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Research Article

IN-SILICO DESIGNING AND SCREENING OF NOVEL PYRIDOINDOLE DERIVATIVES AS CREATIN KINASE INHIBITORS: A QSAR MODELING AND DOCKING APPROACH

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ABSTRACT
Pyridoindole derivatives have been used to correlate the half maximal inhibiting concentration (-log IC_{50}) with the partition co-efficient, molecular weight, McGowan volume and topological polar surface area for developing the quantitative structure activity relationship (QSAR) model as primary screening for novel designed pridoindole derivatives. We have used multiple linear regression (MLR) for developing QSAR model. For the validation of the developed QSAR model, statistical analysis such as cross validation test, standard deviation, quality factor, fischers test, root mean square deviation (RMSD), variance; and internal validation such as Y-randomization test have been performed and all the tests validated this QSAR model with fraction of variance r^2 = 0.8202 and LOO-CV variance q^2= 0.8222. Eight novel pyridoindole analogues have been designed and their half maximal inhibiting concentration (-log IC_{50}) has been calculated with the developed QSAR model. It was found that the calculated half maximal inhibiting concentration (-log IC_{50}) of these analogues were within the same range as of the training set. Further, all the screened derivatives gone through a second screening via docking analysis (TARGET-Creatin kinase, PDB id 3DRB) which shows better docking score as compared to the stobadin, a creatin kinase inhibitor. In this 2 tier screening, 3 novel designed molecules (RA5, RA6, and RA7) out of 8 have passed both the screening levels. The results suggested that the screened novel pyridoindole analogues could be developed as good creatin kinase inhibitors.

Keywords: *Pyridoindole Analogues, QSAR, MLR, Docking, Creatin Kinase Inhibitors*

INTRODUCTION
Relative preservation of neuronal structure or function is commonly known as neuroprotection (Casson, 2012). It aims to prevent and slow down the disease progression and some secondary injuries by slowing the loss of neurons (Seidl, 2011). Despite non-similarity in symptoms of CNS disorder, the neurodegenerative mechanisms are same, oxidative stress is one of them. Creatin kinase is an enzyme expressed by various tissues and cell types. It catalyses the conversion of creatine and consumes adenosine triphosphate to create phosphocreatine and adenosine diphosphate (Dunnett, 1999).
Based on the various scientific researches it is clear that pyridoindole stobadine may protect nervous structures against oxidative stress (Stolc, 1999) whereas Stolc and co-workers said that stobadine is a well-known antioxidant, free radical scavenger, and neuroprotectant (Stolc, 2006), which preventes damage to Ca^{2+} sequestering systems in endoplasmic reticulum and synaptosomes induced by lipid peroxidation initiators.
On studying the above mentioned literature about the antioxidant behavior of pyridoindole promoted us to theoretically develop moieties and screen them by the means of *in-silico* resources.
The QSAR studies are perfect tool for understanding the drug design process in terms of their chemical-pharmacological activity interaction. QSAR studies can focus on mechanism of action of ligands with human, bacteria, virus, membranes, enzymes etc. The QSAR methodology comprises of computationally derived descriptors to correlate with pharmacological activities. These descriptors are principally of four types such as electronic, stearic, hydrophobic and topological indices (Verma, 2010). The descriptors used for developing the QSAR model are AlogP, molecular weight, McGowan volume and topological polar surface area (TPSA) (Hou, 2003).

Research Article

Rational drug design helps to facilitate and fasten the drug designing process, which involves various methods to identify novel compound, out of them one method is the docking of molecules with the receptor (Sharma, 2011). Docking procedures allows virtually screening a data-base of compounds and predict the strongest binder based on various scoring functions. It gives way in which two molecules such as drugs and an enzyme receptor fit together and dock to each other well (Shiva, 2010; Ajeet, 2012; Ajeet, 2013).

Molecular docking techniques are used in modern drug design to help understand drug–receptor interaction. It has been shown in the literature that these computational procedures can strongly support and help the design of new, more potent drugs by revealing the mechanism of drug–receptor interaction (Shiva, 2010; Ajeet, 2012; Ajeet, 2013).

In the present study, we developed a QSAR model on a series of pyrido-indole analogues with respect to their creatin kinase nhibition. Further, these analogues were passed with the model and screened molecules have been docked with the catalytic domain of creatin kinase for second line of screening based on ligand protein interaction.

MATERIALS AND METHODS

All the bioactivity values and information about 2D structure of pyrido-indole derivatives (Figure 1) were taken from literature (Stolc, 2008). IC_{50} is referred as the molar concentration of a compound that inhibits 50% growth of bacteria (Verma, 2010). -log IC_{50} is subsequent variable that comprises the bioactivity parameter for the QSAR model. In order to calculate the 2D molecular descriptors, PaDEL descriptor software (Yap, 2011) which incorporate CDK library for descriptor calculation has been used after optimitizing the pyrido-indole derivatives. For the development of QSAR model, MLR (multiple linear regression) (Verma. 2010) has been employed and all were validated through statistics.

The novel pyrido-indole molecules have been designed and optimized through ChemDraw Ultra 7.0. and their bioactivity values have been calculated from developed QSAR model by putting the descriptor values in the QSAR equation. The docking studies (Shiva, 2010; Ajeet, 2012; Ajeet, 2013) of 8 novel pyrido-indole derivatives have been performed with the AutoDock Vina.

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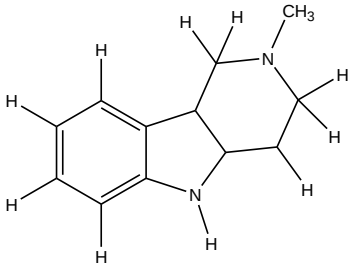

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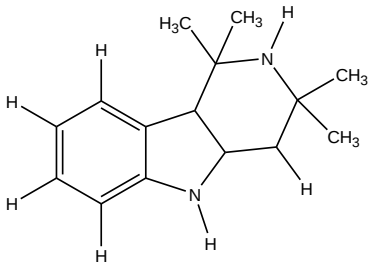

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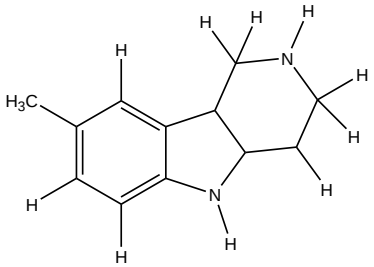

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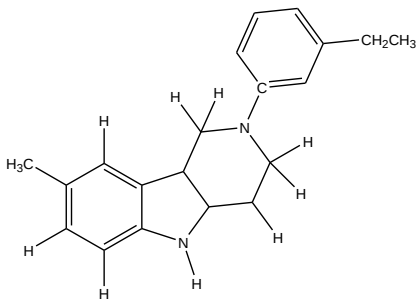

