ORIGINAL RESEARCH PAPER

Biotechnology

MOLECULAR DOCKING OF MACELIGNAN A POLYPHENOLIC DERIVATIVE ISOLATED FROM LEUCAS CEPHALOTES &LEUCAS ASPERA ON COLORECTAL CANCER RECEPTOR PROTEIN AND ITS ADMET PREDICTION.

KEY WORDS: Macelignan, vascular endothelial growth factor – 2, cytochrome P450, caspase-3, epidermal growth factor receptor

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ABSTRACT

The aim of the present study is to analyze the molecular aspects of the lignan macelignan, against colorectal cancer receptors, namely vascular endothelial growth factor 2(VEGF-2), cytochrome P450(CYP), caspase-3, BAX, BCl -2, CDK – 2 and epidermal growth factor receptor (EGFR). Macelignan identified as lignan, a polyphenolic phytocompound that has been successfully isolated from *Leucas cephalotes* & *Leucas aspera* is traditionally identified as a medicinal herb and several studies have shown that they exert antimicrobial, anticarcinogenic and antiaging property. The docking studies have proven to be an essential tool for opening up the structural diversity of natural products to be used in an orderly manner. Molecular docking was effectively done with Schrodinger Glide software version 2020-4. The binding affinity was found to be in the range of -7.8 to -6.3 kcalmol^{-1}. From the toxicity assessment, which was carried out with pkCSM online server, it was found that the macelignan had no show skin sensitization, hepatotoxicity and AMES toxicity and that the ADMET behavior was within the acceptable limits of the drug behavior.

1. INTRODUCTION

Colorectal cancer (CRC) is the 3rd most rampant cancer witnessed in both males and females and the second most common in terms of mortality rate. Globally 10% and 9.2% of men and women are diagnosed with CRD and has caused over 500,000 deaths annually.[1]. CRC results from the atypical division and progressive growth of colon cells. This abnormal cell division forms polyps, which may be benign or cancerous. The cause of these abnormal divisions is yet to be fully understood[2].

Like most cancers, CRC does not show any significant symptoms in infancy, which makes it difficult to detect early. However, the commonly associated symptoms include changes in stool frequency, rectal bleeding, abdominal pains, weakness, and weight loss[3]. CRC is detected by sigmoidoscopy or colonoscopy while treatment proceudres include surgery, radiation therapy and drug treatments, viz; chemotherapy, targeted therapy, and immunotherapy [4], [5]. The apoptosis suppression is often associated with an increased expression of anti-apoptotic proteins and a decreased countenance of pro-apoptotic proteins [6], [7]. Few important examples of carcinogenic macromolecules are B-cell lymphoma 2 (Bcl-2), vascular endothelial growth factor receptor 2 (VEGFR-2), cyclin-dependent protein kinase 6 (CDK-6), CDK-2, [8], Bcl-2 associated X protein(BAX) [9], EGFR [10], Caspase-3[11], cytochrome P450[12]. All of these receptors/proteins can be used as potential therapeutic targets for cancer therapy.

Macelignan also termed as Anwulignan is classified as a polyphenolic belongs to the class of lignans compound. Macelignan found in the nutmeg mace of Myristica fragrans is gaining importance as a new source in treating various diseases. Macelignan has been shown to hold a variety of pharmacological activities, including antibacterial, antiinflammatory, anticancer, antidiabetic, and hepatoprotective activities; Recently, studies have also reported neuroprotective activities [13].

Various invitro, *Invivo*, and *Insilco* methods were employed to evaluate the anticancer potential of drugs molecules. Among these methods, docking has been widely used as a tool in drug discovery and designing for cancer [14], [15]. Molecular docking is used as a striking framework to understand biomolecular interactions of drug for drug design and discovery as well as for mechanistic investigation by placing a ligand mainly at the preferred binding site of the target specific region of the receptor (DNA/protein) primarily in a non-covalent manner to form a constant composite of potential efficacy and specificity. The core objective of molecular docking is to obtain ligand-receptor complex with optimized conformation and with the purpose of having reduced binding free energy[16], [17]. An insilico analysis is performed by docking a molecule to envisage its action with the selected target cell. Docking is an effort to complement the ligand, a small molecule within the receptor that is a large protein molecule[18]. In addition, evaluating docking activity which is one of the principal stages in drug design, is the evaluation of the pharmacokinetic attributes of a compound under study. ADMET analysis using an animal model is expensive, therefore molecular modelling is used to predict the chemical properties, pharmacokinetic properties (ADME), and compound toxicity [19], [20].

