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Computational screening of anticancer drugs targeting miRNA155 synthesis in breast cancer

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miRNAs have been identified to play a crucial role in carcinogenesis through their binding to various regulatory proteins. One such causative molecule identified as miRNA155, which when overexpressed is responsible for carcinogenesis and also leads to telomere fragility. miRNA155 levels in the blood are used for early screening of cancer. Several anticancer drugs such as doxorubicin have been identified, which act by binding to DNA and DNA binding enzymes to check their expression levels. In this study doxorubicin and its similar compounds were used to analyze their binding with miR155 DNA for inhibition of miRNA155 synthesis and their binding energies were calculated. Based on the docking, ADME, and toxicity results Morpholinyl Doxorubicin was used for molecular dynamics studies and was identified as a potential drug candidate.

Keywords: ADME, Anticancer drugs, Chemotherapeutic drugs, Doxorubicin, Molecular dynamics, Toxicity prediction

miRNAs are 18–24 nucleotides in length[1,2] and play crucial roles in biological phenomena including cellular proliferation, development, apoptosis, tumorigenesis, and stress response. Abnormal miRNA expression has been analyzed in numerous solid tumors, such as breast cancer[3,4], due to their dysregulated expression in cancer cells and are stable in blood[5-7]. In breast cancer, several studies have supported an abnormal expression of miRNA-155 (MIR-155) in patients with the disease[8]. The overexpression of MIR-155 has been identified as a breast cancer risk factor[9], which is associated with different clinical and pathological markers, tumour subtype, metastasis events, and invasive properties of breast cancer, poor survival rates, alongside high tumour grade, advanced stage, and lymph node metastasis[8]. miR155 is involved in controlling several mechanisms of cell survival, cell growth, radio/chemo-resistance[8,10,11], inhibiting different target genes including FOXO3A, VHL, and SOCS1.

Various treatment plans for cancer patients are available, depending upon the type and the stage identified during diagnosis[12]. One such drug, that has been identified to be effective against cancer is doxorubicin[13,14]. Doxorubicin has exhibited great potential for the treatment of breast cancer and is considered as one of the most effective Food and Drug Administration-approved chemotherapeutic drugs[15]. The mode of action of doxorubicin includes its binding to DNA associated enzymes and also intercalation between base pairs of the DNA double helix[16,17], which results in a variety of cytotoxic effects that happen in concurrence with anti-proliferation, resulting in DNA damage[17,18]. Other doxorubicin actions include free radical generation which causes further DNA damage, inhibition of macromolecule production, DNA unwinding/separation, and increase in alkylation[19,20].

In recent times for designing rational drugs, the *in silico* methods have gained attention, because of their efficiency, cost- effectiveness, and time- saving capabilities. The drug design process involves screening of potential drug- like lead compounds through molecular docking and then the ADMET (absorption, distribution, metabolism, excretion, and toxicity) analysis of the screened molecules[21]. These models have inexpensive and high-throughput optimization ability for parallel analysis of bioavailability and activity along with the safety in the process of rationalized drug development. Because of the costly ADMET experimental procedures, *in silico* method for ADMET analysis in early drug discovery has become a way of choice[22].

In this study the molecular binding of miR155 DNA with doxorubicin and its similar compounds is analyzed for their binding affinity and as these compounds can bind to and inhibit the expression of DNA. ADMET analysis of all the selected drugs has been done for predicting suitable drugs for cancer treatment.

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Materials and Methods

DNA Sequence

The gene sequence of miR155 for *Homo sapiens* was retrieved from gene bank database with accession number NR_030784.1 in the FASTA format from the NCBI website (https://www.ncbi.nlm.nih.gov/)[23]. The retrieved sequence is ′CTGTTAATGCTAATCGTGA TAGGGGTTTTTGCCTCCAACTGACTCCTACA TATTAGCATTAACAG′.

DNA structure

The pdb structure file for the retrieved sequence was generated into B type helix using the online tool make-na server available at http://casegroup.rutgers.edu/casegr-sh-2.2.html[24]. After conversion, the pdb file for the given DNA sequence was downloaded and saved.

Ligands

Doxorubicin is reported to bind to DNA, hence it was selected for the studies, and similarity search was done on Pubchem and molecules showing more than 95% similarity were selected for the study. The 3 dimensional (3D) structure files for Doxorubicin and its similar compounds were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/)[25]. Thenames and structures of the retrieved compounds are represented in (Table 1). After saving the structure files these were converted into pdb format necessary for analyzing molecular binding studies using the tool Open Babel[26].

