International Journal of Medicine and Pharmaceutical Science (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 7, Issue 1, Feb 2017, 29-46

DESIGNING OF GLUTAMATE RECEPTOR INHIBITORS OF QUINAZOLINONE DERIVATIVES BY A COMPARATIVE QSAR ANALYSIS AND \MOLECULAR MODELING STUDIES

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ABSTRACT

An attempt was made to develop a two Dimensional Quantitative Structure–Activity Relationship (2D-QSAR) and molecular docking studies on a series of quinazolinone derivatives acting as glutamate receptor inhibitors for correlating the chemical composition of quinazolinone analogs and estimation of their anticonvulsant activity using Multiple Linear Regression (MLR) Analysis. New Chemical Entities (NCEs) were designed using results of pharmacophore profiling from known anticonvulsants. Binding affinities of designed NCEs were studied on Glutamate receptor using docking studies and their ADMET properties were also predicted. Finally, most promising compounds were selected from molecular modeling studies. 12 compounds showed significant Glutamate receptor inhibiting activity compared to standard ligand bound with glutamate receptor (PDB: 1GR2). These four basic strategies (pharmacophore mapping, QSAR, docking & ADMET screening) were implemented to evaluate the performance of derivatives. Although predicted Ki through QSAR model showed mild activity against glutamate receptor, but conclusively, compounds 22, 15 & 8 were observed to be most feasible to act against glutamate receptor for anticonvulsant activity.

KEYWORDS: ADMET, Anticonvulsant, Docking, Epilepsy, Glutamate, Pharmacophore, QSAR & Quinazolinone

Received: Dec 12, 2016; **Accepted:** Jan 11, 2017; **Published:** Jan 18, 2017; **Paper Id.:** IJMPSFEB20174

Original Article

INTRODUCTION

Epilepsy, a neurological, non-communicable, pervasive disease signalized by the unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons that affects more than 60 million people worldwide according to epidemiological studies [1,2.] In developed countries is approximately 50 per 100,000 while that of developing country is 100 per 100,000, in 2013 about 22 million people have been suffering from epilepsy (WHO, 2006). The presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients[3]. Many conventional antiepileptic drugs like phenytoin, sodium valproate and carbamazepine reported several serious side effects notably neurotoxicity with them [4, 5]. The greater numbers of antiepileptic drugs are consumed life long, concomitant administration of other drugs predisposes to the risk of drug interaction. However, newer antiepileptics like gabapentin, vigabatrin, lamotrigine, *etc* are used supplemental to the conventional agents[6].

Anticonvulsant drugs are estimated to be effective in treating 90% of the epileptic patients only. However, all the conventional and newer anticonvulsant drugs which are currently approved, and are already in use, have dose-related toxicity and idiosyncratic side effects[7]. Thus, it is essential to investigate for new

antiepileptic agents with lower toxicity and fewer side effects. A wide variety of compounds has been designed for this purpose[8].

The reason behind epilepsy has been defined on the basis of single gene defect, interaction of multiple genes as well as an environmental factor[9]. Most of the interacting genes are known to be involved with ion channels, enzymes, GABA, and G protein-coupled receptors [10]. The majority of antiepileptic drugs reduce the release of excitatory glutamate by blocking sodium or calcium channels, activation of Gamma-Amino-Butyric-Acid (GABA), inhibition of glutamate receptor and activation of peroxisome proliferator-activated receptor alpha. At present, the most commonly used antiepilepsy/anticonvulsant therapy for synaptic transmission are through the concomitant use of drugs that belongs either to the class of GABA activator (GA), $Na^{+/}Ca^{++}$ inhibitor (NCaI), Glutamate receptor inhibitor (GRI), and PPAR-alpha activator (PPARα) *etc*. [11,12].

Synaptic transmission in the mammalian central nervous system is mediated by 'L-glutamate' on three classes of ionotropic glutamate receptors, namely AMPA, NMDA and Kainate. GRIs work through these receptors. GRI (Glutamate receptor inhibitor) are structurally diverse group of compounds which binds to the Glutamate receptor and interacts with a specific allosteric non-substrate binding pocket site. Currently, drugs used to treat epilepsy under GRI for anticonvulsant therapy are Perampanel [13, 14].

As quinazolinone backbone has shown a variety of biological activities it appears as an ideal frame for designing of anti-epileptic leads as glutamate receptor inhibitor. They may act as anticonvulsant potential lead as well as possess efficacy against epilepsy. Crystal structure analysis of glutamate receptor (PDB: 1GR2, 4PE5) showed that most of GRI bound to the glutamate receptor. One of the wings of this GRI interact with a hydrophobic pockets formed mainly by the side chains of tyrosine, phenylalanine, threonine, arginine, serine, glutamic acid. Recently, pharmacophore mapping, 2D/3D QSAR and docking guided optimization of identification of novel compounds have been used as important strategies in the discovery of new anticonvulsants. ADMET screening of newly designed molecules provide a pre-clinical trial scaled analysis for their bioavailability and drug-like possibilities. These approaches are inexpensive and more practical than discovering novel compounds. The present work was focused on computer-aided design of GRI containing quinazolinone nucleus with simultaneous goals of enhanced performance against glutamate receptor. All the New Chemical Entities (NCEs) were designed on the basis of pharmacophore components of well known anticonvulsants from literature survey. In order to gain molecular interaction insights, docking studies of NCEs are carried out targeting glutamate receptor. The possible activity of NCEs can be obtained from two-dimensional (2D) Quantitative Structure–Activity Relationship (QSAR) studies using Multiple Linear Regression (MLR) Analysis. ADMET properties are used to estimate the drug like pharmacokinetic profile of the designed NCEs. The most promising compounds can be selected on the basis of results of molecular modeling studies. After confirmation of molecular interaction, their activity and ADMET screening of derivatives were performed for cross-evaluation of their performance for identification of most possible novel compounds[15-20].

MATERIAL AND METHODS

Raw Data

Raw data for anticonvulsant activity was collected from literatures and databases. Anticonvulsant activity was collected in terms of Ki (nM) value against glutamate receptor. Bioactivity in term of Ki nM (inhibition constant) was transformed to (natural) log Ki (nM) for normalisation of data set. Crystallographic structure of glutamate receptor was

collected from PDB database.

Pharmacophore Profiling of Compounds

Pharmacophore properties of anticonvulsant compounds were identified through literatures. These properties were used for designing of quinazolinone derivatives. The pharmacophore profiles of designed molecules were judged in comparison of positive control compounds.

Structural Modelling and Optimization

Chem Bio Draw Ultra v12.0 modeling suite (CambridgeSoft Corp., UK) was used for sketching of compounds under study. Molecule's geometry cleaning and energy minimization was performed by Discovery studio 3.5 client (Accelrys USA). It was also used for conversion of 2D to 3D structure.

Docking Simulation Parameters

Auto Dock Vina 4.2 was used for virtual high throughput screening of compounds against glutamate receptor. Docking of known positive control was used for identification of best possible binding site of query molecules. During docking simulation process, ligand was set to flexible mode, while the protein set to rigid form. All other docking simulation parameters were set to default mode[21].

