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Computational Simulation-Based Virtual Screening, Synthesis and In Vitro Biological Screening of a "Chalcone Hit" as Anti-Inflammatory and Antidiabetic Agents

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Abstract

The main objective of this proposed project is to identify a "chalcone hit" that has the strength to prevent the catalytic biological function mediated by an inflammatory drug target cyclooxygenase 2 (COX-2) and an antidiabetic drug target dipeptidyl peptidase IV (DPP-IV) target enzymes. The x-ray crystallographic structures (COX-2, PDB ID: 1PXX and DPP-IV, PDB ID: 2ONC) were used in the structure-based virtual screening process, where, a database of bioactive chalcones extracted from PubChem database studied for their in-silico target binding affinity against COX-2 & DPP-IV, the "chalcone hit" specific to the individual targets were conventionally synthesised and characterised using physical and spectral methods. In addition, the "chalcone hit" of a specific target was tested using in vitro bioassay protocols. The compounds (Pub Chem CID 5730821) was identified as virtual hit against COX-2, likewise, the compound (Pub Chem CID 262537) was identified as virtual hit against DPP-IV which was exhibited bioactivity in vitro experiment. Among the database of chalcones, we could have identified and selected the top ranked best fit "chalcone hits" which were synthesised further and evaluated in an in vitro COX-2 & DPP-IV enzyme-based assays on their respective hit compound was found to be having COX-2 & DPP-IV inhibitory potential..

Keywords: *virtual screening, molecular docking, chalcone, cyclooxygenase 2, dipeptidyl peptidase IV*

1. Introduction

Chalcones are flavonoid precursors, chemically it is an α,β-unsaturated ketone that forms the central core moiety responsible for various types of biological activities [1]. Chalcones are naturally occurring compounds which can be conveniently synthesised in the laboratory via. Simple aldol condensation reaction between acetophenone and aldehyde [2]. The synthetic feasibility of chalcones in chemical laboratories triggered synthetic chemists to explore the chemical diversity of novel chalcone derivatives as potential bioactive agents, certainly various types of new classes of synthetic chalcones were identified with observed biological activity profiles [3]. The bioactivity spectrum of chalcones includes anticancer [4], antiviral [5], antidiabetic [6], antibacterial [7], antifungal [8], antitubercular [9], antimalarial [10], cytotoxic [11], vasorelaxants [12], cyclooxygenase-2 (COX-2) [13], 5-lipoxygenase (5-LO) [14], dipeptidyl peptidase IV (DPP-IV) Inhibitor [15] and other bioactive profiles [16].

Non-steroidal anti-inflammatory drugs (NSAIDs) which are cyclooxygenase (COX) enzyme inhibitors in a competitive manner, approved by USFDA for rheumatoid arthritis, a class of enzymes which is responsible for the conversion of arachidonic acid substrate to inflammatory mediator products specifically prostaglandins (PGs). COX-2 is the key enzyme for the therapeutic intervention of inflammatory potential of NSAIDs, produced by inhibiting its catalytic activity, whereas the undesired side effects are due to COX-1 inhibitory activity [17]. More specific and selective COX-2 inhibitors useful to reduce the adverse effects associated with NSAIDs which inhibits both COX-1 & COX-2 [18]. Rofecoxib, valdecoxib, and celecoxib respectively are some of the clinically used COX-2 inhibitors developed with limited side effects and improved safety profile [19]. Due to the adverse cardiovascular side effects rofecoxib has been withdrawn from the market recently and currently scientists are exploring to find better ligands towards selective and more specific COX-2 inhibitory. In the same order, chalcones were initially identified as potential anti-inflammatory

properties further based on its synthetic feasibility and chemical diversity has gained remarkable attention from the related scientific communities, chalcones derivatives prepared from natural or derived from semi-synthetic or synthetic origin [20]. There are number of reports published on the modulation of anti-inflammatory drug targets such as cyclooxygenase (COX-1 & COX-2) [21]; lipooxygenase (5-LOX, 12-LOX, & 15-LOX) [22]; interleukins (IL) [23]; prostaglandins (PGs) [24]; MCP-1 (Monocyte Chemoattractant Protein-1) [25]; leukotriene D4 (LTD4) [26]; ICAM-1 (Intracellular Cell Adhesion Molecule-1) [27]; VCAM-1 (Vascular Cell Adhesion Molecule-1) [28]; nitric oxide synthase (NOS) [29] and nuclear factor-κB (NF- κB) [30] respectively.

Structure-based virtual screening strategy has become an integrated computer-aided drug discovery and development tool for performing virtual screening simulations in research and development units at pharmaceutical industry [31]. Molecular docking is a target-based computational approach to dock a group of chemical libraries into the catalytic active enzyme-binding cavity of the drug-target using a standalone or online-server based docking software, to identify virtual "hit molecules" predicted to be having potential binding at the catalytic site of a target protein [32]. This technique provide scientist with a set of molecules which can further plan to be synthesised in the laboratory and used for the *in vitro* or *in vivo* bioassay testing [33]. The ligands obtained from docking-based virtual screening protocols could be further carried forward into the phases of drug discovery pipeline, this will certainly improve the chance of getting a lead molecule that has the potential to become a drug candidate in the later stages of drug discovery. In the present study, we have performed molecular docking studies using a database of chalcones to identify the "best-fit ligand" which is a "chalcone hit" against a selected an anti-inflammatory therapeutic drug target COX-2 [34]. We have presented the structure-based molecular docking integrated virtual screening results and discussion based on the binding energy, binding interactions of the "best fit ligand". Subsequently, it was proposed worthwhile to synthesise and test using *in vitro* assay to confirm the bioactivity profile of the "best fit ligand".

