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Molecular Docking studies of D2 Dopamine receptor with Risperidone derivatives

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Abstract:
In this work, 3D model of D2 dopamine receptor was determined by comparative homology modeling program MODELLER. The computed model's energy was minimized and validated using PROCHECK and Errat tool to obtain a stable model structure and was submitted in Protein Model Database (PMDB-ID: PM0079251). Stable model was used for molecular docking against Risperidone and their 15 derivatives using AutoDock 4.2, which resulted in energy-based descriptors such as Binding Energy, Ligand Efficiency, Inhib Constant, Intermol energy, vdW + Hbond + desolv Energy, Electrostatic Energy, Total Internal Energy and Torsional Energy. After that, we have built quantitative structure activity relationship (QSAR) model, which was trained and tested on Risperidone and their 15 derivatives having activity value pKi in μM. For QSAR modeling, Multiple Linear Regression model was engendered using energy-based descriptors yielding correlation coefficient r2 of 0.513. To assess the predictive performance of QSAR models, different cross-validation procedures were adopted. Our results suggests that ligand-receptor binding interactions for D2 employing QSAR modeling seems to be a promising approach for prediction of pKi value of novel antagonists against D2 receptor.

Keywords: Schizophrenia, Docking, AutoDock, Risperidone analogues, D2 dopamine receptor.

Background:
Schizophrenia is a severe form of mental illness affecting ~0.001% of the adult population globally in the age group 15-35 years **[1]**. Schizophrenic patients have delusions, hallucinations, disorganized speech and bizarre behaviour as positive symptoms and have poverty of speech, blunted affect, social withdrawal, absence of normal emotional feelings and expressions, lack of energy, interest, motivation as well as absence of purpose of life as negative symptoms **[1-4]**. Neuroleptics are used to treat Schizophrenia and divided into two main classes such as typical neuroleptics (Chlorpromazine, Promazine, Haloperidol, Phenothiazines etc) and atypical neuroleptics (Clozapine, Melperone, Risperidone, Olanzapine, Quetiapine etc). Typical neuroleptic acts by blocking the action of the neurotransmitter dopamine at the level of D2 receptor. D2 receptor blockade is the main target for antipsychotic drugs, because there is a higher density of D2 in schizophrenic brains **[5-7]**. The lower level of dopamine stimulation mainly reduces positive symptoms. However, decreased dopamine level results in increased acetylcholine levels that may cause side effects called extrapyramidal symptoms (EPS) **[5, 7, 8]**. Atypical neuroleptics have effect on positive symptoms as well as on negative symptoms that's why the atypical neuroleptics are more effective than typical neuroleptics **[8]**. Five dopamine receptors D1, D2, D3, D4, and D5 have been identified and each of the receptors contains about 400 amino acids, and they have seven regions spanning the neural membrane **[9, 10]**. When drugs block dopamine receptors in the basal ganglia, the symptoms of schizophrenia are reduced. Their function is to bind to

dopamine secreted by presynaptic nerve cells and this binding triggers change in the metabolic activity of the postsynaptic nerve cells **[11]**.

Risperidone is second generation antipsychotic agent used for the treatment of Schizophrenia. In a large population of elderly patients the use of risperidone is associated with a lower risk of extrapyramidal side effects compared to First Generation Antipsychotics **[12, 13]**. In this work we had made a Homology model for D2 dopamine receptor of Homo sapiens. The computed model's energy was minimized and reliability of model was checked by Ramachandran plot and ERRAT value. Stable model was docked with the risperidone and their 15 antagonists of D2 dopamine receptor. The docking results were further used for the QSAR modelling; Multiple Linear Regression (MLR) model was engendered using energy-based descriptors yielding correlation coefficient r^2 of 0.513.

Methodology:

Retrieval of target protein Sequence

Amino acid sequence of D2 dopamine receptor (ID: P14416) of *Homo Sapiens* was obtained from uniprot database (http://www.uniprot.org/uniprot/).

Template Searching

To find a suitable template protein for the modeling of the target D2 dopamine receptor BLAST **[14]** program was used against the PDB database. By the BLAST search, we selected structure of the human dopamine D3 receptor in complex with Eticlopride (PDB Id: 3PBL) as template protein for query sequence and has sequence identity 50%.