Docking

Molecular docking of selected ligands with miR155 DNA

Molecular binding studies were performed using AutoDock Tools 1.5.6[27]. The process included initial processing of DNA and ligand files and saving them into pdbqt format. In the next step, the grid was setup onto the DNA molecule and grid parameter file was saved. Then after running AutoGrid, the docking was performed and the results were analyzed using AutoDock Tools for estimation of binding energies, hydrogen bonds formed, and bond length of formed hydrogen bonds.

ADME and Toxicity analysis

The ADME analysis of the selected compound was performed using the online tool ALOGPS 2.1 available at http://www.vcclab.org/lab/alogps/[28]. This

Table 1 — Docking results of selected ligands with miR155 DNA

S. No.	Ligand	Chain	Base	Bond Length (A°)	Binding Energy (kcal/mol)
1	Doxorubicin	B	A39	2.199	−10.94
2	Doxorubicin-N-4-hydroxyphenoxyacetamide	B	G33	2.03	−6.21
3	Nemorubicin	B	G30 G33	1.989 2.167	−9.14
4	YM4 Doxorubicin Analog	B	A32	1.965	−9.03
5	Epirubicin	B	A40	2.201	−10.41
6	Mra-CN	A B B	G31 G33 G34	1.693 2.199 2.031	−7.64
7	Cyanomorpholinoadriamycin	B	G30	2.167	−8.77
8	YM1 Doxorubicin Analog	B	G34 G34	1.934 2.087	−10.54
9	Morpholinyl Doxorubicin	B	A36	2.098	−9.33
10	Adriamycin	B	A38	1.913	−9.44
11	Oxazolocyanomorpholino Adriamycin	A B	G31 G34	2.199 1.773	−10.7
12	Lys(6)-LHRH-doxorubicin	B	G31 G33	2.081 1.919	−3.21
13	Adriamycinone	B	G33	2.164	−7.18
14	Pirarubicin	B	A36	1.988	−9.57
15	Cyanomethyladriamycin	B	A39	2.003	−9.47
16	NSC169534	B	G30 G31 A32	2.114 1.822 2.08	−10.79

tool was used to calculate the logP and logS values of the compounds, conferring to their absorption and distribution in the body, respectively. The selected compounds were further subjected to toxicity prediction for the parameters including subcellular localization, category of acute oral toxicity, effects on blood- brain barrier, carcinogenicity, and LD50 values using the online tool admetSAR available at http://lmmd.ecust.edu.cn/admetsar1/home/[29].

Molecular dynamics Analysis

The final identified compound was then analyzed for molecular dynamics simulation analysis using NAMD[30]. For this purpose, the molecule-DNA complex was saved as pdb file and then NAMD tool was used for molecular dynamics analysis with a binding time of 10ns. The plots for potential energy and RMSD (root mean square deviation) were plotted.

Results and Discussion

miR155 DNA

The retrieved FASTA sequence for the miR155 gene was converted into a double helix structure with B type helix and saved as pdb structure file as represented in supplementary (Fig. 1).

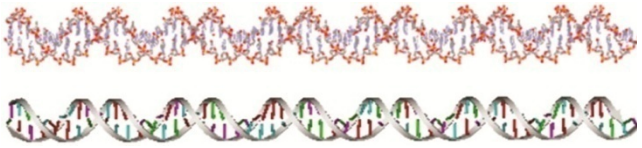

Fig. 1 — Generated 3-dimensional structure of miR155 DNA

Docking

Binding of miR155 DNA with selected ligands

The molecular binding studies of the DNA with Doxorubicin and its similar compounds were performed using AutoDock Tools 1.5.6. The docking programme has been employed frequently as a very quick and reliable method for screening of the phytochemicals potency against various disorders. Autodock executes this task by identifying the most similar component having a strong binding affinity towards the specific target with the lowest binding energy and predicts the binding orientation and conformation[31].The results of the molecular docking studies along with binding energies, hydrogen bond formation, and bond length of the formed hydrogen bond are represented in (Table 1).

From the (Table 1) it is evident that the ligands 5, 8, 11 and 16 having energy values of −10.41, −10.54, −10.7 and −10.79 kcal/mol, respectively, show the binding energies comparable to the binding energy of doxorubicin having an energy value of −10.94 kcal/mol, thus exhibiting the capability to bind to and damage miR155 DNA to inhibit its expression.

