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Research Article

Pyrazolic Chalcone Derivatives Targeting Cyclin Dependant Kinase: *In-Silico* Molecular Docking, ADME and Druglikeness Studies

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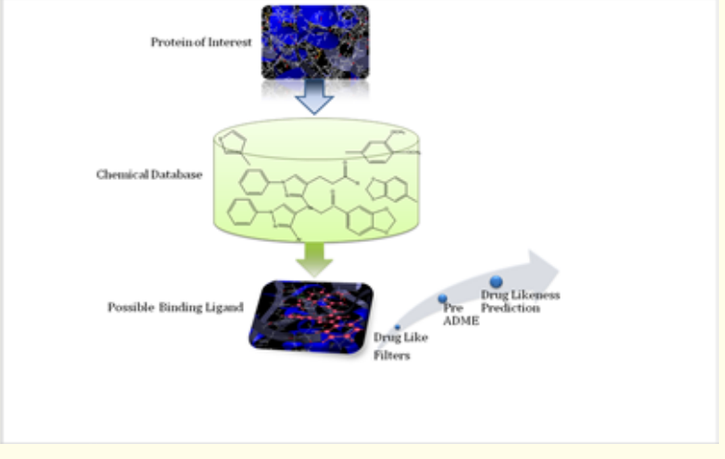
Abstract

Pharmaceutical research has successfully incorporated a wealth of molecular modelling methods, within a variety of drug discovery programs, to study complex biological and chemical system. A series of derivative with pyrazolic chalcone moiety has been studied for the anticancer activity i.e. hepatocellular carcinoma. In the present study molecular docking has been performed on 1,3-diaryl-2-propen-1-ones, naturally derived flavonoids, to identify the key structural features for binding with cyclin dependant kinase receptor. Studies revealed that residues LYS89A, LYS33A are playing a key role in determining the affinity of the active inhibitors through hydrogen bonding. Further drug like properties were evaluated using preADMET tool version 2.0 and values were found to be in ranges predicted by preADMET. The results obtained will be used in the designing of new chalcone derivatives.

Graphical Abstract



Keywords: Chalcones; Hepatocellular carcinoma; Pyrazole; Docking; Pre ADME

Abbreviation

CDK: Cyclin Dependant Kinase; HCC: Hepatocellular Carcinoma; BBB: Blood Brain Barrier; MDCK: Maden Darby Canine Kidney

Introduction

Cancer is characterized by uncontrolled tumour cell proliferation resulting from aberrant activity of various cell cycle proteins and which can then invade adjoining parts of the body and spread to other organs [1]. In 2017, 1,688,780 new cancer cases and 600,920 cancer deaths are projected to occur in the United States. For all sites combined, the cancer incidence rate is 20% higher in men than in women, while the cancer death rate is 40% higher [2]. Among all the cancers Hepatocellular carcinoma (HCC) is the 6th generally normal malignancy and the second most successive reason for growth related mortality [3]. Cyclin-dependent kinases (CDKs) are one of the key cellular kinases involved in cell cycle to control the development of cancer as shown in figure 1 [4].

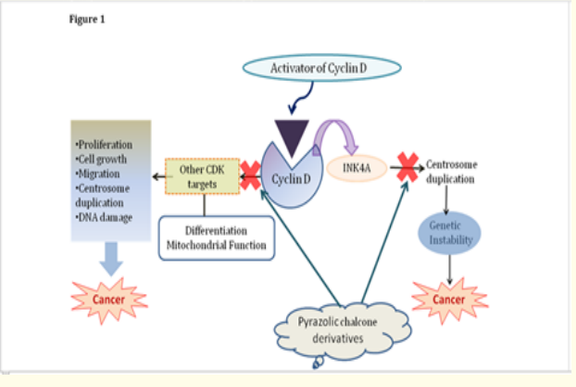


Figure 1: Role of cyclin D in cancer development.