Sequence alignment

The sequence alignment of target sequence with template was performed by using dynamics programming based align2D module in Modeller 9.11 software **[15]**. 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 D2 dopamine receptor was constructed using Modeller 9.11 software **[15]**. Alignment of query with template protein was used as input for model single script in Modeller program and five comparative models were generated. These models were validated with the help of Modeller objective function and DOPE score, which were the statistical parameter for the assessment of model using the standard Modeller energy function. The constructed models were subjected to constraint energy minimization with a harmonic constraint of 200 kJ/mol/A˚, 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. Energy computations were done with the GROMOS96 implementation of SWISS-pdbViewer (http://iqc.ethz.ch/gromos) **[16]**. The qualities of models were checked with the help of Procheck **[17]** and Errat **[18]** tools.

Docking

To gain better insight for the interactions between Risperidone and their derivatives with D2 dopamine receptor molecular docking studies were carried out **[19]**. AutoDock Software along with a graphical user interface, AutoDock Tools (ADT) was utilized to generate grids, calculate dock score and evaluate the conformations. ADT requires the receptor and ligand coordinates in either Mol2 or PDB format **[20-23]**. The receptor PDB file was transformed into the PDBQT format file containing the receptor atom coordinates, partial charges and salvation parameters. The ligand file was transformed into a PDBQT file, merged nonpolar hydrogen atoms and torsions were defined. The grid calculations were set up and maps were calculated with the programme AutoGrid. All docking runs were performed using the Lamarckian genetic algorithm and the obtained dock score were reported in kcal/mol. Docking of Resperidone and their 15 antagonists on the predefined and experimentally characterized binding pocket, where the residues ASP78, ILE148, THR376, and TYR380 were being particularly important.

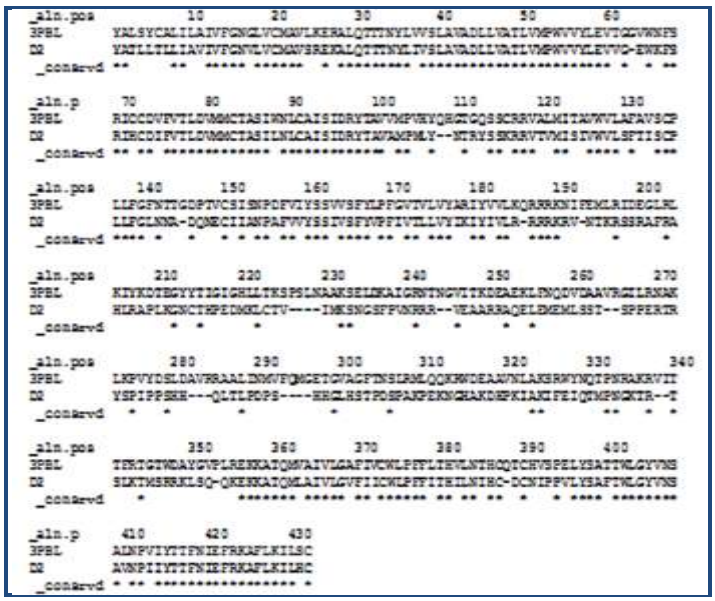

Figure 1: The sequence alignment of the query protein and the template protein using align 2D script of modeller software. Target and template have 50% sequence identity.

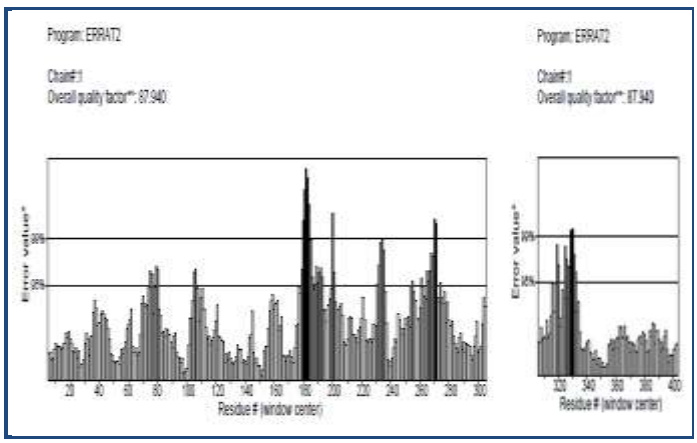

Figure 2: Errat plot for D2 dopamine receptor structure. Error values for residues as predicted by ERRAT for D2 dopamine receptor. Y axis presents the error value and X axis presents the

amino acid sequences of D2 dopamine receptor. An error value exceeding 99% confidence level indicates poorly modelled regions. The overall quality factor assigned to D2 dopamine receptor is 87.940.