Chemical Descriptors and QSAR Modelling Parameters

To screen out potential leads against glutamate receptor, a total of known anticonvulsant compounds with low to high Ki (nM) values were collected in the raw data set from PubChem database of NCBI[22.] To select the compounds for model development, pharmacophore features of control and query compounds were matched. Only best selected compounds were used for model building. Molecular descriptors were calculated through PaDEL-Descriptors software[23]. After removing zero values descriptors, the descriptors were selected through data reduction through removal of highly inter-correlated descriptors followed by forward selection and backward elimination procedures. Finally, a total of 17 known anticonvulsant compounds with experimental Ki and two molecular descriptors were found to be involved in the model building using multiple-linear-regression (MLR) method. QSAR model robustness and prediction quality were represented by high regression coefficient (R^2) value. Cross-validation of QSAR model was done by LOO (Leave-one-out) approach. The applicability domain of derived QSAR model was indicated by cross-validation regression coefficient (R^2_{CV}). Evaluation of model was also performed through residual plot.

Evaluation of Pharmacokinetic Behaviour through Lipinski's Rule of Five and ADME Parameters

Potential leads may fail to clear the clinical trial approval through FDA due to unmatched standard pharmacokinetic properties. The key pharmacokinetic properties were represented by 'admetSAR' as used by Drug Bank database[24]. Besides this, Lipinski's rules of five[25] along with other physicochemical properties were used to explain the pharmacokinetic behaviour of compounds. TPSA and MW (cutoff= ≤500) were used to evaluate the fractional absorption of compounds. Bioavailability of compounds were evaluated by topological PSA (polar surface area) (cutoff= ≤140 $Å^2$). These descriptors also represent the passive membrane transport. For estimation of fractional absorption, sum of H-bond donors and acceptors was used. Additionally, number of rotatable bonds also used as a measure of bioavailability. The pharmacokinetic behaviour of drug distribution depends on membrane permeability (estimated by Caco-2 cell line), blood-brain barrier and distribution (volume). Excretion ability of compounds from the body is evaluated on the basis of

logP (octanol/water) and molecular weight. Renal clearance is indicated by negative lipophilicity of molecule. Metabolism of compounds in liver was evaluated on the basis of logP value (hydrophobic condition) and topological polar surface area of molecules. Lipophilicity of molecule also provided indications about absorption and metabolic process. Majority of oral bio-available drugs (90%) follow the Lipinski's rule of five; therefore the designed molecules were also studied for oral bioavailability of active anticonvulsant drugs through rule of five. These chemical properties for drug-likeness were calculated for quinazolinone derivatives and further evaluated for compliance with a standard drug.

Pharmacophore Distance Map

Structure-based pharmacophore distance map was prepared for quinazolinone derivatives, where were found to be mild active against glutamate receptor and GABA respectively. Distance map was prepared on the basis of six properties from the designed molecules. The properties were as: hydrogen bond acceptor, hydrogen bond donor, hydrophobic, Negative ionizable component, Positive ionizable and ring aromatic. The maps were drawn through manual process by measuring the average inter-component distance from the designed molecules. The designed glutamate receptor binders showed only five-property based pharmacophore, as shown in following figure 6.

RESULTS AND DISCUSSIONS

Preliminary quantitative structure-activity relationship (SAR) studies revealed high structure-activity relationship *(i.e.* pharmacophore) features for anticonvulsant activity. Based on features identified from pharmacophore, molecules were designed on the nucleus of quinazolinone. Pharmacophore features were also used for data collection for QSAR model building using multiple linear regression (MLR) method. Since the QSAR approach is a well established as lead optimization method, therefore the designed molecules were screened through QSAR model to predict the Ki value of new anticonvulsant compounds derivatives, therefore indicating the activity range. The binding affinity of known anticonvulsant target glutamate receptor was studied through docking simulation so that to identify the possible binding site and to explain the drug-target activity relationship by using the crystallographic complex structure of glutamate receptor. Finally the designed molecules were processed for ADMET screening for estimate the pharmacological behaviour of designed molecules. Results of pharmacophore, QSAR based Ki prediction, docking, and ADMET screening were analysed to receive conclusive information to predict the possibilities about designed molecules for anticonvulsant activity.

Pharmacophore Profiling for Anticonvulsant Activity through Glutamate Receptor Inhibition

The known quantitative structure-activity-relationship studies revealed possible pharmacophore features for anticonvulsant activity. The designed compounds possess the pharmacophore essential for anticonvulsant activity. The pharmacophore proposed contains: (i) hydrophobic domain (HPD) of newer anticonvulsants considering the most potent of semicarbazones as anticonvulsant recently, it was planned to prepare new semicarbazones with quinazoline scaffold; (ii) hydrogen bonding donors (HBD) (iii) two electron donor system (D); and (iv) distal aryl ring which affects pharmacokinetics (PKS). All these elements are present in other clinically effective drugs or their metabolites.

To understand the pharmacophore behavior of glutamate inhibitor for anticonvulsant activity, model building molecules were observed. Observations were based on the sub-structural relationships considering the electron flow. It was found that the following pharmacophore components are available for anticonvulsant activity through inhibition of glutamate receptor: hetero-non-aromatic 5-point ring, an amine group, a hydroxyl group, imine group, carboxylic acid

group, and benzene ring. Our designed molecules did not contain these pharmacophore components. This observation also represents the comparatively mild potential of designed molecules in comparison of existing inhibitors of glutamate receptor.

Design of New Chemical Entities (NCEs) Containing Quinazolinone Nucleus

The findings of pharmacophore studies provided the overall substitution pattern (electrostatic, steric and hydrophobic pattern) required around the quinazolinone nucleus. Hypotheses shown in literature were also considered for optimization of quinazolinone derivatives as shown in figure 3. Pharmacophore features signified the importance of quinazolinone nucleus for the anticonvulsant activity of compounds. This information had helped a lot in optimizing quinazolinone pharmacophore and designing of NCEs containing quinazolinone ring for potent anticonvulsant activity. Substitution pattern around quinazolinone pharmacophore showed in Figure 2 was used for the manual design of NCEs. Designed compounds were passed through Lipinski's screen to ensure drug like the pharmacokinetic profile of the designed compounds in order to improve their bioavailability. The parameters used as Lipinski's filters are: Number of hydrogen bond acceptor (A) (<10), number of hydrogen bond donor (B) (<5), number of rotatable bond (R) (<10), Clog P (X) (<5), molecular weight (W) (<500 g/ mol) and polar surface area (S) is (<140 Å).

We had designed twenty-one compounds containing quinazolinone nucleus with substitution pattern shown in figure 4. All these compounds were subjected for further studies to sort out the compounds with good binding affinity for glutamate receptor and having good ADME properties.

Molecular Docking Studies on Glutamate Receptor

To understand the binding behavior of studied compounds named '5 to 25' against glutamate receptor-based anticonvulsant activity were processed for docking studies. The docking studies suggested that designed compounds inhibit the glutamate receptor activity by high binding affinity in comparison to control. Later, orientations and binding affinity of tested derivatives were explored. The binding affinity allowed the derivatives to be compared with standard control 'Kainate'.

