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Pharmacophore mapping based virtual screening, molecular docking and ADMET studies of ROCK II inhibitors

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1. Introduction

Multiple Sclerosis (MS) is an “immune mediated disease” and not auto-immune, due to the reason that it is unknown for which antigen immune cells are activated. These activated immune cells attack the myelin layer of nerve cell and form a scar (Sclerosis) (www.nationalmssociety.org). It is seen that people suffering from multiple sclerosis have increased from 2 million to 2.3 million, and yet there is no cure. So there is a need to design potential drug like molecules. Rho kinase has been recently seen to play a role in MS, where its inhibition induces myelin regeneration. Rho kinase belongs to the serine-threonine family and has 160 kDa of molecular weight. With two isoforms namely ROCK-1 and ROCK-2 it has a vital role to play in the physiological function of cell contraction, proliferation and migration (Hirooka et al., 2004). The inhibition of Rho kinase has been proven useful in various medical conditions like multiple sclerosis (Luo et al., 2014; Kjoller and Hall, 1999), inflammatory disorders (LoGrasso and Feng, 2009), myocardial ischemia (Satoh et al., 2007), hypertension (Uehata et al., 1997), erectile dysfunction (Bivalacqua et al., 2004), glaucoma (Waki et al., 2001), cancer migration (Nakajima et al., 2003a, 2003b) and spinal cord injury (Chan et al., 2005). This has led to the discovery of various classes of ROCK inhibitors with basic scaffolds of isoquinolines (Chan et al., 2005), pyridines (Chen et al., 2014), benzodioxane amides (Takami et al., 2004) chromane amides (Feng et al., 2008), azaindoles (Chen et al., 2008), benzimidazoles, benzoxazoles (Schirok et al., 2008a), benzothiazoles (Sessions et al., 2008), indazoles (Yin et al., 2009), aniline-ureas (Feng et al., 2007) and tetrahydroisoquinolines (Yin et al., 2010). With these many molecules only one has been marketed, Fasudil (HA-1077), which structurally is composed of an isoquinoline (Fang et al., 2010) and homopiperazine ring connected by a sulphonyl group (Dong et al., 2010). Rho kinase-2 inhibition, from recent studies has proven to be quite effective in the treatment of multiple sclerosis. There is a need to design and synthesize new compounds with good pharmacokinetic properties and low IC50. To achieve this aim computer aided drug design like pharmacophore modeling and docking were employed. To further optimize designed lead, their ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties were predicted through online available software, and changes were made to make it less toxic (Gramatica, 2007).

2. Materials and computational methods

2.1. Dataset selection

Eight structurally diverse and highly potent molecules were used to generate ligand based Pharmacophore, which are shown in Table 1 (Feng et al., 2008; Chen et al., 2008; Sessions et al., 2008; Yin et al., 2009; Schirok et al., 2008b). The tripos force field function of Sybyl X was used to minimize the energy of molecules. After, energy minimization partial atomic charge was calculated using the Gasteiger Huckel method. Every other parameter for computation of minimization was kept as default.

2.2. The pharmacophore generation

GALAHAD module of Sybyl X was used to generate pharmacophore. GALAHAD uses the genetic algorithm to align molecules and also considers multiple parameters for scoring like pharmacophoric overlap, energetics and steric similarity. It has a mode of keeping conformational flexibility, after which models are generated as hypermolecules. Each hypermolecule contains features from each molecule in training set. The selected model then can be used as a probe for screening different databases. For the generation of the pharmacophore, all the parameters were kept as default except population size, which was entered as 40 and maximum generations was changed to 80. Finally, 20 models were generated considering specificity and Pareto ranking, from which top 3 models are shown in Table 2.

2.3. Validation of pharmacophore model

GH (Güner Henry) scoring method and ROC (Receiver Operating Characteristics) curve analysis are generally used for validation of pharmacophore. They help in determining reliability and accuracy of the pharmacophore model (Patel et al., 2002; Thangapandian et al., 2011).

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Table 1
Training set molecules used to develop pharmacophore hypothesis for ROCK-2 inhibitors.