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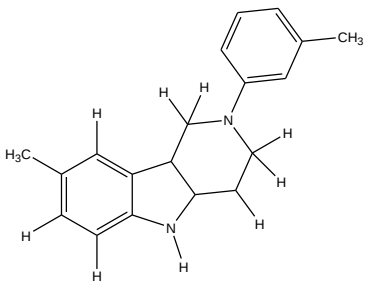

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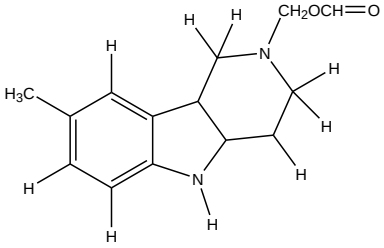

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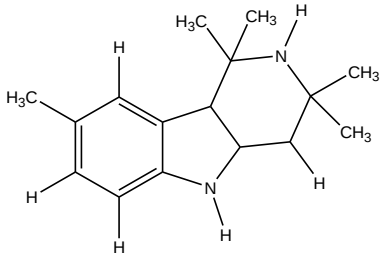

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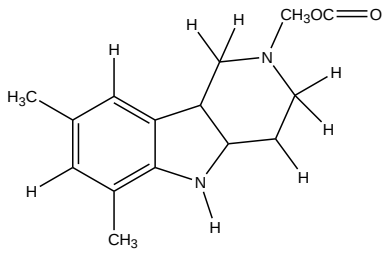

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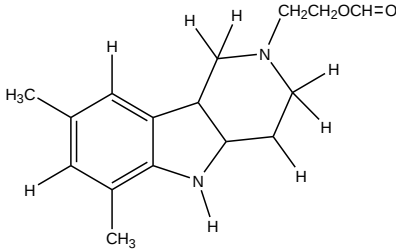

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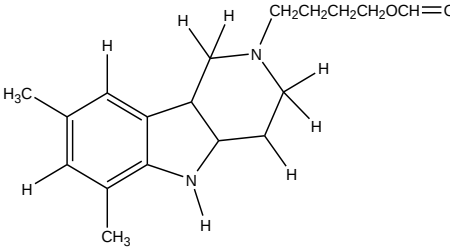

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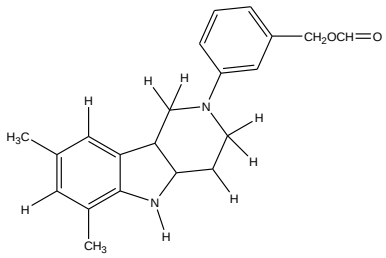

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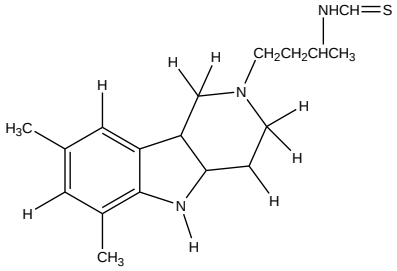

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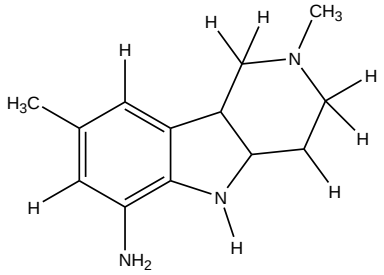

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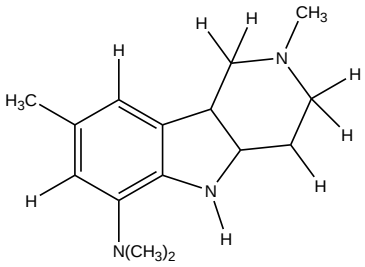

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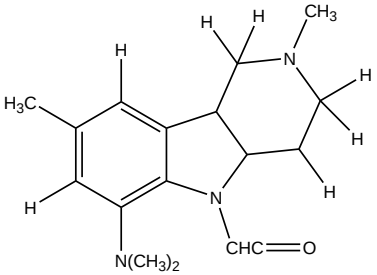

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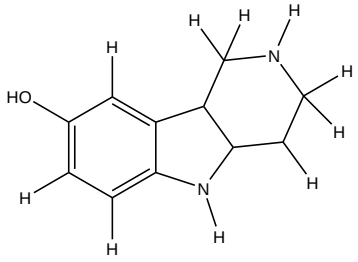

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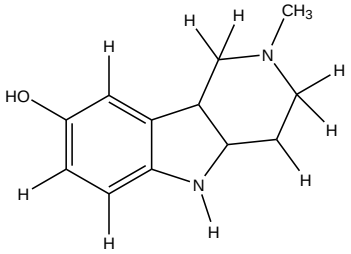

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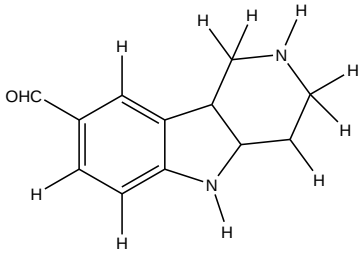

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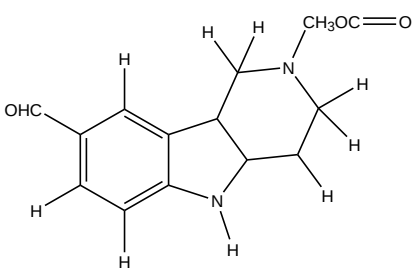

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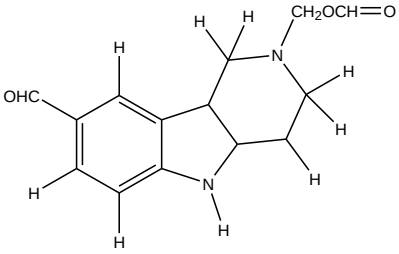

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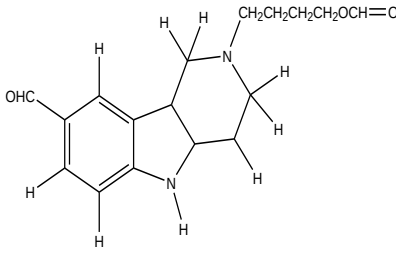

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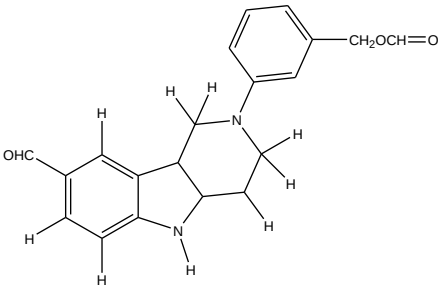