Docking Studies

Molecular docking tool, AutoDock-Vina software was used for studying binding modes of the designed compounds into the binding pocket of glutamate receptor. AutoDock-Vina was found to produce the least number of inaccurate poses and results near to native co-crystallized structures [26.] These studies helped to sort out the designed compounds with good binding affinity against glutamate receptor. The docking score in terms of Kcal/mol and other results of docking studies of designed compounds of quinazolinone series are presented in Table 1.

Binding Affinity

Binding affinity is shown in a negative value, which indicates the measure of the stability of ligand-Protein interaction. The binding affinity of the standard compound Kainate (PDB: 1GR2) was found to be -8.2 kcal/mol. The binding affinity of the designed NCEs "10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 5, 6, 7, 8, 9" was found to be -8.5, -9.7, -8.6, -8.4, -9.4, -9, -8.4, -8.3, -9.1, -8.3, -8.7, -9.2, -8.8, -8.7, -8.6, -9.3, -8.9, -9.1, -9.4, -9.1, and -9 kcal/mol respectively. The close analysis of these results suggests that the designed NCEs have a comparable binding affinity with the standard compound. Overall interacting residues can be visualized from Table-1.

Contacts

Docking studies were analyzed in reference to known target (Glutamate receptor) binding ligand control IRG2. Literature based information shows that binding pocket of control ligand at target bears tyrosine, glutamic acid, proline, threonine, arginine, and serine residues majority of hydrophobic nature. Control ligand binds with a binding affinity of -8.1 kcal/mol. In reference of control ligand, query compound shared common residues tyrosine, glutamic acid, proline, threonine, arginine, and serine. Out of the tested ligand compounds, "10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 5, 6, 7, 8, 9" ligands were found to be possibly good interactivity with target Glutamate receptor.

QSAR Model Based Prediction of Glutamate Targeted Anticonvulsant Activity

QSAR studies

All QSAR studies were performed in Weka software. A series of 22 compounds of quinazolinone derivatives tested for their anticonvulsant activity was selected for QSAR Studies. 17 Compounds were used for model building. The model was cross evaluated with Leave-One-Out-Cross validation (LOOCV) method. Selection of molecules in the training set and their cross validation is a key and important feature of any QSAR model. Therefore the care was taken in such a way that biological activities of all compounds validation result must *lie* within the maximum and minimum value range of biological activities of the training set of compounds. A Uni-column statistics for the training set and the validated result were generated to check the correctness of selection criteria for training set molecules. The maximum and minimum value in training and set were compared in a way that:

- The maximum value of LN Ki (nM) of query compound should be less than or equal to the maximum value of LN Ki (nM) of the training set.
- The minimum value of LN Ki (nM) of query compound should be higher than or equal to the minimum value of LN Ki (nM) of the training set.

This observation showed that validation was interpolative and derived within the minimum–maximum range of training set. The mean and standard deviation of LN Ki (nM) values of sets of training and test provide insights to the relative difference of mean and point density distribution of two sets. Several 2D QSAR models were generated for a training set of 17 compounds using MLR method. The best QSAR model was selected on the basis of the value of statistical parameters like R^2 (square of the correlation coefficient for a training set of compounds), and R^2_{cv} (LOO cross-validated R^2). The QSAR model was validated through LOOCV method. Statistical results generated by 2D QSAR analysis showed that QSAR model has good cross-validation predictability. Prior studies of anticonvulsant lead identification and optimization showed an important part of QSAR application in drug discovery. Activity was predicted on the basis of derived QSAR model for newly designed quinazolinone derivatives. The QSAR model development accuracy was represented by R^2 (= 0.924) (*i.e.,* 92.4%) and activity prediction accuracy denoted by R^2_{CV} (= 0.889) (*i.e.,* 88.9%) (Table 2, 3) (Figure 7, 8). Two chemical descriptors namely, ATSc4 and VCH.7 well allied with experimental anticonvulsant activity. Derived QSAR model equation as:

Predicted LN Ki (nM) = 6.7103 * D2 + 9.2198 * D3 + 5.4257

(**Here D2:** ATSc4, D3: VCH.7; these are molecular descriptors calculated from PaDEL-Descriptor software)

[R^2 = 0.924 and R^2_{CV} = 0.889]

Where, R^2= regression coefficient and R^2_{CV}= cross-validation regression coefficient. QSAR results suggest that compounds '8, 15 and 22' possess good potency for anticonvulsant activity.

Compliance with Pharmacokinetics Properties and Toxicity Estimation (ADME/T)

Prediction of the ADME parameters prior to the experimental studies is one of the most important aspects of the drug discovery and development of the drug molecule. The drug may fail to reach the market phase if those properties are not fulfilled by the drug candidate. Taking into consideration the above-mentioned aspects, the ADME profile of the designed NCEs was studied using the tool admeSAR. In addition to predicting molecular properties, provides the ranges for comparing the properties of the molecules with those of majority of known drugs. The range of values that cause a molecule to be flagged can be similar or dissimilar to other known drugs. Lipinski's rule of five and adme-SAR physical descriptors and pharmaceutically relevant properties of quinazolinone analogs were analyzed, among which significant descriptors were reported here required for predicting the drug-like properties of molecules. These properties were (Table 4 and also in supplementary table S1)

Glutamate receptor's control inhibitors were observed to bear the properties of crossing the Blood-brain-Barrier (BBB), being intestinal absorbable, easy accessibility to the cells, the non-inhibitor substrate of plasma proteins, being safe from being metabolized through CYPs and don't have carcinogenic properties. Results revealed that quinazolinone derivative (5 to 25) followed the screening through Lipinski's rule of five for oral bioavailability, while 18 and 25 showed an acceptable violation of 1 and 2. Here, the hydrophilicity of studied compounds was measured by logP value. Rule of five screening results indicate mild hydrophilicity of quinazolinone derivatives and so there is the good average possibility of absorption or membrane permeability due to their logP values less than 5 (Table 4). LogP also associated with blood–brain barrier used to calculate the membrane permeability. The derivatives showed less efficiency of membrane permeability than control compound. The low aqueous solubility of derivatives may significantly affect its absorption and distribution. Higher doses may be required for bioavailability. All derivatives showed higher lipo affinity than control compound. Intestinal permeability has been found to be comparatively lower than control compound. Derivatives 18 and 25 also have a molecular weight >500.

Toxicity Indicated by Quinazolinone Derivatives at High Doses/Long Term use

If administered in high doses or used therapeutically in long term, the toxicity risk assessment screening results indicated (Table 5). One noticeable component is that no any derivative showed carcinogenicity. It indicates the safe trials of these compounds for further lead optimization. The prior studies related to cases of accumulation and its toxicity also supported the predicted results. Still, there is a scope for further lead optimization based on these calculated ADMET parameters.

