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Research Article

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In Silico drug designing studies on Human Protein Kinases inhibitors

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ABSTRACT

The key protein / enzyme involved in cancerous process are Human Protein kinases, which are considered as major therapeutic targets for cancer drug development. Several inhibitors have reported positively for Human protein Kinases in dysregulation of cancerous process. Chalcones, present in various plant species as open-chain flavonoids are reported to have anticancer properties. In the present study we designed various chalcones analogues and studied their binding efficiency of their analogues with Human Protein kinases through Insilico methods. By our virtual screening and docking result, we found that the Compound H and Compound J have highest binding affinity with the Human Protein Kinases and also we predicted the binding site.amino acid residues and the nature of hydrogen bonding. However more invivo experimental validation of our results with animal models will enlighten the development of more potent drugs from chalcone derivatives for treatment of cancer.

Key words: *Human Protein Kinases, Chalcones derivatives, Binding interation, molecular docking*

INTRODUCTION

One of the main challenges in modern drug discovery and research against cancer is to discover effective, safer chemotherapeutic agents targeting various metabolic byproducts involved during the progression of cancer. Presently researcher involving Cancer drug discovery always focused on the newly synthesized therapeutic agents that act on specifically targeted key molecules involved in the cancerous process [1–4] One such key molecule involved in cancerous process is Protein kinases which is considered as major therapeutic targets and several inhibitors target against this kinase have reported positively in dysregulation of cancerous process. CK2, a serine / threonine protein kinase which is ubiquitous, highly conserved made up of a heterotetrameric holoenzyme, functionally acts as a dimer containing two catalytic subunits (a and / or a 0) and a regulatory b subunits [5]. The major function of this enzyme CK2 is to phosphorylate intermediates which are having an important role cell division, differentiation and at transcription level. Over expression, non regulation or dysregulation were reported in many types of cancer and also in hematological malignancies and hence it is a good target for rational drug therapies [6]. The heterocyclic carbazole motif, a privileged scaffold found in many natural and synthetic carbazole derivatives is reported to have diverse biological functions.

Chalcones are synthesized in a various plant species as open-chain flavonoids. The structure of chalcones and its derivatives consist of two aromatic rings connected by a 3 -carbon α,β,-unsaturated carbonyl system and chemically

known are 1,3- diphenyl-2-propen-1-ones [7]. Chalcone and its derivatives are reported and proved in various researches that it has various biological activities that includes anticancer activity [8]. For more than three decades the anticancer activity of chalcones has been investigated and reported in various reports [9]. But very few reports explain the basic mechanism of chalcones for the cytotoxic or anti cancer activities which include cell cycle regulation, inhibition of angiogenesis, tubulin polymerization, nuclear factor-kappa B (NF-κB), and protein kinases [10 – 13]. The success of naturally occurring chalcones as one of the probable anticancer agents has given the inspiration for numerous novel synthetic chalcones development.

Such efforts generally have three important strategies. First, structural manipulation of both aryl rings, second, the replacement of aryl with heteroaryl/ alicyclic/ steroidal scaffolds and third, molecular hybridization through conjugation with other active pharmacological scaffolds for increasing the anticancer properties [14,15]. So many studies revealed chalcones and their analogue has anticancer properties. But yet many studies have to be performed on chalcones and their analogues with targeted proteins or enzymes related to cancer. Our study is focused on the designing various chalcones analogues and their binding efficiency of their analogues with Human Protein kinases.

The list of 13 Chalcones derivatives structures are listed in the Fig.1 and their properties are listed in the Table – 1.