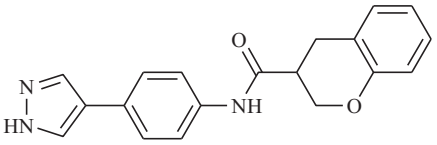
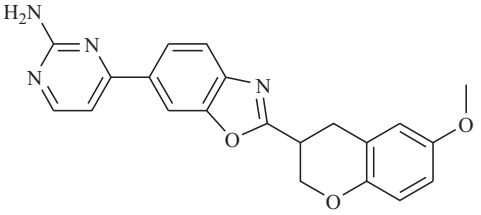
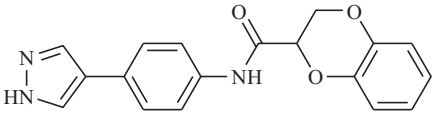
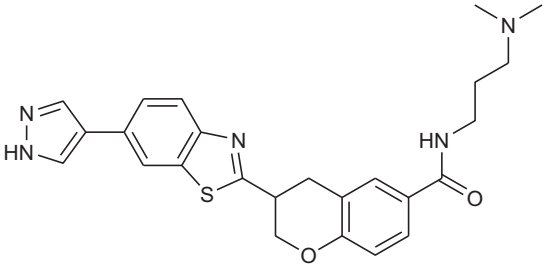
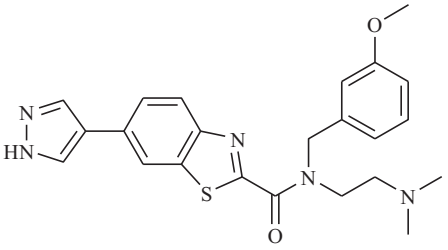
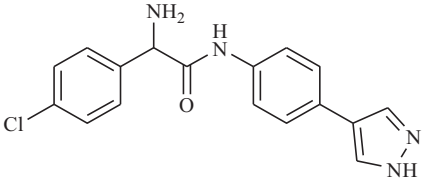
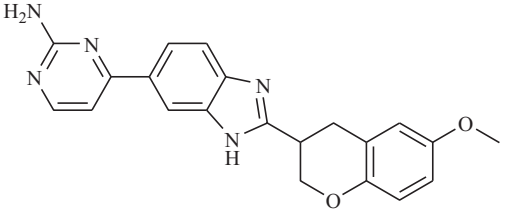
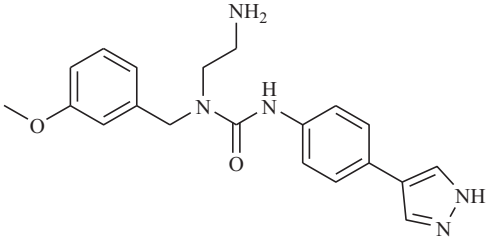
Sr. No.	Structure	Chemical Class	IC_{50} Value
1		Chromane – 3-amide	2 nM
2		Benzoxazole	2 nM
3		Pyrazole	1.5 nM
4		Benzothiazole	0.9 nM
5		Benzothiazole	0.4 nM
6		Tetrahydroisoquinoline	1 nM
7		Benzimidazole	2 nM
8		urea	1 nM

Table 2
Results of the pharmacophore hypothesis generated by GALAHAD.

Models	Specificity[a]	Hits[b]	Features[c]	Pareto Ranking[d]	Energy
Model_03	5.205	7	5	0	1.84
Model_05	4.94	7	5	0	3.56
Model_06	5.030	8	4	0	4.74

[a] Specificity measures the number of false positives.
[b] Hits is the number of molecules that matched during the search.
[c] Molecular framework responsible for a drug's biological activity.
[d] Pareto Ranking shows the hierarchical position of conformer generated.

Table 3
Statistical Parameter for GH Scoring Method.

Sr. No.	Parameter	Value
1	Total molecules in database (**T**)	234
2	Total number of Decoys (**D**)	208
3	Total Number of actives in database (**A**)	26
4	Total Hits (Ht)	30
5	Active Hits (Ha)	24
6	% Yield of actives [(Ha/Ht)*100]	80%
7	% Ratio of actives [(Ha/A)*100]	92.3%
8	Enrichment factor (E) [(Ha*D)/(Ht*A)]	6.4
9	False Negatives [A - Ha]	2
10	False Positives [Ht - Ha]	6
11	Goodness of Hit Score	0.729

In Güner-Henry (GH) Scoring method, 26 diverse actives were taken from various published literature and 208 decoy molecules were downloaded from the DUD database (Patel et al., 2002). In a total, 234 molecule database was screened against top scored Model_03 after generation of conformers. False positive and true positive rates were calculated. For further validation enrichment ratio, % yield and GH score were formulated as shown in Table 3. The value of the GH score should be between 0.7 and 1. If it is 0 then it is considered as a null model and if it is 1 then it is said to be an ideal model.