In the present study macelignan was screened for its binding affinity for colorectal receptor molecules through *Insilico* approach towards finding the best lead that can be a novel antagonist in inhibiting the progression of CRC.

MATERIALS AND METHODOLOGY

I. Insilco study

a. Ligand preparation

PDB file of macelignan was given as an input to SCFBio software to interpret the drug-likeness of macelignan. The 3D crystallographic structure of macelignan was obtained from PUBCHEM in sdf format. The ligand structure was prepared by LigPrep wizard of Maestro. OPLS3e force field was selected. pH 5-9 was set to generate states, possible tautomers were generated and specified chiralities were retained. The output format was set to maestro [21].

b. Receptor Preparation

Protein preparation wizard of Maestro software was used for protein preparation. The receptor structures namely 4SO0 (BAX), 2O2F (BCl-2), 5I9B (Caspase-3), 4NZ2 (CDK-2), 1M17 (EGFR), 2OH4 (VEGFR), were taken from Protein Data bank [22]. Bond orders were assigned, zero-bond orders to metals and sulphide bridges were created, missing side chains and loops were filled using Prime, water molecules beyond 5 Å were deleted and hetero states at 7 +/- 2 were generated. H-bonds assignment was optimised based on sample water orientations using PROPKA. Finally, the protein structure was minimized in the OPLS3E force field to the default Root Mean Square Deviation (RMSD) value of 0.30 [23].

c. Site prediction

The possible binding sites on the receptor were predicted using SiteMap from the system tray. The option for potential high ranked receptor binding sites was checked and partial input charges were also used. The ligand binds to the receptor

within the region of the grid. The size of the grid was adjusted if necessary [24]–[26].

d. Docking

Docking studies of macelignan against the common colorectal cancer receptor was carried out with the software version 2020-4 of Schrodinger Glide [27]–[29]. Docking step was set up in the ligand docking tool under the tasks bar. On a well-defined receptor grid, flexible docking using the extra precision (XP) feature of Glide module was performed with sample nitrogen inversions, sample ring conformations, adding epic state penalties to docking score [30].

e. Post-docking analysis

The docked poses were displayed and analysed in the Poseviewer. The hydrogen bonds and other interactions were seen, the tabulated results were examined along with the 2D diagram of the ligand-receptor interaction. The best scored pose was selected for each receptor.

Pharmacokinetic prediction

The lower molecular weight pharmacokinetic and toxicity properties of macelignan were predicted using online server pkCSM. Macelignan's Smiles string was entered into the server and ADMET prediction model selected [31].

RESULTS AND DISCUSSION

Validation of ligand molecule

Optimization of 7 receptors resulted in minimum energy of its structure and also the amino acid residues of all receptors were visualized in sterically favorable regions. The minimized energy of each receptor is shown in Table-1.

Table 1: Target Receptor Energy after Optimization

Target	PDB ID	Energy – OPLS3
Vascular Endothelial Growth Factor receptor – 2 (VEGFR)	2OH4	-1383.98
Cyclin Dependent Kinase – 2 (CDK-2)	3EZV	-1875.39
Epidermal Growth Factor Receptor (EGFR)	1M17	-967.37
B-cell Lymphoma 2 (BCl-2)	2O2F	-188.94
Bcl-2 Associated X protein (BAX)	4S0O	-1475.35
Cytochrome P450(CYP450)	4NZ2	-4172.49
Caspase -3	5I9B	-1429.48

Analysis of Molecular docking

Molecular docking studies of the selected ligand against different receptors were carried out using Schrodinger Glide software version 2020-4. The molecular dock scores (MDS) and the interaction represented as Glide Score & Glide Energy, are shown in the table below. The best docked positions of the receptor with macelignan as ligand in 2D & 3D form are shown below in Fig. (1-7)