Fig. 1: Various Chalcones derivatives

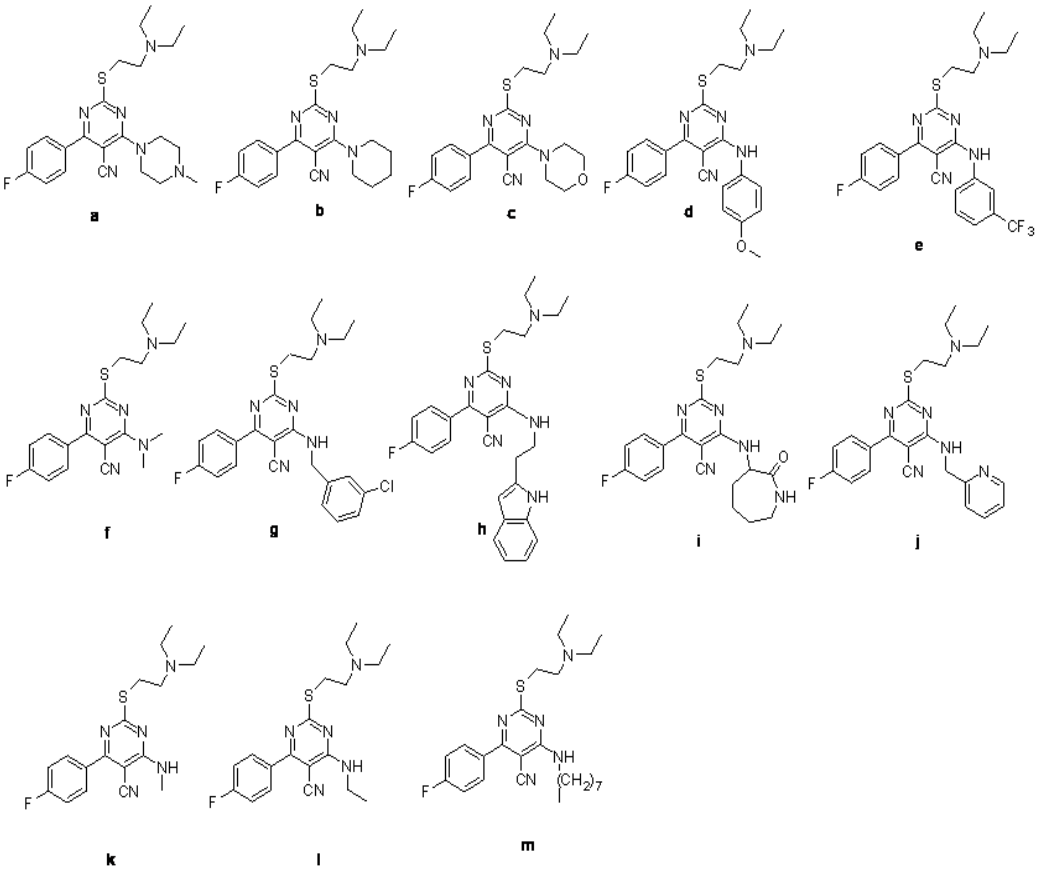


Table – 1: Properties of Various Chalcones derivatives

Derivative	Molecular formula	Formula weight	Composition	Molar Refractivity (cm^3)	Molar Volume (cm^3)	Parachor (cm^3)	Index of Refraction	Surface Tension (dyne/cm)	Density (g/cm^3)	Polarizability (10^{-24} cm^3)	RDBE	Monoisotopic Mass (Da)
A	$C_{22}H_{29}FN_6S$	428.56	C(61.66%),H(6.82%),F(4.43%), N(19.61%), S(7.48%)	120.28 ± 0.4	342.5 ± 5.0	962.6 ± 6.0	1.619 ± 0.03	62.3 ± 5.0	1.25 ± 0.1	47.68 ± 0.5	11	428.21
B	$C_{22}H_{28}FN_5S$	413.55	C(63.89%),H(6.82%),F(4.59%), N(16.93%), S(7.75%)	116.39 ± 0.4	336.2 ± 5.0	936.3 ± 6.0	1.608 ± 0.03	60.0 ± 5.0	1.22 ± 0.1	46.14 ± 0.5	11	413.20
C	$C_{21}H_{26}FN_5OS$	415.52	C(60.70%),H(6.31%),F(4.57%), N(16.85%), O(3.85%),S(7.72%)	113.51 ± 0.4	326.1 ± 5.0	916.5 ± 6.0	1.612 ± 0.03	62.3 ± 5.0	1.27 ± 0.1	44.99 ± 0.5	11	415.18
D	$C_{24}H_{26}FN_5OS$	451.55	C(63.84%),H(5.80%),F(4.21%), N(15.51%), O(3.54%), S (7.10%)	126.13 ± 0.4	355.8 ± 5.0	999.8 ± 6.0	1.626 ± 0.03	62.3 ± 5.0	1.26 ± 0.1	50.00 ± 0.5	14	451.18
E	$C_{24}H_{23}F_4N_5S$	489.53	C(58.88%),H(4.74%),F(15.52%), N(14.31%), S(6.55%)	124.75 ± 0.4	364.9 ± 5.0	1003.2± 6.0	1.599 ± 0.03	57.0 ± 5.0	1.34 ± 0.1	49.45 ± 0.5	14	489.16
F	$C_{19}H_{24}FN_5S$	373.49	C(61.10%),H(6.48%),F(5.09%), N(18.75%), S(8.59%)	104.54 ± 0.4	306.6 ± 5.0	845.5 ± 6.0	1.597 ± 0.03	57.8 ± 5.0	1.21 ± 0.1	41.44 ± 0.5	10	373.17
G	$C_{24}H_{25}ClFN_5S$	470.00	C(61.33%),H(5.36%),Cl(7.54%), F(4.04%), N(14.90%), S(6.82%)	129.22 ± 0.4	361.1 ± 5.0	1018.4± 6.0	1.634 ± 0.03	63.2 ± 5.0	1.30 ± 0.1	51.22 ± 0.5	14	469.15
H	$C_{27}H_{29}FN_6S$	488.62	C(66.37%),H(5.98%),F(3.89%), N(17.20%), S(6.56%)	140.51 ± 0.4	379.0 ± 5.0	1087.6 ± 6.0	1.663 ± 0.03	67.7 ± 5.0	1.28 ± 0.1	55.70 ± 0.5	16	488.21
I	$C_{23}H_{29}FN_6OS$	456.57	C(60.50%),H(6.40%),F(4.16%) N(18.41%),O(3.50%),S(7.02%)	124.54 ± 0.4	358.4 ± 5.0	1009.7 ± 6.0	1.611 ± 0.03	62.9 ± 5.0	1.27 ± 0.1	49.37 ± 0.5	12	456.21