Due to undesirable side effects of the currently used antidiabetic drugs, always there is a room for research in the areas related to the antidiabetic drug discovery and development [35]. The underlying pathogenesis of T2DM has projected several perspectives of therapeutic interventions in which scientist can provide their contribution to identify new chemical ligands as potential target-specific agents, some of the well-established drug targets includes dipeptidyl peptidase 4 (DPP-4) [36]; AMP (Adenosine Monophosphate)- AMPK (Activated Protein Kinase) [37]; PTP1B (Protein Tyrosine Phosphatase 1B) [38]; GLUT4 (Glucose Transporter Type 4) [39]; α-glucosidase [40]; α-amylase [41]; peroxisome proliferator-activated receptor-gamma (PPARγ) [42]; aldose reductase (ALR) [43]; and sodium glucose cotransporter 2 (SGLT2) [44]. Chalcones have already been identified as potential antidiabetic agents [45], several structure-activity relationship studies have been completed and provided insight to develop as new class of antidiabetic drugs [46].

Dipeptidyl peptidase IV (DPP-IV) is a biological enzyme and well-established antidiabetic therapeutic drug target which is present in blood as solubilized form or linked to membranes in tissues [47]. The main function is to cleave the biological peptides, this target has been identified as one of the most significant targets where it is closely associated with the pathogenesis of various diseases such as T2DM, cancer and rheumatoid arthritis [48]. DPP IV stimulates the glucose uptake of the muscle forms vital strategy in the treatment of type-2-diabetes mellitus (T2DM) [49]. The USFDA approved DPP-IV inhibitor saxagliptin act by forming a covalent bond with the target protein at the active binding site of DPP IV, however this is a reversible complex [50].

In the subsequent study protocol, we have also performed molecular docking studies using the same database of chalcones to identify the "best-fit ligand" which is a "chalcone hit" against a selected an antidiabetic therapeutic drug target DPP-IV. We have also showed the computer-aided molecular docking integrated virtual screening results and discussion based on the binding energy, binding interactions of the "best fit ligand". Subsequently, it was proposed worthwhile to synthesise and test using *in vitro* assay to confirm the bioactivity profile of the "best fit ligand".

2. Materials and Methods

2.1. Computational Software

Computer Aided Drug Design (CADD) opensource softwares which are applied in various steps involved in the process of ligand-protein reverse docking study includes Accelrys Draw™ [51],

Open Babel™ [52], ArgusLab™ v 4.0 [53] respectively, iGemdock v 2.1 is the major software used in the present study to perform molecular docking simulation investigations [54]. The relevant softwares listed above were used to perform molecular energy studies, ligand preparation, protein target preparation, active site detection and simulated docking study.

2.2. Target Protein Preparation

The crystallographic structure (X-ray) of COX-2 & DPP-IV drug-target enzymes were extricated from the website http://www.rcsb.org/pdb, a database of proteins. The choice of target proteins depends on a few variables for example structure ought to be controlled by diffraction X-ray studies, and goals ought to be between 1 - 2.5 Aº, it ought to contain a co-solidified ligand; the chose protein ought not have any breaks in their protein 3D structure. Moreover, we have also considered ramachandran graph measurements as the significant channel for protein determination that none of the deposits should introduce in denied locales. At long last, the resultant objective proteins with co-solidified ligands were set up to perform sub-atomic docking reenactment convention. The crystallographic structure acquired from PDB are appeared in the accompanying Table 1 & 2 [55].

Table 1. COX-2 X-ray structures (ligand-protein complexes) used in the target selection. The RMSD values among the two poses obtained from the native ligand and docking ligand were listed and the H-bond creating amino acid residues of COX-2 target proteins downloaded from PDB are indicated.

PDB ID	Co-ligand	Residues (H-Bond)	RMSD
1CVU	Arachadonic acid	Ser 530, Tyr 38	1.22
1CX2	S58 (LID)	Arg 120, His 90	1.01
1DDX	Prostaglandine G2	-	4.63
1PXX	Diclofenac	Ser 530, Tyr 385	0.40
3PGH	Flurbiprofen	Tyr 355, Arg 120	0.40
4COX	Indomethacin	Tyr 355	0,78
6COX	S58 (LID)	Gln 192, Leu 352, Ser 353, H 90, Arg 120	5.83

Table 2. DPP-IV X-ray structures (ligand-protein complexes) used in the target selection. The RMSD values among the two poses obtained from the native ligand and docking ligand were listed and the H-bond creating amino acid residues of DPP-IV target proteins downloaded from PDB are indicated.

PDB ID	Co-crystallized ligand	H-Bond residues	RMSD
2ONC	2-({2-[(3r)-3-Aminopiperidin-1-Yl]-4-Oxoquinazolin- 3(4h)-Yl}methyl)benzonitrile	Glu 205, Tyr 631	1.66
1NU6	2-(Acetylamino)-2-Deoxy-A-D-Glucopyranose	Asn 92, Asn75	2.01
5YP4	LYS-PRO	Glu 209, Asn 614, Asn 6 Ser 613, Tyr 645	1.92
3WQH	Anagliptin	Glu 205	2.40
4LKO	3-(Aminomethyl)-4-(2,4-Dichlorophenyl)-6-(2-Methoxyethyl)-2-Methyl-6,7-Dihydro-5h-Pyrrolo[3,4- B]pyridin-5-One	Glu 205, Glu 206, Tyr 54 Tyr 662	1.85
4J3J	N-[(3r)-3-Amino-4-(2,4,5-Trifluorophenyl)butyl]-(Trifluoromethyl)-3,4-Dihydropyrrolo[1,2-	Glu 205, Glu 206, Tyr 58 Tyr 662, Asn 710	2.73

A]pyrazine- 2(1h)-Carboxamide

2.3. Chalcone database preparation

A database of 100 chalcones were selected by applying various types of screening filters for curation as won in the following Figure 1, the curated dataset of 100 chalcones which were officially forming part of NIH molecular library [56], these 100 bioactive chalcones (chemical names and CID codes were shown in Table 3) were initially downloaded directly from PubChem (https://pubchem.ncbi.nlm.nih.gov/) database as an SDF file along with 3D coordinates, further these ligands were transformed into software compatible file format "MOL2" using Open Babel (www.openbabel.org) software and subjected for ligand preparation using Argus Lab (www.arguslab.com) and finally carried forward into the docking study using iGEMDOCK software.