The same database of 234 molecules was used for ROC analysis and ROC curve was plotted using SPSS (Statistical Package for the Social Sciences) software. From the obtained results of hits, a graph of sensitivity v/s 1-specificity was plotted. The pharmacophore model is only declared as validated if AUC in the graph is > 0.5. This can only be obtained if the model retrieves most of the actives and lesser inactive from the dataset. Sensitivity measures the number of true positives, i.e. the actual number of actives retrieved and specificity measures false positives.

$$Sensitivity = \frac{Numberoftruepositives}{(NumberoftruePositives + Numberoffalsenegatives)}$$

$$Specificity = \frac{Numberoftruenegatives}{(Numberoftruenegatives + Numberoffalsepositives)}$$

2.4. Virtual screening and designing molecules

Potential leads are needed to be discovered to overcome the disadvantages of already available potent molecules and also to design a new class. This is possible by screening a different database over obtained pharmacophore. Virtual screening has proved to be beneficial in scaffold hopping; many new drugs have been synthesized and marketed with the use of this technology. The unity search module of Sybyl X was used to perform the virtual screening. All the parameters were set as default except priority, which was turned up to high. The IBS database was screened against validated pharmacophore and Lipinski's rule of five and other filters like removing counter ions, bad fragments, etc. were applied on obtained hits. Hits were ranked on the basis of Qfit. The Qfit value represents “pharmacophoric match between query and the hit compound” (Fei et al., 2013). Few hits retrieved after virtual screening that scored Qfit value more than 80 were analyzed structurally and they were found to contain benzthiazole moiety.

2.5. Docking

To identify the binding pose of a molecule, docking was performed. The binding pose of a standard was compared with the test molecule, so that essential amino acids can be identified. GOLD and Surflex module of Sybyl X were used to dock compounds and from the score most potential candidates were identified. The kinase domain of Rho kinase-2 was utilized for docking, which was obtained from RCSB (Research Collaboratory for Structural Bioinformatics) protein data bank (PDB id: 2F2U) with a resolution of 2.43 Å. The Surflex-Dock module of Sybyl X gives a consensus score (CSscore) which comprises of polar, entropic, solvation and repulsive terms. In this method, first the protein structure was prepared by removing substructures and extracting the structure of docked ligand (fasudil). The protein structure was energy minimized in the presence of Tripos force field and partial atomic charge of Gasteiger-Huckel. The protomol was generated at the site of protein-ligand interaction. The designed molecules were then docked at the protomol site. The Following is the information obtained after performing docking.

a. **Total score** which is the Consensus score given as -log (*Kd*).
b. **Polar value** defines hydrogen bonding and can be useful when excluding compounds without hydrogen bonding.
c. **Crash value** is the degree of inappropriate penetration by the ligand into the protein and of interpenetration between ligand atoms (self-clash) that are separated by rotatable bonds. Crash scores close to 0 are favourable.

Docking in GOLD gives you the GOLD score (Chowdhury et al., 2011)which comprises of protein-ligand hydrogen bond energy (external H-bond), protein-ligand van der Waals (vdw) energy (external vdw), ligand internal vdw energy (internal vdw), ligand torsional strain energy (internal torsion) and optionally, a fifth component, ligand intra-molecular hydrogen bond energy (internal H-bond), may be added. Docking study was performed stepwise, directed in the GOLD wizard. The hydrogen and water molecules were removed from the uploaded protein structure. Fasudil was extracted as a standard molecule from chain ‘A’ and that area of extraction was defined as the binding site. The GOLD template was selected as Chemscore-kinase and Score was selected as a GOLD score. The run was performed at the slow speed to produce more accurate results.