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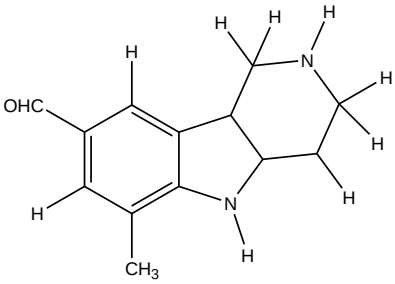

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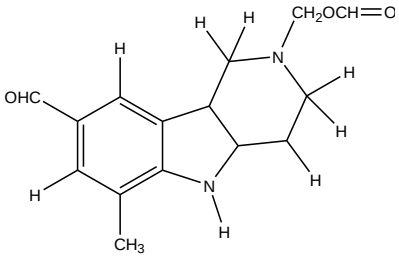

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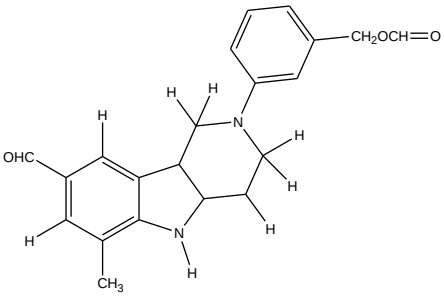

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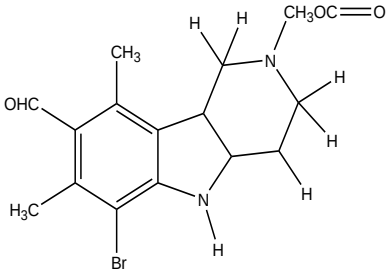

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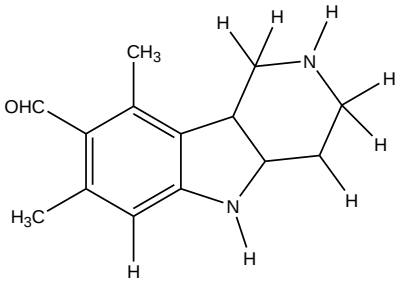

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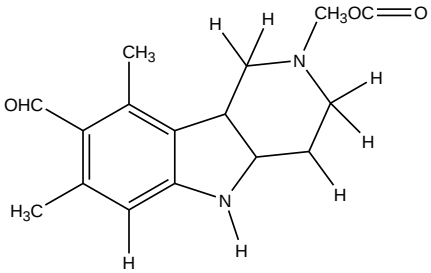

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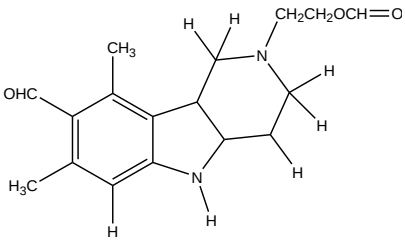

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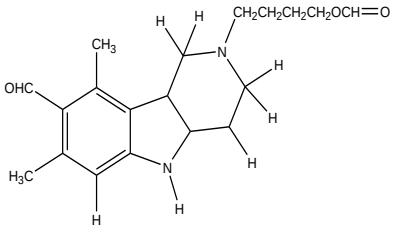

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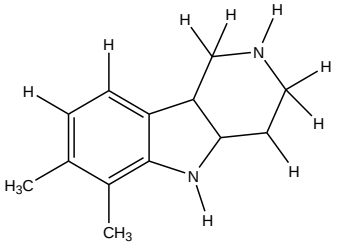

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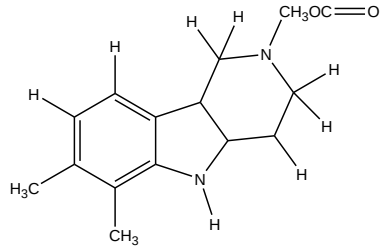

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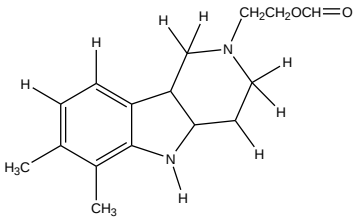

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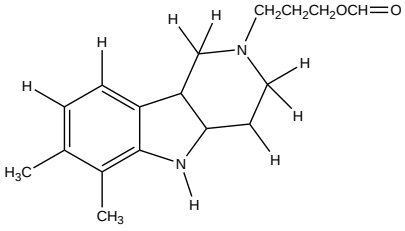

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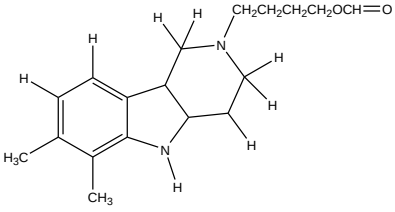

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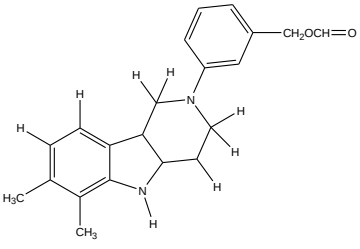