Table 2: Glide score & Glide energy of Docked molecules

Receptors	PDB Id	No. of H-bonds	Interacting amino acids	Molecular Docking score (kcal/mol)	Glide emodel
VEGFR 2	2OH4	0; 2 pi-pi bonds	Lys 866	-8.44	-44.47
CDK-2	3EZV	2	Asn 3, Phe 4	-7.197	-46.86
EGFR	1M17	2	Met 769, Glu 738	-7.089	-52.012
BCL-2	2O2F	1	Glu 133	-7.058	-38.51
BAX	4S0O	3	Asn 106, Phe 105, Leu 47	-6.45	-48.64
CASPASE-3	5I9B	1	Asn 35	-5.169	-46.85
CYP 450	4NZ2	1	Asn 204	-4.95	-52.15

Binding of Macelignan with VEGFR 2

It was found that macelignan was best docked to VEGFR-2 as shown in Fig. 1 and showed the highest binding affinity with a Glide score of -8.44 and a Glide energy of -44.47 and good interaction with the active site residue of Lys866 with 2 pi-pi interactions.

a

b

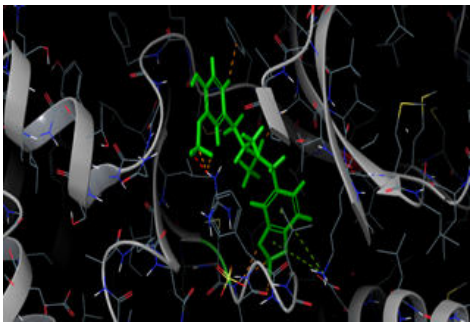
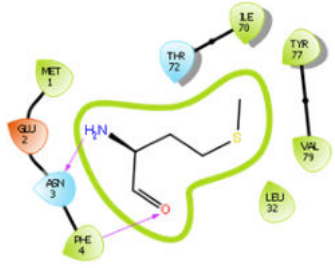


Fig.1 Illustration of docked complexes between macelignan &VEGFR receptor (a) 2D interaction diagram (b)3D form. The residual amino acids are shown as thin lines & ligand as thick lines with fixed colour. The H-bonds are represented as blue lines and halogen bonding as orange lines.

Binding of Macelignan with CDK-2

The second-best docking pose of Macelignan was visualized with the CDK-2 receptor as shown in Fig.2 the interaction with 2 H bonds was observed with Asn 3 & Phe 4 amino acid residue. The Glide energy obtained was -46.86 and the Glide score was -7.197. The outer poseview also indicates the presence of a steric interaction as yellow line. This was found to be a distinct binding position as the binding affinity was characteristically concentrated towards the NH2 end, as seen in 2D form in Fig. 2(a).

a



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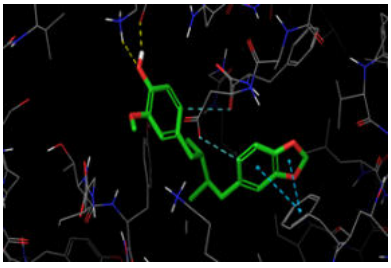
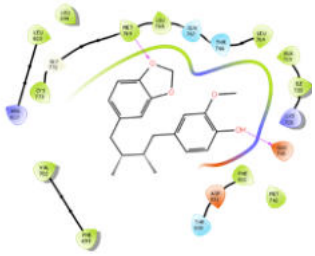


Fig.2 illustration of docked complexes between macelignan & CDK2 (a)2D form (b)3D form. The residual amino acids are shown as thin lines & ligand as thick lines with fixed colour. The H-bond are represented as yellow lines, steric hindrance as blue lines.

Binding of Macelignan with EGFR

It can be seen from Fig. 3 that macelignan docks well with EGFR with 2 H bonding which shows a good residual interaction with Met 769 & Glu738. The Glide energy & Glide score obtained were -52.012 & -7.089 respectively.

a



b

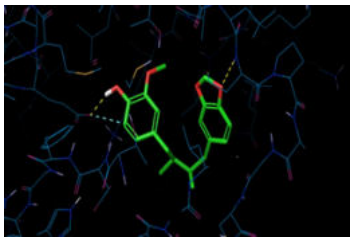
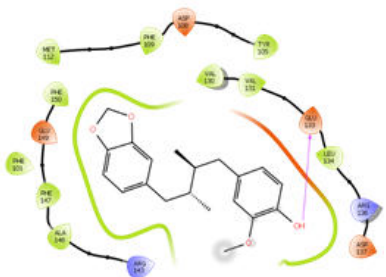


Fig.3 Illustration of docked complexes between macelignan & EGFR (a)2D form (b)3D form. The residual amino acids are shown as thin lines & ligand as thick lines with fixed colour. The H-bond are represented as yellow lines, steric hindrance as blue lines.