J	$C_{23}H_{25}FN_6S$	436.54	C(63.28%),H(5.77%),F(4.35%), N(19.25%),S(7.35%)	122.19 ± 0.4	343.2 ± 5.0	976.8 ± 6.0	1.630 ± 0.03	65.6 ± 5.0	1.27 ± 0.1	48.44 ± 0.5	14	436.18
K	$C_{18}H_{22}FN_5S$	359.46	C(60.14%),H(6.17%),F(5.29%), N(19.48%),S(8.92%)	99.68 ± 0.4	291.6 ± 5.0	807.4 ± 6.0	1.599 ± 0.03	58.7 ± 5.0	1.23 ± 0.1	39.51 ± 0.5	10	359.15
L	$C_{19}H_{24}FN_5S$	373.49	C(61.10%),H(6.48%),F(5.09%), N(18.75%),S(8.59%)	104.31 ± 0.4	307.7 ± 5.0	847.5 ± 6.0	1.593 ± 0.03	57.4 ± 5.0	1.21 ± 0.1	41.35 ± 0.5	10	373.17
M	$C_{18}H_{22}FN_5S$?	359.46	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

MATERIALS AND METHODS

Preparation of macromolecule HPK:

The protein data bank (PDB) is a database which has 27-year history, containing information on more than 9000 three-dimensional structures of proteins as well as biological macromolecules that are experimentally determined. Data can be easily submitted through PDB's using mm CIF or PDB format, those data are conveniently examined through PDB's WWW-based tool 3DB Browser. Its Collaborative centers established worldwide helps to assist in deposition, archiving, and distribution of data's. HPK is the key molecule involved in cancerous process.3-Dimensional (3D) structures of the HPK was obtained from Protein Data Bank (PDB) [16]. 3D structures of HPK were visualized through Py-MOL viewer [17].

Preparation of ligands or chemical derivatives:

Co-crystallized ligands were identified and their derivatives were obtained from NCBI PubChem Compound database [18] and constructed using chemsketch [19]. Hydrogen atoms were added to all the derivatives and their gasteiger atomic partial charges were computed. A geometrical optimization of all the chalcones derivatives was performed to find flexible conformations of the compounds during the docking using chimera[20].