Complete Molecule evaluation Profile

To evaluate the performance of compounds, four basic strategies: pharmacophore mapping, QSAR, docking & ADMET screening, were implemented. Although predicted Ki was showed mild activity against glutamate receptor, but conclusively, 22, 15 & 8 were observed to be most feasible to act against glutamate receptor in table 5.

CONCLUSIONS

We report here the establishment of 2D-QSAR model and docking study on a series of quinazolinone

derivatives as glutamate inhibitors. The performance of quinazolinone derivatives for anticonvulsant activity against glutamate receptor, four basic strategies: pharmacophore mapping, QSAR, docking & ADMET screening were implemented. Although predicted Ki was showed mild activity against glutamate receptor, but conclusively, compounds 22, 15 & 8 were observed to be most feasible to act against glutamate receptor. The correlation of the results obtained from docking and QSAR studies lead to better understanding of the structural requirements for enhanced activity. The obtained results can be used as a guideline to design and predict new potent glutamate inhibitors, which could be an effective way to find novel leads for the development of the anticonvulsant drug.

CONFLICT OF INTEREST

The authors report no conflict of interest.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Late Prof. S.N. Pandeya for the research guidance he has provided during this project. One of the authors is thankful to Dr. Om Prakash IIIT, Allahabad, for providing necessary facilities and support to complete this research work.

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APPENDICE-1

List of Figures

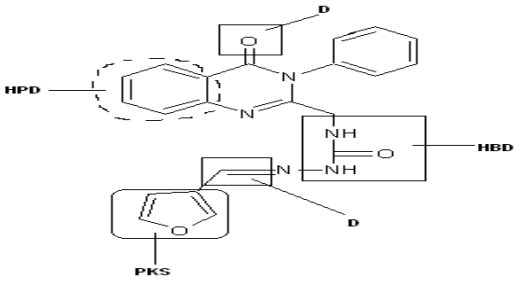
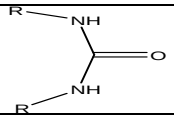
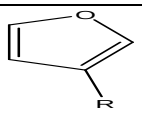
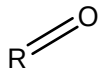


Figure 1: Example Showing Pharmacophore Components at Quinazolinone Derivative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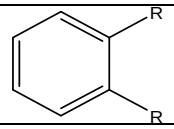
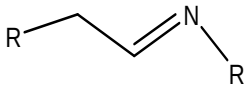
		
HPD	**D**	

Figure 2: Pharmacophore Components used for Designing of Quinazoline Derivatives

Pharmacophore components in quinazolinone derivatiove are as: HBD (Hydrogen Bonding Domain), PKS (A diastral aryl ring which affects pharmacokinetics), HPD (Hydrophobic domain), D (Electron donor system)

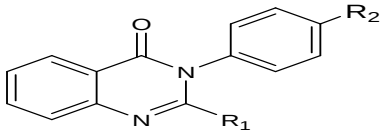
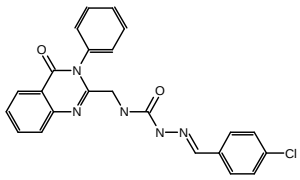
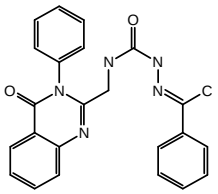
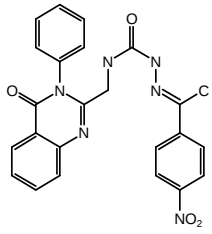
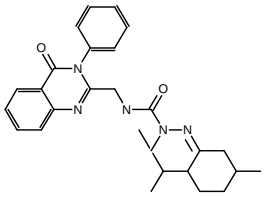
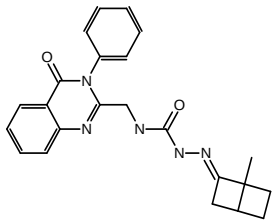
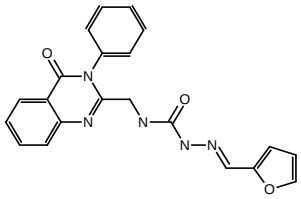
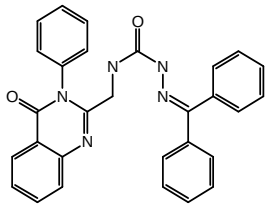
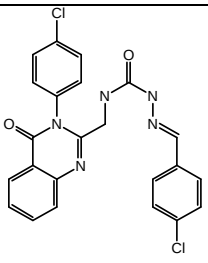
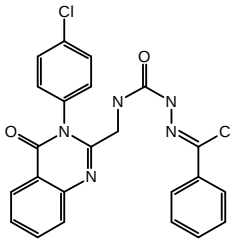
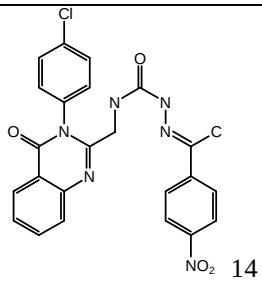
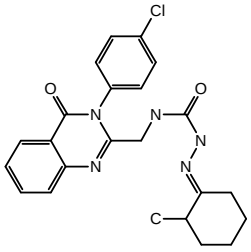
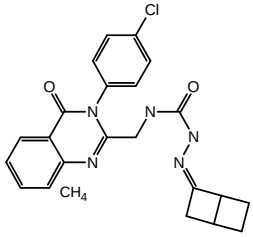
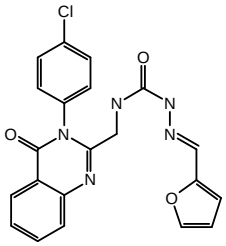
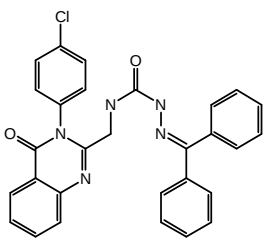
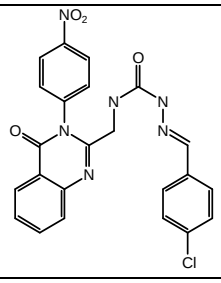
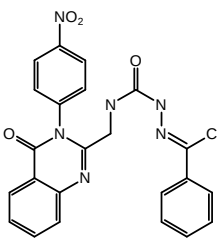