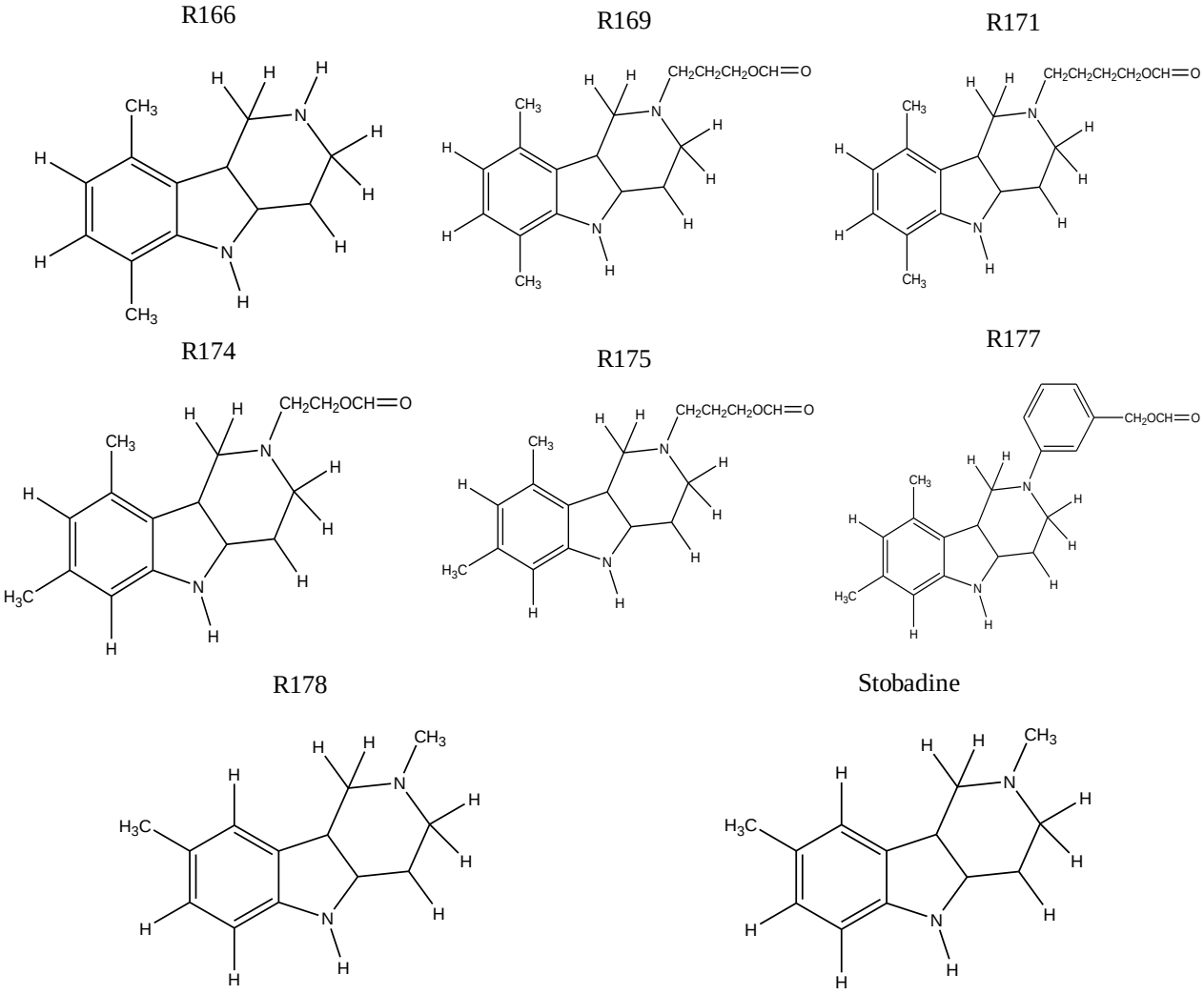


Figure 1: Structures of pyridoindole derivative used as training set for QSAR model

Statistical Parameters

Fraction of variance (r^2): The value of fraction of variance may vary between 0 (means model without explanatory power) and 1 (means perfect model). QSAR model having $r^2 > 0.6$ will only be considered for validation (Verma, 2010).

Cross-validation Test (q^2): A QSAR model must have $q^2 > 0.5$ for the predictive ability (Verma, 2010).

Standard deviation (s): The smaller s value is always required for the predictive QSAR model.

r^2-q^2 < 0.3: The difference between r^2 and q^2 should never be exceeding by 0.3. A large difference suggests the following: presence of outliers, over-fitted model, and presence of irrelevant variables in data (Verma, 2010).

Quality factor (Q): Over fitting and chance correlation, due to excess number of descriptors, can be detected by Q value. Positive value for this QSAR model suggests its high predictive power and lack of over fitting.

Fischer statistics (F): The F value of QSAR model was compared with their literature value at 95% level.

Model Validation

The QSAR model validation was carried with statistical analysis and with internal validation.

Docking Studies

The designed novel pyrido-indole derivatives were docked into catalytic domain of creatin kinase. For this study, X-ray crystal structure of creatin kinase was taken from protein data bank with PDB id 3DRB.

RESULTS AND DISCUSSION

From the data (Table 1), QSAR equation 1 have been developed and 95% confidence intervals are given in parenthesis as follows-

-log IC50=1.875051(±0.5859609)- 0.1894421(±0.1680057)(ALogP)- 0.0055141(±0.008751)(MW)+2.362933 (±1.09011)(MG)+ 0.0022559 (±0.0097043)(TPSA) Eq. (1)

Where, MW- Molecular weight, MG- McGowan volume, TPSA-Topological polar surface area

Table 1: Descriptors used to derive QSAR equation along with bioactivities for the inhibition by pyridoindole analogues

Training set code	-log IC_{50}			ALogP	MW	MG	TPSA
	Obs.	Pred.	Diff.				
R102	3.778	4.552264	-0.77426	-0.1652	188.1313	1.5442	15.27
R105	4.852	5.065406	-0.21341	1.2793	230.1782	1.9669	24.06
R106	4.641	4.589048	0.051952	-0.2547	188.1313	1.5442	24.06
R107	6.126	5.813027	0.312973	1.2468	292.1939	2.4338	15.27
R108	5.71	5.493891	0.216109	1.5819	278.1782	2.2929	15.27
R109	4.787	5.097506	-0.31051	0.0244	246.1368	1.9004	41.57
R111	5.388	5.236492	0.151508	1.7257	244.1939	2.1078	24.06
R115	5.551	5.420449	0.130551	0.5808	260.1524	2.1069	49.41
R116	5.804	5.420449	0.383551	0.5808	260.1524	2.1069	49.41
R119	6.263	6.14132	0.12168	-0.0875	302.1994	2.464	41.57
R121	6.287	6.14022	0.14678	1.2379	336.1837	2.6491	41.57
R123	5.799	6.323739	-0.52474	0.5452	317.1926	2.6099	59.39
R124	5.509	5.161805	0.347195	-0.9155	217.1578	1.7849	41.29
R125	5.62	5.438399	0.181601	0.0522	245.1891	2.0667	18.51
R129	5.428	5.81457	-0.38657	0.1754	285.1841	2.3212	26.79
R131	4.905	4.620781	0.284219	-1.2642	190.1106	1.462	44.29
R132	4.673	4.755083	-0.08208	-0.7283	204.1262	1.6029	35.5
R133	4.914	4.745643	0.168357	-1.0891	202.1106	1.5599	41.13
R136	5.282	5.405957	-0.12396	-0.7	260.1160	1.9817	66.48
R137	5.487	5.254101	0.232899	-0.81	260.1160	1.9161	58.64
R140	6.024	6.126828	-0.10283	-1.3683	302.1630	2.3388	58.64
R142	6.246	6.125727	0.120273	-0.0429	336.1473	2.5239	58.64
R143	5.328	4.91673	0.41127	-0.6427	216.1262	1.7008	41.13
R144	5.12	5.425187	-0.30519	-0.3636	274.1317	2.057	58.64
R145	6.563	6.296814	0.266186	0.4035	350.1630	2.6648	58.64
R146	5.491	5.646278	-0.15528	0.6455	366.0579	2.4385	66.48
R147	5.059	5.087817	-0.02882	-0.1963	230.1419	1.8417	41.13
R148	5.509	5.748131	-0.23913	0.1928	288.1473	2.2635	66.48
R149	5.925	6.349729	-0.42473	0.1005	318.1943	2.5883	58.64
R151	6.474	6.469001	0.004999	-0.4755	330.1943	2.6206	58.64
R158	4.91	4.760135	0.149865	0.1917	202.1469	1.6851	24.06
R159	5.348	5.420449	-0.07245	0.5808	260.1524	2.1069	49.41
R160	5.609	5.520894	0.088106	0.4885	274.1681	2.1822	41.57