Docking study:

iGEMDOCK (A Generic Evolutionary Method for molecular DOCKing) integrated the computer based virtual screening, structure based molecular docking, post screening, analysis and 3D structure visualization steps. The virtual screening and docking studies were carried out using IGEMDOCK docking program[21]. 3D structural coordinates of HPK was implemented and imported through the GEMDOCK user interface to carry out the computer aided docking study of chalcones derivatives. In the pre docking analysis, the output path was set. GEMDOCK default data parameters included the population size (n = 150), number of solutions (s = 15) and generation (g = 60) to compute the probable ligand binding conformation for HPK protein. Then the docking calculation was initiated using GEMDOCK algorithm scoring function. After docking run, the individual binding conformation pose of each derivatives were analyzed and their binding interaction with the target protein HPK was observed. In the post docking analysis, the best binding pose and total binding energy of each derivative was analyzed. The binding affinity, best binding pose and total binding energy values were saved in output folder. A detailed interaction of Protein – ligand binding site was analyzed and visualized using 3D macromolecule visualizes PyMOL [17].

RESULTS AND DISCUSSION

The 3D **structure coordinates** of human protein kinase-CK2 is optimized and 13 compounds are energy minimized to have minimal potential energy using chimera. Evaluation of binding conformation of 13 analogs with HPK-CK2 is performed using iGEMDOCK. From docking study, we listed binding affinity of 13 compounds based on ligand binding energy (Table. 2).

Table – 2: Docking results for Human protein kinase

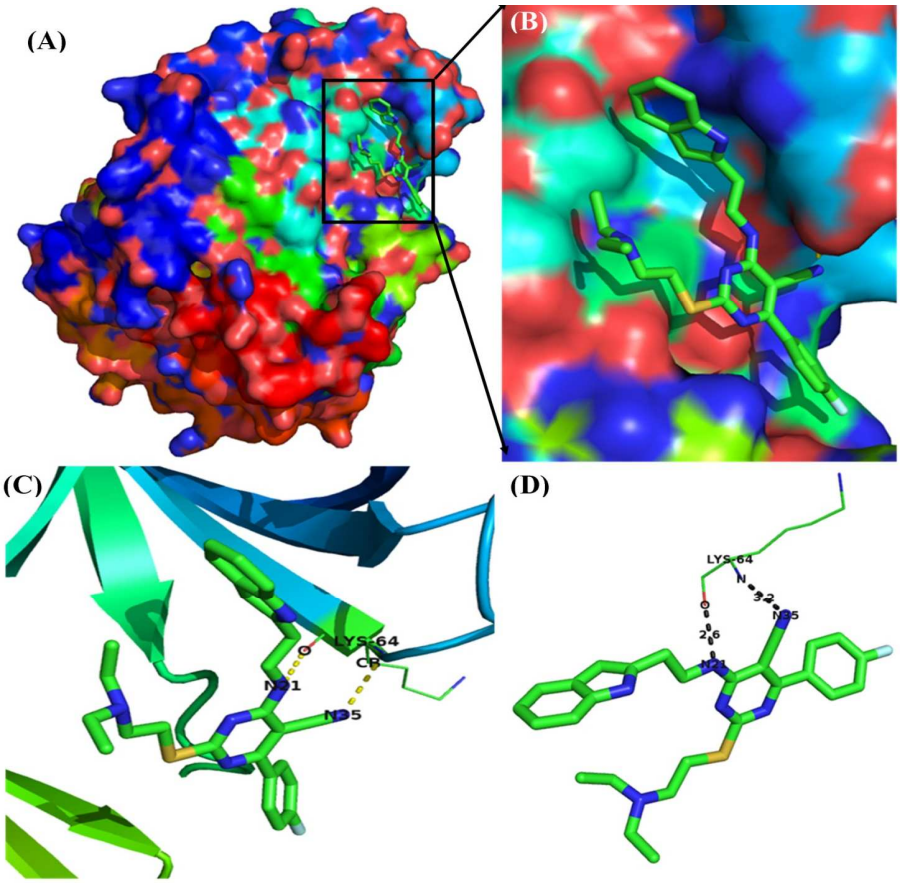
Ligand	Binding energy kca/mol	Ligand efficiency kcal/mol	H-bond formed	H-bond residues	H-bond distance
A	-7.47	-0.25	2	(Lys68)Nz-N30 (Asp175)NH-OD1	3.6 1.8
B	-7.07	-0.24	1	(Val116) N-N15	2.9
C	-7.27	-0.21	1	(Val116) N-N21	3.3
D	-7.88	-0.25	1	(Asp175)OD 1-NH	1.8
E	-6.68	-0.2	2	(Val116)O-H39 (Val116)N-N27	2.7 3.1
F	-6.07	-0.23	1	(Leu45)O-NH	1.9
G	-7.09	-0.23	1	(His160)NE 2-N21	3.5
H	**-8.7**	**-0.3**	**2**	**(Lys64)N-N36 (Lys64)O-N21**	**3.2 2.6**
I	-7.98	-0.25	1	(Asn189)OD 1-N27	3.3
J	**-8.2**	**-0.26**	**1**	**(Asp175) OD1-N21**	**3.2**
K	-6.32	-0.25	2	(Val116)N-N25 (Val116)O-H44	2.9 2.6
L	-6.32	-0.25	2	(Val116)O-H45 (Val116)N-N26	2.3 3.0
M	-5.41	-0.17	1	(Leu45)O-NH	2.1