The cyclins were named because of their periodic, cell cycle-dependent pattern of expression. The synthesis of individual cyclins, and consequent CDKs activation at specific cell cycle stages, coordinates the sequential completion of DNA replication and cell division [5-6]. These kinases also underlie the checkpoints that halt cell cycle progression in response to DNA damage and defects in the mitotic spindle. Therefore, as indicated in figure 1 cell cycle regulators CDK are considered attractive targets in cancer therapy [7].

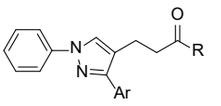
Molecules based on natural products have a relevant role in oncology drug discovery, and several natural product-derived compounds present beneficial effects when combined with classical chemotherapeutic drugs [8,9]. Chalcones (1,3-diphenyl-2-propen-1-one) are natural occurring intermediates of flavonoid [10] biosynthesis and diffusely exists in natural plant products, presenting a broad spectrum of biological activities, such as anti-cancer [11], antioxidant, anti-inflammatory, antibacterial and antimalarial [12]. It has been reported in the previous research chalcones derivatives have anticancer potential against the Cyclin dependent kinase enzymes [13].

Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. Rational Drug Design (RDD) helps to facilitate and speedup the drug designing process, which involves variety of methods to identify novel compounds [14]. One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor. Docking is the process by which two molecules fit together in 3D space. Pyrazoles itself represents as a potent scaffold [15-17] widely known for their use antipyretic, anti-inflammatory, antitumor and anticancer [11,18]. Mohammed MA Hawash., et al. have synthesized pyrazolic chalcone derivatives as novel hepatocellular carcinoma therapeutics as CDKs inhibitors [19]. Thus, in the present study we have performed molecular docking studies of these compounds against CDKs receptors by using 2R3F protein

Methodology

Molecular Docking

The process of novel drug discovery and development is generally recognized to be time-consuming, risky and costly. A typical drug discovery and development cycle, from concept to market, takes approximately 14 years and 0.8 to 1.0 billion USD [20]. Rapid developments in combinatorial chemistry and high-throughput screening technologies have provided an environment to expedite the drug discovery process by enabling huge libraries of compounds to be screened and synthesized in short time [21]. Molecular docking is an approach which anticipates the favoured binding orientation of ligand against receptor (Protein) to make a stable complex in order to predict the strength of connection or binding affinity by utilizing scoring functions. With the aim to computationally simulate the molecular identification process and accomplish an optimized conformation, 30 molecules from a series of pyrazolic chalcone derivatives [19] have been docked against CDKs protein (2R3F) in by keeping minimum free energy of the system in order to find the possible interactions between the ligands and receptor protein.



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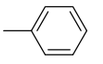
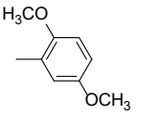
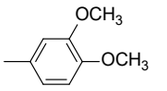
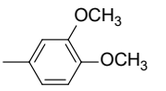
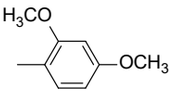
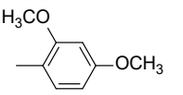
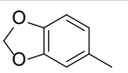
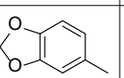
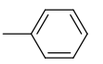
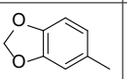
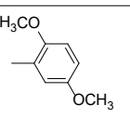
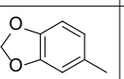
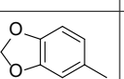
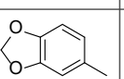
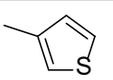
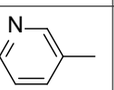
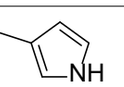
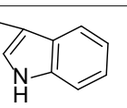
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
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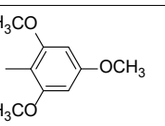
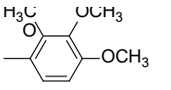
17		
18		