R161	5.909	5.831107	0.077893	0.2005	288.1837	2.3231	41.57
R163	6.032	6.14132	-0.10932	-0.0875	302.1994	2.464	41.57
R165	6.25	6.14022	0.10978	1.2379	336.1837	2.6491	41.57
R166	4.969	4.760135	0.208865	0.1917	202.1469	1.6851	24.06
R169	6.039	5.831107	0.207893	0.2005	288.1837	2.3231	41.57
R171	6.105	6.14132	-0.03632	-0.0875	302.1994	2.464	41.57
R174	5.731	5.520894	0.210106	0.4885	274.1681	2.1822	41.57
R175	6.008	5.831107	0.176893	0.2005	288.1837	2.3231	41.57
R177	6.012	6.14022	-0.12822	1.2379	336.1837	2.6491	41.57
R178	4.234	4.723351	-0.48935	0.2812	202.1469	1.6851	15.27
Stobadin	4.469	4.723351	-0.25435	0.2812	202.1469	1.6851	15.27

Validation of QSAR Model

A quantitative assessment of model robustness has been performed through model validation. All the statistical results of model validation have been given (Table 2).

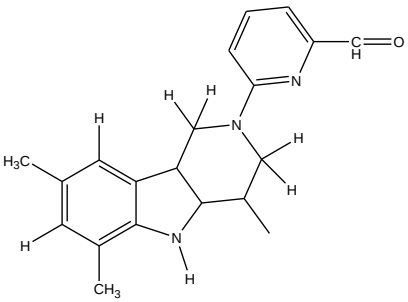
Table 2: Results of statistical validation

r^2	LOO-q^2	LFO-q^2	s	r^2- LOOq^2 < 0.3	r^2- LFOq^2 < 0.3	Q	RMSD	Variance	F
0.8202	0.8222	0.8235	0.59	-0.00197	-0.00327	1.53	0.0396	0.0781	44.47

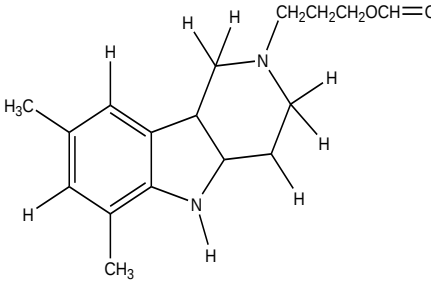
Internal Validation

Bioactivity calculation for test set from developed QSAR model: In this type of internal validation, the test set has to pass the developed QSAR model. (Figure 2, Table 3)

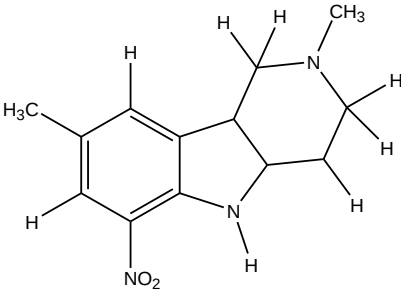
R114



R117



R126



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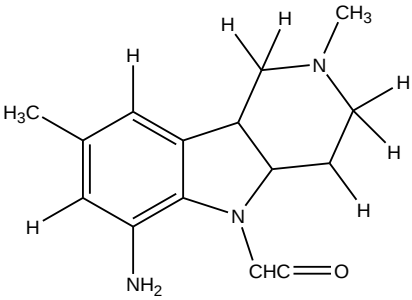
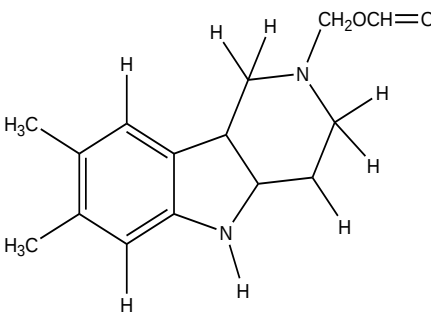
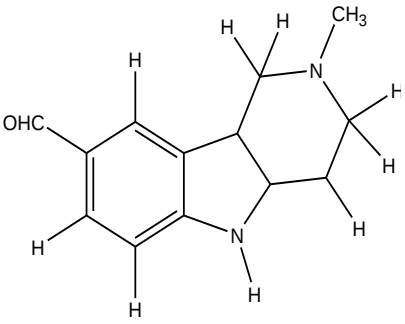


