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Pharmacophore modelling and docking studies of pyrrolidinyl pyridone and pyrazinone analogues as prolyl oligopeptidase(POP) inhibitors

Mohan Babu Jatavath, Lingala Yamini, Sree Kanth Sivan and Vijjulatha Manga*

Molecular Modelling and Medicinal Chemistry Group, Department of Chemistry, University College of Science, Osmania University, Hyderabad

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ABSTRACT

Prolyl oligo peptidase (POP) is a serine endo peptidase that hydrolyses proline containing peptides with less than 30 amino acid length. POP enzyme suspected to be involved in several biological functions such as cell proliferation, differentiation, signal transduction, Parkinson's and Alzheimer's disease. POP inhibitors have been developed to restore the depleted neuro peptide levels encountered in aging or in neurodegenerative disorders. Sequentially to understand the mechanism of action, pharmacophore analyses and docking studies were performed on POP inhibitors. Five point Pharmacophore with pharmacophoric features, two hydrogen bond acceptors (A), one hydrophobic group (H) and two aromatic rings (R) was generated with considerable R^2 and Q^2 values of 0.913 and 0.652 respectively. The results obtained from this study were used for designing of new POP inhibitors. This study further furnishes modest information in the development and investigation of POP inhibitors.

INTRODUCTION

Prolyl oligo peptidase (POP) (EC 3.4.21.26) also known as prolyl endo peptidase (PREP), was identified by Walter et al. in the year 1971 from bovine uterus as an oxytocin cleaving enzyme; later it was linked to the hydrolysis of neuro peptides. It is a serine protease belongs to the family S9 of the serine carboxy peptidase clan. [1] It has a special feature of hydrolysing small proline rich peptides (length less than 30 amino acids) such as neuro peptides, arginine-vasopressin (AVP), neurotensin, oxytocin and substance P (SP) etc. It cleaves the -*Pro-Xaa*- bond at the carboxyl side of a proline residue, where *Xaa* is amino acid other than proline.[2-3] POP family is widely distributed in organisms ranging from bacterial species to mammals.[4] It is found in all tissues, but the highest activities have been measured in the brain.[5-6] Generally, POP is present in neurons, which use gamma-aminobutyric acid (GABA) and acetylcholine (ACh) as neurotransmitters rather than dopamine.[7] Due to its locality and importance in neuropeptide break-down, POP enzyme has associated to CNS diseases related to neuropeptidergic malfunction [8] such as depression, Parkinson's, Alzheimer's diseases[1], amnesia, schizophrenia, trypanosomiasis, bipolar affective disorder etc. In animals, the role of POP was observed to control peptide signalling pathways and has been extensively studied. The inhibitors of POP restore the altered activity of neural network and adjust neuropeptides levels to normal.

The present work highlights pharmacophore modelling and docking analysis of Pyrrolidinyl Pyridone (PP) and Pyrazinone (Py) analogues of POP inhibitors. Pharmacophore [9] is an important model in rational drug design that exemplifies the geometry complementary of drug to the target. Pharmacophore hypothesis collects common features distributed in three-dimensional space that participate in important interactions between drug and active site. Atom based 3D- pharmacophore model [10, 11] and docking was performed with a series of PP and PY analogues of Prolyl oligo peptidase [12] with PHASE [13] and Glide modules respectively. PHASE (Pharmacophore Alignment and Scoring Engine) is a flexible module for pharmacophore identification, assessment, model development [14, 15] database construction and searching [16].

Glide (Grid Based Ligand Docking with Energetics) has been designed to perform an exhaustive search of the positional, orientation and conformational space available to the ligand based on energy of the system. Docking study was applied with a series of hierarchical filters to search for possible sites of the ligand in the active site region of the receptor. The objective of the present study is to fabricate reliable and modest information required for the design of new inhibitors towards POP.

EXPERIMENTAL SECTION

A dataset comprising of 39 molecules (Table-1) was used and its *in vitro* biological activity data reported as IC_{50} values were converted to pIC_{50}. A total of 39 molecules were available with pIC_{50} values, of which 29 molecules were randomly chosen as training set and 10 molecules were selected as test sets to accommodate structural diversity in model generation.