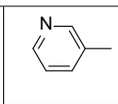
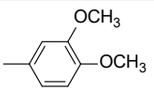
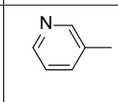
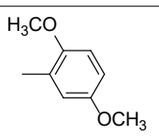
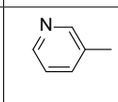
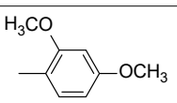
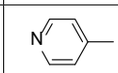
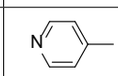
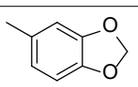
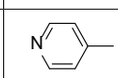
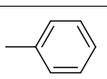
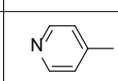
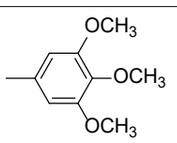
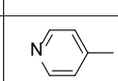
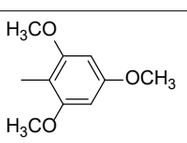
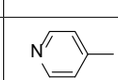
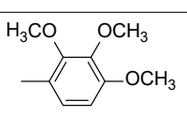
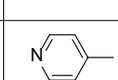
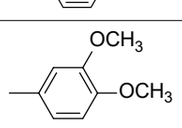
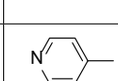
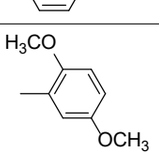
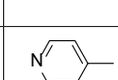
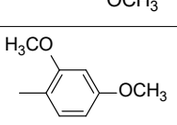
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		

Table 1: Series of various selected derivatives as novel pyrazolic chalcone compound.

Protein Preparation

The 3D Crystal structure, of CDKs from Homo sapiens, with a resolution of 1.5A was retrieved from protein data bank (PDB ID: 2R3F) in pdb format (www.rcsb.org) shown in figure 2. Before docking, the protein structure was prepared and refined by removing the water molecules, followed by addition of hydrogen atoms, deleting the ligand and co-factors present in crystal structure. The external ligand was then extracted from the protein and protein was saved in .mol format.

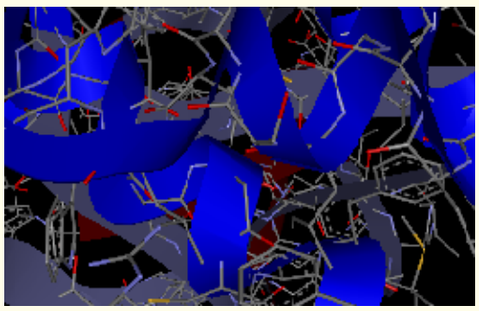


Figure 2: Structure of cyclin dependant 2 kinase receptor (2R3F); containing unwanted protein chains, water molecules.

Ligand preparation

One of the important determinants for a successful docking is the structure of the ligand. The 2D structure of the ligands were prepared by using chem draw ultra 8.0, followed by their conversion into 3D. During refinement, energy minimisation of the co-crystallized complex was carried out using Merck Molecular Force Field (MMFF) method and analytical gradient and the conformations with lowest energy were selected for further studies.

Ligand Docking

The evolution of bio and chemo informatics associated with the development of specialized software and increasing computer technologies has produced a great interest in theoretical *in silico* methods, bridging chemical and biological space is the key to drug discovery and development. Vlife MDS 4.6 is a comprehensive and integrated software package for computer aided molecule and drug discovery. Mohammed MA Hawash., *et al.* have synthesized 30 pyrazolic chalcone derivatives and are reported to have potent CDKs inhibitory activity. Docking and preADME studies on given set of molecules will help us in identifying hits or leads amongst available selected hepatocellular carcinoma therapeutic targets, as well as give important structural important feature in lead optimization process. Lower energy conformers of 30 compounds of pyrazolic chalcones derivatives were docked on the receptor 2R3F as shown in figure 3, using advanced docking programme. Docking studies were implemented via docking score and single best pose was generated as the output for particular ligand. After docking simulation, the best docked conformer of ligand was checked for various interactions in the binding sites like hydrogen bonding, hydrophobic bonding, aromatic interactions and Vander wail's interaction. The 2R3F docking complexes of pyrazolic chalcone derivative were subjected to analysis of simulate binding (D-score).

