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Research Article

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A Molecular Modelling Study on Derivatives of Pyrazolyl Thiazolinone as Potential EGFR and HER-2 Kinase Inhibitors

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ABSTRACT

EGFR and HER-2 kinase are protein tyrosine kinase receptors (PTKRs) also called Phosphotyrosinkinase receptors play significant role in controlling cell division and differentiation step in cell-cycle. It is also a promising strategy for the development of novel and newer anticancer agents. In pursuit of better anticancer agents, pyrazolyl thiazolinone derivatives were quantitatively studied by using Fujita ban, Hansch and 3D-QSAR analysis. Sequential multiple linear regression analysis was used for building the QSAR model. The QSAR model was generated using 24 compounds as training set. Developed QSAR model was internally validated using leave-one-out method of cross-validation (q^2) and externally validated using test set considering predictive squared correlation coefficient (Pred_r^2). Molecular modelling and QSAR analysis revealed that electronic and steric parameters contribute significantly to its inhibitory activity. Substitution by the methyl group at R1 position increase the lipophilicity and R2 position remain unsubstituted or substituted with bromine group is more favourable for kinase inhibitory activity, whereas substitution at R1 position with more electronegative group contributed negatively. The results of present study may be useful in designing of newer, more potent and more active pyrazolyl-thiazolinone analogs as EGFR and HER-2 kinase inhibitors.

Keywords: EGFR; HER-2; QSAR; SMLR; Descriptor; Anticancer agent

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INTRODUCTION

Cancer still remains one of the most feared diseases in the modern world. According to the World Health Organization, it affected one person in the three and caused a quarter of all deaths in the developed world during the year 2000 [1]. Cancer can be defined as a disease in which group of abnormal cells grow uncontrollably by disregarding the normal rules of cells division. Normal cells are constantly subjected to signals that dictate whether the cells divide, differentiate into another cell or die. Cancer cells develop a degree of autonomy from these signals, resulting in uncontrolled growth and proliferation. If the proliferation is allowed to continue and spread, it can be fatal [2]. Receptor Protein tyrosine kinase (RPTKs) also called phosphotyrosine kinase play significant role in control of cellular growth and differentiation step in human neoplastic disease [3]. EGFR, a transmembrane protein tyrosine kinase (PTK) that is activated by ligand induced dimerization, plays a critical role in regulating cell-proliferation, differentiation and migration [4]. There are four members in EGFR family, namely; EGFR (HER-1/ErbB-1), HER-2 (ErbB-2/neu), HER-3 (ErbB-3) and HER-4 (ErbB-4) [5]. EGFR tyrosine kinase mediated cell growth signalling pathway plays an important role in formation and development of many types of solid tumors including head and neck, lung, breast, bladder, prostate and kidney cancers [6-9]. Therefore EGFR tyrosine kinase represents an attractive target for the development of novel anticancer agents. EGFR and HER-2 are the hottest targets in modern approach of cancer treatment, because their over expression or abnormal activation often cause cell malignant transformation [10]. Compounds which belong to the kinase inhibitory activity of EGFR and /or HER-2 receptor after binding of its inhibitory activity are of an attractive target for the designing of new potential anticancer agents. Several tyrosine kinase inhibitors such as Geftinib (Iressa), Erlotinib (Tarceva), Gleevec and Herceptin (Figure 1) have been approved by USFDA available in the market for treatment of variety of cancers [11,12].