The docking images of different ligands with miR155 DNA are represented in (Fig. 2A & B), where the formed hydrogen bonds are represented by dark

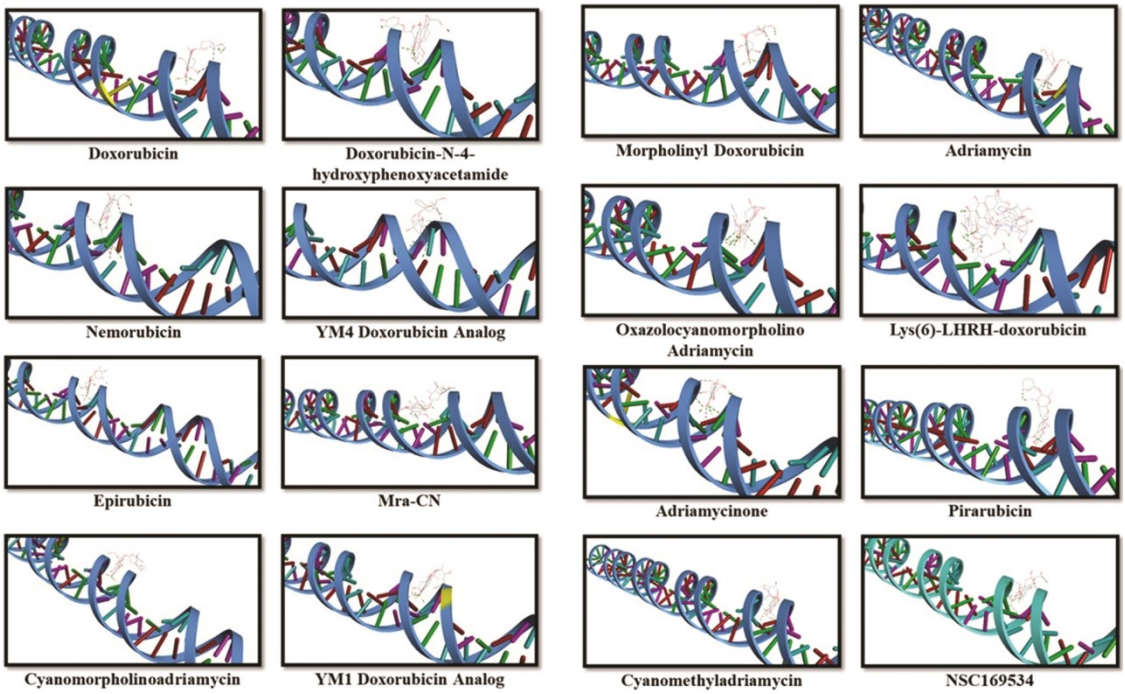

Fig. 2A & B — Docking poses of the selected ligands with miR155 DNA

Table 2 —ADME and Toxicity analysis of selected drug candidates

S. No.	Compound	ADME		Toxicity				
		logP	logS	Subcellular localization	Carcinogenicity	Acute Oral Toxicity	BBB	LD50
1	Doxorubicin	1.41	–2.67	Nucleus	No	III	-	2.664
2	Doxorubicin-N-4-hydroxyphenoxyacetamide	2.21	–3.66	Nucleus	No	III	-	2.676
3	Nemorubicin	1.73	–2.78	Nucleus	No	III	-	3.1607
4	YM4 Doxorubicin Analog	0.46	–3.3	Nucleus	No	III	-	2.5729
5	Epirubicin	1.41	–2.67	Nucleus	No	III	-	2.6644
6	Mra-CN	1.95	–2.59	Nucleus	No	III	-	2.7405
7	Cyanomorpholinoadriamycin	1.95	–2.59	Nucleus	No	III	-	2.7405
8	YM1 Doxorubicin Analog	1.36	–3.34	Nucleus	No	III	-	2.5729
9	Morpholinyl Doxorubicin	1.79	–2.75	Nucleus	No	III	-	2.922
10	Adriamycin	-0.08	–3.17	Nucleus	No	III	-	2.7374
11	Oxazolocyanomorpholino Adriamycin	2.1	–2.38	Lysosome	No	III	-	2.7359
12	Lys(6)-LHRH-doxorubicin	0.95	–4.43	Lysosome	No	III	-	2.7206
13	Adriamycinone	1.09	–2.55	Mitochondria	No	III		2.4516
14	Pirarubicin	2.06	–3.32	Nucleus	No	III	-	2.6624
15	Cyanomethyladriamycin	1.36	–2.92	Nucleus	No	III	-	2.7125
16	NSC169534	1.41	–2.67	Nucleus	No	III	-	2.6644