Figure 1. Curation filters applied to prepare 100 bioactive chalcones database

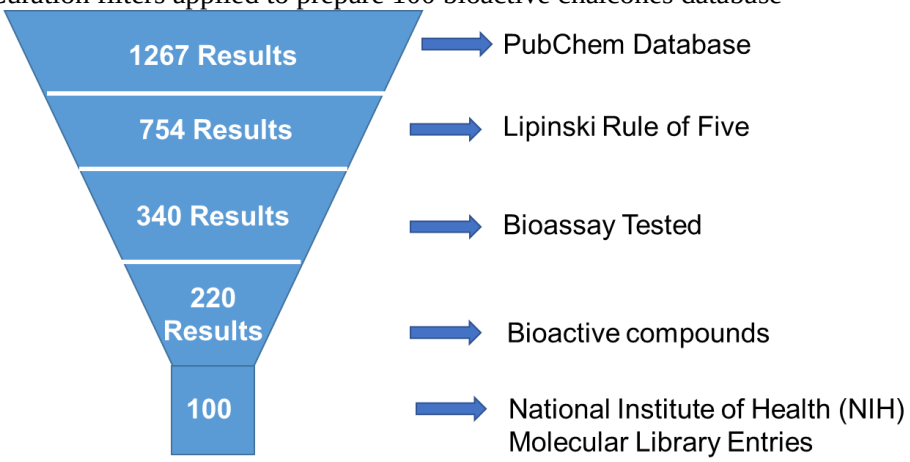


Table 3. CID codes of 100 bioactive chalcone database derived from NIH molecular library.

PubChem CID	PubChem CID	PubChem CID	PubChem CID	PubChem CID
13990811	6154317	5709228	5357660	5318998
12560199	6065576	5706277	5357218	5316793
11473265	6063342	5468166	5356384	5302715
10862032	5980988	5467477	5356340	5291959
10107266	5978982	5461154	5356121	5282362
10065951	5973690	5383464	5356057	5282361
10022050	5965183	5378514	5355888	5281294
9862769	5953849	5377854	5354779	5281222
9840805	5936200	5377516	5354494	5280960
6636248	5908055	5377374	5348046	5270542
6478421	5837330	5377323	5346086	643182
6475718	5822497	5377022	5346051	641819
6474668	5811533	5377008	5346032	641785
6474295	5743234	5376979	5346030	638278
6438825	5737668	5376916	5337942	638277
6438092	5730821	5375849	5337611	638276
6386019	5712162	5373273	5331296	637760
6304520	5712116	5372946	5322052	268760
6253344	5711223	5369664	5319688	262537

6229068	5709318	5367146	5319471	95472

2.4. Software validation

iGEMDOCK v 2.1 programming approval was completed by utilizing X-ray beam structures (1PXX & 2ONC) stored with diclofenac and (2-({2-[(3r)-3-Aminopiperidin-1-Yl]-4-Oxoquinazolin- 3(4h)-Yl}methyl)benzonitrile) co-solidified ligands, the complexes were gotten from the website http://www.rcsb.org/pdb, a database of proteins. The Root Mean Square Deviation (RMSD) between the X-beam co-solidified ligand compliance and the docked adaptation were COX-2 1.84 A^o and DPP-IV 1.66 A^o respectively confirmed that these parameters for docking protocol work was acceptable in repeating X-ray beam precious solidified structure.

2.5. Molecular docking

Sub-atomic docking strategy was utilized to dock the database of 100 bioactive chalcones against 1PXX & 2ONC utilizing iGEMDOCK to distinguish the "chalcone hit" against chose target COX-2. iGEMDOCK needs the receptor and ligand facilitates in either PDB or MOL2 group. Molecular docking has been conducted utilizing the option standard protein-ligand docking convention. The active site was characterized by crystallographic ligands of 1PXX & 2ONC. The default settings were utilized for all the numbers and docking run was completed.

2.6. Chalcone synthesis

The best fit chalcone of each target has been synthesised by reacting equimolar concentrations of substituted acetophenone with substituted benzaldehyde through a conventional Claisen-Schmidt condensation type of reaction to form an α,β-unsaturated ketone bridge between ring A and ring B of a chalcone basic structure, further the compound was purified and proceeded to physical (melting point) and spectral characterisation (FT-IR, ^{1}H NMR and Mass) further to confirm the chemical structure of the synthesised chalcone. Subsequently, the synthesised “chalcone hit” has been carried forward to the next step for screening its COX-2 inhibitory potency [57].

2.7. *In vitro* COX-2 screening

COX-2 screening has been performed using a screening assay kit (Cayman Ann Arbor, MI, USA). The screening assay was conducted based on the guidelines and instructions given by the manufacturer, as per the details reported in the literature [58].

2.8. *In vitro* DPP-IV screening

DPP-IV screening has been performed using a screening assay kit (Cayman Ann Arbor, MI, USA). The screening assay was conducted based on the guidelines and instructions given by the manufacturer, as per the details reported in the literature [59].