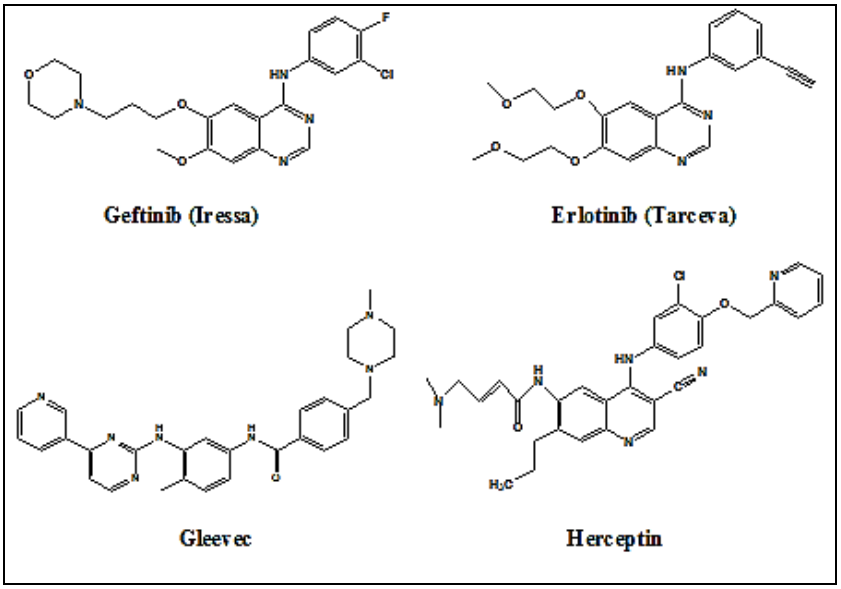


Figure 1: Chemical structures of some approved drug

Pyrazoline template is one of the privileged structural fragments in modern medicinal chemistry considering its broad pharmacological spectrum and affinity for various biotargets in the class of heterocyclic compounds. Some of the drugs available in the market which possesed pyrazole moiety as a basic nucleus such as celecoxib [13], deracoxib [14], etoricoxib and atorivodone [15]. Pyrazol and their derivatives exhibit wide spectrum of biological activities such as anti-inflammatory, antipyretic, analgesic [16], antimicrobial [17], anticancer [18], sodium-channel-blocker [19], antitubercular [20], antiviral [21], anti-hypertensive [22], anti-diabetic [23] etc. In recent, Ducray et al. studied 3-alkoxy-1H-pyraozolo[3,4-d] pyrimidines analogues and reported their potential EGFR and HER-2 receptor tyrosine kinase inhibitory activity [24].

Thiazoline ring system is a core structure in various heterocyclic compounds exhibit a wide range of pharmacological activities like as antitumor [25], anticonvulsant [26] antimicrobial [27], antiviral [28], antifungal [29], anti-inflammatory [27], and so on. Rather than thiazolidinone motif have been reported to exhibit marked antitumor activities on different biotarget such as phosphatase of a regenerating liver (PRL-3) [30], nonmembrane protein tyrosine phosphatase(SHP-2) [31], sphingosine kinase(sk) [32], JNK-stimulating phosphatase-1(JSP-1) [33], tumor necrosis factor TNFα [34], antiapoptotic biocomplex Bcl-X_L-BH3 [35], integrin$\alpha_v\beta_3$ [36]. In modern days various significant advancements has been made by computational chemistry led new challenges to drug discovery by rational process. Quantitative structure activity relationship (QSAR) which has become a valuable tool for establishing quantitative relationship between biological activity and descriptors representing physicochemical properties of the compounds in a series using statistical methods and it helps to predict the biological activities of newly designed analogues contributing the drug discovery process [37]. In the quest for search of novel and potent anticancer agents, QSAR study has been carried out on pyrazolyl-thiazolinone derivatives which were reported as potential inhibitors of EGFR and HER-2 protein tyrosine kinase receptors.

EXPERIMENTAL SECTION

Data Set

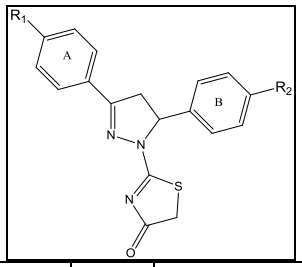
In the present study EGFR and HER-2 kinase receptor inhibitory activity data of pyrazolyl thiazolinone derivatives (36 compounds) were taken from the published work of Zhu et al. [4]. The biological activity data (IC_{50} in µM) were converted into negative logarithmic dose (-logIC_{50} or pIC_{50} in mole) to reduce skewness of data set for QSAR studies (Table 1). Subsequently inhibitory activity data is taken as dependent variable, which exhibit correlation with independent parameter.

Molecular Modelling

A Molecular modelling study was carried out to classical QSAR method Fujita-ban and subsequently Hansch approach. Fujita ban analysis is a non-parametric approach and modification of free Wilson technique. Initially series was subjected to QSAR analysis via Fujita ban approach using simple regression technique in order to find out whether or not the addition of substituent is of significance in improving the correlation between the structures of compounds with their biological activities. Indicator variable 1 and 0 was included for structural variation due to presence and absence of substitution at position R1 and R2. Hansch approach is a parametric

approach was carried out to establish the correlation between kinase inhibition and various substituents of pyrazolyl-thiazolinone derivatives. Values of various substituent constants such as hydrophobicity (π), steric (molar-refractivity or MR), hydrogen acceptor (HA), hydrogen donor (HD), electronic (field effect or $\mathcal{F}$, resonance or $\mathcal{R}$ and hammett's constant or σ) were taken into account from the reported data [38,39] and verloop sterimol parameter (*L, B_1, B_2, B_3,B_4*) were taken from the literature [40].