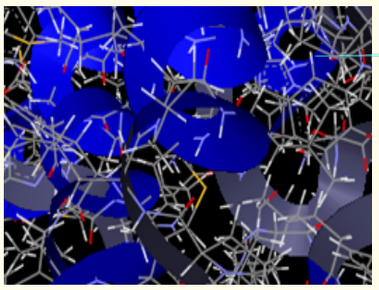


Figure 3: Structure of cyclin dependant 2 kinase receptor (2R3F); containing required protein chains and hydrogen bonds.

In-silico ADME prediction

For the compound to be effective as a drug, a molecule must reach its target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biologic events to occur. Drug development involving assessment of absorption, distribution, metabolism and excretion (ADME) is increasingly earlier in the discovery process, at a stage when considered compounds are numerous but access to the physical samples is limited. In that context, computer models constitute valid alternatives to experiments. Here, ADME properties were determined using preADMET tool version 2.0 software (preadmet.bmdrc.kr) to predict their drug like properties. The compounds were evaluated considering, predictive absorption for BBB (blood brain barrier), MDCK (Maden Darby Canine Kidney), Caco-2 cell, HIA (human intestinal absorption), and plasma protein binding [22] counting different features represented their 'druggable' pharmacokinetic profile.

Drug likeliness Prediction

Drug-likeness assesses qualitatively the chance for a molecule to become an oral drug with respect to its bioavailability based on its structural or physicochemical properties inspections. In the present study, Lipinski's rule, Comprehensive Medicinal Chemistry (CMC) like rule, MDDR (Drug Data Report) like rules and World Drug Index (WDI) rule have been used to predict the drug likeness of newly synthesized compounds. The Lipinski's Rule of Five states that for chemical compound to be a successful oral drug, it should fall within the following criteria: (i) Number of hydrogen bond donors (sum of OHs and NHs) ≤ 5, (ii) Number of hydrogen bond acceptors (sum of Os and Ns) ≤10, (iii) molecular weight ≤ 500, (iv) partition coefficient (LogP value) should be ≤ 5 [23].

Another rules to predict the drug-likeliness working on similar lines is CMC-like rules that covers the parameters like log P (-0.4-5.6), molecular weight (160-180), molar refractivity (40-130) and total number of atoms (20-70). Whereas in MDDR-like rules, descriptors used are the number of the rings, the number of rigid bonds and the number of rotatable bonds. The probability of finding a 'drug-like' compound is in its ranges (No. Rings ≥ 3, No. Of rigid bonds ≥ 18, No. Of rotatable bonds ≥ 6), the probability of finding a 'nondrug-like' compound is higher in the ranges (No. Rings ≤ 2, No. Rigid bonds ≤ 1, No. Rotatable bonds ≤ 5). WDI –like rules is based on compound that have molecular properties within 90% upper bound found in the WDI [24].