3. Results and Discussion

3.1. Molecular docking

The docking reenactment method was performed utilizing iGEMDOCK program with 100 bioactive chalcones (appeared in Table 3) against 1PXX protein target. Each compound has been docked into restricting site locale of 1PXX shaped by Leu 352, Trp 387, Val349, Ala 527, Ser 539, Leu 384, Tyr 348, Gly 526, Met 522, and Tyr 385 deposits separately. The least vitality docked compliance of the best populated group (best cluster) announced in this virtual screening was bearing PubChem CID: 5730821 displayed complete restricting vitality (docking score) with - 134.67 kcal/mol which is moderately progressively stable authoritative in correlation with the standard co-solidified ligand diclofenac having detailed with - 88.32 kcal/mol. Table 4 sums up the consequences of the docking scores (all out energies) between the chose chalcone database and known inhibitor of target 1PXX. The information in Table 4 shows that compound (PubChem CID: 5730821) displayed best restricting proficiency against COX-2 (1PXX) with docking score (- 134.67 kcal/mol). Among 100 chalcones the majority of the mixes indicated better restricting energies against COX-2 (1PXX) target protein than the standard diclofenac which can be decided from Table 4 the scope of docking scores for these engineered chalcones (- 86.88 to - 134.67 kcal/mol) and the outcomes are utilized to

comprehend the status of restricting limit of all the chalcones to protein target chose for virtual screening philosophy. So as to fortify this methodology our investigations conveyed forward by looking at the coupling associations of the "chalcone hit" and co-solidified ligand inside the dynamic restricting site district of protein target COX-2 (1PXX) and H-bond communicating buildups are additionally perceived inside the coupling pocket of 1PXX as appeared in Figure 2 and Figure 3. From the Figures 2 and 3, it was seen that the best fit ligand recognized in the investigation could likewise figure out how to frame a H-bond with the synergist amino corrosive buildup Ser 530, the equivalent has additionally been shaped by the local co-solidified ligand diclofenac this shows the arrangement of H-bond with Ser 530 [60] is increasingly pivotal for a compound to display its inhibitory potential, anyway so as to affirm this speculation we may require radiolabeled ligand restricting test contemplates utilizing ligand restricting area of the COX-2 objective protein.

The docking simulation technique was performed using iGEMDOCK program with 100 bioactive chalcones (shown in Table 3) against DPP-IV (2ONC) target protein. Each compound was docked into the enzyme site of 2ONC constituted by GLU 205, GLU 206, GLU 206, PHE 357, TYR 547, TRP 629, SER 630, TYR 631, VAL 656, TYR 662, TYR 666, ASN 710, VAL 711 amino acids. The docked conformation with lowest energy among the most populated cluster reported in this virtual screening was bearing PubChem CID: 262537 exhibited total binding energy with -127.219 kcal/mol which is relatively more stable binding in comparison with the standard co-crystallized ligand 2-({2-[(3r)-3-Aminopiperidin-1-Yl]-4-Oxoquinazolin- 3(4h)-Yl}methyl)benzonitrile having reported with -115.80 kcal/mol. Table 5 encapsulates the results of the docking energies concerning the selected chalcone database and standard inhibitor of target DPP-IV. The data in Table 5 shows PubChem CID: 262537 exhibited best binding efficiency against DPP-IV (2ONC) with docking score (-127.219 kcal/mol). Among 100 chalcones most of the compounds showed better binding energies against DPP-IV (2ONC) target protein than the co-crystallized ligand which can be judged from Table 5 the range of docking scores for these synthetic chalcones (-85.92 to -127.219 kcal/mol) and the outcomes are utilized to comprehend the status of binding ability of all the chalcones to protein target chose for virtual screening system. So as to reinforce this methodology our examinations conveyed forward by analyzing the binding properties of the "chalcone hit" and co-solidified ligand inside the dynamic active site locale of protein target DPP-IV (2ONC) and H-bond collaborating buildup residues are additionally perceived inside the binding pocket of 2ONC as appeared in Figure 2 and Figure 3. From the Figures 2 & 3, it was observed that the best fit ligand identified in the study could also managed to form a H-bond with the catalytic amino acid residues Tyr 666, Gly 632, Ser 628, which are different from the ones observed from the native co-crystallized ligand 2-({2-[(3r)-3-Aminopiperidin-1-Yl]-4-Oxoquinazolin- 3(4h)-Yl}methyl)benzonitrile this indicates that the formation of H-bond with Glu 205, Tyr 631 [61], thus the new interactions may have some binding role for the active binding of a chalcone hit molecule to provide the inhibitory properties more critical for a compound to exhibit its inhibitory potential, however in order to confirm this hypothesis we may need radiolabeled ligand binding assay studies using ligand binding domain of the DPP-IV target protein. This study would provide a preliminary screening to understand the binding energy of a ligand that has best-fit potential against 2ONC.

Table 4. Docking scores (kcal/mol) of chalcone database against 1PXX (COX-2 target protein).

PubChem CID	Docking Score kcal/mol	PubChem CID	Docking Score kcal/mo	PubChem CID	Docking Score kcal/mo	PubChem CID	Docking Score kcal/mo	PubChem CID	Docking Score kcal/mo
95472	-102.92	5319471	-119.80	5367146	-94.90	5709318	-102.52	6229068	-103.12
262537	-129.34	5319688	-106.19	5369664	-103.46	5711223	-114.50	6253344	-109.59
268760	-119.53	5322052	-104.72	5372946	-99.99	5712116	-106.55	6304520	-108.44
637760	-90.57	5331296	-106.91	5373273	-119.01	5712162	-101.12	6386019	-117.29
638276	-96.64	5337611	-102.26	5375849	-94.27	**5730821**	**-134.67**	6438092	-112.05
638277	-99.06	5337942	-86.88	5376916	-99.59	5737668	-95.42	6438825	-132().4
638278	-104.54	5346030	-105.18	5376979	-98.04	5743234	-121.02	6474295	-103.27