Binding of Macelignan with BCL-2

Macelignan docked to BCL-2 in the position of antagonist with a Glide energy of -38.51 and Glide Score of -7.058 and showed a good interaction with the rest of the active site residue of Glu133 with 1 H bonding, however solvent exposure end at O terminal region was observed characteristically.

a



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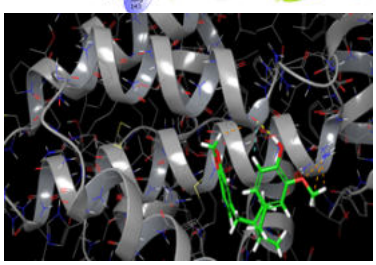


Fig.4 Illustration of docked complexes between macelignan & BCL-2 (a)2D form (b)3D form.

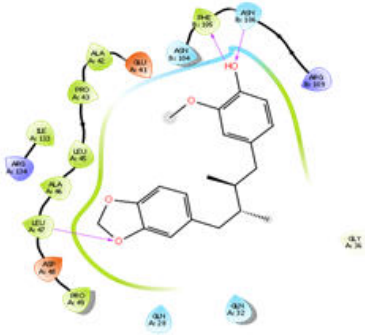
The residual amino acids are shown as thin lines & ligand as thick lines with fixed colour. The H-bond are represented as blue lines, steric hindrance as yellow and halogen bonding as orange lines.

Binding of Macelignan with BAX

Macelignan showed a satisfactory interaction with the BAX receptor with 3 H bonds and Glide score of -6.45 & -48.64 Glide energy respectively.

Binding was observed for LeuA 47 on chain A and PheB 105 & AsnB 106 on chain B active site residue.

a



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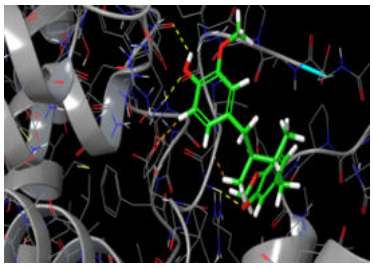
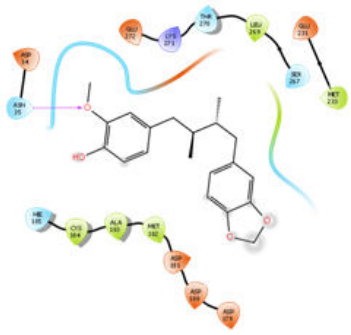


Fig.5 Illustration of docked complexes between macelignan & BAX (a)2D form (b)3D form. The residual amino acids are shown as thin lines & ligand as thick lines with fixed colour. The H-bond are represented as blue lines, steric hindrance as yellow and halogen bonding as orange lines.

Binding of Macelignan with CASPASE-3

Caspase-3 when docked to macelignan exhibited a Glide score of -5.169 &Glide energy of -46.859 and 1 H bonding interaction at the Asn 35 position of amino acid residue was observed as shown in Fig.6.

a



b

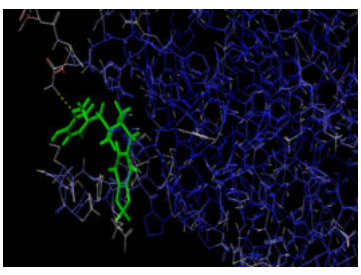
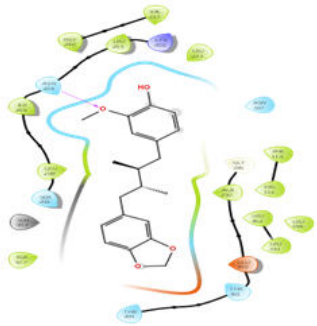


Fig.6 Illustration of docked complexes between macelignan & Cas3 receptor (a)2D form (b)3D form. The residual amino acids are shown as thin lines & ligand as thick lines with fixed colour. The H-bond are represented as blue lines, steric hindrance as yellow and halogen bonding as orange lines.