Table 1: Common structure and inhibitory activity data (IC_{50}) of pyrazolyl-thiazolinone analogs as anticancer agents



S.N.	COMPOUND	R1	R2	IC_{50}(μM)		pIC_{50}(M)	
				EGFR	HER-2	EGFR	HER-2
1	E1	H	H	3.38	5.12	5.471	5.291
2	E2	H	F	4.86	6.35	5.313	5.197
3	E3	H	Cl	3.49	4.83	5.457	5.316
4	E4	H	Br	1.35	3.05	5.87	5.516
5	E5	H	Me	3.03	4.64	5.519	5.333
6	E6	H	OMe	4.27	6.21	5.37	5.207
7	E7	F	H	8.14	10.53	5.089	4.978
8	E8	F	F	16.92	18.12	4.771	4.742
9	E9	F	Cl	10.92	13.16	4.962	4.881
10	E10	F	Br	4.79	6.24	5.32	4.906
11	E11	F	Me	8.36	10.26	5.078	4.989
12	E12	F	OMe	10.69	12.43	4.971	4.906
13	E13	Cl	H	5.34	7.05	5.272	5.152
14	E14	Cl	F	14.21	16.42	4.847	4.785
15	E15	Cl	Cl	8.16	9.96	5.088	5.002
16	E16	Cl	Br	2.28	3.84	5.642	5.416
17	E17	Cl	Me	6.67	8.13	5.176	5.09
18	E18	Cl	OMe	8.58	10.34	5.067	4.985
19	E19	Br	H	3.2	4.87	5.495	5.312
20	E20	Br	F	6.48	8.14	5.188	5.089
21	E21	Br	Cl	4.12	5.87	5.385	5.231
22	E22	Br	Br	2.03	3.65	5.693	5.438
23	E23	Br	Me	5.58	7.04	5.253	5.152
24	E24	Br	OMe	7.96	9.27	5.099	5.033
25	E25	Me	H	1.08	2.24	5.967	5.65
26	E26	Me	F	2.01	3.53	5.697	5.452
27	E27	Me	Cl	1.66	3.11	5.78	5.507
28	E28	Me	Br	0.24	1.07	6.62	5.971
29	E29	Me	Me	1.16	2.52	5.936	5.599
30	E30	Me	OMe	4.24	6.23	5.373	5.206
31	E31	OMe	H	2.37	4.12	5.625	5.385
32	E32	OMe	F	5.95	8.04	5.225	5.095
33	E33	OMe	Cl	5.35	7.59	5.272	5.12
34	E34	OMe	Br	1.26	2.75	5.9	5.561
35	E35	OMe	Me	5.46	7.35	5.263	5.134
36	E36	OMe	OMe	8.89	10.48	5.051	4.98

Further 3D QSAR studies was performed using ChemOffice ultra, version 8.0 [41] and regression analysis was carried out by employing VALSTAT software [42]. All structures of pyrazolyl-thiazolinone derivatives were drawn using chemdraw and transferred to chem3D to convert them into 3D structures. The energy minimization of the molecule was done using molecular mechanics (MM2) until the root mean square (RMS) gradient value became smaller than 0.01 kcal/mol A^0. Further Minimized molecule was carried out to re-optimization by semi-empirical AM1 (Austin model) Hamiltonian method available in MOPAC module until the root mean square gradient value became smaller than 0.01 kcal/molA^0 for calculating partial atomic charges and electron density on various atoms. The descriptor values for all the molecules were calculated using "compute properties

module" of program. All calculated descriptors were taken into account as independent variable and inhibitory activity data was taken as dependent variable.

Generation of Training and Test Set

To evaluate the QSAR model, data sets were divided into training and test set using random selection method. Training set is used to develop the QSAR model for which biological activity data are known. Test set is used to challenge the QSAR model developed based on the training set to assess the predictive power of the model which is not included in model generation.

Regression Analysis and Model Validation

VALSTAT software was used to generate the QSAR model by employing Sequential Multiple Linear regression analysis. In SMLR the program searched for all permutation and combination sequentially for the data set. The data was transferred to statistical program in order to establish the correlation between physicochemical parameter and biological activity. The main objective of analysis is to determine the best variables which produce the most significant linear QSAR models linking the structure of the compounds.

