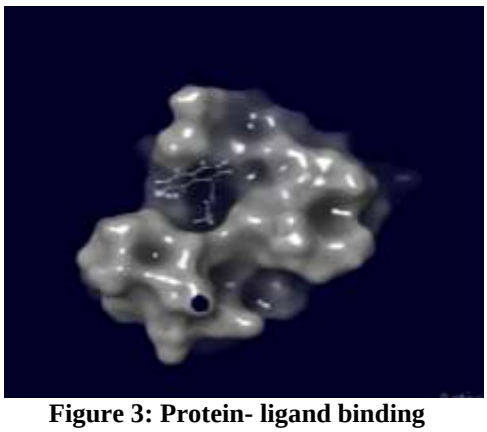

Figure 3: Protein- ligand binding

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SCHEME OF SYNTHESIS

The compounds were synthesized as per the following schemes

Step 1

0.5g of 4-Aminoacetophenone was dissolved in 20 ml of Dichloromethane which is then allowed to react with equimolar concentration of Decanoyl chloride at 0℃ for about 2 hours. The progression of reaction was monitored using TLC.Using rotary evaporator the solvent was evaporated and the unreacted starting material is removed by washing with water.[12]

Scheme of synthesis of N-(4-acetylphenyl)decanamide

Step 2

0.5g N-(4-Acetylphenyl) decanamide and 0.2ml Anisaldehyde were taken and grinded using mortar and pestle, to this freshly prepared ethanolic potassium hydroxide was added drop wise and triturated until a visible change was observed. The reaction mixture was then washed well with an ethanol-water mixture in the ratio (25:75) until neutral to litmus paper. The solid separated was filtered, washed with water, and recrystallized from ethanol-water mixture. Completion of reaction was confirmed by TLC.[13]

Scheme of synthesis of (E)-N-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)decanamide

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Figure 4: Synthesised product

Characterization of Synthesized Compound
Thin Layer Chromatography

The purity of the synthesized Chalcone was monitored by Thin Layer Chromatography (TLC) on pre-coated silica G plates and visualized by using UV chamber. The solvent system used; n-Hexane**:** Ethylacetate (2:3). Melting points of derivative was determined by capillary fusion method using Melting point determination apparatus. Solubility of the derivative in Water, Ethanol, Ethyl acetate, Chloroform, Dimethyl Sulfoxide (DMSO) was tested.The characterization of synthesized derivative was done by using IR Spectroscopy.[14, 15]

IN VITRO ANTIOXIDANT ACTIVITY

2ml aliquot of DPPH methanol solution (25µg/ml.) was added to 0.5 ml. sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.[16]

$$\%\text{RSA} = \frac{\text{A0} - \text{As}}{\text{A0}} \times 100$$

Where,
A_0-Absorbance of control
A_S-Absorbance of sample

RESULTS

Chalcones are prepared by computational softwares by adding anisaldehyde with various ketones and these prepared chalcones undergo docking with human aldose reductase enzyme PDB ID 1Z3N. Docking score of various chalcones are given below in the table

SI. No	COMPOUND	DOCKING SCORE
1		-9.991
2		-9.864
3		-9.527

4		-9.447
5		-9.293
6		-9.034
7		-9.032
8		-8.848
9		-8.754
10		-8.555

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11		-8.552
12		-8.540
13		-8.181
14		-7.962
15		-7.863
16		-7.793
17		-7.733

