International Journal of Scientific and Innovative Research 2017; 5(2) : 39 - 46
P-ISSN 2347-2189, E- ISSN 2347-4971

MOLECULAR DOCKING AND QSAR STUDIES OF ANTIPSYCHOTIC RISPERIDONE DERIVATIVES AGAINST SEROTON IN 5-HT_{2A} RECEPTOR

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ABSTRACT

Mental disorders have become highly prevalent due to ambitious lifestyle, urbanization, and stressful environment which incorporate depression, schizophrenia, bipolar disorder, obsessive-compulsive disorder, Alzheimer's disease, anxiety and so on. Among all the mental health issues, schizophrenia is the one of the exceptionally extreme, chronic debilitating problem with disturbance in thought, perception and emotion. In this work, we built 3D homology model of 5-HT_{2A} Serotonin receptor using comparative homology modeling program MODELLER. The computed model was optimized by using molecular dynamics approaches and validatedby PROCHECK and Errat tool in order to obtain a stable model structure. After that we docked risperidone derivatives collected from literature with model structure of 5-HT2A Serotonin receptor using the program AutoDock 4.2, which resulted in energy-based descriptors such as Binding Energy, Intermolecular Energy, Internal Energy, Torsional Energy, vdW + Hbond + desolv Energy and Electrostatic Energy. Docked complex structure was analyzed in molecular dynamics simulation to validate stable interaction between ligand and receptor at the microscopic level. To do this we used CHARMM 22 force field from NAMD incorporated in visual molecular dynamics (VMD 1.9.2) and then evaluating the stability of complex structure by calculating RMSD values. After that a quantitative structure activity relationship (QSAR) model was built using energy-based descriptors as independent variable and pKi value as dependent variable of known risperidone derivatives with 5-HT_{2A} Serotonin receptor, yielding correlation coefficient r^2 of 0.7746. This result will be helpful in designing of more potent inhibitors against 5-HT_{2A} Serotonin receptor prior to their synthesis.

***Keywords*:** Schizophrenia; Homology modeling; Risperidone derivatives; 5-HT_{2A} Serotonin Receptor; Antipsychotic agents.

INTRODUCTION

Schizophrenia is described by positive and negative symptoms that can impact a patient's thoughts, perceptions, speech, affect, and behaviors, affecting more than 21 million people worldwide (WHO, 2015). Schizophrenia is likewise characterized by disorganized idea, which is manifested in speech and behavior. Schizophrenia etiology indicates that many factors are involved, namely genetic factors, (Levy et al., 2010; Alaerts and Del-Favero, 2009) alterations in chemical transmission (dopamine, serotonin etc.), (Lipska et al., 1993; 2004) obstetrical complications (Sorensen et al., 2004; Ho and Magnotta, 2010) and viral Infections (Brown and Derkits, 2010). There is no satisfactory remedy available for prevention of the schizophrenia. Currently available marketed drugs like clozapine, chlorpromazine, risperi done, halo-peridol and olanzapine have nano-molar affinities for serotonin 5-HT_{2A} and dopamine D_2 receptors (Arnt and Skarsfeldt, 1998) yet have some adverse effects such as dizziness, neuroleptic malignant syndrome, diabetes, sexual dysfunction, agitation, sedation and weight gain. To treat positive as well as negative symptoms of Schizophrenia atypical antipsychotics drugs focused more on 5-HT_{2A} receptor instead of D2 dopamine to avoid side

effects called extra pyramidal symptoms (EPS). Neurotransmitter serotonin (5-hydroxy-tryptamine, 5-HT) an ancient neurotransmitter, involved in several neurophysiological and behavioral functions, acts by interacting with multiple receptors (5-HT1-5-HT7) (Anbazhagan et al., 2010). Alterations in serotonergic signalling have also been implicated in various psychiatric disorders (Anbazhagan et al., 2010).

Risperidone is a second-generation extremely potent anti-psychotic agent used to treat schizophrenia with good tolerance in patients. In a large population of elderly patients the use of risperidone is associated with a lower risk of EPS compared to first generation antipsychotics drugs (Bishop and Pavuluri, 2008; Vasilyeva et al., 2013). This drug decreases the negative symptoms by acting on the serotonergic and noradrenergic receptors, while their effects on the dopaminergic pathway (Avram et al., 2011) with lower side effects reduce the positive symptoms. In this work, we had made a homology model for 5-HT_{2A} Serotonin receptor of Homo sapiens. Risperidone derivatives were docked with computed model of 5-HT_{2A} Serotonin receptor. Molecular dynamics (MD) simulation studies were performed on inhibitor – protein complex and after that, we have built QSAR model using energy-based descriptors as independent variable and pKi value as dependent variable of known risperidone derivatives with 5-HT2A Serotonin receptor, using Multiple Linear Regression.