The binding pose for each ligand molecule into the HPK-CK2 target enzyme is analyzed and the one having lowest ligand binding energy with HPK-CK2 among the different poses are generated. The lower energy scores represent better target protein-ligand binding affinity compared to higher energy score. Among the 13 analogs, compound H and J are found to have lower ligand binding energy value than other analogs. Analog "H" has least binding energy score with HPK-CK2 (binding energy value=-8.7 kcal/mol) and compound J has ligand binding energy value of -8.2 kcal/mol. We further analyzed the docked pose for finding the binding mode of compound H and compound J in to HPK-CK2 to validate the reasonable binding conformations.

Docking of compound – H into HPK-CK2:

Docking simulation of compound - H is performed for HPK-CK2. From the docking study, we observed that compound – H has best binding affinity with the cancer target protein HPK-CK2. Interaction analysis of binding mode of compound – H in HPK-CK2 reveals that it forms two hydrogen bonds with polar residue Lys64. Compound H is found to form a hydrogen bond by interaction with NH group of Lys64 with (bond distance= 3.2 Å). Another hydrogen bond is observed between the atom N21 and the oxygen moiety of Lys64 (Fig 1). A close-up view of binding mode. Close up view of ribbon model Interaction of compound – H with Human protein kinase-CK2. The interacted amino acids residues, hydrogen bond networks in the binding pocket and the distance (in Angstrom units) of bonds are all shown in Fig.1.