The (±) data within parenthesis are the standard deviation, associated with coefficient of descriptors in regression equation. Validation is a crucial step in any QSAR modelling method. It is needed to establish the predictiveness of a model on unseen data and to help determine the complexity of an equation that the amount of data justifies. Model is validated both internally (least square fit R^2, Leave-one out-cross validation or q^2, γ randomization, boots-trapping r^2_{bs}) and externally (predictive ability of correlation coefficient r^2_{pred}). High value of q^2 alone is not sufficient criteria for selection of QSAR model. The best significant model was selected on the basis of various statistical parameter such as correlation coefficient(r), standard error of estimation(SE), Sequential Fischer test(F), bootstrapping squared correlation coefficient(r^2_{bs}), standard-deviation(S), cross validated squared correlation coefficient(r^2_{CV} or q^2), S_{PRESS}, S_{DEP}, r^2pred, randomize biological activity data set (chance) and test for outliers (z score) which confirms the robustness and applicability of QSAR equation on the structural analogues.

RESULTS AND DISCUSSION

Generation of Model for EGFR Receptor

In present study a data set of 36 compounds were taken into consideration to develop the QSAR model between physicochemical parameter as independent variable and inhibitory activity as dependent variable. Fujita ban approach is used to find out the de novo contribution of substituent to the scaffold. Several Statistical significant Quadra-variant equations were generated. Equation 1 was considered as best QSAR model on the prior of Statistics.

$$pIC_{50} = [5.282(\pm 0.077)]- F1\ [0.370(\pm 0.152)]+Me1[0.538(\pm 0.172)]+H2\ [0.223 (\pm 0.154)]+ Br2[0.494(\pm 0.152)] \quad \text{..... Eq. (1)}$$

$$n = 24,\ r = 0.933,\ r^2 = 0.870,\ q^2 = 0.794,\ F = 31.762,\ std. = 0.128$$

contribution of parameters to model is

$$F1:Me1:H2:Br2::1.659:1.808:1:2.212$$

Fujita ban model explains best squared correlation coefficient (r^2=0.870) between descriptors, which explains 87% variantion in biological activity. Value of Fischer test F=31.762 which is >99.9% statistically significant and squared correlation coefficient (q^2=0.794) suggested good predictive ability of model. At R1 position substitutuion with small group is favourable for activity, whereas R2 position is unsubstituted or less electronegative group significantly increase its inhibitory activity.

Further Hansch approach was taken into account to see the effect of substituents to the biological activity of molecules. In various significant equations, eq.(2) was selected as best QSAR model at 95% significance level.

$$pIC_{50} = [5.145(\pm 0.530)] - R1\mathcal{F}\ [1.444(\pm 0.515)]+ R1Biv\ [0.392(\pm 0.294)]+ R2\mathcal{F}\ [0.031(\pm 0.033)]+ R2\ \sigma_m\ [1.053(\pm 0.616)] \quad \text{.....Eq. (2)}$$

$$n = 24,\ r = 0.852,\ r^2 = 0.725,\ q^2 = 0.562,\ F = 12.532,\ std. = 0.229$$

contribution of parameters to model is

$$R1F:R1Biv:R2F:R2\sigma_m::1.934:4.540:1:1.618$$

This model gives us the idea about effect of steric (field effect, verloop's parameter) and electronic descriptors(hammett's constant σ) to the biological activity. Eq.(2) showed squared correlation coefficient r^2=0.725 which accounts 72% variance in activity. The cross validated squared correlation coefficient (q^2=0.562), Standard error of prediction (S_{DEP}=0.257), predictive squared correlation coefficient (r^2_{pred}=0.729) suggest good internal predictability of model.

The series was also carried out to QSAR study using 3D descriptors via Chem-Office ultra software. SEQ-MLR techinique was used to develop the QSAR model. Amongst all equations, Eq. (3) was selected as best significant model (Table 2).

pIC_{50} = [-18.145(±5.829)]+ $N_{1,4}$VDW[2.949(±0.926)]+ Y[0.001(±0.0001)+ MR [4.721(±1.319)]-P [2.056(±0.800)] .....Eq.(3)

n = 24, r = 0.914, r^2 = 0.835, q^2 = 0.742, F = 23.994, std. = 0.185

contribution of parameters to model is

N1,4VDW:Y:MR:P::7.097:1:20.876:2.872

Quadra variant model (3) has squared correlation coefficient (r^2=0.835) which explains 83% variance in inhibitory activity. Randomization test is also followed which ensures that model is not due to chance. Chance value of 0.001correspondance to 0.1% chance of fortuitous correlation. The predictive ability of the model is significantly explained by cross validated squared correlation coefficient (q^2=0.742), F-test (F=23.994), S_{PRESS}=0.232 and S_{DEP}=0.206 (Figures 2 and 3).