R130



R134



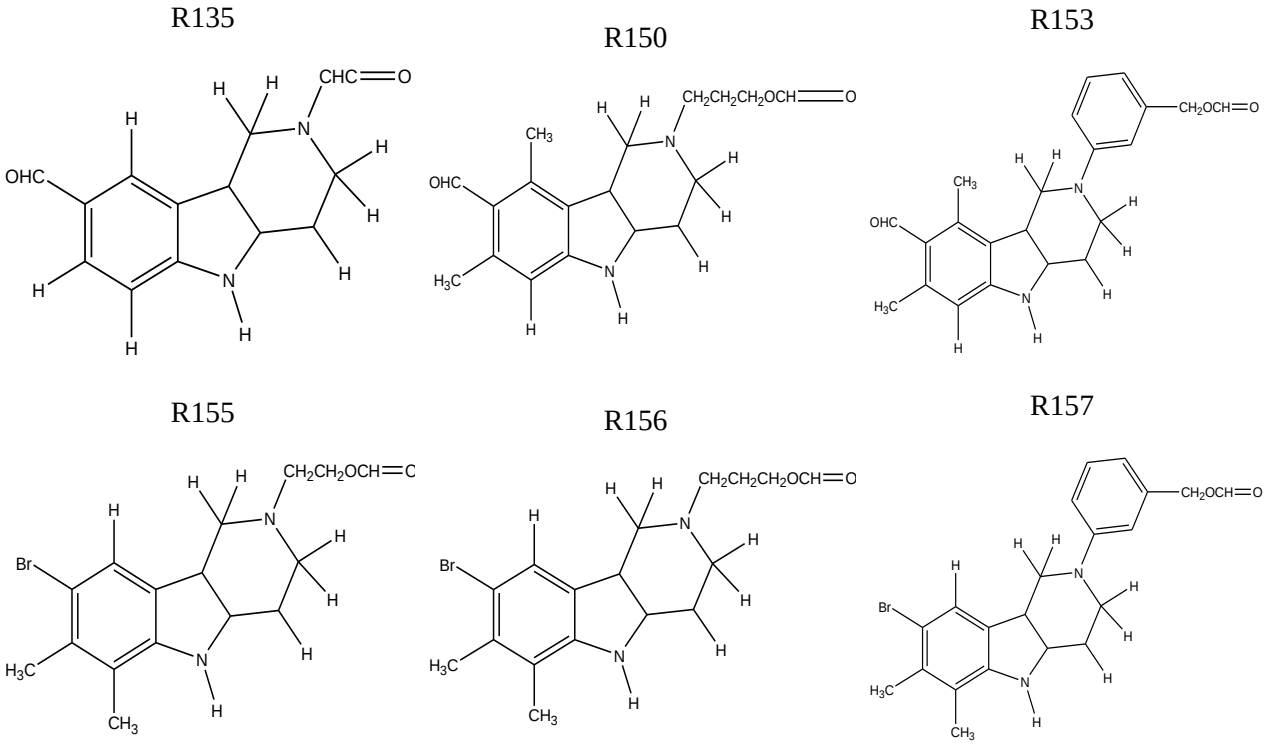


Figure 2: Structures of test set

Table 3: Calculated -log IC50 for test set from developed QSAR model

Test set	ALogP	MW	MG	TPSA	Observed -log IC50	Pred. -log IC50	Diff.
R114	0.7466	321.1841	2.5493	44.7	4.793	6.2388	-1.4458
R117	0.2005	288.1838	2.3231	41.57	6.084	5.9664	0.1175
R126	0.4945	247.1321	1.8593	58.41	1.684	5.0543	-3.3703
R128	0.7339	280.0575	1.8601	15.27	1	4.7601	-3.7601
R130	0.4708	260.1525	2.0413	41.57	5.948	5.3899	0.5580
R134	-0.5532	216.1263	1.7008	32.34	4.698	4.9808	-0.2828
R135	-0.9659	242.1055	1.8144	49.41	4.543	5.2314	-0.6884
R150	-0.1875	316.1787	2.4797	58.64	6.098	6.3038	-0.2058
R153	0.8499	364.1787	2.8057	58.64	5.759	6.6378	-0.8788
R155	0.9412	352.0786	2.3572	41.57	5.955	5.5875	0.3674
R156	0.6532	366.0943	2.4981	41.57	6.002	5.9047	0.0972
R157	1.6906	414.0943	2.8241	41.57	5.944	6.2386	-0.2946

Y-Randomization Test: This technique is used to establish the QSAR model robustness. For this test, the dependent variable vector is randomly shuffled, and a new QSAR model is developed using the unchanged independent variable. This process was repeated for five times. The statistical data of r^2 for five runs are given (Table 4). The values r^2<0.6in Y-randomization test confirm the robustness of this QSAR model (Verma, 2010).

Table 4: Results of internal validation: Y-randomization test (5 runs)

No. of Y-randomization	First	Second	Third	Fourth	Fifth
r^2	0.1221	0.4409	0.1221	0.1221	0.1221

A comparison (MLR plots) of observed values and predicted values of -log IC50 for pyridoindole derivatives used for development of QSAR equation is shown in Figure 3 and radar graph is shown in Figure 4.

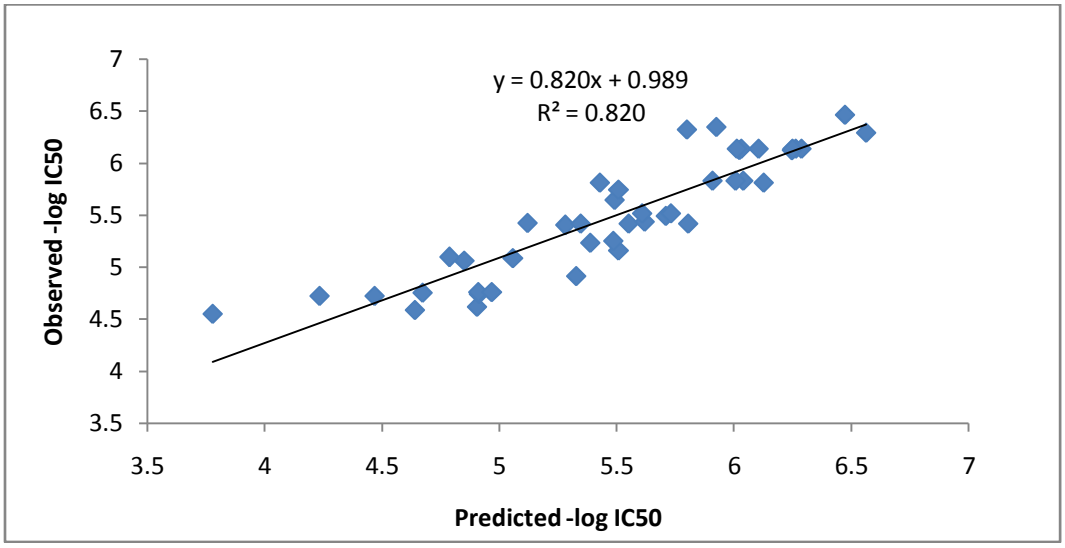


Figure 3: Multiple linear regression plot for QSAR study

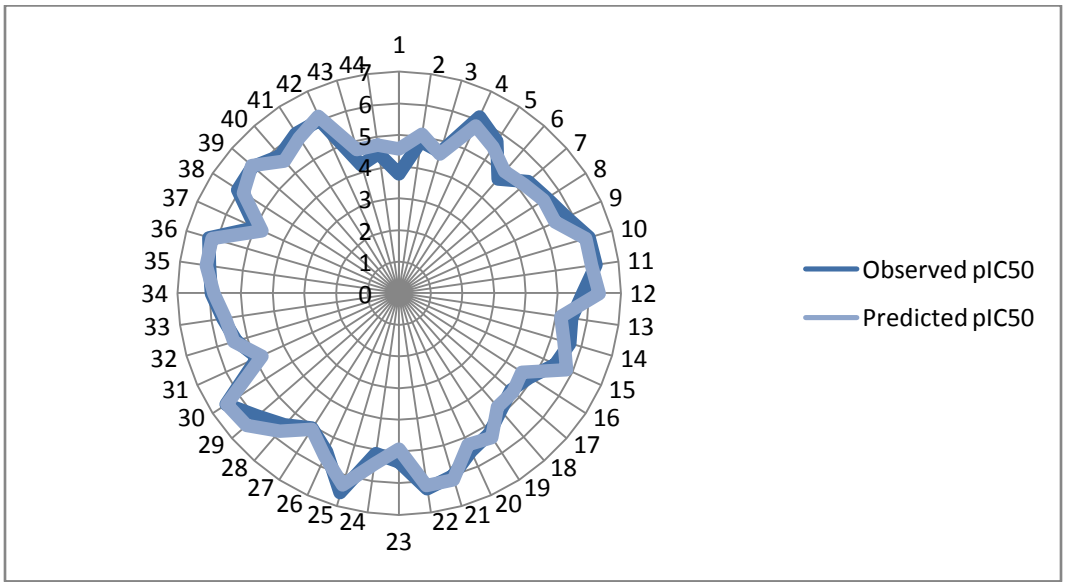


Figure 4: Radar graph between observed and predicted bioactivities (pIC50 ~-log IC50)

Designing and optimization of novel pyridoindole derivatives

The novel pyridoindole derivatives (Figure 5) were designed and their energy minimization for highest stability was performed. Details of calculated descriptor values and predicted bioactivity through derived QSAR model have been given (Table 5).