Computational details for 3D QSAR

The 3D conversion and minimization was performed using LigPrep [17] with OPLS2005 force field [18] incorporated in PHASE. Pharmacophore model development requires all-atom 3D structures that are realistic representations of the experimental molecular structure something close to the putative binding mode. Hence, conformers were generated using a rapid torsion angle search followed by minimization of each generated structure using OPLS2005 force field, with implicit distance dependent dielectric solvent model. A maximum of 1000 conformers were generated per structure using a pre process minimization of 100 steps and post process minimization of 50 steps. Each minimized conformer was filtered through a relative energy window of 10 kCal/mol and RMSD of 1.00 Á.

C***reating pharmacophore sites***

Each ligand structure is represented by a set of points in 3D space, that facilitate non covalent binding between the ligand and its target receptor. PHASE provides a built-in set of six pharmacophore features, hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), negatively ionizable (N), positively ionizable (P), and aromatic ring (R). Based upon the structural similarity and the common pharmacophoric features PHASE generates the possible pharmacophore sites for the data set. These generated features were further assigned for geometrical entities of data set to define physical chareteristics.

Finding a common pharmacophore

Pharmacophore from all conformations of the ligand in the active site are examined and those pharmacophore that contain identical sets of features with very similar spatial arrangements are grouped together and gives rise to a common pharmacophore. Common pharmacophores are identified using a tree based partitioning technique that grouped together similar pharmacophore according to their inter site distances, i.e., the distances between pairs of sites in the pharmacophore. Active and inactive thresholds of pIC_{50} 5.4 and 5.1, respectively, were applied to the training set for developing the common pharmacophore hypotheses. After applying default feature definitions to each ligand, common pharmacophore containing sites were generated for scoring the hypothesis.

Scoring Hypotheses

In the score hypotheses step, common pharmacophore are examined, and a scoring procedure is applied to identify the pharmacophore that yields the best alignment of the active set ligands. This pharmacophore provides a hypothesis to explain how the active molecules bind to the receptor. The scoring procedure provided a ranking of different hypotheses and allows making rational choices about hypotheses which is most appropriate for further investigation. Scoring with respect to actives was conducted using default parameters for site, vector, and volume terms. Ligand activity, expressed as $-\log_{10}(IC_{50})$, was incorporated into the score with a weight of 1.0, rest with default values. Hypotheses that emerged from this process were subsequently scored with respect to inactive, using a weight of 1.0. The inactive molecules were scored to observe the alignment of these molecules with respect to the pharmacophore hypothesis to enable making a decision on the selection of the hypothesis. Larger is the difference between the scores of active and inactives better is the hypothesis at distinguishing the actives from inactives.

Building QSAR model

PHASE provides the means to build QSAR models using the activities of the ligands that match a given hypothesis. PHASE QSAR models are based on PLS regression, applied to a large set of binary valued variables. QSAR models were generated based on PLS regression, and applied to a set of binary variables. Independant variables for QSAR model were derived from a grid space occupied by training set of ligands. Based on the type of grid space occupied, atom types were classified as: D: hydrogen-bond donor, H: hydrophobic or nonpolar, N: negative ionic, P: positive ionic, W: electron-withdrawing (includes hydrogen-bond acceptors), X: miscellaneous (all other types).

Atom based QSAR models were generated by taking all atoms of the molecule that matched to the hypothesis. Atom-based QSAR models were generated for AAHRR41 hypothesis using the 29 member training set and a grid spacing of 1.0Å. QSAR models containing one to five PLS factors were generated. A model with five PLS factor was considered as the best statistical model and validated by predicting activities of test set molecules.