MATERIALS AND METHODS

Protein preparation

Amino acid sequence of 5-HT2A Serotonin receptor (ID: P28223) in Homo sapiens was retrieved from Uniprot database (The UniProt Consortium, 2015).

Template Searching

The primary amino-acid sequence was used to search for a suitable template, which was carried out by a homology search via Basic Local Alignment Search Tool (Altschul et al., 1997) against the Protein Data Bank (PDB) to generate a 3D coordinate structure. The homology search showed the crystal structure of Chimeric protein of 5-ht2b-bril in complex with Ergotamine (PDB Id: 4IB4) was found to be the best hit based on query coverage, identity, E-value, and high similarity with the "A" chain, so was therefore considered as the template for homology modeling.

Sequence alignment

The sequence alignment of target and template was generated using the align2D module in Modeller software (Sali and Blundell, 1993). Default parameters were applied and the aligned sequences were inspected and adjusted manually to minimize the number of gaps and insertions.

Homology modeling and structure refinement

Homology model of the target protein was constructed using a restrained-based approach in MODELLER using a model-single module (Sali and Blundell, 1993). We built five 3D structures; among those, the best ones were judged by low discrete optimized protein energy (DOPE) and Modeller objective function. The constructed model was subjected to constraint energy minimization with a harmonic constraint of 200 kJ/mol/Å, applied for all protein atoms, using the steepest descent and conjugate gradient technique to eliminate bad contacts between protein atoms. Computations were done in vacuo with the GROMOS96 43B1 parameters set, without reaction field. An energy computation was done with the GROMOS96 implementation of SWISS- pdb Viewer (http://iqc.ethz.ch/gromos) (Guex and Peitsch, 1997). The quality of model was checked with the help of PROCHECK (Laskowski et al., 1993) and Errat (Bowie et al., 1991) tools.

Inhibitors dataset

Fifteen experimentally known risperidone derivatives were obtained from the literature (Avram et al., 2011). The 3D structure of known risperidone and their 15 derivatives were built using PubChem Sketcher v2.4 (https://pubchem.ncbi.nlm.nih.gov/edit2/index.html?cnt=0). All the ligands were subjected to energy minimization using the HyperChem software (HyperChem Release 7.5).

Molecular docking

Docking of risperidone derivatives screened from literature (Avram et al., 2011) against 5-HT_{2A} serotonin receptor structure were done using molecular docking program AutoDock 4.2 (Morris et al., 2009). Gasteiger charges are added to the ligand and maximum 6 numbers of active

torsions are given to the lead compounds using AutoDock tool (http://autodock.scripps.edu /resources/adt). Kollman charges and the solvation term were added to the protein structure. The Lamarckian genetic algorithm implemented in Autodock was used for docking.

Molecular dynamics simulations

Molecular dynamics simulations were done using the NAMD (NAnoscale Molecular Dynamics program; v2.7) graphical interface module (Phillips et al., 2005) incorporated visual molecular dynamics (VMD 1.9.2) (Humphrey et al, 1996). The protein-ligand complex was immersed in the center of a 50 Å box of water molecules where all water molecule atoms (H-O-H) were closer than 1.5 Å and a CHARMM (Chemistry at HARvard Macromolecular Mechanics) 22 parameter file for proteins and lipids; phi and psi cross-term map correction were used in the force field for complexes. A protein structure file (psf) stores structural information of the protein, such as various types of bonding interactions. The psf was created from the initial pdb and topology files using psfgen package of VMD. After running psfgen, two new files were generated protein pdb and protein psf and by accessing PSF and PDB files; NAMD generated the trajectory DCD file. After the simulations, the results were analyzed in VMD by calculating the Root mean square deviation (RMSD) of the complex.

2D QSAR

Risperidone and their 15 antagonists of 5-HT_{2A} serotonin membrane receptor were used for 2D QSAR studies. Using MLR, the QSAR model was developed with six types of energybased independent variables such as Binding Energy (BE), Intermolecular Energy (IME), Internal Energy (IE), Torsional Energy (TorE), vdW + Hbond + desolv Energy (VdwE) and electrostatic energy (EE) and one dependent variable activity pKi in uM with the help of different cross-validation procedures values.