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18		-7.706

Table no.1 docking score of compounds

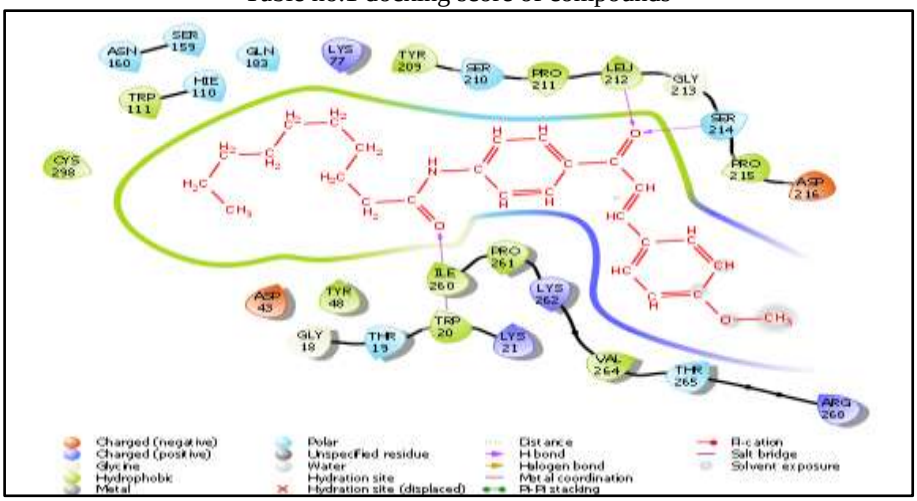

Figure 5: Interaction diagram of ligand molecule with protein 1Z3N

From the above docking results E)-N-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)decanamide has highest docking score and this compound is \ synthesised and the physicochemical properties of prepared compound is represented in the table below

Molecular Formula:	$C_{26}H_{33}NO_3$
Molecular weight:	407.55
Melting point	95-98C^0
Rf value	0.82
TLC profile	nhexane: ethyl acetate (2:3)

Table.no. 2 physicochemical parameters

figure no. 6 synthesized product

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Synthesized chalcones undergo characterisation by IR spectroscopy it was found out that NH stretching at 3308cm^{-1} C=O stretching at 1671cm^{-1} and aromatic C=C at 1466cm^{-1}

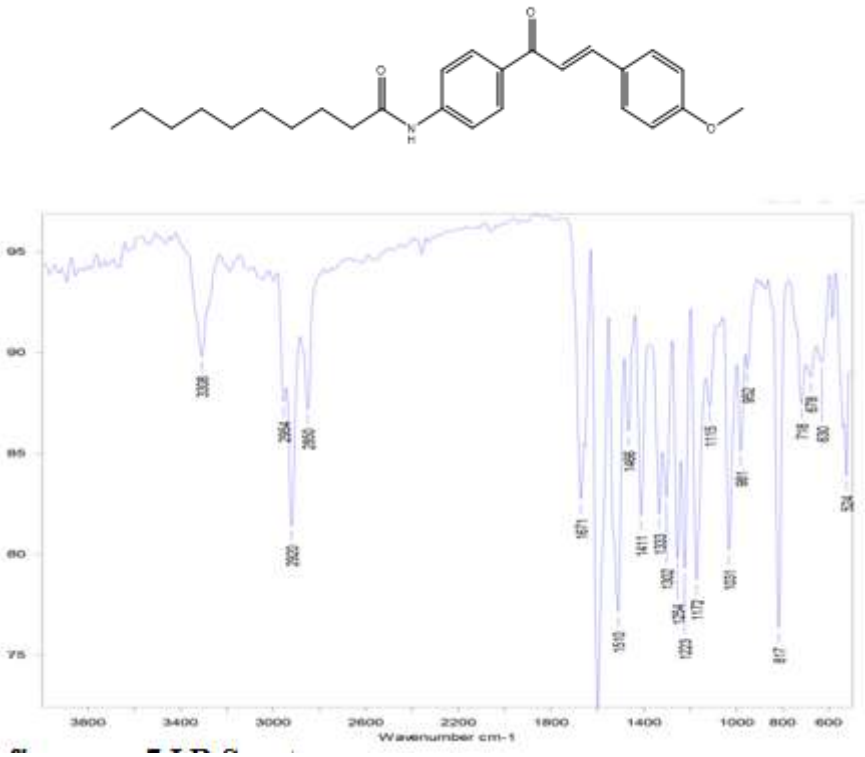

Figure no. 7 I R Spectrum

IN VITRO ANTIOXIDANT ACTIVITY

The synthesized compound was screened for its free radical scavenging activity by DPPH method. The freshly prepared solution exhibits a deep blue color with the absorption maximum at 517nm. This deep blue color generally fades when antioxidant is present in the solution. The percentage radical scavenging of the compound was 19.9824% at a concentration of 50µg/ml whereas that of the standard ascorbic acid was 89.4642% at 50µg/ml concentration. The IC50 value of the compound was calculated using "y= mx+c" formula, which was 32.850µg/ml and that of the standard ascorbic acid was 30.4396µg/ml.