Binding of Macelignan with CYP

According to the observations from Fig.7, the docking between Macelignan & CYP 450 showed 1 H bonding and binding affinity with a Glide score of -4.95 & Glide energy of -52.157 was obtained. Although CYP450 showed maximum binding energy due to lowest binding score and high steric interactions the receptor is considerably weak in its interacting ability.

a



b

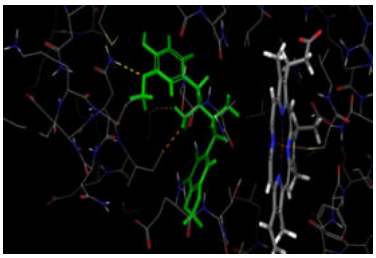


Fig .7 Illustration of docked complexes between macelignan & CYP450 (a)2D form (b)3D form. The residual amino acids are shown as thin lines & ligand as thick lines with fixed colour. The H-bond are represented as blue lines, steric hindrance as yellow and halogen bonding as orange lines.

These docking studies suggest that macelignan possess a distinct binding pose with the 7 identified receptors

Pharmacokinetic interaction study

The values received from the SCFbio server showed that Macelignan did not violate any rule. Macelignan has a molecular weight of 328 DA, 1 HBD and 4 HBA, the value of the octanol/water partition coefficient (logP) was 4.18 and the molar refractivity power was 92.95. This suggests that macelignan does not violate the Lipinski Rule of Five and therefore it can be predicted that this compound can be easily absorbed and have high permeability [31].

A strong correlation between chemical descriptors and ADMET was observed for macelignan. Macelignan has low molecular weight less than 500KDa along with its ADMET parameters as seen in Table 3 obtained from the pkCSM on-line tool. It presents the data showing that skin permeability is an important consideration in transdermal drug delivery where the K_p value is within the permissible limit (log Kp > -2.5). it can therefore be predicted that the molecule has good skin permeability [31].

It can also be seen from Table.3 that the Caco-2 permeability value (lop Papp) of Macelignan is 1.393. The Caco-2 monolayer cell is widely used as an invitro model of the human mucosa to predict the absorption of an orally administered drug [31]. From the pkCSM predictive model for Caco-2 it can be predicted that macelignan has high permeability.

VD_{ss}. BBB and CNS permeability from the data in Table.3 show that Macelignan meets the criteria for the active ingredient distribution, since all values are within the framework of the defined standards (log VDss < - 0.15; -1<log BB< 0.3; - 3 < log PS < -2). From the data it is also correlated that macelignan molecule tends to be metabolised from the detoxification enzymes present in the body.

From Table.3 it has been observed that the log CLTOT value for macelignan was -0.036 log ml/min/kg, a permissible limit for the hepatic & renal clearance of the drug from the body after administration of appropriate dose and its bioavailability.

The Toxicity property was determined from the AMES test and the hepatotoxicity test, from the Table.3 it was observed that macelignan is non-toxic.

CONCLUSION

From the study carried out it was concluded that Macelignan has a good ADMET profile and proves that it could be a potential lead candidate for the treatment of colorectal cancer. The present research carried out can also be considered as a leap in the novel drug development specifically in carcinogenesis, The Insilco experiment performed provides mechanistic evidence to substantiate that macelignan is a potential lead and further, requires Invitro & In vivo studies to demonstrate the efficiency of ligand binding to the identified receptor.

Table 3. ADMET properties of Macelignan

Molecule	Absorption			Distribution			Metabolism		Excretion	Toxicity		
	Intestinal abs	Skin Permeability	Caco2 permeability	VDss	BBB permeability	CNS permeability	CYP2D6 inhibitior	CYP3A4 inhibitior	Total Clearance	AMES toxicity	Hepato toxicity	Oral Rat Acute Toxicity (LD50)
Macelignan	93.61	-2.707	1.393	0.376	-0.2	-1.76	No	No	-0	No	No	2.086

Conflict of Interest

The Authors have no potential conflict of interest to declare.