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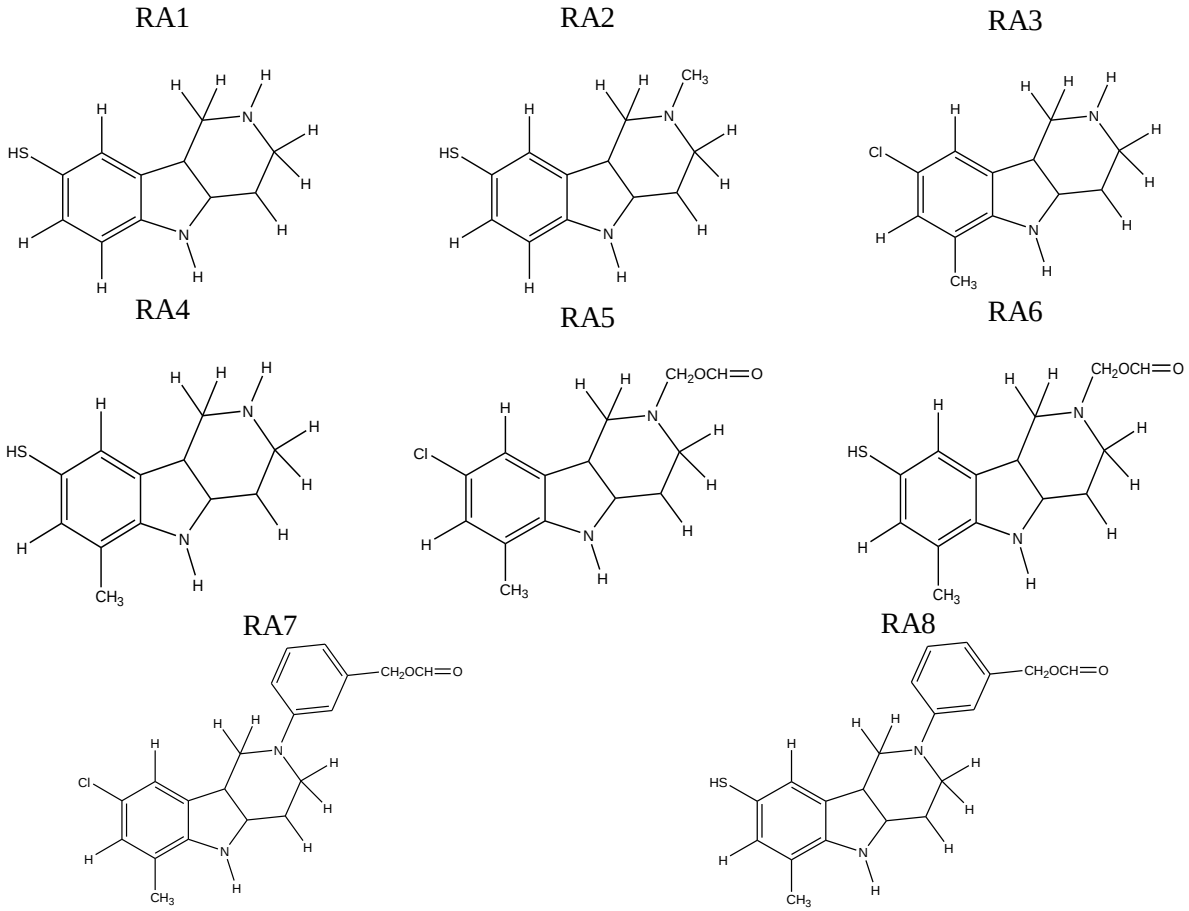


Figure 5: Novel designed pyridoindole derivatives

Table 5: Predicted -log IC50 of novel designed pyridoindole derivatives

Novel designed derivatives	-log IC50 Predicted	ALogP	MW	MG	TPSA
RA1	4.791437	-0.6369	206.0878	1.5668	62.86
RA2	4.935299	-0.101	220.1034	1.7077	54.07
RA3	4.727621	0.114	222.0924	1.6666	24.06
RA4	4.969795	-0.1905	220.1034	1.7077	62.86
RA5	5.261208	0.3931	280.0979	2.0228	41.57
RA6	5.503382	0.0886	278.1089	2.0639	80.37
RA7	6.171694	1.1602	356.1292	2.6306	41.57
RA8	6.413867	0.8557	354.1402	2.6717	80.37

Docking Results

Binding site analysis

The experimental analysis of binding site shows that Arg 130, Arg 96, Gly 331, Arg 252, Ser 128, Ser 285, Thr 322 and Thr 224 could be the catalytic site residue present in the structure of creatin kinase.

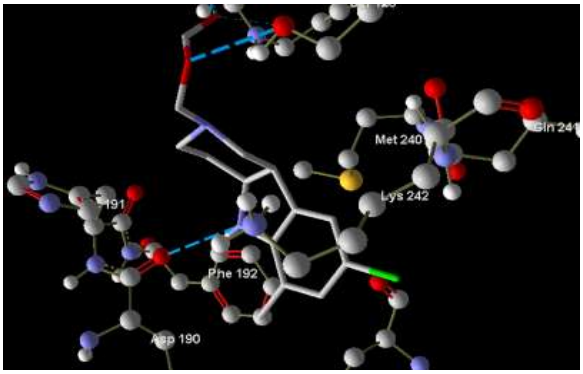
Docking studies of novel pyridoindole derivatives with creatin kinase

Docking studies showed that derivatives RA5, RA6 and RA7 were docked by overlapping with stobadin; a pre-existing creatin kinase inhibitor. The best pose interaction energy was found to be as -6.6 Kcal/mol, -6 Kcal/mol and -6.9 Kcal/mol. Here, negative values for interaction energy would reflect the positive docking approach. Number of hydrogen bonds and other binding details (Table 6) and docking images (Figure 6) are given.