Docking studies

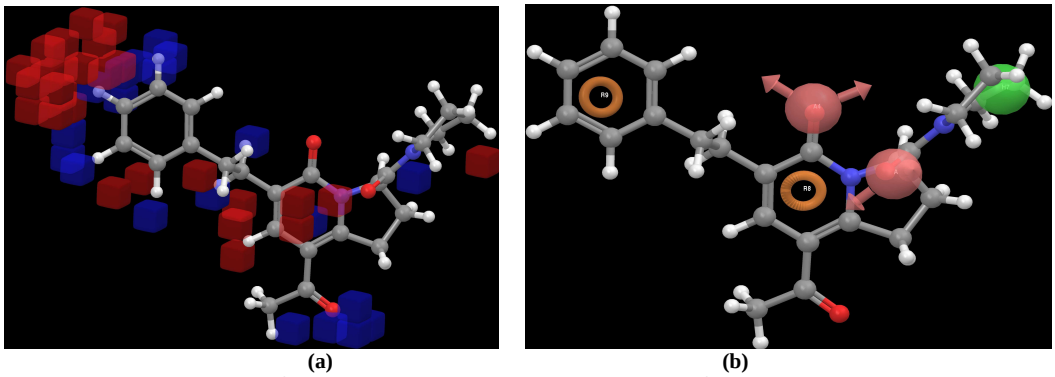
Target protein preparation and docking:

The three dimensional crystal structure of Prolyl oligopeptidase(POP) (PDB Id: 3DDU) was downloaded from the Protein Data Bank PDB) (http://www.rcsb.org)**.** Before docking the ligands into the protein active site, the protein was prepared using protein preparation wizard of Schrodinger's molecular docking software. In this protein preparation all water molecules and hetero atoms were removed and hydrogen atoms were added to the protein. The active site of the protein was defined for generating the grid. The ligands were then docked into the prepared grid, for which "standard precision mode" was selected. No constraints were defined.

RESULTS AND DISCUSSION

A set of 39 molecules having affinity towards POP were used for 3D QSAR study. Fine grained conformational sampling and scoring techniques were utilized to identify common pharmacophore required for critical binding with the receptor. During the conformational sampling each hypothesis is accompanied by a set of aligned conformations. This reflects the relative orientation of molecules likely bind to the receptor. Later known activity data was combined with the aligned conformations of the hypothesis to generate a 3D QSAR model, governed by molecular structure and activity. Pharmacophore models were derived using 29 molecules training set and 10 molecules test set of PP and PY analogues of POP (Table 1). AAHHR features (Figure 1) were selected for creating sites and further used in model generation. This five featured hypothesis subjected to stringent scoring function.

For each ligand, one aligned conformer based on the lowest Root Mean Square Error (RMSE) of atom coordinates from those of the reference was superimposed on AAHRR41. Then fitness scores for all ligands were observed on the best scored pharmacophore model AAHRR41. The greater the fitness score, the greater will be the activity prediction of the molecule. Fitness score also contains a distance term, which measures the distance that separates the feature on the molecule from the centroid of the hypothesis feature. The generated QSAR model is checked for its validity and predictive nature on set of 10 molecules and obtained a correlation coefficient of 0.913 between experimental verses predicted activity. A scatter plot of experimental verses predicted values were plotted for the set of 39 molecules and the same was represented in figure 2.

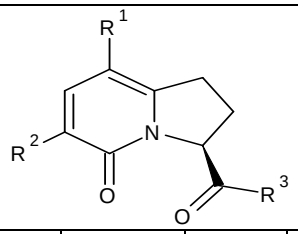


(a) **(b)**

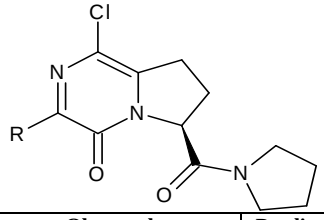
Figure 1: (a) Pictorial representation of the cubes generated using the QSAR model of most active molecule (21). Blue cubes indicate favourable regions,while red cubes indicate unfavourable region for the activity. (b) PHASE generated pharmacophore model AAHRR41 of most active molecule (21) illustrating hydrogen bond acceptor (A4; pink), hydrophobic group (H7; green), aromatic ring (R 8; orange) features

Actual and predicted activity values of test set molecules (Table-1) exhibited a correlation of 0.913 as R^2 value and 0.652 Q^2 respectively with reported POP inhibitory activity against the model AAHRR41. For a reliable model, the squared predictive correlation coefficient should be >0.6. [19, 20] The results of this study reveal that model AAHRR41 is reliable and valid for further investigation of POP activity. The diagrammatic representation of the generated fields of QSAR model (figure-1a) revealed the favor and disfavored regions of the data set. The productive information obtained from the analysis guided to design new molecules with improved interactions and predictive activity (table-3).