2.6. In-silico ADMET prediction

In *silico* ADMET prediction can help in knowing absorption, distribution, metabolism, excretion and toxicity pattern of a candidate prior to synthesis. This can save both time and cost for designing of a molecule. It is very helpful in lead optimization, wherein physicochemical properties are required to be improved (Ju et al., 2007).

OSIRIS (http://www.organic-chemistry.org/prog/peo/) and PreADMET (https://preadmet.bmdrc.kr/adme) software were used to predict *In silico* pharmacokinetic properties and toxicities. In an OSIRIS window, molecules were drawn and different toxicities like mutagenicity, tumorigenicity, irritating effect, reproductive effects, as well as parameters like TPSA, drug likeliness, and the drug score of given compounds were predicted. In PreADMET study, molecules were taken in.mol format and their ADMET properties were predicted.

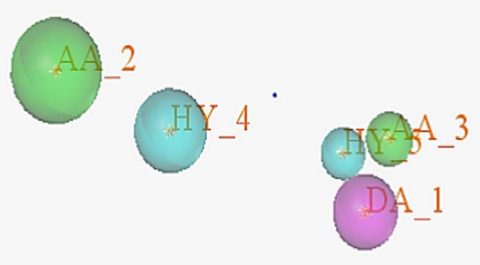


Fig. 1. Three Dimensional Pharmacophore Hypothesis Generated by GALAHAD.

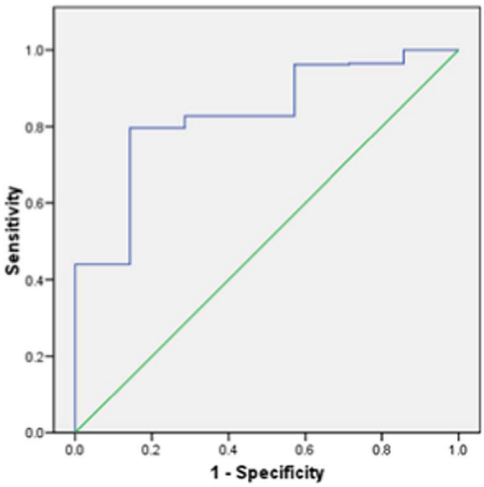


Fig. 2. ROC Curve of the Pharmacophore Model_03.

Table 4
ROC Parameters of Pharmacophore Model.

Sr No.	Parameter	Value
1	False positive rate (**FP**)	.028
2	True Positive rate (**TP**)	.920
3	Precision	0.8
4	Recall	0.920
5	Accuracy	0.982
6	F-measure	0.855
7	Sensitivity	**0.923**
8	Specificity	**0.971**

3. Results and discussion

3.1. Pharmacophore generation

Pharmacophore analysis by GALAHARD resulted into the generation of 20 models. All the models were analyzed for their specificity, the number of hits and features. Model_3 which scored the highest specificity of 5.205 and lowest energy of 1.84 Kcal with 5 features; one donor atom, two acceptor atoms and two hydrophobic features was selected for validation before using it for virtual screening. The result table shows the number of features, hits, specificity, Pareto ranking and energy (Table 2). Specificity indicates how well the features are generated for that specific set of a training set, which in turn helps in decreasing the number of false positives obtained during validation. Specificity should be greater than 5. Pareto Ranking shows a hierarchical position of conformer generated by the genetic algorithm for the generation of hypothesis, '0' shows that it is a child and no further conformers can be generated and '1' depicts a parent, where further modifications can be done. With Pareto ranking 0 and specificity greater than 5, this five featured model 3 can be considered as an optimum model. A three dimensional pharmacophore hypothesis is shown in Fig. 1.

3.2. Validation of the pharmacophore

Guner-Henry score defines how well actives are retrieved from a total dataset which consists of active molecules with inactive decoy molecules (Fei et al., 2013). With the GH score '1', the model can be considered as an ideal model and '0' shows a null model. Practically GH score greater than 0.6 indicates an acceptable model for the virtual screening. The results of Unity search of this dataset against the query model_3 gave 30 hits which were found to map on all five features of the pharmacophore model. Out of 30 hits, 24 molecules were active, while only 6 were inactive decoy molecules. Thus, model 3 was found excellent in retrieving actives from the inactives. All the parameters required for GH score were calculated and is shown in Table 3. Model_03 yielded GH score of 0.729, henceforth it was considered reliable for the virtual screening of chemical databases.