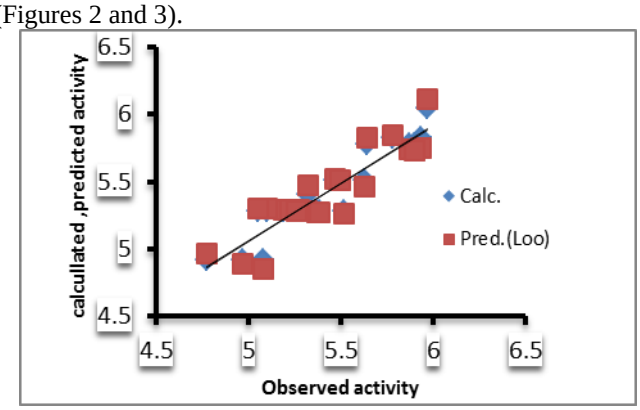


Figure 2: A plot between observed vs. calculated vs. predicted activity of model-1

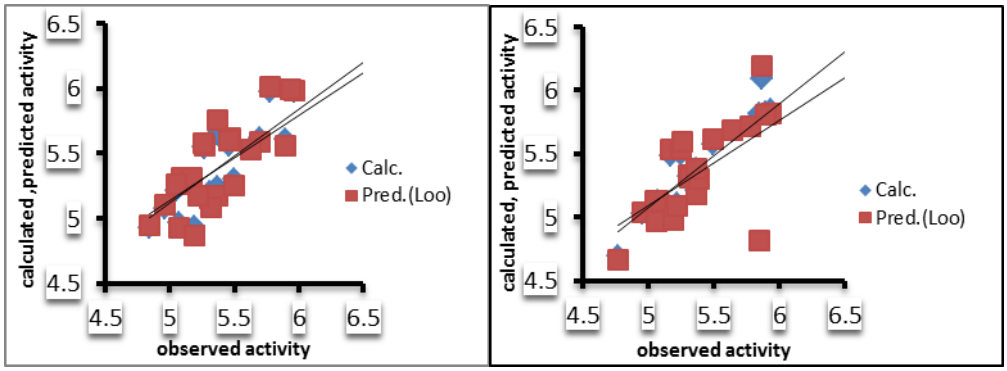


Figure 3: Plot of observed vs. calculated vs. predicted activity of model-2 and model-3

Generation of Model for HER-2 Receptor

To develop the model several approaches of QSAR such as Fujita ban, Hansch and 3D QSAR was employed using sequential multiple regression analysis. The best model was selected on the basis of statistical parameter.

pIC_{50} = [5.180(± 0.090)]- F1 [0.277(± 0.186)]-Cl1[0.169(± 0.144)]+Me1 [0.299 (± 0.166)]+Br2[0.368(± 0.144)] ..... Eq. (4)

n = 24, r = 0.886, r^2 = 0.786, q^2 = 0.659, F = 18.400, std. = 0.135

contribution of parameters to model is

F1:Cl1:Me1:Br2::1:1.22071:1.43897:2.21311

Fujita ban expression suggested that electronwithdrwaing group at R1 position of HER-2 receptor not favourable to its inhibitory activity, whereas substitution with electrondonating and lesser bulky group is conducive to its inhibitory activity. Eq.(4) showed better correlation coefficient (r=0.886) which explains 88% variance in activity. Model has squared correlation coefficient (r^2=0.786), cross validated squared correlation coefficient (q^2=0.659), S_{PRESS}=0.170 and S_{DEP}=0.152 which explains good internal consistency as well as predictivity of the model. Model exhibit internal significance level better than >99.9% with F value=18.400 and lower value of standard deviation s=0.135. Model was further analyzed for outlier by using z-score value. There was not found any outlier which suggested that model is able to explain structurally diverseifed molecule.

pIC_{50}= [5.311(±0.164)] + R1MR[0.029(±0.019)]- R1F[0.855(±0.318)]+ R2MR [0.020(±0.022) + R2R[0.685(±0.367)] .....Eq.(5)

n = 24, r = 0.848, r^2 = 0.719, q^2 = 0.566, F = 12.174, std. = 0.129

contribution of parameters to model is

R1MR:R1F:R2MR:R2R::1.55606:2.121:1:1.38151

Table 2: Observed, calculated and predicted IC_{50} (by the LOO method) of training set of pyrazolyl-thiazolinone derivatives as EGFR inhibitors