Table-1: Structure, biological activity, predicted activity and fitness score data of PP and PY molecules used in PHASE analysis



Molecule	R^1	R^2	R^3	Expt. pIC50	Pred. pIC50	Fitness Score	Dockscore
1	C(O)Me	OCH_2CF_3	Pyrrolidine	6.233	6.4	1.43	-5.112
2	C(O)Me	OCH_2-4-F-Ph	Pyrrolidine	7.275	7.41	1.43	-6.157
3	C(O)Me	OCH_2-4-CF_3-Ph	Pyrrolidine	7.552	7.35	1.4	-7.514
4	C(O)Me	OCH_2-4-OMe-Ph	Pyrrolidine	6.853	6.66	1.45	-6.614
5	C(O)Me	OCH_2-4-Cl-Ph	Pyrrolidine	7.318	7.71	1.4	-5.937
6	C(O)Me	OCH_2-4-CN-Ph	Pyrrolidine	6.106	6.43	1.45	-6.344
7	C(O)Me	OCH_2-3,5-F-Ph	Pyrrolidine	7.657	7.43	1.43	-6.82
8	C(O)Me	OCH_2-3,4-F-Ph	Pyrrolidine	8.154	7.79	1.41	-6.535
9	C(O)Me	OCH_2-3-Cl-4-F-Ph	Pyrrolidine	8.096	7.81	1.42	-5.977
10	C(O)Me	OCH_2-3,4-Cl-Ph	Pyrrolidine	7.552	7.71	1.42	-5.329
11	SO_2Ph	OCH_2-3,5-F-Ph	Pyrrolidine	8.397	8.27	1.42	-5.308
12	SO_2Ph	OCH_2-4-CF_3-Ph	Pyrrolidine	7.958	7.94	1.33	-.6.654
13	SO_2Ph	OCH_2-4-^{t}Bu-Ph	Pyrrolidine	6.568	6.6	1.37	-5.493
14 t	H	OCH_2-4-F-Ph	Pyrrolidine	7.657	7.47	1.48	-6.644
15 t	H	OCH_2-3,5-F-Ph	Pyrrolidine	7.657	7.52	1.49	-5.776
16	H	OCH_2-3,4-F-Ph	Pyrrolidine	7.744	7.47	1.47	-6.846
17 t	H	OCH_2-2,5-F-Ph	Pyrrolidine	7.193	6.63	1.5	-6.881
18	H	OCH_2-4-CF_3-Ph	Pyrrolidine	7.468	7.4	1.46	-6.678
19	H	OCH_2-4-^{t}Bu-Ph	Pyrrolidine	7.221	7.43	1.41	-5.772
20	H	OCH_2-3,4-F-Ph	Pyrrolidine	7.508	7.67	1.5	-6.799
21	C(O)Me	$(CH_2)_2$Ph	Pyrrolidine	8.698	9.01	1.55	-6.747
22 t	C(O)Me	$(CH_2)_2$-4-F-Ph	Pyrrolidine	8.522	9.01	1.56	-6.205