CONCENTRATION (µg/ml)	ABSORBANCE		% RSA	
	Derivative	Ascorbic Acid	Derivative	Ascorbic Acid
10	0.551	0.493	1.607	11.9642
20	0.536	0.322	4.285	23.8000
30	0.514	0.251	8.214	55.1785
40	0.446	0.194	20.357	65.3571
50	0.324	0.059	42.142	89.4642

Table.no:3 Antioxidant activity

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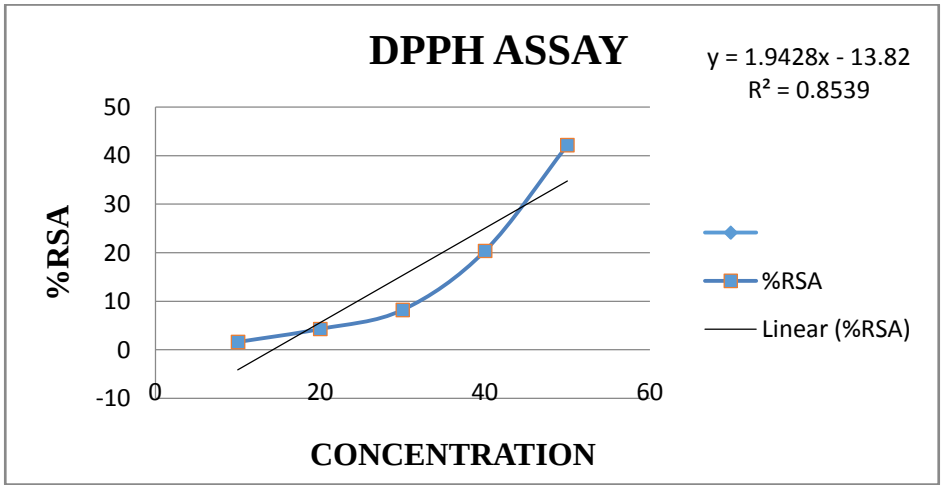

Figure 8. DPPH free radical scavenging assay of synthesized compound

III. DISCUSSION

Molecular docking study was to identify whether the designed compounds possess antioxidant activity. Docking has been performed with the protein Human aldose reductase (PDB ID: 1Z3N) and selected ligands. The result of molecular docking in terms of the docking score against the selected target was tabulated. From this data, compounds having high docking scoreThe synthesis of (E)-N-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)decanamide was successfully carried out according to the synthetic procedure.The TLC results revealed a single spot, confirming the purity of the synthesized derivative and R_f value of compound was found to be 0.82. Melting points of derivative was determined by capillary fusion method using Melting point determination apparatusand the melting point was found to be within the range 95-98℃.The spectral analysis of the derivative was carried out and data was interpreted. IR spectrum shows 3308 cm^{-1} (NH stretching), 1671 cm^{-1} (C=O stretching for amide), 1466 cm^{-1} (C=C stretching.The synthesized compound was screened for its free radical scavenging activity by DPPH method.The percentage radical scavenging of the compound was 19.9824% at a concentration of 50µg/ml whereas that of the standard ascorbic acid was 89.4642% at 50µg/ml concentration. The IC50 value of the compound was calculated, which was 32.850µg/ml and that of the standard ascorbic acid was 30.4396µg/ml.

IV. CONCLUSION

The purpose of the study was to computationally design an antioxidant chalcone. Structure-based drug design is employed to design the chalcone derivative. Molecular docking was carried out by using Human aldose reductase protein (PDB ID:1Z3N). Drug with the highest docking score was synthesized by Claisen-Schmidt condensation and characterized by TLC, IR Spectroscopy and the various physical properties of the compound such as solubility and melting point were determined. Antioxidant activity of synthesized compound was estimated by DPPH Radical scavenging activity by UV-Visible Spectroscopy.

The study confirms the in vitroantioxidant potential of the synthesized compound, with results lower than the standard ascorbic acid.

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