2D QSAR study

Risperidone and their 15 antagonists of D2 dopamine receptor were used for 2D QSAR studies. Using MLR, the QSAR model was developed with eight types of independent variables Binding Energy, Ligand Efficiency, Inhib Constant, Intermol energy, vdW + Hbond + desolv Energy, Electrostatic Energy, Total Internal Energy and Torsional Energy and one dependent variable activity (*ki*) with the help of different cross-validation procedures values.

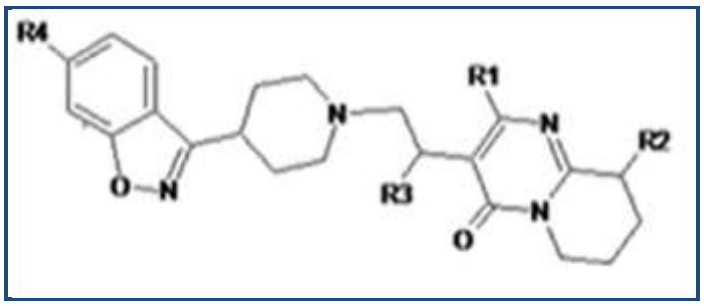

Figure 3: Risperidone Derivatives: On the basis of four different groups at R1, R2, R3 and R4 positions

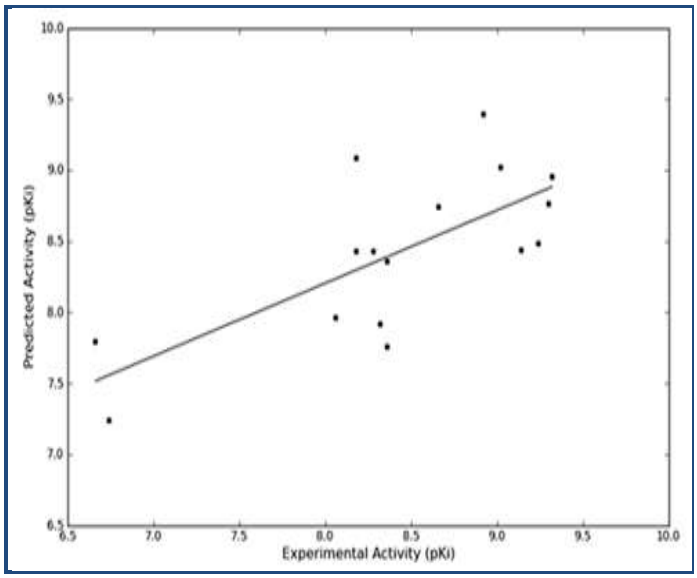

Figure 4: Depict the experimental and predicted pKi value in X and Y direction respectively with r2= 0.513 using MLR.

Results & Discussion:

The sequence alignment of the query and template was shown in **(Figure 1)**. The query sequence was made up of 443 residues; however, the structure of template was containing 481 residues. Using manual editing query was modelled from residue number 23 to 443 and after alignment the sequence identity was 50%. The result of alignment was employed to build new homology model. After the optimization and energy minimization process, the best model was selected among five 3D models generated for D2 protein on the basis of Modeller scores and the Molecular Objective Function of the selected model was 2174.2141 kcal/mol and the DOPE score result was -46843.125002141 kcal/mol. Energy minimization of 3D structure is vital for providing the maximum stability to the protein. Ramachandran plot drawn through PROCHECK **[17]** program validated the model with 91.6% of total residues in most favoured regions, 7.0% residues in additional allowed regions, 0.5% in the generously allowed regions and 0.8% 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 crystallographic model building and refinement. The program works by analysing the statistics of non-bonded interactions 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 **[18]**. This was extremely useful in making decisions about reliability. After the errat the overall quality factor was 87.940 which have been shown in the **(Figure 2)**.

On the basis of R groups **(Figure 3)** at four different positions, Risperidone and their 15 derivatives were taken from literature **[19]** is shown in **Table 1 (see supplementary material)**. Docking studies predicted the interaction of ligands with protein and residues involved in these complexes. In this work, optimal interactions and the best autodock score were used as criteria to interpret the best conformation among the 15 conformations, generated by AutoDock program.

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 15 times with each inhibitor as a test molecule. Once a model was constructed, goodness about the fit and statistical significance was assessed using the statistical parameters **[24]**.