Result and Discussion

Molecular Docking

Computational docking is widely used for the study of protein–ligand interactions and for drug discovery and development. Docking simulations studies were performed on 30 molecules of pyrazolic chalcone derivatives using GRIP docking in BIOPREDICTA module with swift and accurate feature. Cocrystal structure of the protein was identified with eight cavities (active sites), but in the present studies cavity number 1 was used for docking studies where the external ligand ((5-(2,3-dichlorophenyl)-N-(pyridin-4-ylmethyl)pyrazolo[1,5-a]pyrimidin-7-amine)*) was present. Important amino acid residues present and involved in the binding active site were LYS20A, HIS84A, LYS89A, LYS33A, PHE82A, GLU8A, LYS20A, ILE10A, LEU83A, LYS89A etc. In the present studies GRIP docking module has been used for docking studies, which essentially requires a set of ligands with its conformers to be docked into a receptor cavity. Using the information regarding receptor cavity and ligand position, it generates a grid around the reference ligand in the active site or the whole active site itself. Thus in order to postulate a hypothetical binding model for the interaction of these 30 pyrazolic molecules, they have been docked on the same cavity with a motive to find the similarity and type of interactions involved in comparison to external ligand, after following the protocol of removal of water molecules and addition of hydrogen atoms to maintain valency. Results in the form of Docking scores which are fast approximate mathematical method used to predict the strength of non-covalent interaction between two molecules. Compiled in the table 2 and figure 4(a-e), 5(a-e), 6(a-e). The 2D and 3D and binding pocket representation of the ligand-receptor interactions of the selected five compounds 4, 12, 23, 28 and 29, molecules are selected on the basis of low IC_{50} w.r.t. cyclin -CDK receptor respectively. Grip values docked pose of the fitted ligands were visualized extending deep into the active site pocket and showing several interactions such as Vander Waal's, hydrophobic contacts and π-π stacking interactions and hydrogen bonds with the key residues of the active site figure 4(a-e) All the compounds have been found to bound best with the cyclin -dependent kinase 2 receptor and afforded high dock score from -67.76 to -50.42. Further docking behaviour was found in comparison to the reference external ligand majorly by interacting with common amino acid residues LYS89A, ILE10A, VAL18A, GLY13A and strong hydrophobic bonds. Amongst all the docked ligand, compound 4 has shown greater affinity for CDKs receptor with highest dock score of -67.76. The high score of compound 4 can be attributed to its strong hydrogen bonds between nitrogen of ring B, and carbonyl oxygen with amino acid residue LYS33A, LYS89A at the distance of 2.49Ă and 2.23Ă, respectively. Hydrophobic interactions also been observed in compound 4 with amino acid residue HIS84A, LYS20A with the distance 4.48Ă and 4.88 Ă respectively (Table 3). Complementarily shape, and 60-70% similarity of residues with reference could be also contributory factors for their great binding and decreased energy.

Compound No	D-Score
1	-65.94
2	-48.08
3	-33.67
4	-67.76
5	-49.99
6	-45.43
7	-65.72
8	-59.97
9	-44.91
10	-59.34
11	-62.76
12	-63.04
13	-51.43
14	-58.70
15	-58.24
16	-48.26
17	-44.27
18	-26.63
19	-51.23
20	-32.53
21	-30.69
22	-53.66
23	-50.42
24	-46.93
25	-17.74
26	-42.34
27	-32.61
28	-65.25
29	-59.65
30	-56.66

Table 2: Docking score of the compound.

Compound No	D-Score	No. of Residues	Hydrophobic Interaction	Hydrogen Bonding
4	-67.76	4	LYS20A,HIS84A	LYS89A, LYS33A
12	-63.04	5	GLN131A, VAL18A,GLY13A	LYS33A, LYS89A
23	-50.42	5	LYS20A,ILE10A, PHE82A	LYS89A, LEU83A
28	-65.25	6	PHE82A, ,GLU8A,LYS20A, ILE10A	LEU83A, LYS89A
29	-59.65	7	GLN131A,GLY134,GLU12A,LEU134A,ALA144A,ASN132A	LYS33A

Table 3: Type of interactions involved.