641785	-104.89	5346032	-96.14	5377008	-93.16	5811533	-102.52	6474668	-110.24
641819	-97.11	5346051	-101.06	5377022	-91.90	5822497	-107.64	6475718	-130.40
643182	-104.86	5346086	-101.97	5377323	-105.72	5837330	-104.76	6478421	-114.16
5270542	-103.84	5348046	-93.32	5377374	-98.88	5908055	-104.45	6636248	-126.46
5280960	-105.97	5354494	-105.05	5377516	-100.56	5936200	-120.48	9840805	-130.06
5281222	-110.75	5354779	-105.49	5377854	-105.09	5953849	-114.49	9862769	-129.50
5281294	-111.75	5355888	-104.93	5378514	-106.79	5965183	-99.44	10022050	-130.88
5282361	-93.26	5356057	-99.27	5383464	-98.73	5973690	-105.20	10065951	-117.23
5282362	-94.29	5356121	-109.06	5461154	-111.79	5978982	-114.40	10107266	-113.31
5291959	-111.68	5356340	-118.52	5467477	-101.20	5980988	-103.57	10862032	-127.72
5302715	-102.15	5356384	-99.12	5468166	-107.90	6063342	-114.30	11473265	-127.49
5316793	-106.12	5357218	-98.73	5706277	-105.60	6065576	-107.43	12560199	-123.54
5318998	-121.48	5357660	-98.68	5709228	-111.91	6154317	-112.26	13990811	-112.31

Table 5. Docking scores (kcal/mol) of chalcone database against 2ONC (DPP-IV target protein).

PubChem CID	**Docking Score kcal/mol**	**PubChem CID**	**Docking Score kcal/mol**	**PubChem CID**	**Docking Score kcal/mol**	**PubChem CID**	**Docking Score kcal/mol**	**PubChem CID**	**Docking Score kcal/mol**
95472	-95.284	5319471	-125.321	5367146	-96.1065	5709318	-100.49	6229068	-96.3776
262537	**-127.219**	5319688	-98.995	5369664	-99.94	5711223	-100.343	6253344	-103.572
268760	-110.692	5322052	-98.2287	5372946	-94.4339	5712116	-108.662	6304520	-99.1262
637760	-89.821	5331296	-102.399	5373273	-109.652	5712162	-96.2615	6386019	-113.259
638276	-93.6151	5337611	-106.418	5375849	-92.684	5730821	-120.457	6438092	-103.029
638277	-101.04	5337942	-85.9223	5376916	-95.8712	5737668	-97.1868	6438825	-116.522
638278	-95.6565	5346030	-100.583	5376979	-98.1214	5743234	-111.689	6474295	-100.638
641785	-102.176	5346032	-94.6433	5377008	-92.0406	5811533	-100.35	6474668	-101.875
641819	-94.7493	5346051	-102.643	5377022	-90.8263	5822497	-99.0643	6475718	-114.606
643182	-105.119	5346086	-100.57	5377323	-92.469	5837330	-99.752	6478421	-108.548
5270542	-96.4411	5348046	-90.9082	5377374	-94.7823	5908055	-100.817	6636248	-112.036
5280960	-101.053	5354494	-103.845	5377516	-96.2189	5936200	-108.023	9840805	-118.248
5281222	-102.192	5354779	-104.375	5377854	-98.7596	5953849	-108.166	9862769	-117.669
5281294	-106.897	5355888	-103.93	5378514	-108.284	5965183	-101.477	10022050	-120.764
5282361	-91.4587	5356057	-95.1962	5383464	-96.7972	5973690	-102.046	10065951	-108.209
5282362	-90.9575	5356121	-100.499	5461154	-108.846	5978982	-105.737	10107266	-102.781
5291959	-98.8472	5356340	-99.2928	5467477	-94.0573	5980988	-101.156	10862032	-118.366
5302715	-99.1872	5356384	-95.078	5468166	-100.866	6063342	-108.509	11473265	-124.701
5316793	-104.923	5357218	-95.1265	5706277	-95.0383	6065576	-99.5224	12560199	-122.726
5318998	-122.065	5357660	-93.3496	5709228	-106.294	6154317	-103.682	13990811	-105.655

Figure 2. Ligand binding profile of co-crystallized ligand (diclofenac) at the binding site region of 1PXX.

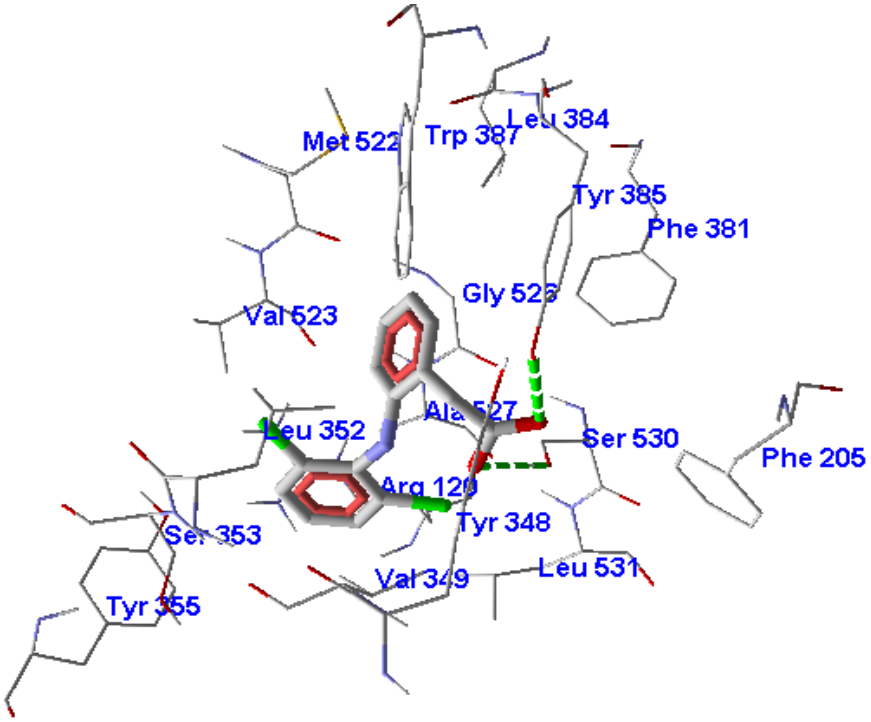


Figure 3. Ligand binding profile of chalcone hit (Pub Chem CID 5730821) at the binding site region of 1PXX.