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REFERENCES

[1] L. Simon, A. Imane, K. K. Srinivasan, L. Pathak, and I. Daoud, "In Silico Drug-Designing Studies on Flavanoids as Anticolon Cancer Agents: Pharmacophore Mapping, Molecular Docking, and Monte Carlo Method-Based QSAR Modeling.," Interdiscip. Sci., vol. 9, no. 3, pp. 445–458, Sep. 2017, doi: 10.1007/s12539-016-0169-4.

[2] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2020," CA. Cancer J. Clin., vol. 70, no. 1, pp. 7–30, Jan. 2020, doi: 10.3322/caac.21590.

[3] M. Olde Bekkink, C. McCowan, G. A. Falk, C. Teljeur, F. A. Van de Laar, and T. Fahey, "Diagnostic accuracy systematic review of rectal bleeding in combination with other symptoms, signs and tests in relation to colorectal cancer," Br. J. Cancer, vol. 102, no. 1, pp. 48–58, Jan. 2010, doi: 10.1038/ sj. bjc. 6605426.

[4] F. T. Kolligs, "Diagnostics and Epidemiology of Colorectal Cancer," Visc. Med., vol. 32, no. 3, pp. 158–164, 2016, doi: 10.1159/000446488.

[5] I. Mármol, C. Sánchez-de-Diego, A. Pradilla Dieste, E. Cerrada, and M. Rodriguez Yoldi, "Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer," Int. J. Mol. Sci., vol. 18, no. 1, p. 197, Jan. 2017, doi: 10.3390/ijms18010197.

[6] M. Govindarasu, M. Palani, and M. Vaiyapuri, "IN SILICO DOCKING STUDIES ON KAEMPFERITRIN WITH DIVERSE INFLAMMATORY AND APOPTOTIC PROTEINS FUNCTIONAL APPROACH TOWARDS THE COLON CANCER," Int. J. Pharm. Pharm. Sci., vol. 9, no. 9, p. 199, Jul. 2017, doi: 10.22159/ ijpps. 2017 v 9i9.20500.

[7] J. Lin, C. Page, X. Jin, A. O. Sethi, R. Patel, and G. Nunez, "Suppression activity of pro-apoptotic gene products in cancer cells, a potential application for cancer gene therapy.," Anticancer Res., vol. 21, no. 2A, pp. 831–9, 2001, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/11396172.

[8] A G Wingren, "CDK2 (cyclin dependent kinase 2)." 2017, [Online]. Available: http://atlasgeneticsoncology.org/Genes/GC_CDK2.html.

[9] Z. Liu, Y. Ding, N. Ye, C. Wild, H. Chen, and J. Zhou, "Direct Activation of Bax Protein for Cancer Therapy," Med. Res. Rev., vol. 36, no. 2, pp. 313–341, Mar. 2016, doi: 10.1002/med.21379.

[10] J. H. Park, Y. Liu, M. A. Lemmon, and R. Radhakrishnan, "Erlotinib binds both inactive and active conformations of the EGFR tyrosine kinase domain," Biochem. J., vol. 448, no. 3, pp. 417–423, Dec. 2012, doi: 10.1042/BJ20121513.

[11] B.-Y. Park et al., "Increase of Caspase-3 Activity by Lignans from Machilus thunbergii in HL-60 Cells," Biol. Pharm. Bull., vol. 27, no. 8, pp. 1305–1307, 2004, doi: 10.1248/bpb.27.1305.

[12] C. Rodriguez-Antona and M. Ingelman-Sundberg, "Cytochrome P450 pharmacogenetics and cancer," Oncogene, vol. 25, no. 11, pp. 1679–1691, Mar. 2006, doi: 10.1038/sj.onc.1209377.

[13] S. Paul, J. K. Hwang, H. Y. Kim, W. K. Jeon, C. Chung, and J.-S. Han, "Multiple biological properties of macelignan and its pharmacological implications," Arch. Pharm. Res., vol. 36, no. 3, pp. 264–272, Mar. 2013, doi: 10.1007/s12272-013-0048-z.