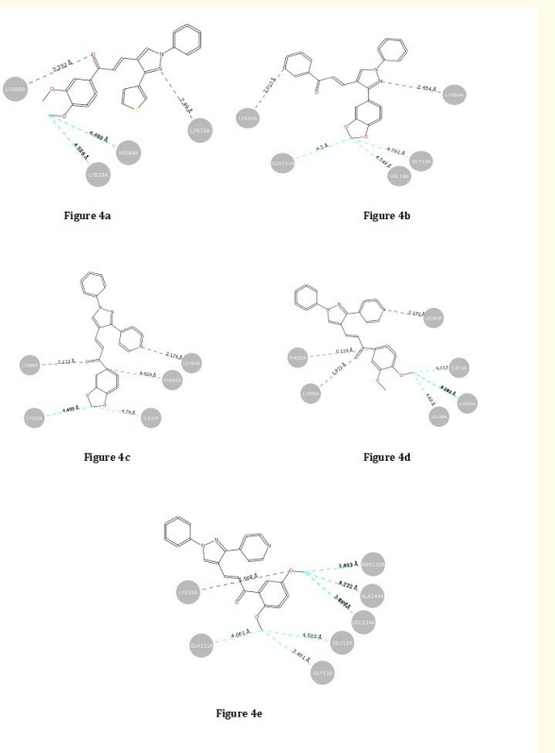


Figure 4a

Figure 4b

Figure 4c

Figure 4d

Figure 4e

Figure 4 (a-e)

(4a) 2D representation showing the binding orientation of the compounds 4 into the active side of 2R3F.

(4b) 2D representation showing the binding orientation of the compounds 12 into the active side of 2R3F.

(4c) 2D representation showing the binding orientation of the compounds 23 into the active side of 2R3F.

(4d) 2D representation showing the binding orientation of the compounds 28 into the active side of 2R3F.

(4e) 2D representation showing the binding orientation of the compounds 29 into the active side of 2R3F.

----------: Hydrophobic Interactions

----------: Hydrogen Bonding

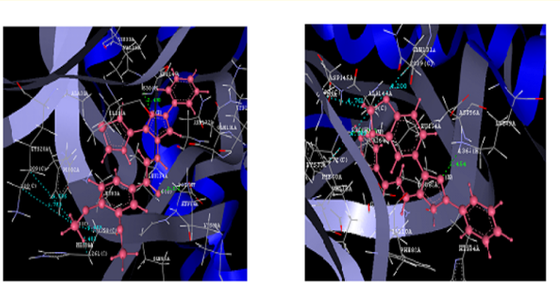


Figure 5a

Figure 5b

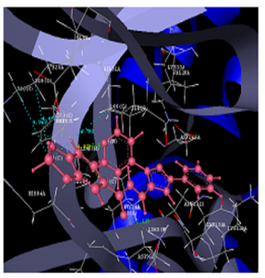


Figure 5c

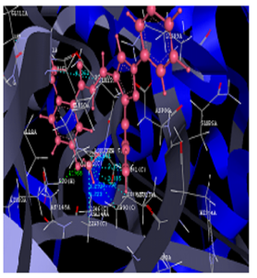


Figure 5d

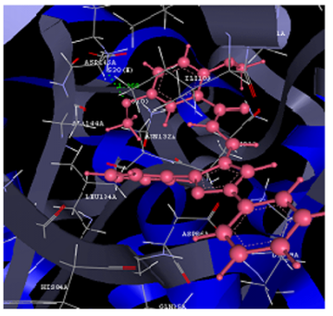


Figure 5e

Figure 5 (a-e)

(5a) 3D representation showing the binding orientation of the compounds 4 into the active side of 2R3F.

(5b) 3D representation showing the binding orientation of the compounds 12 into the active side of 2R3F.

(5c) 3D representation showing the binding orientation of the compounds 23 into the active side of 2R3F.

(5d) 3D representation showing the binding orientation of the compounds 28 into the active side of 2R3F**.**

(5e) 3D representation showing the binding orientation of the compounds 29 into the active side of 2R3F.