green lines. The bond length of the formed hydrogen bonds was measured. Ligand 5 formed hydrogen bond with the 40th adenine of chain B, with the hydrogen bond length being 2.201 A°. Similarly, ligand 8 formed two hydrogen bonds with G34 of chain B having bond lengths of 1.934 and 2.087 A°, respectively. Ligand 11 exhibited binding with G31 and G34 of chains A and B of miR155 DNA with hydrogen bond lengths of 2.199 and 1.773 A°, respectively. Whereas, ligand 16 exhibited binding with G30, G31, and A32 of chain B of miR155 DNA having hydrogen bond lengths of 2.114, 1.822, and 2.08 A°, respectively. All other ligands have higher binding energies thus showing comparatively lower binding affinities with miR155 DNA. Out of these ligands, 11 is showing binding with both the chains/helices of miR155 DNA and also having binding energy compared to the binding energy of doxorubicin which indicates its intercalation between two strands of the DNA and thus can act as a more effective candidate for inhibiting the expression of oncogenic miR155 RNA.

ADME and Toxicity analysis

The ADME analysis was carried out to further screen the selected molecules for and their logP and logS values, representing the lipophilicity and drug solubility in water.

The lipophilicity of the potential drug candidates is calculated in terms of logP, which represents the partition of the drug in water and octane at equilibrium. This parameter signifies the absorption of the drug into the cells. The safe range of logP values is regarded as 0-5[32]. It is evident from (Table 2) that except Adriamycin, all other ligands exhibit positive logP values falling in the safe limit (logP < 5). The aqueous solubility of a compound impacts the distribution of the drug thus leading to lower distribution of the drug with a decrease in solubility and also most of the marketed drugs have logS value higher than –4[33]. All the selected ligands exhibited logS values in an acceptable range.

The toxicity evaluations of potential drug candidates are necessary to analyze their harmful and deleterious effects on the human body. The selected molecules were analyzed for their carcinogenicity, subcellular localization, acute oral toxicity, blood- brain barrier penetration capability, and LD50 values (Table 2)[29].

During the analysis it was observed that all the selected ligands are negative for their carcinogenicity and blood- brain barrier penetration, hence the further screening can be done Based on their subcellular localization and LD50 values. Also, all the ligands exhibited category III of acute oral toxicity, thus

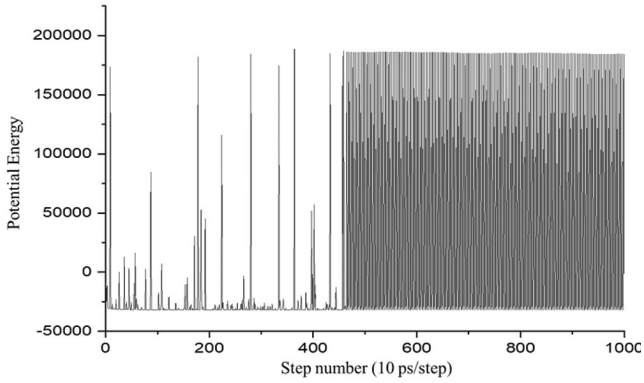

Fig. 3 — Potential energy variations in the formed complex during molecular dynamics simulation

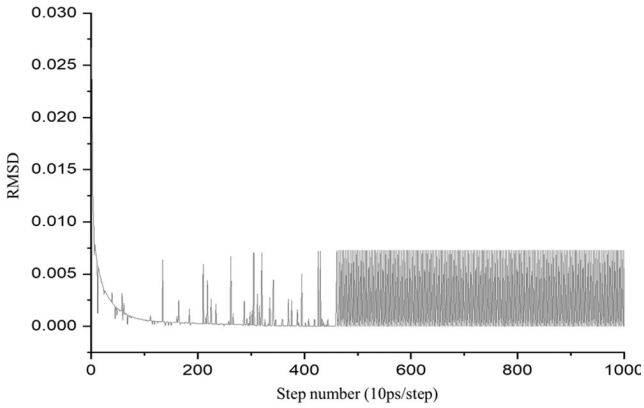

Fig. 4 — Variations in RMSD values during molecular dynamics

signifying that their LD50 values lie in the range of 500 mg/kg to 5000 mg/kg body weight. Out of all the screened compounds, the ones showing subcellular localization in the nucleus and the compounds with the highest LD50 values can be forwarded for further studies.