[14] S. Tabassum, M. Zaki, M. Afzal, and F. Arjmand, "Synthesis and

characterization of Cu(II)-based anticancer chemotherapeutic agent targeting topoisomerase I□: In vitro DNA binding, pBR322 cleavage, molecular docking studies and cytotoxicity against human cancer cell lines," Eur. J. Med. Chem., vol. 74, pp. 509–523, Mar. 2014, doi: 10.1016/j. ejmech. 2013.12.046.

[15] S. N. e Zahra, N. A. Khattak, and A. Mir, "Comparative modeling and docking studies of p16ink4/Cyclin D1/Rb pathway genes in lung cancer revealed functionally interactive residue of RB1 and its functional partner E2F1," Theor. Biol. Med. Model., vol. 10, no. 1, p. 1, Dec. 2013, doi: 10.1186/1742-4682-10-1.

[16] A. M. Dar and S. Mir, "Molecular Docking: Approaches, Types, Applications and Basic Challenges," J. Anal. Bioanal. Tech., vol. 08, no. 02, 2017, doi: 10.4172/2155-9872.1000356.

[17] R. Rohs, "Molecular flexibility in ab initio drug docking to DNA: binding-site and binding-mode transitions in all-atom Monte Carlo simulations," Nucleic Acids Res., vol. 33, no. 22, pp. 7048–7057, Dec. 2005, doi: 10.1093/nar/gki1008.

[18] J. Li et al., "In Silico Discovery of Potential VEGFR-2 Inhibitors from Natural Derivatives for Anti-Angiogenesis Therapy," Int. J. Mol. Sci., vol. 15, no. 9, pp. 15994–16011, Sep. 2014, doi: 10.3390/ijms150915994.

[19] K. Tsaioun, "Evidence-based absorption, distribution, metabolism, excretion (ADME) and its interplay with alternative toxicity methods," ALTEX, pp. 343–358, 2016, doi: 10.14573/altex.1610101.

[20] G. Moroy, V. Y. Martiny, P. Vayer, B. O. Villoutreix, and M. A. Miteva, "Toward in silico structure-based ADMET prediction in drug discovery.," Drug Discov. Today, vol. 17, no. 1–2, pp. 44–55, Jan. 2012, doi: 10.1016/j.drudis.2011.10.023.

[21] "Schrödinger Release." Schrödinger, LLC, New York, NY, 2020, 2020.

[22] G. M. Sastry, M. Adzhigirey, T. Day, R. Annabhimoju, and W. Sherman, "Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments.," J. Comput. Aided. Mol. Des., vol. 27, no. 3, pp. 221–34, Mar. 2013, doi: 10.1007/s10822-013-9644-8.

[23] "Schrödinger Release." , Schrödinger, LLC, New York, 2020.

[24] T. Halgren, "New method for fast and accurate binding-site identification and analysis.," Chem. Biol. Drug Des., vol. 69, no. 2, pp. 146–8, Feb. 2007, doi: 10.1111/j.1747-0285.2007.00483.x.

[25] "Schrödinger Release." Schrödinger, LLC, New York, NY, 2020, New York, 2020.

[26] T. A. Halgren, "Identifying and Characterizing Binding Sites and Assessing Druggability," J. Chem. Inf. Model., vol. 49, no. 2, pp. 377–389, Feb. 2009, doi: 10.1021/ci800324m.

[27] T. A. Halgren et al., "Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening.," J. Med. Chem., vol. 47, no. 7, pp. 1750–9, Mar. 2004, doi: 10.1021/jm030644s.

[28] R. A. Friesner et al., "Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein□Ligand Complexes," J. Med. Chem., vol. 49, no. 21, pp. 6177–6196, Oct. 2006, doi: 10.1021/jm051256o.

[29] R. A. Friesner et al., "Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy.," J. Med. Chem., vol. 47, no. 7, pp. 1739–49, Mar. 2004, doi: 10.1021/jm0306430.

[30] "Schrödinger Release 2020-4." Schrödinger, LLC, New York, NY, 2020, New York, 2020.

[31] D. E. V. Pires, T. L. Blundell, and D. B. Ascher, "pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures," J. Med. Chem., vol. 58, no. 9, pp. 4066–4072, May 2015, doi: 10.1021/acs.jmedchem.5b00104.