Molecule	R	Observed pIC50	Predicted pIC50	Fitness Score	Dockscore
23t	Cl	5.508	6.86	2.18	-5.495
24	OPh	6	6.07	2.2	-6.430
25	OCH_2-4-F-Ph	7.397	7.42	1.46	-6.329
26	OCH_2-3,4-Cl-Ph	7.468	7.45	1.57	-6.587
27	OCH_2-3,4-F-Ph	7.259	7.37	1.47	-7.068
28 t	OCH_2-3-Cl-4-F-Ph	7.366	7.21	1.59	-6.788
29	$O(CH_2)_2$-3,4-Cl-Ph	5.657	5.53	1.36	-7.156
30	OCH_2-4-Pyridyl	6.096	6.17	1.99	-6.154
31 t	$O(CH_2)_2$-4-F-Ph	6.045	6.68	1.06	-7.430
32	OCH_2-Cyclohexyl	7.154	7.06	2.11	-6.194
33	OCH_2-4-CF_3-Ph	6.602	7.11	1.43	-6.628
34	OCH_2-2,3,5-F-Ph	7.397	7.06	3	-7.118
35	OCH_2-3-Cl-Ph	7.522	7.29	1.6	-6.198
36 t	$NH(CH_2)_2$Ph	7.096	7.48	1.11	-6.335
37 t	$NHCH_2$Ph	7.92	7.67	1.34	-6.574
38	$NHCH_2$-4-F-Ph	7.769	7.67	1.33	-6.720
39 t	$NHCH_2$-3,4-F-Ph	7.823	7.2	1.39	-7.297

t= test set molecules, ^{t}Bu = *tertiary butyl,* Ph = *Phenyl*

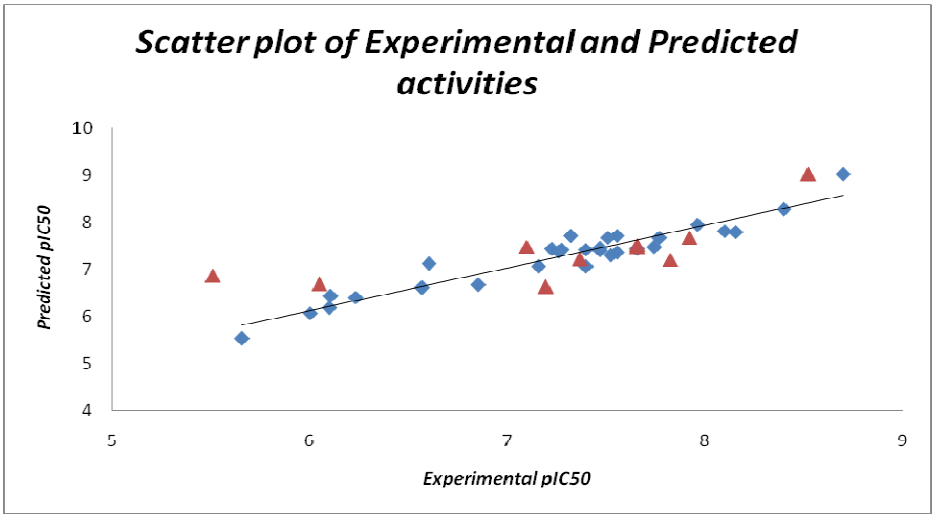
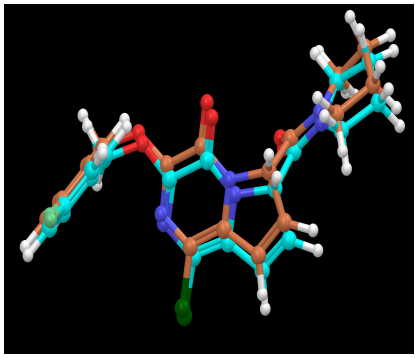
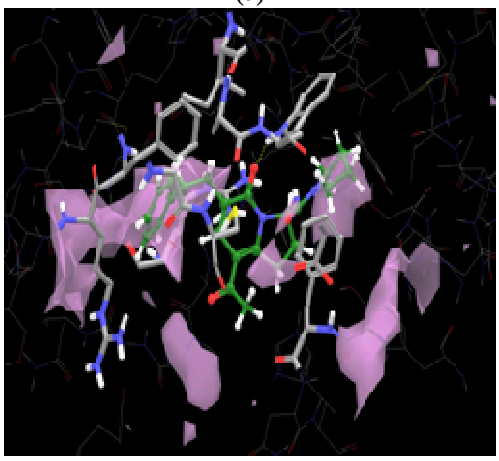