Figure 3: Scaffold used in Designing of Quinazolinone Derivatives

 5	 6	 7	 8
 9	 10	 11	 12
 13	 14	 15	 16
 17	 18	 19	 20

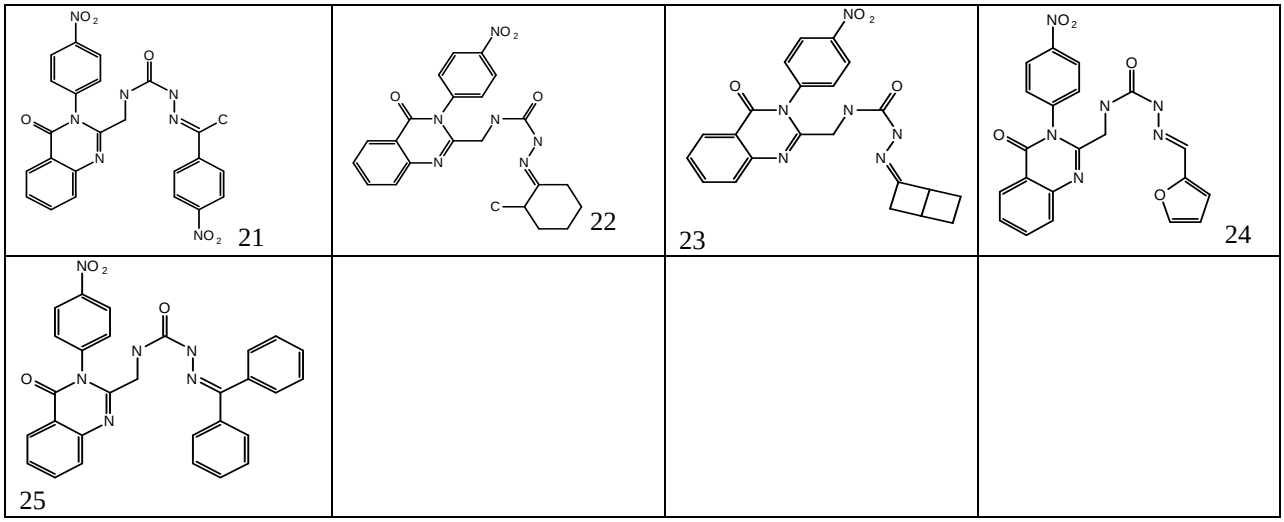


Figure 4: Designed Quinazolinone Derivative

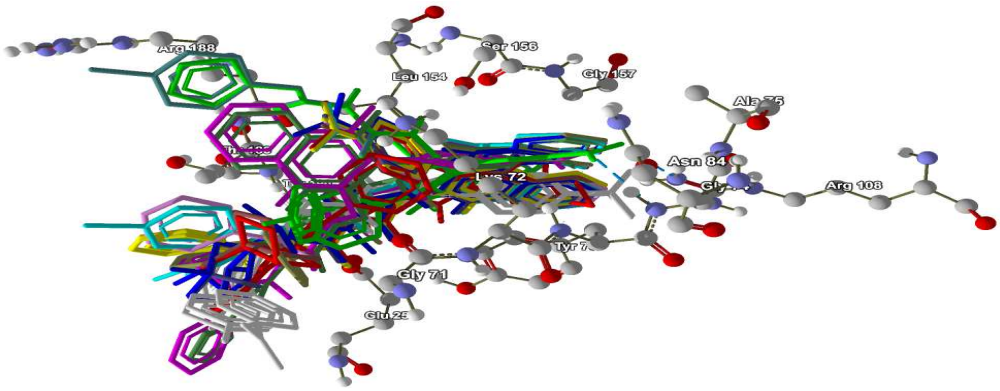


Figure 5 A: Residual Interaction of Glutamate Receptor with '5 to 25' & Docking Results of Quinazolinone Derivatives against Glutamate Recepto

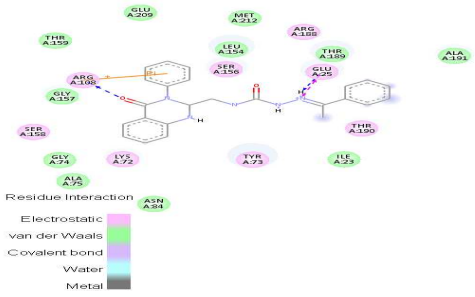


Figure 5.A: 5 at Glutamate Receptor PDB: 1GR2

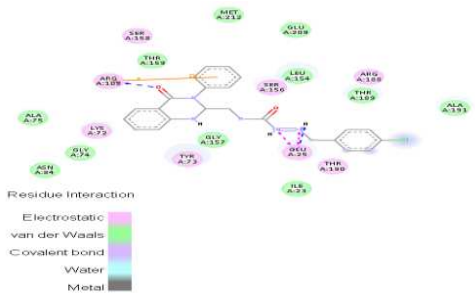


Figure 5.B: 6 at Glutamate receptor (PDB: 1GR2)

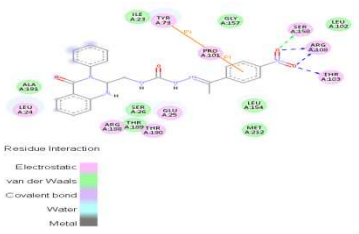


Figure 5.C: 7 at Glutamate Receptor (PDB: 1GR2

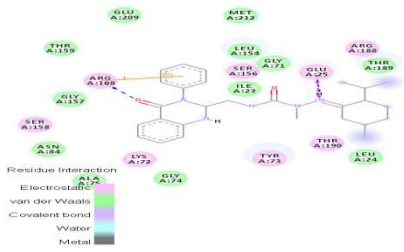


Figure 5.D: 8 at Glutamate Receptor (PDB: 1GR2)

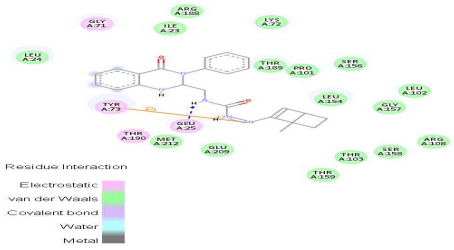


Figure 5.E: 9 at Glutamate Receptor (PDB: 1GR2)

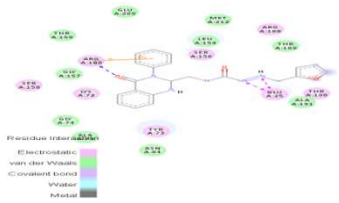


Figure 5.F: 10 at Glutamate Receptor (PDB: 1GR2)

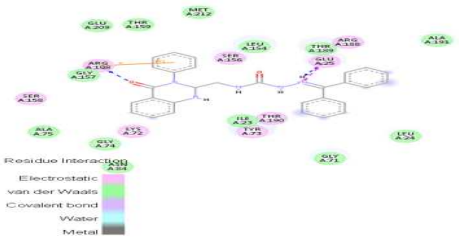


Figure 5.G: 11 at Glutamate Receptor (PDB: 1GR2)

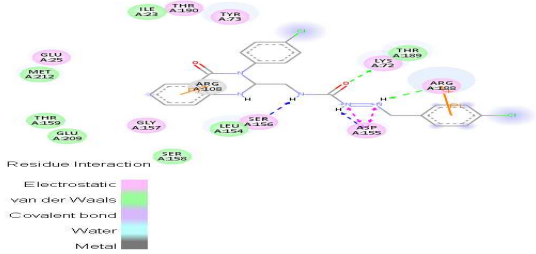


Figure 5.H: 12 at Glutamate Receptor (PDB: 1GR2)

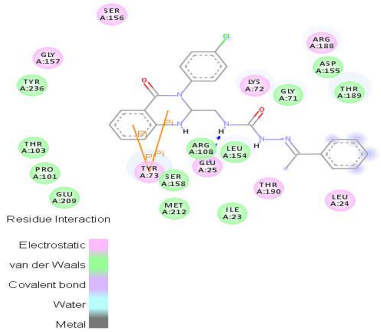


Figure 5.I: 13 at Glutamate Receptor (PDB: 1GR2)