Fujita- ban Model-1				Hansch Model-2				3D-QSAR Model-3			
Compd.	**Obs.**	**Calc.**	**Pred.$_{(Loo)}$**	**Compd.**	**Obs.**	**Calc.**	**Pred.$_{(Loo)}$**	**Compd.**	**Obs.**	**Calc.**	**Pred.$_{(Loo)}$**
1	5.471	5.505	5.518	**1**	5.471	5.569	5.62	**2**	5.313	5.328	5.333
2	5.313	5.282	5.279	**2**	5.313	5.208	5.155	**4**	5.87	6.097	6.193
4	5.87	5.776	5.74	**3**	5.457	5.565	5.6	**6**	5.37	5.214	5.18
5	5.519	5.282	5.26	**6**	5.37	5.243	5.175	**7**	5.089	5.095	5.097
6	5.37	5.282	5.274	**9**	4.962	5.082	5.11	**8**	4.771	4.695	4.67
8	4.771	4.912	4.964	**10**	5.32	5.149	5.087	**9**	4.962	5.031	5.044
10	5.32	5.405	5.47	**14**	4.847	4.93	4.951	**10**	5.32	5.314	5.313
11	5.078	4.912	4.85	**15**	5.088	5.287	5.312	**11**	5.078	5.127	5.134
12	4.971	4.912	4.89	**17**	5.176	5.297	5.312	**14**	5.847	5.822	4.816
16	5.642	5.776	5.825	**18**	5.067	4.965	4.934	**16**	5.642	5.678	5.685
19	5.495	5.505	5.509	**19**	5.495	5.307	5.254	**17**	5.176	5.482	5.533
20	5.188	5.282	5.29	**20**	5.188	4.945	4.873	**18**	5.067	4.99	4.967
21	5.385	5.282	5.273	**25**	5.967	5.98	5.985	**19**	5.495	5.581	5.619
23	5.253	5.282	5.284	**26**	5.697	5.619	5.593	**20**	5.188	5.028	4.976
24	5.099	5.282	5.298	**27**	5.78	5.976	6.013	**21**	5.385	5.326	5.306
25	5.967	6.043	6.113	**28**	6.62	6.043	5.865	**23**	5.253	5.492	5.534
27	5.78	5.819	5.843	**29**	5.936	5.986	5.995	**27**	5.78	5.728	5.715
29	5.936	5.819	5.752	**30**	5.373	5.654	5.761	**28**	6.62	6.117	5.979
31	5.625	5.505	5.459	**31**	5.625	5.547	5.532	**29**	5.936	5.846	5.822
32	5.225	5.282	5.287	**32**	5.225	5.186	5.178	**30**	5.373	5.384	5.387
33	5.272	5.282	5.287	**33**	5.272	5.543	5.566	**32**	5.225	5.11	5.088
34	5.9	5.776	5.73	**34**	5.9	5.61	5.557	**34**	5.9	5.834	5.814
35	5.263	5.282	5.283	**35**	5.263	5.553	5.578	**35**	5.263	5.555	5.597
36	5.051	5.282	5.302	**36**	5.051	5.221	5.263	**36**	5.051	5.099	5.135

This model has squared correlation coefficient (r^2=0.719) which explains 72% variance in activity. Hansch approach suggested that substitution with bulkier group at both R1 and R2 position is optimum for its activity. The LOO cross validated squared correlation coefficient (q^2=0.566), S_{PRESS}=0.161 and S_{DEP} =0.143 suggested good internal predictive ability and as well as internal consistency of the model. Bootstrapping squared correlation coefficient (r^2_{bs}=0.766) explains model robustness. Model also exhibit internal significance level better than >99.9% with F value =12.174 (Figures 4 and 5) (Table 3).

pIC_{50}= [9.851(±3.368)]-T[5.458(±4.016)]+X[0.001(±0.001)]+HOMO [0.331(±0.355)]+E[0.001(±0.001)] .....Eq.(6)

n = 24, r = 0.856, r^2 = 0.732, q^2 = 0.591, F = 12.997, std. = 0.115

contribution of parameters to model is

T:X:HOMO:E::1:4.08169:14.5714:10.7514

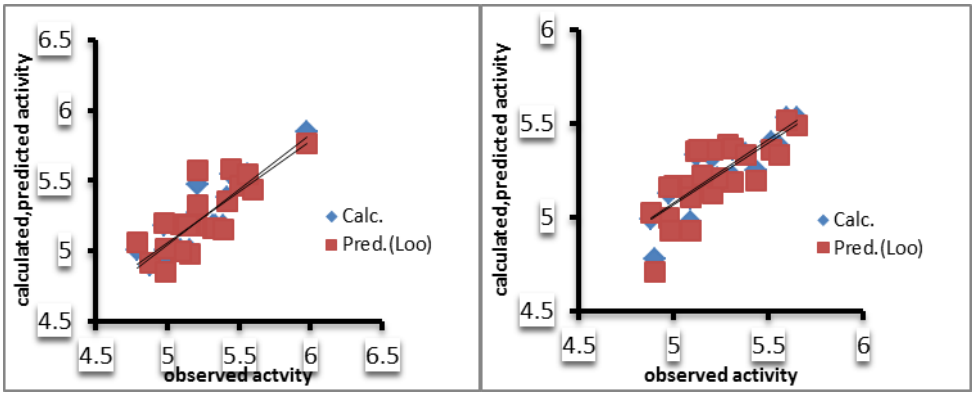


Figure 4: Plot of observed vs calculated vs predicted activity of model-4 and model-5