Figure 2: Scatter plot of Experimental and Predicted activities of training and test set molecules. (Test set is represented as triangles and training set is represented as squares)

Docking studies were carried out using Glide [21] module in Schrödinger to identify the favorable interactions between ligand and the receptor molecule. Applied docking methodology was validated by re-docking the crystal ligand into the generated grid and obtained the RMSD of 0.687Å (Figure 3a). Ligand poses were generated based on combined results of position and orientation of the ligand relative to the receptor. These generated poses from hierarchical filtering were analyzed for their interaction with the receptor. The initial and final stage of docking algorithm generated a energy minimized docking complex with best non bonded ligand - receptor interaction energy. These were further subjected to the scoring function to obtain dock score of the molecule. POP inhibitor (molecule-31) showed hydrogen bond interactions with Trp 595 (Figure-3b), of the protein active site.



(a)



(b)

Figure 3: (a)Superimposition of crystal structure pose (cyan) on docked pose (Orange) of co-crystallized ligand. The RMS deviation is 0.687 Å (b) Dock pose of most active molecule 21 showing hydrogen bond interactions with active site amino acid Trp 595

New molecule design:

Detailed Pharmacophore analysis empowered us to identify structural requirements for observed inhibitory activity. New molecules were designed based on docking and pharmacophore results. Molecules were designed with a referred substitution at R3 of PP molecules (figure 4) with napthyl, ortho substituted benzene and substituted napthyl groups. These designed molecules were docked into the active site and they showed similar interactions with comparable dock score and predicted activity with respect to the most active molecule 21. Figure 5 shows dock pose of newly designed molecule N1, that shows two hydrogen bond interactions with the active site amino acids Arg 643 and Trp 595. Among this, the less substituted simple napthyl derivative has showed a dock score. Whereas molecule N3 showed good predicted activity and fitness score (table 2) which is in the range of highly active molecule. Based on the predicted activity, dock score and fitness score these were considered as moderately active towards POP inhibition. Designed molecules showed predictable similarity in their activity and docking interactions with reference molecule.

Table 2: Predicted activity, Dock score and Hypothesis fitness score of newly designed molecules

Molecules	Predicted Activity	Dock Score	Fitness Score
N1	7.562	-8.78	1.337
N2	7.963	-8.004	1.667
N3	8.120	-7.545	1.801
N4	7.858	-7.263	1.586
N5	7.571	-7.262	1.551