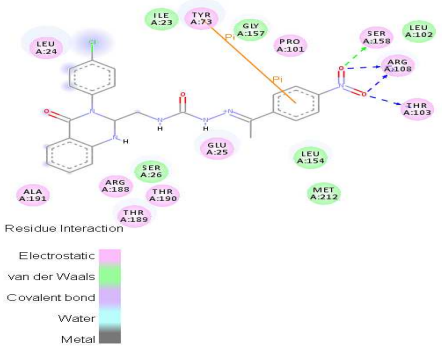


Figure 5.J: 14 at Glutamate Receptor (PDB: 1GR2)

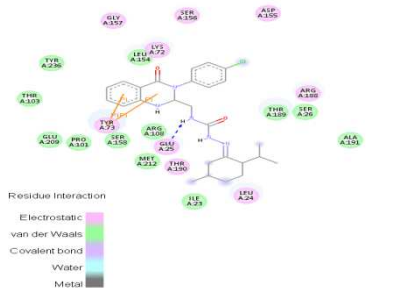


Figure 5.K: 15 at Glutamate Receptor (PDB: 1GR2)

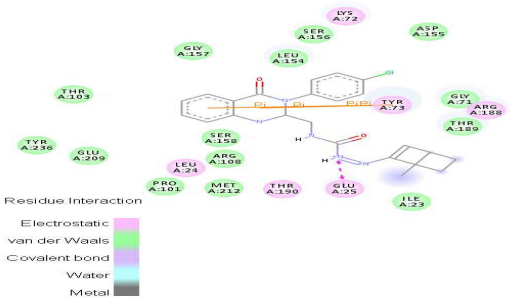


Figure 5.L: 16 at Glutamate Receptor (PDB: 1GR2)

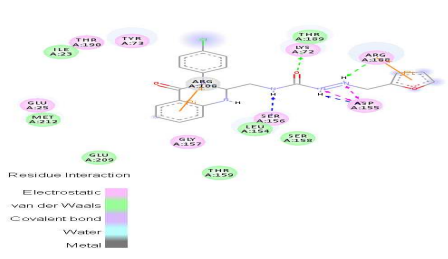


Figure 5.M: 17 at Glutamate Receptor (PDB: 1GR2)

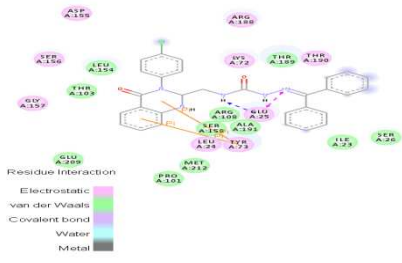


Figure 5.N: 18 at Glutamate Receptor (PDB: 1GR2)

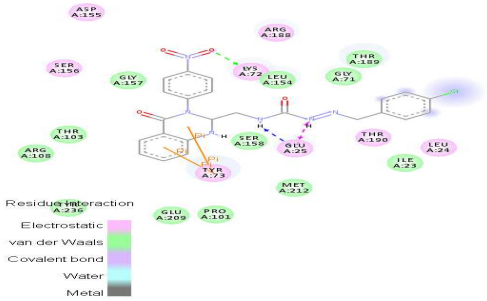


Figure 5.O: 19 at Glutamate Receptor (PDB: 1GR2)

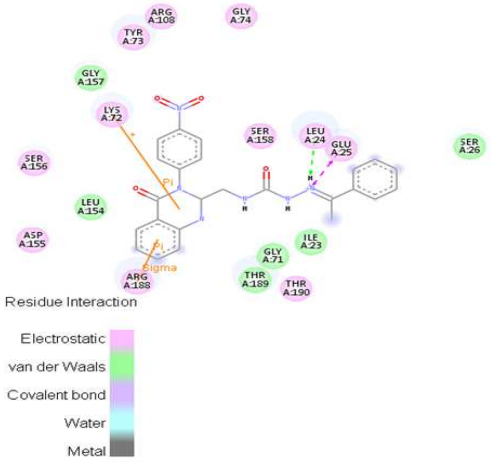


Figure 5.P: 20 at Glutamate Receptor (PDB: 1GR2)

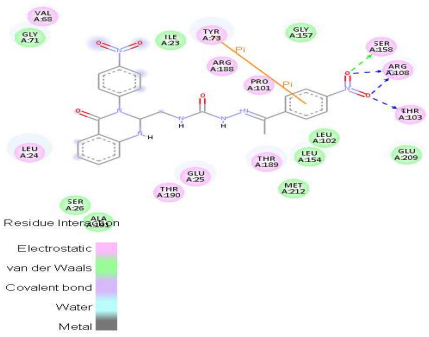


Figure 5.Q: 21 at Glutamate Receptor (PDB: 1GR2)

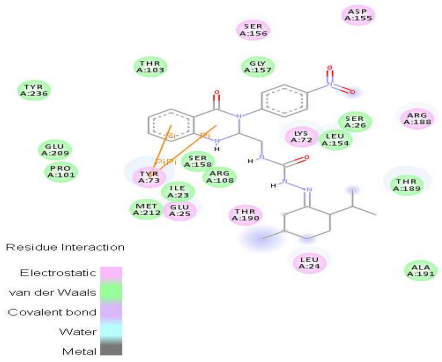


Figure 5.R: 22 at Glutamate Receptor (PDB: 1GR2)

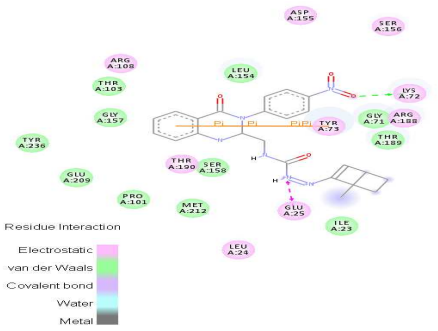


Figure 5.S: 23 at Glutamate Receptor (PDB: 1GR2)

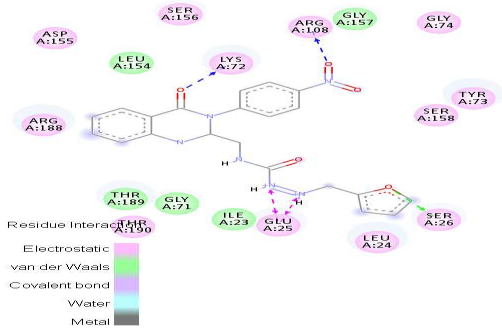


Figure 5.T: 24 at Glutamate Receptor (PDB: 1GR2)

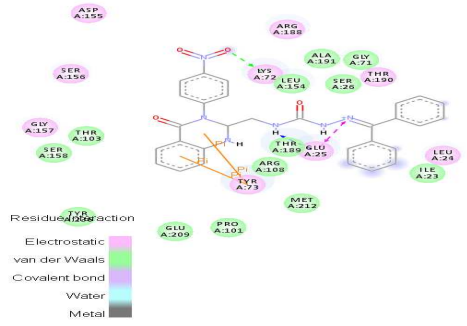


Figure 5.U: 25 at Glutamate Receptor (PDB: 1GR2)