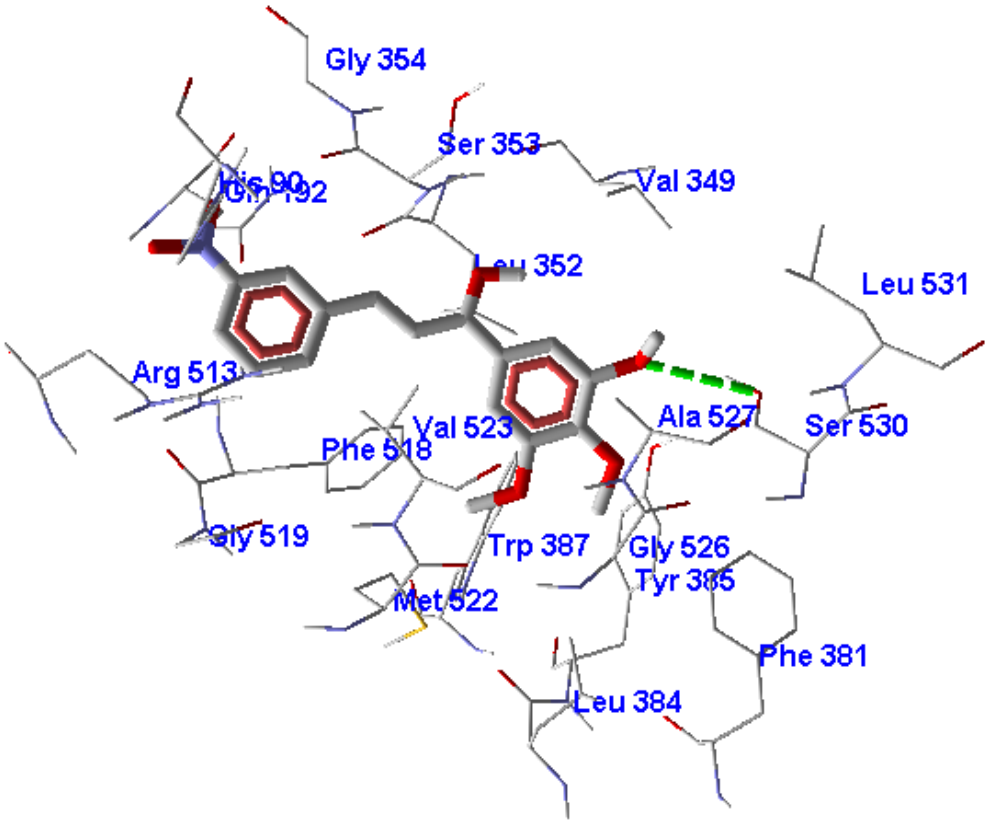


Figure 4. Ligand binding profile of co-crystallized ligand (2-({2-[(3r)-3-Aminopiperidin-1-Yl]-4-Oxoquinazolin- 3(4h)-Yl}methyl)benzonitrile) at the binding site region of 2ONC.