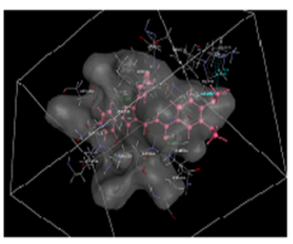


Figure 6a

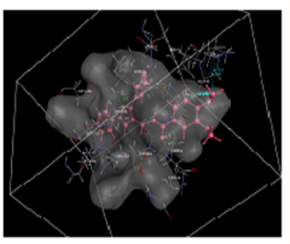


Figure 6b

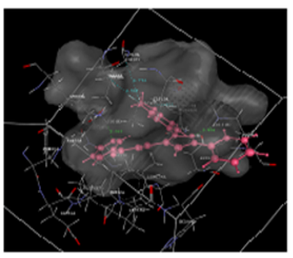


Figure 6c

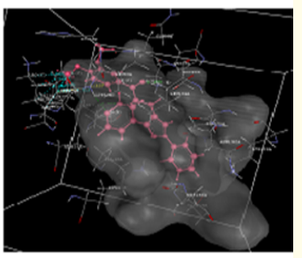


Figure 6d

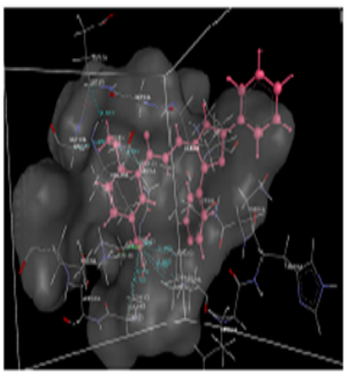


Figure 6e

Figure 6 (a-e)

(6a) 3D representation showing the binding orientation of the compounds 4 into the active side of 2R3F; gray surface represent pocket site of the protein.

(6b) 3D representation showing the binding orientation of the compounds 12 into the active side of 2R3F; gray surface represent pocket site of the protein.

(6c) 3D representation showing the binding orientation of the compounds 23 into the active side of 2R3F; gray surface represent pocket site of the protein.

(6d) 3D representation showing the binding orientation of the compounds 28 into the active side of 2R3F; gray surface represent pocket site of the protein.

(6e) 3D representation showing the binding orientation of the compounds 29 into the active side of 2R3F; gray surface represent pocket site of the protein.

In-silico ADMET prediction

Number of the drugs under clinical trials couldn't see the clinics due to the of failure at the stage of pharmacokinetic assessment. Recent analysis by Kennedy covers the different causes by which 198 NCEs failed in clinical development and found that the most prominent cause of the failures was associated with poor pharmacokinetic (PK) and ADME properties [25]. Although lack of efficacy could also be one of the main reasons for terminations, the unsatisfactory PK/ADME, toxicology and adverse effects accounts for up to two-thirds of the total failures. Initial screening of hits and leads before their clinical testing will not only decrease the rate of failure, but it reduces the cost of drugs discovery program. Taking into consideration, a preliminary predictive *in-silico* pharmacokinetic study of the selected series of compounds was undertaken using online server preADMET (http://preadmet.bmdrc.org/). Incorporation of such tools as a part of the drug design process can screen molecules that are more likely to exhibit satisfactory ADME properties. The server calculated the parameters such as human intestinal absorption (HIA%), cellular permeability Caco-2 *in vitro,* cell permeability Maden Darby Canine Kidney (MDCK), % plasma protein binding, blood brain barrier (logPS) (Table 4).

Compound No.	HIA (%)	Caco-2 (nm/sec)	MDCK	BBB (logPS)	Plasma protein binding (%)
4	97.69	50.99	0.11	0.50	95.72
12	97.45	47.32	0.61	0.10	95.98
23	97.45	48.27	0.16	0.19	99.27
28	97.44	51.38	0.07	0.47	94.93
29	97.44	54.33	0.06	0.10	96.70

Table 4: Pre-ADME prediction.