RESULTS AND DISCUSSION

The sequence alignment of the query and template was shown in fig.1. The query sequence was made up of 471 residues; however, the structure of template was containing 430 residues. Using manual editing query was modeled from residue number 6 to 359 and alignment has 62% sequence identity. The result of alignment was employed to build new homology model.

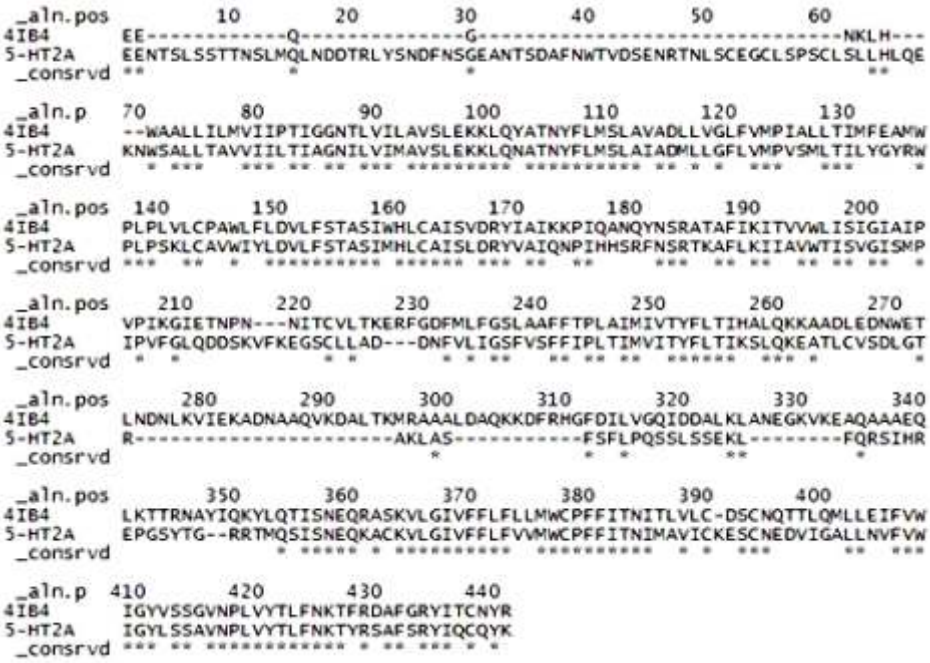


Figure 1: The sequence alignment of the query protein and the template protein using align2Dscript of modeller software. Target and template have 62% sequence identity.

After the optimization and energy minimization process, the best model was selected among five 3D models generated for 5-HT_{2A} serotonin protein based on Modeller objective function and DOPE score, which are 2109.74561 kcal/mol and -45325.50391 kcal/mol, respectively. Ramachandran plot was drawn through PROCHECK (Laskowski et al., 1993) program validated the model with 87.7% of total residues in most favoured regions, 9.0% residues in additional allowed regions, 2.2% in the generously allowed regions and 1.1% in the disallowed regions. ERRAT (http://nihserver. mbi.ucla.edu, http://www.doe-mbi.ucla.edu/services/errat.html) is a protein structure verification algorithm that is especially well-suited for evaluating the progress of model building and refinement. The program works by analyzing the statistics of non-bonded inter-actions between different atom types. A single output plot was produced by errat program that gave the value of the error function vs. position of a 9-residue sliding window. By comparison with statistics from highly refined structures, the error values have been calibrated to give confidence limits (Bowie et al., 1991). This was extremely useful in making decisions about reliability. After the errat the overall quality factor was 81.163 which have been shown in the fig. 2.