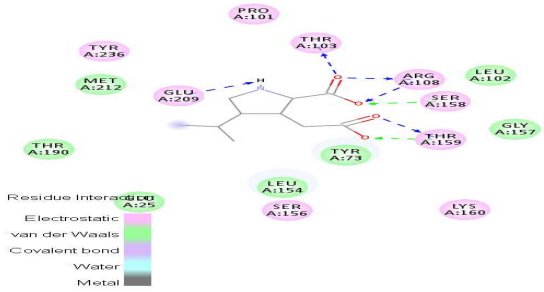


Figure 5.V: Kainate at Glutamate Receptor (PDB: 1GR2)

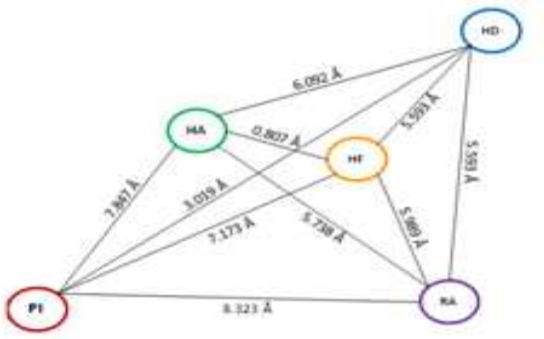


Figure 6: Five Property Based Pharmacophore Distance Map of Series-1 Designed Molecules Against Glutamate Receptor. Here the Components are as: Hydrogen Bond Acceptor (HA), Hydrogen Bond Donor (HD), Hydrophobic (HF), Positive Ionisable (PI) and Ring Aromatic (RA) Components. The inter-Component Distance has been Providing in Å.

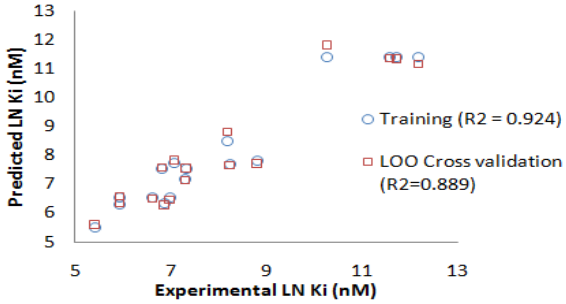


Figure 7: Regression Plots Showing Model Training as Well as Cross-Evaluation Results through Leave-One-Out Cross Validation

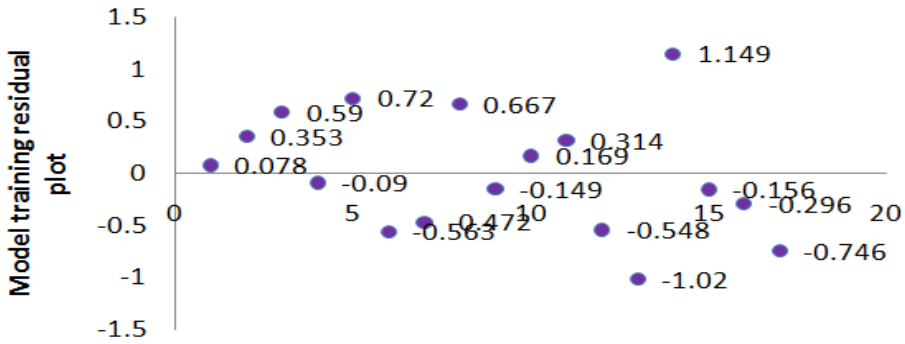


Figure 8: Residual Plot of Model Representing Error Distribution of QSAR Model

Table 1: Docking Results and Evaluation of Query Molecules in Reference of Known Glutamate Receptor Binder at PDB: 1GR2

		Binding Affinity (kcal/mol)	Interacting Residues	Comparison with Control
Control	Kainate (PDB: 1GR2 bound)	-8.1	Tyrosine, Glutamic Acid, Proline, Threonine, Arginine, And Serine	
Query	10	-8.5	Arginine, Glutamic Acid	Stronger
Query	11	-9.7	Arginine, Glutamic Acid	Stronger
Query	12	-8.6	Arginine, Serine	Stronger
Query	13	-8.4	Tyrosine (Pi-Pi Interaction), Arginine	Stronger
Query	14	-9.4	Tyrosine (Pi-Pi Interaction), Srginine, Serine, Threonine	Stronger
Query	15	-9	Tyrosine (Pi-Pi Intercation), Glutamic Aicd	Stronger
Query	16	-8.4	Tyrosine, Glutamic Acid	Stronger
Query	17	-8.3	Arginine, Serine	Stronger
Query	18	-9.1	Tyrosine (Pi-Pi Intercation), Glutamic Acid	Stronger
Query	19	-8.3	Tyrosine (Pi-Pi Intercation), Glutamic Acid	Stronger
Query	20	-8.7	Arginine (Sigma-Pi Bond), Glutamic Acid	Stronger
Query	21	-9.2	Tyrosine (Pi-Bond), Serine, Arginine, Threonine	Stronger
Query	22	-8.8	Tyrosine (Pi-Bond)	Stronger
Query	23	-8.7	Tyrosine (Pi-Bond), Glutamic Acid	Stronger
Query	24	-8.6	Arginine, Glutamic Acid, Serine	Stronger
Query	25	-9.3	Tyrosine, Threonine, Glutamic Acid	Stronger
Query	5	-8.9	Arginine, Glutamic Acid	Stronger
Query	6	-9.1	Arginine, Glutamic Acid	Stronger
Query	7	-9.4	Tyrosine (Pi-Pi Interaction), Serine, Arginine, Threonine	Stronger
Query	8	-9.1	Arginine, Glutamic Acid	Stronger
Query	9	-9	Threonine, Glutamic Acid	Stronger

Table 1.1: Residual Interaction with '5 to 25'

Interacting Residue	ID	Residual Energy (Kcal/Mol)
ALA	75	-2.3718
ARG	108	-3.71365
ARG	188	-4.52691
ASN	84	-1.20303
ASP	70	-0.45587
ASP	155	-0.40623
GLU	25	-6.10361
GLY	71	-6.37509
GLY	74	-6.32285
GLY	157	-5.48117
ILE	23	-1.61984
LEU	24	-0.58823
LEU	154	-7.82805
LYS	72	-14.0569
MET	212	-0.53573
SER	156	-11.4711
SER	158	-0.58833