Docking and QSAR results of Risperidone and their 15 derivatives with D2 receptor are shown in **Table 2 (see supplementary material)** in which the Antagonist potency (Observed pK_i and Predicted pK_i) are mentioned. A graphical representation between experimental pK_i vs Predicted pK_i is shown in **(Figure 4)**.

Conclusion:

Flexible docking of ligand from chemical database to receptor is an emerging approach and is widely used in drug discovery to reduce the cost and time. Risperidone and their fifteen known derivatives having inhibitory value pKi with D2 dopamine were used for 2D QSAR study. Using MLR analysis, a QSAR based model was generated having eight descriptors namely Binding Energy, Ligand Efficiency, Inhib Constant, Intermol energy, vdW + Hbond + desolv Energy, Electrostatic Energy, Total Internal Energy and Torsional Energy, which accomplished correlation coefficient r^2 value 0.513.

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Supplementary material:

Table 1: Risperidone and their 15 derivatives having different groups

Molecule Name	Groups			
	R1	**R2**	**R3**	**R4**
Risperidone	CH_3	H	H	F
RisA	C_2H_5	H	H	F
RisB	$(CH_3)_2CH$	H	H	F
RisC	CH_3	$(CH_3)_2$	H	F
RisD	$(CH_3)_2CH$	H	OH	F
RisE	C_6H_{13}	H	H	F
RisF	$H_3C\text{-}NH_2$	H	H	F
RisG	$CH_2N(CH_3)_2$	H	H	F
RisH	OH	H	H	F
RisI	C_4H_7	H	H	F
RisJ	C_4H_7	H	NH_2	F
RisK	CH_3	H	C_6H_5	F
RisL	C_4H_7	H	H	OH
RisM	C_4H_7	H	H	Cl
RisN	C_4H_7	H	H	COOH
RisO	C_4H_7	CH_3	H	F

Table 2: Docking Results of D2 Dopamine Receptor with Risperidone and their derivatives with activity (pK_i).

Molecule Name	BE	LEf	InCo	InE	VDHE	ElecE	TotE	TorE	Experimental pK_i	Predicted pK_i
Risperidone	-10.58	-0.35	17.48	-11.78	-11.19	-0.58	-1.02	1.19	8.180	9.083
RisA	-10.17	-0.33	34.82	-11.67	-11.13	-0.54	-1.05	1.49	9.240	8.482
RisB	-9.98	-0.31	48.34	-11.47	-11.28	-0.19	-1.34	1.49	9.300	8.762
RisC	-10.2	-0.32	33.24	-11.4	-10.82	-0.58	-0.79	1.19	8.920	9.393
RisD	-8.66	-0.26	446.49	-10.45	-9.98	-0.47	-0.61	1.79	9.020	9.018
RisE	-9.55	-0.27	100.33	-12.23	-11.97	-0.27	-1.51	2.68	6.740	7.238
RisF	-10.83	-0.35	11.49	-12.62	-10.83	-1.79	-0.13	1.79	8.060	7.960
RisG	-9.77	-0.3	68.75	-11.56	-10.47	-1.09	-0.98	1.79	9.140	8.436
RisH	-9.24	-0.31	169.27	-10.73	-10.32	-0.41	-1.17	1.49	8.280	8.427
RisI	-9.99	-0.3	47.21	-11.78	-11.4	-0.38	-1.42	1.79	8.180	8.427
RisJ	-8.39	-0.25	706.54	-10.48	-9.37	-1.11	-2.55	1.49	8.360	8.357
RisK	-9.87	-0.27	57.88	-11.37	-11.17	-0.2	-1.95	1.49	9.320	8.953
RisL	-10.75	-0.33	13.25	-12.84	-12.57	-0.26	-1.64	2.09	8.320	7.915
RisM	-10.44	-0.32	22.35	-12.23	-11.89	-0.34	-1.62	1.79	8.360	7.756
RisN	-7.61	-0.22	2.65	-9.99	-7.86	-2.13	-1.44	2.39	8.660	8.740
RisO	-10.41	-0.31	23.45	-12.2	-11.68	-0.52	-1.68	1.79	6.660	7.792

BE: Binding Energy; LEf: Ligand Efficiency; InCo: Inhib Constant; InE: Intermol energy; VDHE: vdW + Hbond + desolv Energy; ElecE: Electrostatic Energy; TotE: Total Internal Energy; TorE: Torsional Energy; Ris: Risperidone.