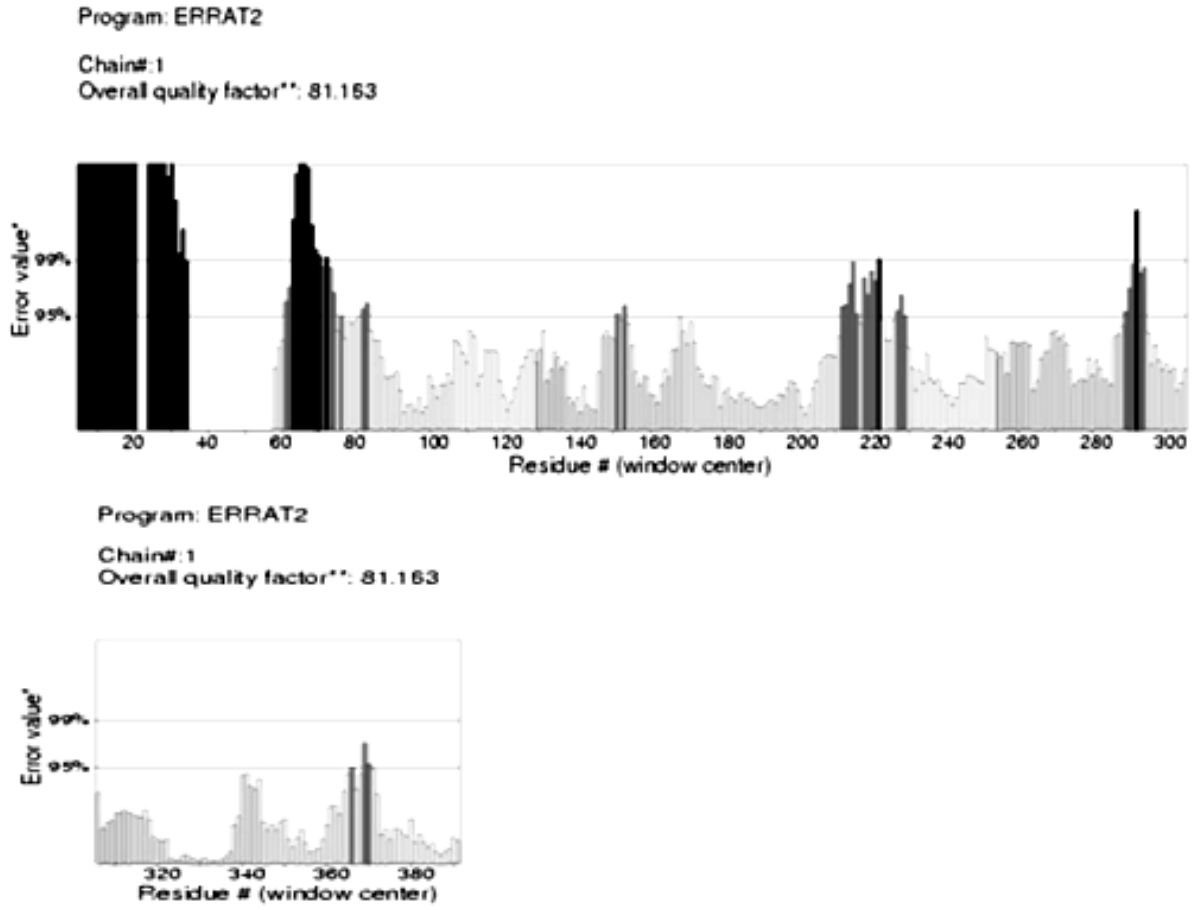
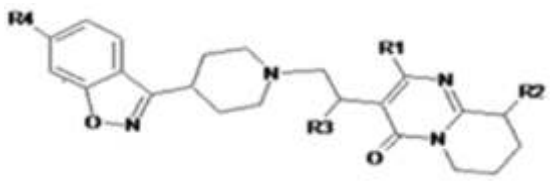


Figure 2: Errat plot for 5-HT_{2A} serotonin receptor structure. Error values for residues as predicted by ERRAT for 5-HT_{2A} serotonin receptor. Y-axis presents the error value and X-axis presents the amino acid sequences of 5-HT_{2A} serotonin receptor. An error value exceeding 99% confidence level indicates poorly modeled regions. The overall quality factor assigned to 5-HT_{2A} serotonin receptor is 81.163.

Based on R1, R2, R3 and R4 groups at different positions, risperidone derivatives of 5-HT_{2A} serotonin receptor were retrieved from literature (Avram et al., 2011) and were shown in table 1.