ROC curve (Fig. 2) shows a performance of the pharmacophore model by detecting how well it retrieves true positives. Here, the ROC curve gave AUC (Area under the curve) of 0.857 with a standard error of 0.034, which depicts a fair and significant model. Sensitivity and specificity of pharmacophore model_03 were found to be 0.923 and 0.971 respectively, which shows that the greater number of true positives were retrieved rather than false positives. The value of other parameters calculated is shown in Table 4.

Table 5
Docking score of designed molecules using surflex dock & GOLD suite.

Sr. No.	Compound	IUPAC Nomenclature	GOLD Score	CS score
1	**Fasudil**	5-(1,4-diazepam – 1-ylsulfonyl)isoquinoline	48.621	5.1246
2	**RIK – 1**	N-(benzo[d]thiazol – 2-ylmethyl)benzamide	52.764	5.2064
3	**RIK – 2**	N-(benzo[d]thiazol – 2-ylmethyl) – 3-chlorobenzamide	56.305	5.0138
4	**RIK – 3**	N-(benzo[d]thiazol – 2-ylmethyl) – 3-nitrobenzamide	56.263	5.7635
5	**RIK – 4**	N-(benzo[d]thiazol – 2-ylmethyl) – 3-aminobenzamide	54.274	5.7570
6	**RIK – 5**	N-(benzo[d]thiazol – 2-ylmethyl) – 3-bromobenzamide	57.040	5.1086
7	**RIK – 6**	N-(benzo[d]thiazol – 2-ylmethyl) – 4-chlorobenzamide	58.259	6.5154
8	**RIK – 7**	N-(benzo[d]thiazol – 2-ylmethyl) – 4-nitrobenzamide	57.375	5.5307
9	**RIK – 8**	N-(benzo[d]thiazol – 2-ylmethyl) – 4-aminobenzamide	54.600	4.4181
10	**RIK – 9**	N-(benzo[d]thiazol – 2-ylmethyl) – 4-bromobenzamide	58.313	6.7283
11	**RIK – 11**	N-(benzo[d]thiazol – 2-ylmethyl)pyrazin – 2-amine	49.457	4.6708
12	**RIK – 13**	N-(benzo[d]thiazol – 2-ylmethyl) – 2-(piperazin – 1-yl)ethanamine	57.771	5.0236
13	**RIK – 14**	N-(benzo[d]thiazol – 2-ylmethyl)pyridin – 4-amine	53.910	5.2092
14	**RIK – 15**	N-(benzo[d]thiazol – 2-ylmethyl)thiazol – 2-amine	46.311	4.346
15	**RIK – 16**	N-(benzo[d]thiazol – 2-ylmethyl) – 4-chloropyridin – 2-amine	52.171	5.1964
16	**RIK – 17**	N-(benzo[d]thiazol – 2-ylmethyl) – 5-nitropyridin – 2-amine	51.519	3.2180

Table 6
Docking analysis of designed molecules.

Sr. No.	Compound	Crash Value	Polar	Amino acid interaction
1	**Fasudil**	−1.5430	1.2048	**MET172, ALA119,MET169, ALA231, LEU221, VAL106**
2	**RIK−1**	−0.5364	0.9212	VAL 178, **ALA119,MET169, LEU221, VAL106, ALA231**
3	**RIK−2**	−1.1796	1.5060	ILE98,VAL153,**MET172, ALA119,MET169,LEU221, VAL106**
4	**RIK−3**	−1.1526	0.0105	ILE98, **MET172, ALA119, MET169,LEU221, VAL106**
5	**RIK−4**	−1.3442	0.0125	ILE98,ASP218,VAL153,ASN219, **MET172, ALA119, MET169, LEU221, VAL106**
6	**RIK−5**	−1.1735	1.0123	ILE98,VAL 153, **MET172, ALA119, MET169, LEU221, VAL106**
7	**RIK−6**	−1.6070	1.1034	ILE98,VAL178,VAL 153, **MET172,ALA119,MET169, LEU221, VAL106, ALA231**
8	**RIK−7**	−0.5227	1.0081	ILE98,ARG100,VAL 153, **MET172,ALA119,MET169, LEU221, VAL106, ALA231**
9	**RIK−8**	−0.7281	1.3364	ILE98,TYR171,VAL 153, **MET172,ALA119,MET169, LEU221, VAL106, ALA231**
10	**RIK−9**	−1.2095	1.1584	ILE98, TYR171,VAL 153, **MET172,ALA119,MET169, LEU221, VAL106**
11	**RIK−11**	−1.4569	0.7898	ARG100, ASP231,GLY101, **LYS121, VAL106, ALA231**
12	**RIK−13**	−1.1735	1.0521	ILE98, TYR171,VAL 153, **MET172,ALA119,MET169, LEU221, VAL106**
13	**RIK−14**	−1.3292	0.0100	ILE98,**ALA119, ALA231 LEU221, VAL106**
14	**RIK−15**	−1.2347	0.4639	GLY99,VAL 153, **MET172,ALA119,MET169, LEU221, VAL106**
15	**RIK−16**	−0.6402	0.0525	ILE98,TYR171,VAL 153, **MET172,ALA119,MET169, LEU221, VAL106**
16	**RIK−17**	−1.4835	1.9492	ILE98, TYR171,VAL 153, **MET172,ALA119,MET169, LEU221, VAL106**