Table 3: Observed, calculated and predicted IC_{50} (by the LOO method) of training set of pyrazolyl-thiazolinone derivatives as EGFR and HER-2 inhibitors

Fujita- ban Model-4				Hansch Model-5				3D-QSAR Model-6			
Compd.	**Obs.**	**Calc.**	**Pred.$_{(Loo)}$**	**Compd.**	**Obs.**	**Calc.**	**Pred.$_{(Loo)}$**	**Compd.**	**Obs.**	**Calc.**	**Pred.$_{(Loo)}$**
2	5.197	5.179	5.178	**1**	5.291	5.362	5.388	**1**	5.291	5.34	5.364
3	5.316	5.179	5.164	**3**	5.316	5.358	5.366	**3**	5.316	5.361	5.366
5	5.333	5.179	5.163	**4**	5.516	5.4	5.361	**4**	5.516	5.549	5.559
9	4.881	4.902	4.914	**6**	5.207	5.147	5.125	**5**	5.333	5.339	5.341
10	5.205	5.27	5.323	**7**	4.978	4.991	4.998	**6**	5.207	5.167	5.152
11	4.989	4.902	4.853	**9**	4.881	4.987	5.023	**7**	4.978	5.143	5.167
13	5.152	5.01	4.981	**12**	4.906	4.777	4.707	**10**	5.205	5.22	5.222
14	4.785	5.01	5.058	**15**	5.002	5.152	5.166	**12**	4.906	4.941	4.955
15	5.002	5.01	5.012	**17**	5.09	5.158	5.164	**14**	4.785	4.88	4.917
16	5.416	5.378	5.359	**18**	4.985	5.942	5.189	**15**	5.002	5.21	5.235
17	5.09	5.01	4.994	**19**	5.312	5.213	5.189	**16**	5.416	5.384	5.378
18	4.985	5.01	5.016	**20**	5.089	4.978	4.927	**19**	5.312	5.236	5.221
22	5.438	5.548	5.587	**21**	5.231	5.209	5.205	**20**	5.089	5.038	5.03
23	5.152	5.179	5.183	**22**	5.438	5.251	5.197	**21**	5.231	5.213	5.211
27	5.507	5.479	5.468	**23**	5.152	5.215	5.223	**22**	5.438	5.429	5.426
28	5.971	5.847	5.766	**25**	5.65	5.529	5.486	**24**	5.033	5.039	5.041
29	5.599	5.478	5.435	**29**	5.599	5.532	5.517	**26**	5.452	5.172	5.151
30	5.206	5.478	5.578	**30**	5.206	5.315	5.359	**30**	5.206	5.19	5.186
31	5.385	5.179	5.157	**31**	5.385	5.337	5.328	**31**	5.385	5.29	5.221
32	5.095	5.179	5.189	**32**	5.095	5.102	5.105	**32**	5.095	4.927	4.861
33	5.12	5.179	5.186	**33**	5.12	5.333	5.353	**33**	5.12	5.24	5.274
34	5.561	5.548	5.543	**34**	5.561	5.376	5.334	**34**	5.561	5.488	5.442
35	5.134	5.179	5.185	**35**	5.134	5.339	5.358	**35**	5.134	5.089	5.085
36	4.98	5.179	5.202	**36**	4.98	5.123	5.158	**36**	4.98	5.106	5.168

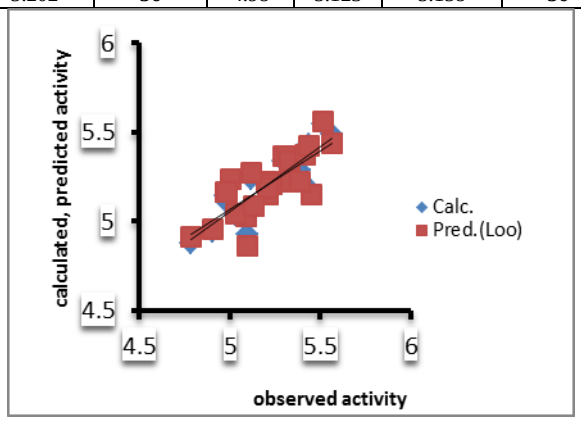


Figure 5: Plot of between observed vs calculated vs predicted activity of model 6