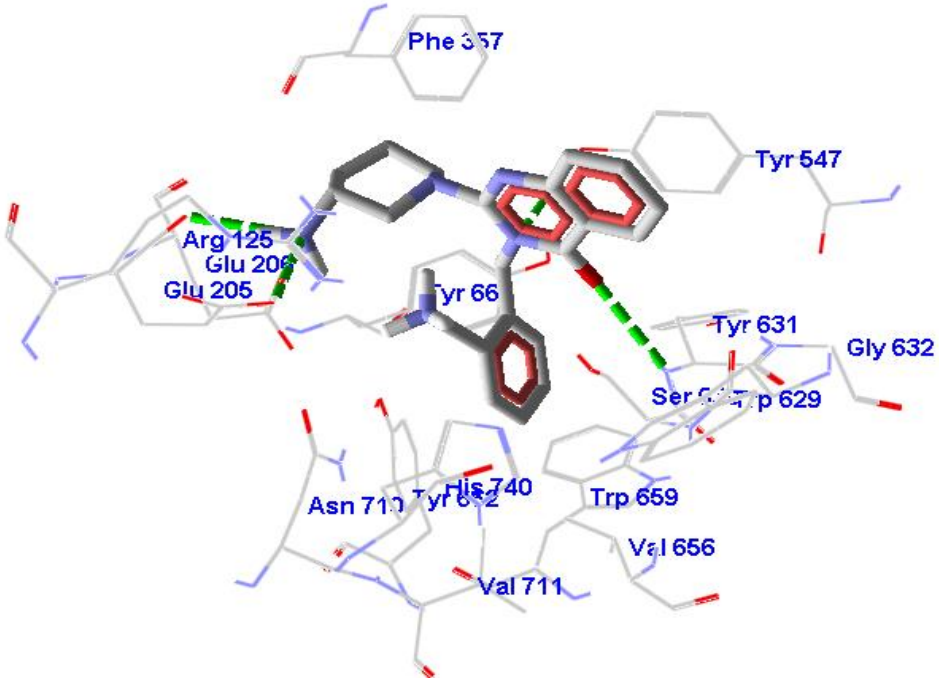
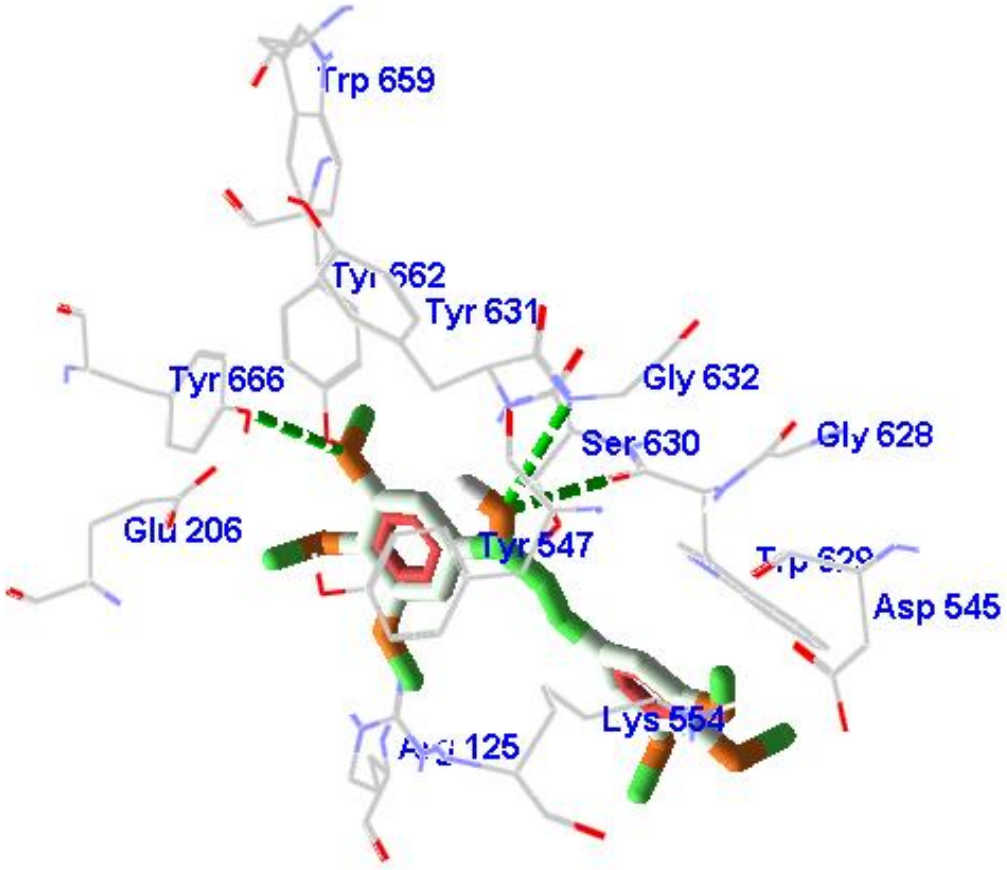
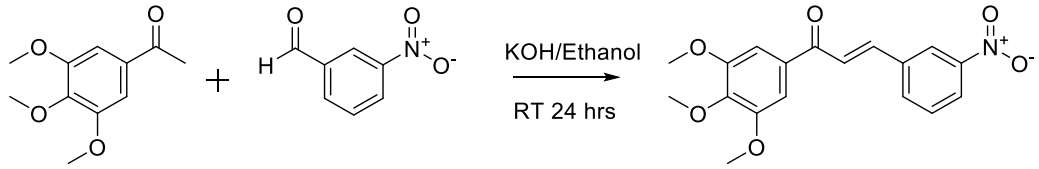


Figure 5. Ligand binding profile of chalcone hit (Pub Chem CID 262537) at the binding site region of 2ONC.



3.2. Chalcone synthesis

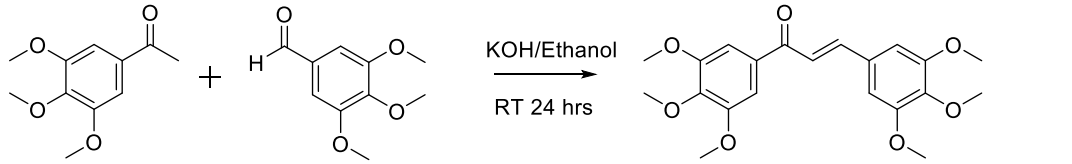
Figure 6. Scheme of synthesis of Pub Chem CID 5730821



3,4,5-trimethoxy acetophenone — 3-nitrobenzaldehyde — (E)-3-(3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-2-propen-1-one

The chalcone was synthesised by reacting equimolar quantities of 3,4,5-trimethoxyacetophenone and 3-nitrobenzaldehyde in ethanol using KOH as base, kept for stirring at room temperature for 24 hrs (Scheme of synthesis shown in Figure 6). The reaction completion was monitored by TLC, the reaction mixture was quenched with 1:1 dilute HCl and performed recrystallisation to obtain the pure yellow colored crystals as final product, m.p.: 195-198 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) 3.96 (s, 3H, Ar-OCH_3), 3.98 (s, 6H, Ar-OCH_3 x 2), 7.32 (s, 2H, Ar-H), 7.60 – 7.55 (m, 2H, Ar-H), 7.86 (d, J = 16 Hz, 1H), 7.91 (d, J = 8 Hz, 1H, Ar-H), 8.21 – 7.99 (m, 1H, Ar-H), 8.52 (s, 1H, Ar-H); FT-IR (cm^{-1}) 1672 cm^{-1} (C=O stretch), 1550 cm^{-1} (C=C stretch), 1152 cm^{-1} (C-O-C stretch); ESI-MS, positive ion mode: m/z 344 $[M+H]^+$. Based on the above spectral data the chemical structure of the compound has been elucidated as (E)-3-(3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-2-propen-1-one, the percentage yield of the product obtained was 43%. The pure crystalline product was used to prepare the dilutions ranging from 100 µM to 0.01 µM concentration to test the potency in an enzyme-based (COX-2) screening assay.