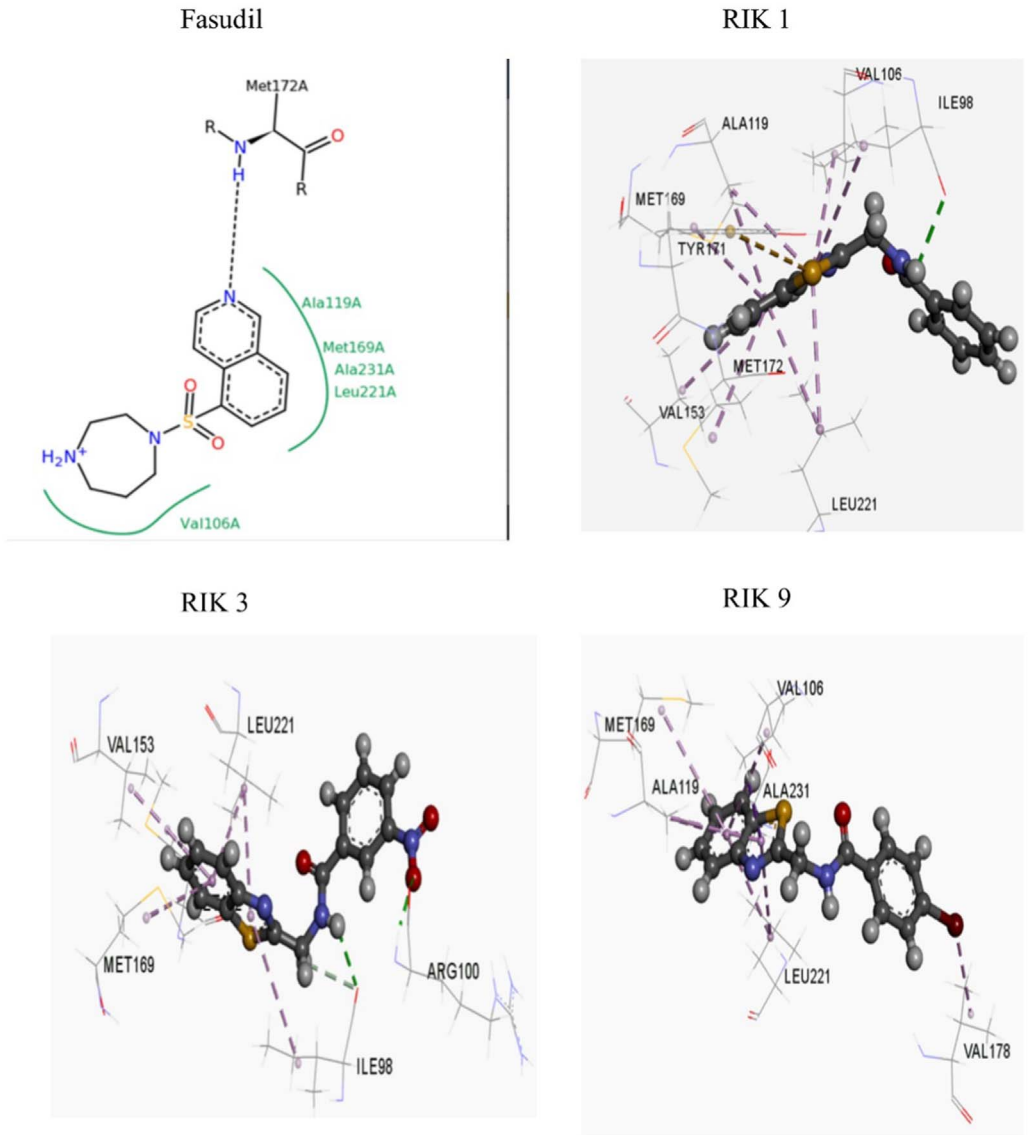


Fig. 3. Binding poses of designed molecules using Surflex Dock.

Table 7
In silico ADME studies of designed molecules.

Comp.	BBB	Caco2 permeability	CyP3A4 inhibitor	Human intestinal absorption	Plasma protein binding	TPSA	Drug likeness
Fasudil	0.237	21.57	No	96.70	17.23	57.79	2.31
RIK−1	1.38	24.64	No	96.17	95.43	70.23	3.41
RIK−2	0.44	47.83	No	96.38	93.65	70.23	5.07
RIK−3	0.037	37.75	No	95.81	96.56	116	−8.69
RIK−4	0.18	4.76	No	95.40	84.58	96.25	3.98
RIK−5	0.48	39.95	No	96.65	90.34	70.23	2.12
RIK−6	0.57	55.95	No	96.38	100	70.23	0.17
RIK−7	0.04	1.54	No	95.81	96.56	96.25	0.31
RIK−8	0.34	5.89	No	95.77	88.45	116	−3.02
RIK−9	0.67	45.67	No	96.82	91.55	70.23	−1.07
RIK−11	0.15	26.32	No	96.84	79.90	78.94	2.31
RIK−13	0.02	22.42	No	94.36	18.20	68.43	4.52
RIK−14	0.01	27.68	No	96.12	84.17	66.05	2.2
RIK−15	1.60	27.55	No	96.73	100	81.4	2.9
RIK−16	0.90	49.46	No	96.27	90.21	66.05	2.35
RIK−17	0.01	3.34	No	95.02	88.89	111.8	−2.93

Below mentioned is the different pharmacokinetic properties and their acceptable range: -.
BBB:- Indicates **BB (C_{brain}/C_{blood})** ratio. Value > 2.0 suggested high absorption to CNS while value < 0.1 indicates very low absorption.
Caco2 permeability: Value of the P_{caco2} (nm/sec) > 70 suggested high permeability while value < 4 indicates low permeability.
HIA: Calculated HIA at pH 7.4: - Value between 0% and 20% indicates poor absorption, 20–70% indicates moderate absorption while 70–100% indicates fair absorption.