Table 1.1: Contd.,		
THR	189	-12.3745
THR	190	-7.53074
TYR	73	-25.9379

Table 2: Model Development & LOO Cross Validation

Dataset	CHEMBL ID	D2	D3	Experimental LN_Ki (nM)	Training predicted	Training Error	CV LOO Predicted	CV LOO Error
Train	106579	-0.10068	0.083036	5.438079	5.516	0.078	5.578	0.14
Train	110915	0.015835	0.082566	5.940171	6.293	0.353	6.596	0.656
Train	317184	0.044173	0.087679	5.940171	6.531	0.59	6.347	0.407
Train	110866	0.044173	0.087679	6.620073	6.531	-0.09	6.521	-0.099
Train	286782	0.09555	0.160209	6.824374	7.544	0.72	7.596	0.772
Train	107990	0.044605	0.064998	6.887553	6.324	-0.563	6.256	-0.632
Train	323142	0.044186	0.087679	7.003065	6.531	-0.472	6.478	-0.525
Train	280179	0.124835	0.160209	7.07327	7.74	0.667	7.804	0.731
Train	110618	0.138566	0.087679	7.31322	7.164	-0.149	7.142	-0.171
Train	415462	0.092181	0.160209	7.352441	7.521	0.169	7.533	0.181
Train	432781	0.274668	0.133869	8.188689	8.503	0.314	8.816	0.628
Train	25875	0.11994	0.160209	8.255828	7.708	-0.548	7.658	-0.597
Train	23255	0.134464	0.160209	8.824678	7.805	-1.02	7.695	-1.13
Train	2115153	0.088826	0.585974	10.27505	11.424	1.149	11.8	1.525
Train	2114116	0.088826	0.585974	11.58058	11.424	-0.156	11.373	-0.207
Train	2115156	0.088826	0.585974	11.71994	11.424	-0.296	11.328	-0.392
Train	2115151	0.088826	0.585974	12.17045	11.424	-0.746	11.18	-0.99

Table 3: Query Prediction through QSAR Model

Data Set	ID	D2	D3	Model Predicted LN Ki (nM)	Z-Score	Calculated Ki (µM)
Query	Kainate (Exp. Ki: 477 to 12221 nM) PDB:1GR2_Glutamate_Control	0.168399	0.200681	8.406	0.152137	4.473829
Query	9	0.09199	0.134052	7.279	-0.37837	1.449538
Query	22	0.156003	0.200681	8.323	0.113067	4.117494
Query	19	0.06611	0.200681	7.72	-0.17078	2.25296
Query	20	0.071504	0.200681	7.756	-0.15383	2.335544
Query	21	0.070689	0.200681	7.75	-0.15666	2.321572
Query	7	0.083085	0.200681	7.833	-0.11759	2.522485
Query	5	0.078506	0.200681	7.803	-0.13171	2.447935
Query	18	0.07172	0.200681	7.757	-0.15336	2.33788
Query	6	-0.00301	0.200681	7.256	-0.3892	1.416579
Query	25	0.084116	0.200681	7.84	-0.11429	2.540205
Query	23	0.071059	0.200681	7.753	-0.15525	2.328548
Query	13	0.07881	0.200681	7.805	-0.13077	2.452836
Query	14	0.077996	0.200681	7.799	-0.13359	2.438163
Query	15	0.16331	0.200681	8.372	0.136132	4.324276
Query	12	0.073417	0.200681	7.769	-0.14771	2.366104
Query	17	0.093333	0.200681	7.902	-0.08511	2.702682
Query	11	0.079027	0.200681	7.806	-0.1303	2.45529
Query	24	0.105728	0.200681	7.985	-0.04604	2.936577
Query	16	0.078366	0.200681	7.802	-0.13218	2.445488
Query	10	0.100639	0.200681	7.951	-0.06204	2.838412
Query	8	0.164106	0.203203	8.4	0.149313	4.447067

Table 4: Lipinski's Rule of Five and Other Parameters for ADME Property Analysis of Molecules

Name	Lipoaffinity Index	No. of HBA	No. of HBD	LogP	No. of Rotatable bonds	Lipinski Failures	Polar Surface Area	Molecular Weight
Kainate	1.484339	5	1	2.01	4	0	46.17	212.0923
10	7.171926	7	2	2.89	4	0	62.88	387.1331
11	11.25937	7	2	3.88	5	0	49.74	473.1852
12	8.866538	7	2	3	4	0	49.74	465.0759
13	9.062381	7	2	3.22	4	0	49.74	445.1306
14	7.70286	7	2	2.89	5	0	95.56	490.1156
15	5.079652	7	2	3.44	4	0	49.74	470.1384
16	6.531924	7	2	3.11	3	0	49.74	432.1227
17	7.257386	7	2	2.78	4	0	62.88	421.0942
18	11.34594	7	2	3.77	5	2	49.74	507.1462
19	7.355385	7	2	2.78	5	0	95.56	476.1
20	7.562317	7	2	3	5	0	95.56	456.1546
21	6.215547	7	2	2.67	6	0	141.38	501.1397
22	3.545965	7	2	3.22	5	0	95.56	481.1624
23	5.019499	7	2	2.89	4	0	95.56	443.1468
24	5.777762	7	2	2.56	5	0	108.7	432.1182
25	9.802674	7	2	3.55	6	2	95.56	518.1703
5	8.778759	7	2	3.11	4	0	49.74	431.1149
6	8.975218	7	2	3.33	4	0	49.74	411.1695
7	7.623879	7	2	3	5	0	95.56	456.1546
8	4.661845	7	1	3.66	4	0	61.42	447.1695
9	6.448959	7	2	3.22	3	0	49.74	398.1617

Table 5: Complete Molecule Evaluation Profile

Compounds	Pharmacophore	QSAR	Docking	ADMET							Final Remark
	Matched Features with Control	Positive Z-Score Considered	Binding Affinity More than Control	BBB+	HIA+	Caco+	pp-Substrate	pp-Non-Inhibitor	no-easy metabolism	No-Carcinogenicity	
Control Kainate (PDB: 1GR2)	5	1	1	1	1	1	1	1	1	1	14
5	2	0	1	1	1	1	1	1	1	1	10
6	2	0	1	1	1	1	0	1	1	1	9
7	2	0	1	1	1	1	0	1	1	1	9
8	**2**	**1**	**1**	1	1	1	0	0	1	1	**9**
9	2	0	1	1	1	1	0	0	1	1	8
10	**3**	0	**1**	1	1	1	1	1	1	1	**11**
11	2	0	1	1	1	1	1	1	1	1	10
12	2	0	1	1	1	1	1	1	1	1	10
13	2	0	1	1	1	1	0	1	1	1	9
14	2	0	1	1	1	1	0	1	1	1	9
15	**2**	**1**	**1**	1	1	1	0	0	1	1	**9**
16	2	0	1	1	1	1	0	0	1	1	8
17	3	0	1	1	1	1	1	1	1	1	11
18	2	0	1	1	1	1	1	1	1	1	10
19	2	0	1	1	1	1	0	1	1	1	9
20	2	0	1	1	1	1	0	1	1	1	9
21	2	0	1	1	1	1	0	1	1	1	9
22	**2**	**1**	**1**	1	1	1	0	1	1	1	**10**
23	**2**	0	**1**	1	1	1	1	1	1	1	**10**
24	**3**	0	**1**	1	1	1	0	1	1	1	**10**
25	2	0	1	1	1	1	0	1	1	1	9