Molecular Dynamics Simulation

Based on the results of autodock, ADME and toxicity analysis, Morpholinyl Doxorubicin was selected for molecular dynamics analysis by using NAMD for 10 nanoseconds (10ps time step, 1000 steps) as a potential candidate that can bind to miR-155 DNA and inhibit its expression. The plots for potential energy and RMSD were plotted (Figs. 3 & 4). The potential energy plot suggests the stabilization of the complex formed and the results were further validated by RMSD values (being in the order of $1*10^{-2}$)[30]. Thus showing the effective binding of the ligand/ drug candidate with the DNA molecule.

Conclusion

The methods of cancer treatments have gained much attention presently due to the low efficacy of the drugs, higher levels of toxicity and side effects. In this light, there is a need to identify new drugs that have the capability to inhibit oncogenes and other carcinogenic agents, thus inhibiting the process of carcinogenesis. In this study, the doxorubicin and its similar compounds were studied for inhibition of oncogenic miR155, over expression of which can lead to breast and other types of cancer. The compound Morpholinyl Doxorubicin was identified as a potential drug candidate based on ADMET and molecular dynamics analysis. The efficacy and effectiveness of these compounds further need to be validated in a wet lab along with their toxicity evaluations both *in vitro* and *in vivo* before preparation of their dosage forms.

Conflict of interest

All authors declare no conflict of interest.

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References

1 Shabaninejad Z, Yousefi F, Movahedpour A, Ghasemi Y, Dokanehiifard S, Rezaei S, Aryan R, Savardashtaki A & Mirzaei H, Electrochemical-based biosensors for microRNA detection: Nanotechnology comes into view. *Anal Biochem*, 581 (2019) 113349.

2 Wang HN, Crawford BM, Fales AM, Bowie ML, Seewaldt VL & Vo-Dinh T, Multiplexed detection of microRNA biomarkers using SERS-based inverse molecular sentinel (iMS) nanoprobes. *J Phys Chem C*, 120 (2016) 21047.

3 Rehman O, Zhuang H, Muhamed Ali A, Ibrahim A & Li Z, Validation of miRNAs as Breast Cancer Biomarkers with a Machine Learning Approach. *Cancers*, 11 (2019) 431.

4 Rodríguez-Martínez A, de Miguel-Pérez D, Ortega FG, García-Puche JL, Robles-Fernández I, Exposito J, Martorell-Marugan J, Carmona-Sáez P, del Carmen Garrido-Navas M, Rolfo C & Ilyine H, Exosomal miRNA profile as complementary tool in the diagnostic and prediction of treatment response in localized breast cancer under neoadjuvant chemotherapy. *Breast Cancer Res*, 21 (2019) 21.

5 Malla RR, Kumari S, Gavara MM, Badana AK, Gugalavath S, Kumar DK & Rokkam P, A perspective on the diagnostics, prognostics, and therapeutics of microRNAs of triple-negative breast cancer. *Biophys Rev*, 11 (2019) 227.

6 Si W, Shen J, Zheng H & Fan W, The role and mechanisms of action of microRNAs in cancer drug resistance. *Clin Epigenetics*, 11 (2019) 25.

7 Tavallaie R, De Almeida SR & Gooding JJ, Toward biosensors for the detection of circulating microRNA as a cancer biomarker: an overview of the challenges and successes. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, 7 (2015) 580.

8 Mattiske S, Suetani RJ, Neilsen PM & Callen DF, The oncogenic role of miR-155 in breast cancer, *Cancer Epidemiol Biomarkers Prev*, 21 (2012) 1.

9 Zeng H, Fang C, Nam S, Cai Q & Long X, The clinicopathological significance of microRNA-155 in breast cancer: a meta-analysis. *Biomed Res Int*, 2014 (2014).

10 Liu J, Huang W, Yang H & Luo Y, Expression and function of miR-155 in breast cancer. *Biotechnol Biotechnol Equip*, 29 (2015) 840.

11 Liu S, Su W, Li Z & Ding X, Electrochemical detection of lung cancer specific microRNAs using 3D DNA origami nanostructures. *Biosens Bioelectron*, 71 (2015) 57.