Plasma protein binding: Value > 90% indicates strong protein binding, i.e. a low amount of drug available for distribution.
TPSA (Topological polar surface area): Value 80 or 100 $Å^2$ indicates good bioavailability.
Drug Likeness: Positive value indicates that the molecule has properties like that of the traded drug.
An *In silico* investigation for the prediction of rat acute toxicity of the design compounds revealed that compounds **RIK-5, 8 and 13** are slightly toxic (class 4). Except **RIK-5, 8 and 13** all other compounds are practically nontoxic (class 5) (Table 8). The results obtained from the software also revealed that almost all compounds found to be safe except RIK-13, which has a medium risk of tumorigenic effect (Table 8).

Table 8
In-silico toxicity studies of designed molecules.

Compound No	Mutagenic Effect	Tumorigenic Effect	Irritant	Reproductive Effect	Acute Rat Toxicity	Drug Score
Fasudil	No	No	No	No	Class 5	0.88
RIK−1	No	No	No	No	Class 5	0.85
RIK−2	No	No	No	No	Class 5	0.78
RIK−3	No	No	No	No	Class 5	0.42
RIK−4	No	No	No	No	Class 5	0.87
RIK−5	No	No	No	No	Class 4	0.74
RIK−6	No	No	No	No	Class 5	0.57
RIK−7	No	No	No	No	Class 5	0.69
RIK−8	No	No	No	No	Class 4	0.44
RIK−9	No	No	No	No	Class 5	0.47
RIK−11	No	No	No	No	Class 5	0.9
RIK−13	No	Medium Risk	No	No	Class 4	0.76
RIK−14	No	No	No	No	Class 5	0.88
RIK−15	No	No	No	No	Class 5	0.84
RIK−16	No	No	No	No	Class 5	0.79
RIK−17	No	No	No	No	Class 5	0.46

Drug Score: It is given out of 1. It determines the overall potential of drug by combining druglikeness, cLogP, logS, molecular weight and toxicity risks.