This model explains the internal predictive ability of model via cross validated squared correlation coefficient (LOO method) (q^2=0.591), predicted sum of square (S_{PRESS}=0.142), Standard error of prediction (S_{DEP} =0.127) as well as externally validated ability via predicted squared correlation coefficient (r^2_{pred}=0.838). Model also exhibit internal significance level better than >99.9% with F value=12.174 and standard deviation s=0.115. Bootstrapping analysis suggested the idea about the model's robustness and statistical confidence. This analysis gives an overview about the contribution of individual molecules to the QSAR model. Bootstrapping squared correlation coefficient (r^2_{bs}=0.764) and standard deviation(S_{bs}=0.116) shows the model is robust and promising. Randomization is a unique method of checking the descriptors used in the model because bioactivities are randomized ensuring the new model is created from bogus data set. It is followed which ensures that model is not due to chance. Chance value of 0.001correspondance to 0.1% chance of fortuitous correlation (Table 4).

Table 4: QSAR statistics of significant equation

Eq. N.	n	R	r^2	s.d.	F	q^2	ICAP*	Pred.r^2	S_{PRESS}	S_{DEP}	Outlier
1	24	0.93	0.87	0.128	31.762	0.794	<0.200	0.726	0.161	0.143	-
2	24	0.725	0.229	0.229	12.532	0.562	<0.267	0.729	0.289	0.257	-
3	24	0.914	0.835	0.185	23.99	0.742	<0.633	0.162	0.232	0.206	-
4	24	0.886	0.786	0.135	17.4	0.659	<0.258	0.838	0.17	0.152	-
5	24	0.848	0.719	0.129	12.174	0.566	<0.519	0.767	0.161	0.143	-
6	24	0.856	0.732	0.115	12.997	0.591	<0.387	0.629	0.142	0.127	-

*The maximum limit of inter-correlation among the descriptors used in generation of equations

CONCLUSION

QSAR analysis was performed on a series of pyrazolyl-thiazolinone derivatives as anticancer agents using molecular modelling program Chemoffice ultra 8.0 and VALSTAT software. QSAR models were developed by using Sequential multiple linear regression method. Fujita ban analysis in QSAR study reveals that absence of fluorine and chlorine group at R1 position in ring A whereas presence of methyl group at R1 position and bromine group at R2 position favourable to its anticancer activity. On the prior of Hansch analysis R1 position is substituted with smaller group having less steric effect with optimum polarity is favourable for its inhibitory activity at both EGFR and HER-2 receptors. Hammet's constant σ, refers to electron withdrawing property and is contributed positively to its inhibitory activity. Substitution with more bulky group at R2 position conducive to its anticancer activity (Figure 6). Further molecular modelling study exhibit that HOMO, Vanderwal force(N1,4VDW), principal moment of inertia on X and Y axis, molar–refractivity(MR), and total energy of molecule (E) contribute positively to its EGFR and HER-2 kinase inhibitory activity, whereas thermodynamic descriptors torsion(T) and partition-coefficient(P) negatively correlate to the model, means the group which decrease the hydrophobicity, may cause increase in biological activity, which is further supported by 2D QSAR, that means substitution with less bulky group at R1 position with optimum polarity greatly enhanced its activity. Principal moment of inertia X and Y describes mass distribution over the molecule on X and Y component in spatial arrangement, contributed positively to the activity suggesting that increases in bulkiness on X and Y component of molecule is favourable to its inhibitory activity, but 2D QSAR study suggested that steric effect is much important at the R1 position, which may helpful in development of more potent inhibitors.

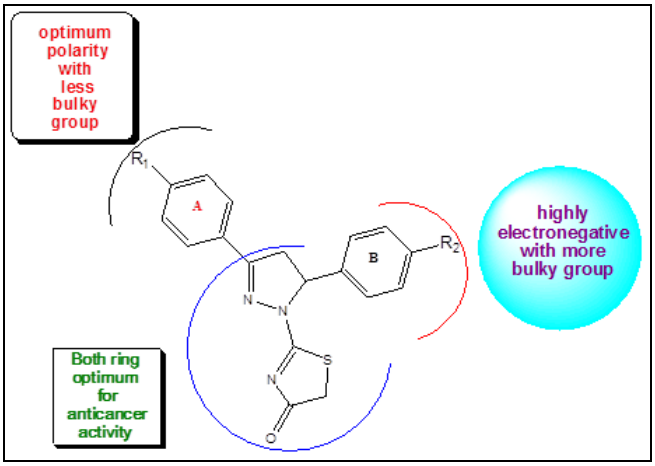


Figure 6: Structural template for anticancer activity

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