12 Paraskevaidi M, Martin-Hirsch PL & Martin FL, Need for early, minimally invasive cancer diagnosis. *Proc Natl Acad Sci U S A*, 116 (2019) 4752.

13 Tacar O, Sriamornsak P & Dass CR, Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. *J Pharm Pharmacol*, 65 (2013) 157.

14 Dehshahri A, Ashrafizadeh M, Afshar EG, Pardakhty A, Mandegary A, Mohammadinejad R & Sethi G, Topoisomerase inhibitors: pharmacology and emerging nanoscale delivery systems. *Pharmacol Res*, 151 (2019) 104551.

15 Szebeni J, Fülöp T, Dézsi L, Metselaar B & Storm G, Liposomal doxorubicin: the good, the bad and the not-so-ugly. *J Drug Target*, 24 (2016) 765.

16 Hilmer SN, Cogger VC, Muller M & Le Couteur DG, The hepatic pharmacokinetics of doxorubicin and liposomal doxorubicin. *Drug Metab Dispos*, 32 (2004) 794.

17 Yun UJ, Lee JH, Shim J, Yoon K, Goh SH, Yi EH, Ye SK, Lee JS, Lee H, Park J & Lee IH, Anti-cancer effect of doxorubicin is mediated by downregulation of HMG-Co A reductase via inhibition of EGFR/Src pathway. *Lab Invest*, 99 (2019) 1.

18 Christowitz C, Davis T, Isaacs A, Van Niekerk G, Hattingh S & Engelbrecht AM, Mechanisms of doxorubicin-induced drug resistance and drug resistant tumour growth in a murine breast tumour model. *BMC cancer*, 19(2019)757.

19 Kumari V&Sangal A, Antimicrobial study of *Arjuna Terminalia* loaded PLGA nanoparticle. *Indian J Biochem Biophys*, 57 (2020) 291.

20 Kumar K&Zakir M, Future prospects of fermentation in unani based drugs. *Indian J Biochem Biophys*, 56 (2019) 347.

21 Mandal S, Moudgil MN & Mandal SK, Rational drug design. *Eur J Pharmacol*, 625 (2009) 90.

22 Wang Y, Xing J, Xu Y, Zhou N, Peng J, Xiong Z, Liu X, Luo X, Luo C, Chen K & Zheng M, *In silico* ADME/T modelling for rational drug design. *Q Rev Biophys*, 48 (2015) 488.

23 MIR155 microRNA 155 [Homo sapiens (human)] - Gene - NCBI. https://www.ncbi.nlm.nih.gov/gene/406947. Accessed 20 March 2017

24 Stroud J, The Make-Na Server. Available at structure.usc. edu/make-na/server. html. Accessed 21 March 2017.

25 Doxorubicin, https://www.https://pubchem.ncbi.nlm.nih. gov/compound/Doxorubicin. Accessed 25 March 2017

26 Singha I, Saxena S, Gautam S, Saha A&Das SK, Grape extract protect against ionizing radiation-induced DNA damage. *Indian J Biochem Biophys*, 57 (2020) 219.

27 Ganeshpurkar A & Saluja A, *In silico* interaction of rutin with some immunomodulatory targets: a docking analysis. *Indian J Biochem Biophys*, 55 (2018) 88.

28 Tetko IV & Tanchuk VY, Application of associative neural networks for prediction of lipophilicity in ALOGPS 2.1 program. *J Chem Inf Comput Sci*, 42 (2002) 1136.

29 Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, Lee PW & Tang Y, admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *J Chem Inf Model*, 52 (2012) 3099.

30 Övey İS&Güler Y, Apoptotic efficiency of capecitabine and 5-fluorouracil on human cancer cells through TRPV1 channels. *Indian J Biochem Biophys*, 57 (2020) 64.

31 Basu A, Sarkar A, Maulik U&Basak P, Three dimensional structure prediction and ligand-protein interaction study of expansin protein ATEXPA23 from *Arabidopsis thaliana* L. *Indian J Biochem Biophys*, 56 (2019) 20.

32 Bhal SK, LogP—making sense of the value. *Advanced Chemistry Development*, (Toronto, ON, Canada), 2007, 1.

33 Tareq Hassan Khan M, Predictions of the ADMET properties of candidate drug molecules utilizing different QSAR/QSPR modeling approaches *Curr Drug Metab*, 11 (2010) 285.