Risperidone derivatives



Sl. No.	Molecule Name	R1	R2	R3	R4
1	Ris	CH_3	H	H	F
2	RisA	C_2H_5	H	H	F
3	RisB	$(CH_3)_2CH$	H	H	F
4	RisC	CH_3	$(CH_3)_2$	H	F
5	RisD	$(CH_3)_2CH$	H	OH	F
6	RisE	C_6H_{13}	H	H	F
7	RisF	$H_3C\text{-}NH_2$	H	H	F
8	RisG	$CH_2N(CH_3)_2$	H	H	F
9	RisH	OH	H	H	F
10	RisI	C_4H_7	H	H	F
11	RisJ	C_4H_7	H	NH_2	F
12	RisK	CH_3	H	C_6H_5	F
13	RisL	C_4H_7	H	H	OH
14	RisM	C_4H_7	H	H	Cl
15	RisN	C_4H_7	H	H	COOH
16	RisO	C_4H_7	CH_3	H	F

Table 1: Risperidone derivatives of 5-HT_{2A} serotonin receptor based on different R1, R2,R3 and R4 groups.

In docking studies of risperidone derivatives with 5-HT_{2A} serotonin receptor, best autodock score was used as criteria to interpret the best conformation among the 30 conformations, generated by AutoDock4.2 program. The docking results of the risperidone derivatives with 5-HT_{2A} serotonin receptor were shown in table 2.

Sl. No.	Molecule Name	Experimental pKi	Predicted pKi	BE	IME	IE	TorE	VdwE	EE
1	Ris	9.76	9.93	-10.54	-11.73	-0.91	1.19	-10 .78	-0.96
2	Ris1	8.98	9.36	-9.2	-10.69	-1.29	1.49	-10 .32	-0.38
3	Ris2	8.44	8.89	-10.25	-11.44	-1.07	1.19	-10 .84	-0.6
4	Ris3	7.06	7.00	-9.64	-12.32	-1.39	2.68	-11 .85	-0.47
5	Ris4	8.84	9.29	-10.47	-12.26	-1.13	1.79	-10 .01	-2.25
6	Ris5	8.96	8.95	-9.26	-10.76	-1.57	1.49	-9.92	-0.84
7	Ris6	8.24	8.21	-9.46	-11.25	-1.85	1.79	-10 .21	-1.04
8	Ris7	8.12	7.66	-9.66	-11.75	-2.09	2.09	-10 .63	-1.12
9	Ris8	8.84	7.78	-10.54	-12.03	-1.68	1.49	-11 .66	-0.37
10	Ris9	6.36	6.89	-8.29	-10.67	-1.82	2.39	-10 .71	0.04
11	Ris10	6.36	7.30	-9.93	-11.72	-1.93	1.79	-11 .57	-0.15
12	Ris11	9.08	8.90	-9.93	-11.72	-0.9	1.79	-10.48	-1.24
13	Ris12	9.16	8.65	-9.23	-10.72	-1.2	1.49	-10.34	-0.38
14	Ris13	9.28	8.73	-8.55	-10.63	-1.3	2.09	-10.12	-0.52
15	Ris14	9.44	9.39	-10.7	-11.89	-0.51	1.19	-10.96	-0.93
16	Ris15	9.06	9.02	-8.21	-9.7	-1.14	1.49	-9.39	-0.31

BE= Binding Energy; IME: Intermolecular Energy; IE= Internal Energy; TorE= Torsional; Energy; VdwE= vdW + Hbond + desolv Energy; EE= Electrostatic energy.

Table 2: Docking results of Risperidone and their derivatives with 5-HT_{2A}serotonin receptor-structure with activity (pKi= - logKi).

Thus from the Complex scoring and binding ability it's deciphered that these compounds are promising inhibitors for 5-HT_{2A} serotonin receptor.

Protein-ligand molecular dynamics simulation

MD simulation is a well-known theoretical technique and is mainly used for evaluating the stability of any predicted model. Therefore, the constructed 3D model of protein-ligand complexes were processed for MD simulation for ps timescale with Langevin dynamics to control the kinetic energy, temperature, and/or pressure of the system. The RMSD values of complexes contain alpha carbon atoms, and all atoms were calculated by taking structure with reference conformation points.

2D QSAR

A QSAR based model has correlation coefficient (r2) of 0.7746 was obtained from risperidone and their 15 derivatives is shown in equation 1.

Predicted pKi = 14.4942 + 25.0236 (BE) + 51.5317 (IME) + 0.7563 (IE) -25.8375 (TorE) -76.1808 (VdwE) - 76.9376 (EE)……...(1)

A graphical representation between experimental pKivs Predicted pKi is shown in Fig.3.