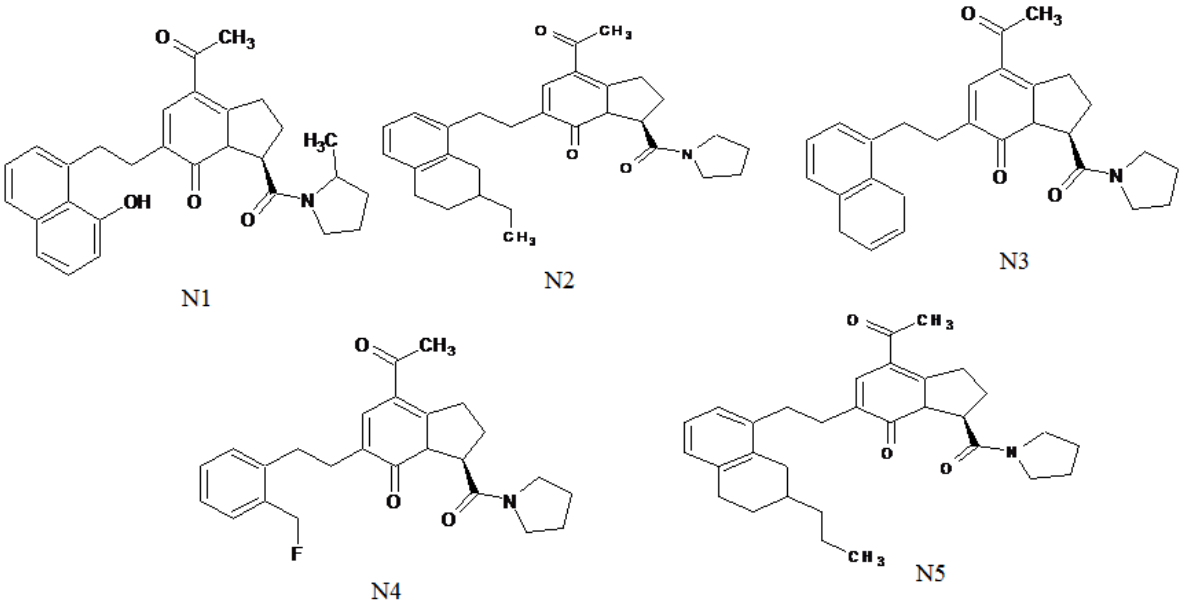


Figure 4: Structure of newly designed molecules

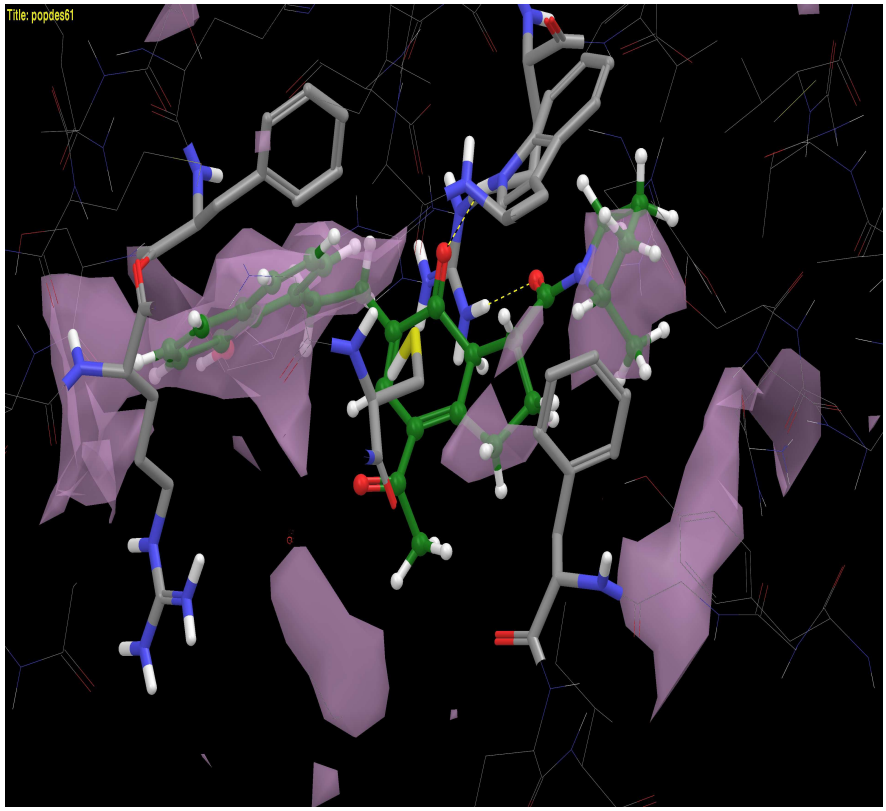


Figure 5: Dock pose of new molecule N1 showing hydrogen bond interactions with active site amino acid Arg 643 and Trp 595

CONCLUSION

This study shows the generation of a pharmacophore model AAHRR41 for Pyrrolidinyl Pyridone and Pyrazinone analogues as potent inhibitors of Prolyl oligopeptidase(POP). Pharmacophore modeling correlates activities with the spatial arrangement of various chemical features. Hypothesis AAHRR41 represents the best pharmacophore model for determining POP activity. This pharmacophore model was able to predict POP activity; the validation and docking results also provide additional confidence in the proposed pharmacophore model. Results suggested that the proposed 3D QSAR model can be useful to rationally design new POP inhibitors and also to identify new promising molecules as POP inhibitors in large 3D database of molecules.

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