3.3. Virtual screening and designing of molecules

Virtual screening of the IBS database (6 lakh molecules) against validated model 3 gave 1.33 lakh hits. After applying various filters like duplicate structures, bad fragments and Lipinski rule of 5, the hit list reduced to “88,955”. Amongst these, 40 molecules exhibited more than 80 Q_{fit} value against Model_03 hence, selected for structural analysis and it was observed that many of them possess benzothiazole moiety. Based on these hit structures and features of the pharmacophore, 15 novel benzothiazole derivatives were designed and subjected to molecular docking to evaluate their binding mode.

3.4. Molecular docking

Results of molecular docking of 15 designed molecules into kinase domain suggested that almost all the interactions of designed molecules with the target matched with the interactions of the standard molecule, i.e. Fasudil with the target. Out of 15 molecules, 7 had both CS score and GOLD score higher than Fasudil as shown in Table 5. Those 7 molecules were compound 1, 3, 4, 6, 7, 9 and 16. The amino acid in bold represent those interactions which are common to both Fasudil and the designed molecules (Tables 5 and 6).

Binding poses of Fasudil, compound 1, 3 and 9 are shown in Fig. 3. It is observed that almost all the compounds have similar interactions with that of Fasudil. Compound 1 forms pi-pi stacking with **TYR171** and all of them share hydrogen bonding and electrostatic interaction with **MET172, ALA119, MET169, ALA231, LEU221, VAL106** (Table 6).

3.5. In-silico ADMET prediction

Results of ADME and toxicity prediction using OSIRIS property explorer and PreADMET software are reported in Tables 7, 8. According to the predicted properties RIK-1 and RIK-15 found to be good CNS acting candidates. RIK-11 has the highest drug score of 0.9, but it has a low BBB permeability, so for multiple sclerosis studies it might not prove to

be an effective as depicted in Table 7. All the compounds were found to have fair absorption from the *in silico* human absorption data. From Caco2 permeability study it was observed that all compounds found to have well predicted permeability except RIK 7 and 17.

Plasma Protein binding study indicated a very low amount of compound to be available for distribution except for the compounds RIK 4, 8, 11, 13, 14 and 17. The topological polar surface area and drug likeliness data suggested that all compounds found to have the predicted activity within the range except compounds RIK- 3, 8 and 17.

4. Conclusion

2.3 million People are right now suffering from Multiple Sclerosis; number even may be high as it is not easily diagnosed. Fasudil which was used as vasodilator now has shown activity against ROCK-2 in EAE models with axonal regeneration. It has IC_{50} of 1.9 micromolar and is the only marketed drug for MS. Hence there is a need to design molecules with low IC_{50} and good pharmacokinetic properties. 8 different structures were taken for the pharmacophore generation with the GALAHAD module of Sybyl. The best model contained 2 hydrophobic, 1 donor atom, 2 acceptor site. This model was validated through GH and ROC method. Virtual screening gave 40 compounds with Q_{fit} value greater than 80% after filtering. Designed benzthiazole derivatives were docked into the kinase domain of ROCK-2 (PDB i.d.: 2F2U). Out of 15 designed molecules 7 had both the CS score and GOLD score higher than Fasudil. Further the designed molecules were screened for ADMET prediction using OSIRIS and ADMETsar software. According to the Organisation for Economic Co-operation and Development (OECD) classification all the designed compounds found to be safe based on *in silico* toxicity prediction. The predicted ADMET studies suggested RIK 16 (N-(benzo[d]thiazol-2-ylmethyl) − 4-chloropyridin-2-amine) to be a promising molecule as Rho kinase 2 inhibitor.

Conflicts of interest

The authors declare no conflict of interest.

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