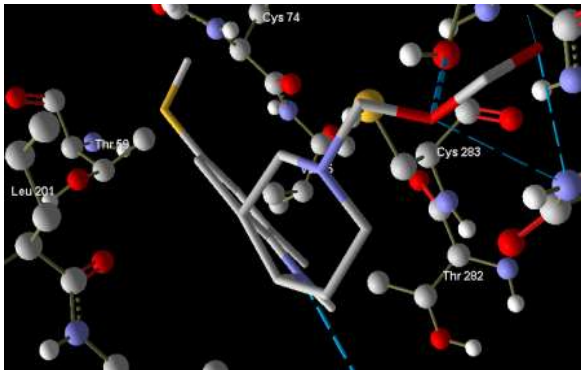
Table 6: Docking results of novel pyridoindole derivatives

Ligand	Receptor	Affinity Kcal/mol	H-bonds	H- Binding Ligand				H- Binding Receptor		
				Elem.	At. ID.	Type	Res.	Elem.	At.ID.	Type
RA1		-5.9	0	-	-	-	-	-	-	-
RA2		-6.6	1	N	06	Donor	Tyr173	O	1298	Acceptor
RA3		-6.8	0	-	-	-	-	-	-	-
RA4		-6.9	2	N	6	Donor	Gly 331	O	2570	Acceptor
				S	16	Donor	Thr 322	O	2516	Acceptor
RA5		-6.6	4	O	19	Accepter	Arg 130	N	977	Donor
	3DRB			O	19	Acceptor	Ser 128	O	960	Both
				O	17	Acceptor	Ser 128	O	960	Both
				N	6	Donor	Asp 190	O	1439	Acceptor
RA6		-6	5	N	6	Donor	Glu 232	O	1791	Acceptor
				O	16	Acceptor	Asn 286	N	2234	Donor
				O	18	Acceptor	Asn 286	N	2234	Donor
				O	16	Acceptor	Ser 285	O	2226	Both
				O	18	Acceptor	Arg 96	N	713	Donor
RA7		-6.9	3	O	25	Acceptor	Thr 224	O	1718	Both
				O	25	Acceptor	Arg 252	N	1954	Donor
				O	25	Acceptor	Arg 252	N	1952	Donor
RA8		-8.3	1	O	22	Acceptor	Asp 195	N	1484	Donor
Stobadin		-6.1	1	N	06	Donor	Gly 171	O	1283	Acceptor

RA5



RA6



RA7

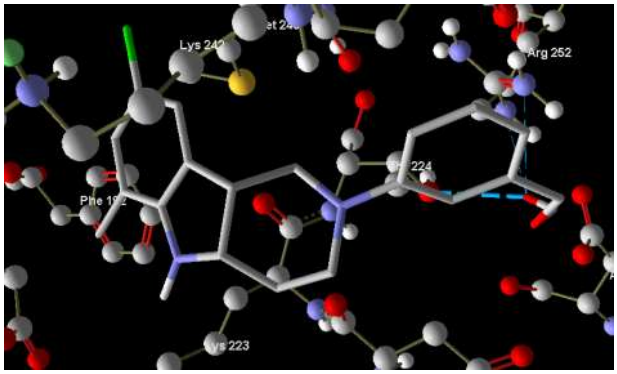


Figure 6: Docked photographs of novel pyridoindole derivatives (RA5, RA6 and RA7 screened by docking studies)

Comparison of docking results with pre-existing creatin kinase inhibitor- Stobadin (reference drug)
On docking studies and docking analysis of stobadin (Figure 7) with the creatin kinase, interacting residues (amino acids) is found as Gly 171.

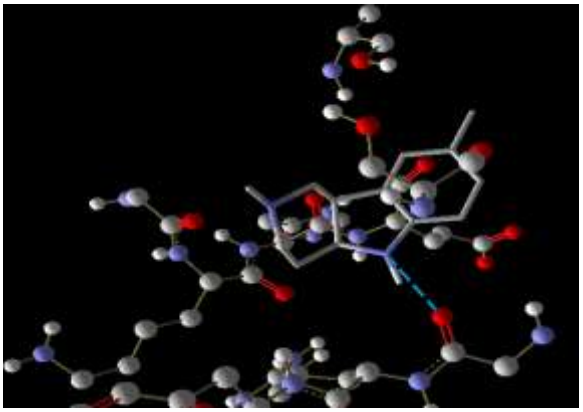


Figure 7: Docking photograph of stobadin with the creatin kinase

On docking analysis, the docked poses of RA5, RA6 and RA7 superimposes the stobadin, a pre-existing creatin kinase inhibitor which can be clearly seen in Figure 8, and the docking analysis shows that it nicely docked with protein.

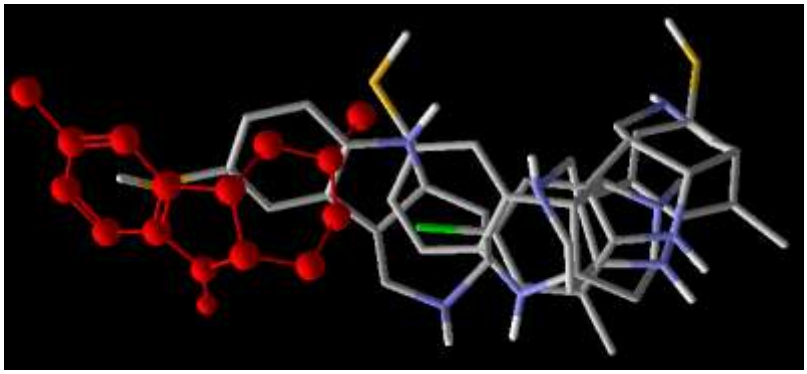


Figure 8: Superimposed docking poses of RA5, RA6 and RA7 (showing with stick model) with the pre-existing ligand stobadin (showing with ball and stick model in red color)

A QSAR model has been developed against creatin kinase inhibition for screening the pyridoindole derivatives with 44 molecules as training set. This QSAR model has been statistically proven. Now, 8 novel pyridoindole derivatives has been designed and toured for the 2 tier screening. These 8 novel derivatives have passed the first screening through QSAR model, after that, all the screened derivatives gone through a second screening via docking analysis (TARGET- Creatin kinase, PDB id 3DRB). In this 2 tier screening, 3 novel designed molecules (RA5, RA6, and RA7) out of 8 have passed both the screening levels.

Conclusion

Computational study comprises of two tier screening (QSAR and docking) of novel pyridoindole derivatives (RA5, RA6 and RA7) proved them potential creatin kinase inhibitors. Although a systemic biochemical study is necessary to confirm the findings. On comparing the chemical structure of pyridoindole derivatives with stobadin, a pre-existing creatin kinase inhibitor; it is concluded that a tricyclic fused system, consisting of pyridine and indole fused rings, which are essential pharmacophoric requirements in designing of creatin kinase inhibitors.

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