Figure 7. Scheme of synthesis of Pub Chem CID 262537



3,4,5-trimethoxy acetophenone — 3,4,5-trimethoxy benzaldehyde — (*E*)-1,3-bis(3,4,5-trimethoxyphenyl)prop-2-en-1-one

The chalcone was synthesised by reacting equimolar quantities of 3,4,5-trimethoxyacetophenone and 3,4,5-trimethoxybenzaldehyde in ethanol using KOH as base, kept for stirring at room temperature for 24 hrs (Scheme of synthesis shown in Figure 7). The reaction completion was monitored by TLC, the reaction mixture was quenched with 1:1 dilute HCl and performed recrystallisation to obtain the pure yellow colored needle shaped crystalline solid obtained as final product, m.p.: 112-115 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.81 (d, J = 16, 1H), 7.42 (d, J = 16, 1H), 7.35 (s, Ar-2H), 6.66 (s, Ar-2H), 3.91 (s, 6H, 2×Ar-OCH_3), 3.88 (s, 3H, Ar-OCH_3), 3.81 (s, 6H, 2×Ar-OCH_3), 3.81 (s, 3H, Ar-OCH_3); FT-IR (cm^{-1}) 1666 cm^{-1} (C=O stretch), 1545 cm^{-1} (C=C stretch), 1150 cm^{-1} (C-O-C stretch); ESI-MS, positive ion mode: m/z 389 $[M+H]^+$. Based on the above spectral data the chemical structure of the compound has been elucidated as (E)-1,3-bis(3,4,5-trimethoxyphenyl)prop-2-en-1-one, the percentage yield of the product obtained was 51%. The pure crystalline product was used to prepare the dilutions ranging from 100 µM to 0.01 µM concentration to test the potency in an enzyme-based (DPP-IV) screening assay.

3.3. *In vitro* COX-2 screening

Based on the activity showed in *in vitro* COX-2 assay data, the chalcone derivative (E)-3-(3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-2-propen-1-one (Pub Chem CID 5730821) exhibited IC_{50}: 52.72 µM, which is not so potent as the standard drug diclofenac which exhibited its COX-2 inhibitory potential at IC_{50}: 0.035 µM. Besides, the chemical structure of the (E)-3-(3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)-2-propen-1-one reveals that the inhibition showed by this compound is mainly due to the flavonoid nature of the ketone bridge connecting ring A and ring B of the chalcone, in addition 3,4,5-trimethoxy group which is a naturally occurring pharmacophore found in many natural products may also contributing factor in the observed activity, nitro group at the 3rd

position of the ring B might improve the selectivity towards the COX-2 receptor which is not forming part of this study.

3.4. *In vitro* DPP-IV screening

Based on the activity showed in *in vitro* DPP-IV assay data, the chalcone derivative (E)-1,3-bis(3,4,5-trimethoxyphenyl)prop-2-en-1-one (Pub Chem CID 262537) exhibited IC_{50}: 37.89 µM, which is not so potent as the standard drug sitagliptin which exhibited its DPP-IV inhibitory potential at IC_{50}: 5.91 µM. Besides, the chemical structure of the (E)-1,3-bis(3,4,5-trimethoxyphenyl)prop-2-en-1-one reveals that the inhibition showed by this compound is mainly due to the flavonoid nature of the ketone bridge connecting ring A and ring B of the chalcone, in addition bis-substituted 3,4,5-trimethoxy groups on each of the rings A and B which is a naturally occurring pharmacophore found in many natural products may also contributing factor in the observed activity, the bis-trimethoxy groups might improve the selectivity towards the DPP-IV receptor which is not forming part of this study.

4. Conclusion

In conclusion, a database of bioactive chalcones was virtually screened against the COX-2 & DPP-IV drug targets using molecular docking protocol, and as a result (Pub Chem CID 5730821) has been identified as a best fit ligand, the same ligand has also been synthesised using conventional organic synthesis methods and tested *in vitro* for its COX-2 inhibitory potential, it has exhibited potential inhibition at IC_{50} value 52.72 µM which confirms the role of chalcone basic nucleus as COX-2 specific ligand. In addition, as a result (Pub Chem CID 262537) has been identified as a best fit ligand, the same ligand has also been synthesised using conventional organic synthesis methods and tested *in vitro* for its DPP-IV inhibitory potential, it has exhibited potential inhibition at IC_{50} value 37.89 µM which confirms the role of chalcone basic nucleus as DPP-IV specific ligand. Further, *in vivo* studies needed to explore to understand the complete mechanism of action.

Conflict of Interest

None of the authors have showed any conflict of interest.

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Abbreviations

µM: Micromolar
2D: Two-dimensional
3D: Three-dimensional
ALR: Aldose reductase
AMPK: Adenosine monophosphate (AMP)-activated protein kinase
CID: Compound Identifier
COX: Cyclooxygenase
DPP-IV: Dipeptidyl peptidase IV
ESI-MS: Electro-spray Ionization Mass Spectroscopy
FT-IR: Fourier Transform Infrared
GLUT4: Glucose transporter type 4
H-bond: Hydrogen bond
ICAM: Intracellular Cell Adhesion Molecule
KOH: P:otassium hydroxide
LOX: Lipooxygenase
LT: Leukotriene
MCP: Monocyte Chemoattractant Protein
NF- κB: Nuclear Factor-κB
NIH: National Institute of Health
NMR: Nuclear Magnetic Resonance

NOS: Nitric Oxide Synthase
NSAIDs: Non-Steroidal Anti-Inflammatory Drugs
PDB: Protein Data Bank
PGs: Prostaglandins
PPARγ: Peroxisome proliferator-activated receptor-gamma
PTP1B: Protein tyrosine phosphatase 1B
RMSD: Root Mean Square Deviation
SDF: Standard Database Format
SGLT2: sodium glucose cotransporter 2
T2DM: Type 2 Diabetes Mellitus
TLC: Thin-layer chromatography
VCAM: Vascular Cell Adhesion Molecule

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