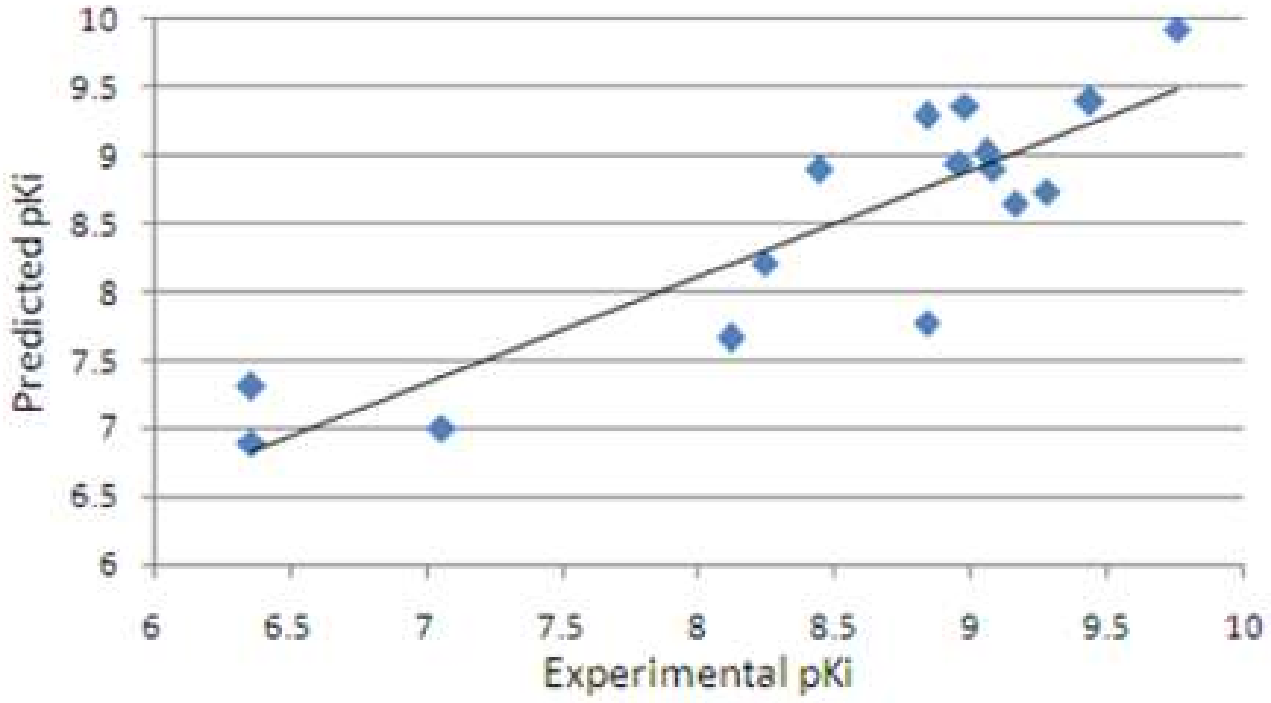


Figure 3: Relationship between experimental (x-axis) and predicted (y-axis) pKi values with an r2 value 0.7746 is shown in a QSAR model developed using multiple linear regression analysis.

Previously, similar study was done on D2 dopamine receptor protein with risperidone and their analogs (Bhargava et al., 2014). To assess the predictive performance of QSAR models, different cross-validation procedures were adopted. First, in leave-one-out strategy (LOOCV), one molecule was removed from the dataset as a test compound and the remaining 15 molecules were used to build the model. This process was repeated 16 times with each inhibitor as a test molecule.

CONCLUSION

The built 3D structure model of 5-HT_{2A} serotonin receptor is reliable for the binding of inhibitors. And developed QSAR model using pKi value of known risperidone derivatives with 5-HT_{2A} serotonin receptor as dependent variable and six energy based descriptorsindependent variable had correlation coefficient r^2 value 0.7746.

CONFLICT OF INTEREST

The authors have no conflict of interest regarding the publication of this paper.

ACKNOWLEDGEMENT

For this work, the authors are grateful to the Bioinformatics Division of Biotech Park, Lucknow, Uttar Pradesh, India for providing computational facilities. The authors are thankful to the Department of Pharmacology and Therapeutics, KGMU, Lucknow, Uttar Pradesh, India for providing necessary facilities for research work. This study is financially supported by the Ragiv Gandhi National Fellowship of University Grants Commission.

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