Fig. 1: Docking results of compound – H in to Human protein kinase-CK2

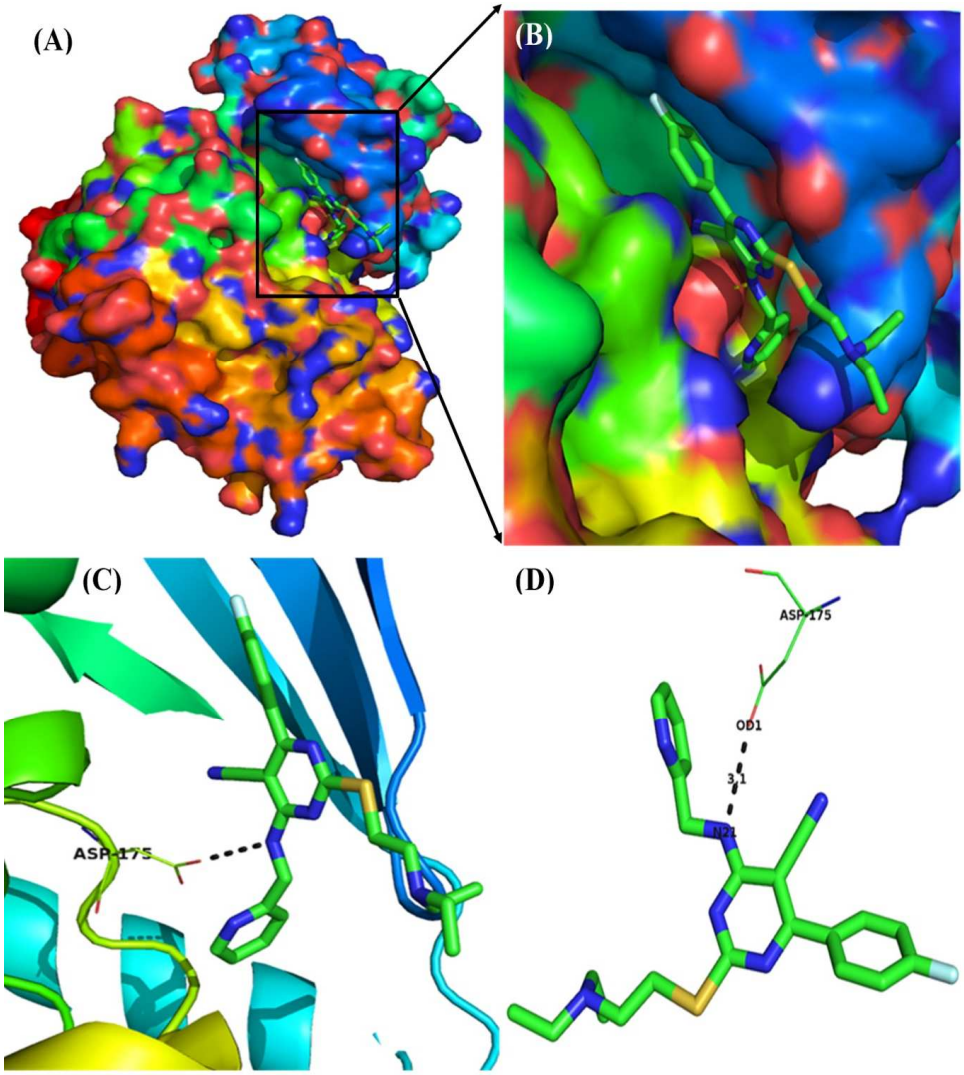


A) Binding mode of compound- H in Human protein kinase-CK2. (B). A close-up view of binding mode. (C) Close up view of ribbon model (D) Interaction of compound – H with Human protein kinase-CK2. Ligand atoms are colored by its type. The interacted amino acids residues, hydrogen bond networks in the binding pocket and the distance (in Angstrom units) of bonds are all shown.

Docking of compound - J into HPK-CK2

Docking studies of chalcone is performed for cancer target enzyme HPK-CK2. In our results on the analog binding conformation modes of chalcone derivatives with HPK-CK2, compound - J shows higher affinity with the HPK-CK2. In examining the binding interaction and position of the compound J with HPK-CK2 ligand binding site predicted by your docking procedure, , it is found that only one strong hydrogen bond is formed. From the analysis of binding pose of compound – J in HPK-CK2, it is found to be formation of hydrogen bond between Oxygen atom of Asp175 and NH of ligand (bond distance=3.2 Å) (Fig 2). A close-up view of binding mode. close up view of ribbon model Interaction of compound H with Human protein kinase-CK2. The , hydrogen bond networks and interacting amino acids residues in the binding cavity and the distance (in Angstrom units) of hydrogen bonds are all shown in Fig.2.

Fig. 2: Docking results of compound – J in to Human protein kinase-CK2



A) Binding mode of compound – J in Human protein kinase-CK2. (B). A close-up view of binding mode. (C) Close up view of ribbon model (D) Interaction of compound – J with Human protein kinase-CK2. Ligand atoms are colored by its type. The interacted amino acids residues, hydrogen bond networks in the binding pocket and the distance (in Angstrom units) of bonds are all shown.

CONCLUSION

Our molecular docking studies explored the possible binding modes of chalcone derivatives with Human protein kinases. It revealed that the chalcone derivatives show higher affinity with Human protein kinases. Especially the compound H and compound J shows best result when compared with other chalcone derivatives. On comparing the binding energy and the binding site residues, we found that all compounds differ either in their binding modes or with the binding site residues for hydrogen bond formation. The conclusion drawn from our virtual screening and docking result was that the Compound H and Compound J have highest binding affinity with the Human Protein Kinases. Though, there are many reports on the in vitro analysis of chalcone derivatives and its anticancer properties, but there are no in silico studies that predict the binding and active regions especially with human protein kinases. Our study is probably the first such attempt to predict the binding site, However validation of our results through invivo and invitro experiments and also with animal models will enlighten hope for the future development of more potent drugs for cancer therapy.

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