For ADMET prediction, 5 molecules from the selected series of 30 molecules were chosen on the basis of low IC_{50} values. The results of ADME prediction are shown in table 4. These properties are presented as a determinant for drug development factors, being the biggest target objectives: good absorption, distribution, metabolism and excretion. Mainly, human intestinal absorption properties, because it is determinant for the drug development that purport to be administered orally. All the compounds under study presented human intestinal absorption values (HIA) in the range of 97.44 to 97.69. The absorption processes are related to the permeation of compounds through biological membrane under the influence of physicochemical characteristics, thus from present observation we can say that good physicochemical properties of the compounds enabled them to qualify HIA% with values > 80 - 100%. The low values for brain barrier indicating no diffusion of these molecules in brain. Further, the cell permeability *in vitro* Caco-2 is an important test to assess intestinal absorption of drugs. Values (47.32 to 54.33) greater than 25 nm/sec in table 4 are indicating the greater cell permeability of all the selected compounds for Caco -2 cells. Analysis of the data in table 4 for MDCK system, indicates low permeability of these inhibitors towards kidney cells with values from 0.06 nm/sec to 0.61 nm/sec. Permeability can be classified into low (< 25 nm/sec) and mean (> 25 to 500 nm/sec). The distribution properties are verified by the % plasma protein binding (%PPB) values, and are found in the range of 95.70 to 99.98. Such inhibitors got the potential to trigger the pharmacological response as a large amount of free drug is available to interact with their receptors. In addition to changing the pharmacological response of molecule the PPB also modifies the renal excretion because only unbound drug is available for glomerular filtration thus increasing excretion and decreasing half-life.

Toxicity of most potent five compounds has been predicted by using preADMET tool and results are listed in the table 5. Data obtained from the toxicity prediction studies indicating that all the derivatives are found to be non-carcinogenic with their negative results for the carcino mouse model. In contrast to this, pyrazolic derivatives have shown positive results against carcino rat model except for compound 4 which is in agreement with the docking studies indicating the strong interaction of compounds 4 with less energy. Further, the Human Ether Related Gene (hERG) channel is associated with cardiac action potential and its blockade result in slowed repolarization and prolongation of action potential duration responsible for sudden death. Present study has indicated the medium level associated with all compounds against hERG inhibition. Thus low docking score with less binding energy along with negative observation for carcino rat and medium risk with hERG not only confirm the non-carcinogenic nature of compound 4 but made it safer for further studies.

Compound No	Carcino_ Mouse	Carcino_ Rat	hERG_ inhibition
4	Negative	Negative	Medium_risk
12	Negative	Positive	Medium_risk
23	Negative	Positive	Medium_risk
28	Negative	Positive	Medium_risk
29	Negative	Positive	Medium_risk

Table 5: Toxicity prediction.

Drug likeliness Prediction

Drug likeness is a qualitative concept used in drug design for how "drug like" a substance is, with respect to factors like bioavailability. It can be estimated from the molecular structure, before its actual syntheses and evaluation. It is not surprising then, that even though the chemical structures of drugs can differ greatly in accordance with the requirement of complementary interactions to diverse target receptors successful drugs on the market today do share certain similarities in their physicochemical properties. Primarily, such characteristics determine the pharmacokinetics of the drug, where favourable ADME (absorption, distribution, metabolism, and excretion) properties are required. Therefore, from the above discussion the result obtained from table 6 showed that all the synthesized compounds used in the study have successfully qualified drug-likeliness properties such as Lipniski's Rule, CMC like rules. Whereas according to MDDR like rules, compound 4, 25, 29 falls in the category of drug likeness except for compound 12 and 27 that exhibit mid structure property.

Compound No	Rule of Five	CMC like Rule	MDDR like Rule	WDI like Rule
4	Suitable	Qualified	Drug-like	90%
12	Suitable	Qualified	Mid-structure	90%
23	Suitable	Qualified	Mid-structure	90%
28	Suitable	Qualified	Drug-like	90%
29	Suitable	Qualified	Drug-like	90%`

Table 6: Drug likeliness prediction.

Conclusion

In conclusion, a series of pyrazolic chalcone derivatives were evaluated for the ligand receptor interaction. Among the series, compound 4 has been well accommodated in the binding pocket of cyclin dependent kinase in comparable orientation with lowest estimated binding energy -67.76 with its hydrogen bonding and hydrophobic interactions. All the compounds signifying the good ADMET profile and drug likeness properties and compound 4 was found to be safe and non-toxic amongst all. The present study suggested that the strategy of predicting CDKs inhibition based on pyrazolic substituted chalcones and their orientation would be useful for developing novel potent CDKs inhibitors.

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