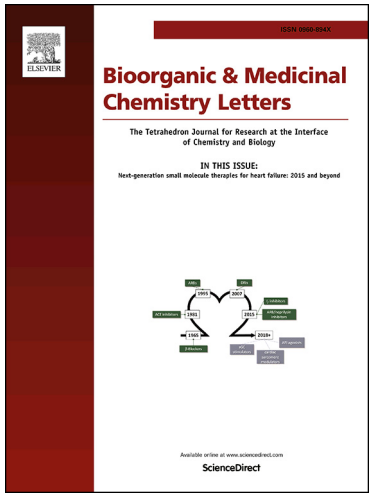

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Synthesis of novel hetero ring fused pyridine derivatives; their anticancer activity, CoMFA and CoMSIA studies

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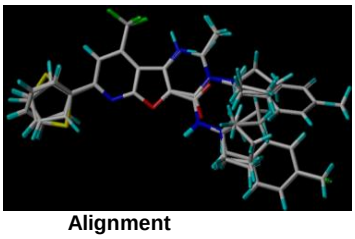
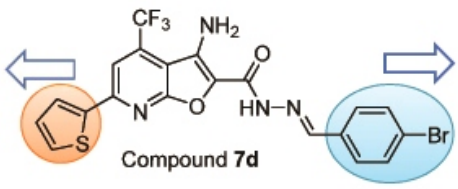
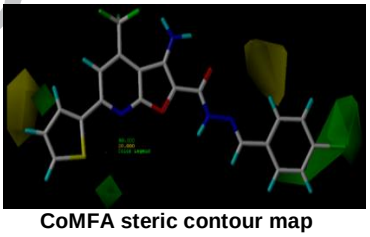


Alignment



Compound **7d**

IC_{50} (μM) = 5.2 ± 0.18
Steric Contribution = 73.6%



CoMFA steric contour map

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ABSTRACT

A series of novel furo[2,3-*b*]pyridine-2-carboxamide **4a-h**/pyrido[3',2':4,5]furo[3,2-*d*] pyrimidin-4(3*H*)-one derivatives **5a-p** were prepared from pyridin 2(1*H*) one **1** via selective O-alkylation with α-bromoethylester followed by cyclization, then reaction with different aliphatic primary amines to obtain **4** and further reaction with triethyl orthoacetate/triethyl orthoformate. Also prepared novel furo[2,3-b]pyridine-2-carbohydrazide Schiff's bases **7a-h** and pyrido [3',2':4,5]furo[3,2-*d*]pyrimidin-4(3*H*)-one derivatives **8a-h** starting from furo[2,3-*b*]pyridine carboxylate derivatives **3** by reaction with hydrazine hydrate to form **6** and reaction with diverse substituted aldehydes and cyclization. Products **4a-h**, **5a-p**, **7a-h** and **8a-h** were screened against four human cancer cell lines (HeLa, COLO205, Hep G2 and MCF 7) and one normal cell line (HEK 293). Compounds **4e, 4f, 4g, 5h, 7c, 7d, 7e** and **7f** showed significant anticancer activity against all the cell lines at micro molar concentration and found to be non-toxic to normal cell line. Studies for HeLa, COLO205 and MCF-7 using CoMFA and CoMSIA. Models from 3D-QSAR provided a strong basis for future rational design of more active and selective HeLa, COLO205 and MCF-7 cell line inhibitors.

Present statistics indicate that the cancer disease is second common cause of death after heart disease and continuously growing as worldwide killer.[1] Although chemotherapy is the backbone for cancer treatment, the use of chemotherapeutics is often limited due to undesirable side effects. Therefore, identification of new agents as targets for the treatment of cancer is very important. Some of the furopyridine and pyrimidi none derivatives (5,6 fused ring systems) found to have biological and chemotherapeutic importance. Furopyridines are structural analogues to indoles, pyrrolopyridines which are frequently employed as core scaffolds and play a significant role in promoting activity.[2,3] Furo[2,3-b]pyridine skeleton is rarely found in naturally occurring alkaloids;[4] however, it showed activity against HIV,[5] CNS disorders,[6] skin diseases[7] and hyperglycemia.[8] Furo[2,3-*b*]pyridine derivatives also demonstrated in vitro activity for tubulin polymerization,[9] Lck[10] and Akt[11] kinase inhibitors. A specific drug Cicletanine, L-754, 394 based on furopyridine, shows an antihypertensive with vasorelaxant, diuretic property[12,13] and potent HIV protease inhibitor. Fluorouracil and Tegafur based on pyrimidinone skeleton used as anticancer agents are outlined in Figure 1.

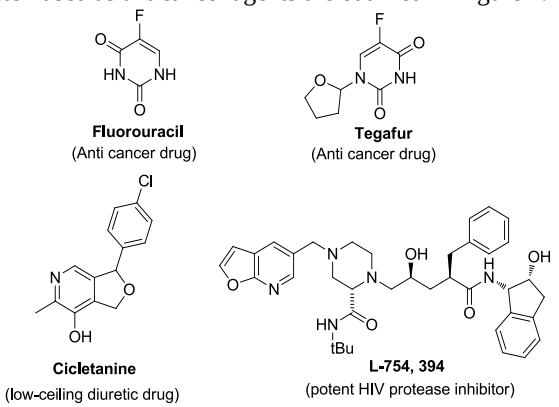

Figure 1. Bio-active compounds based on furopyridine and pyrimidinone scaffolds.

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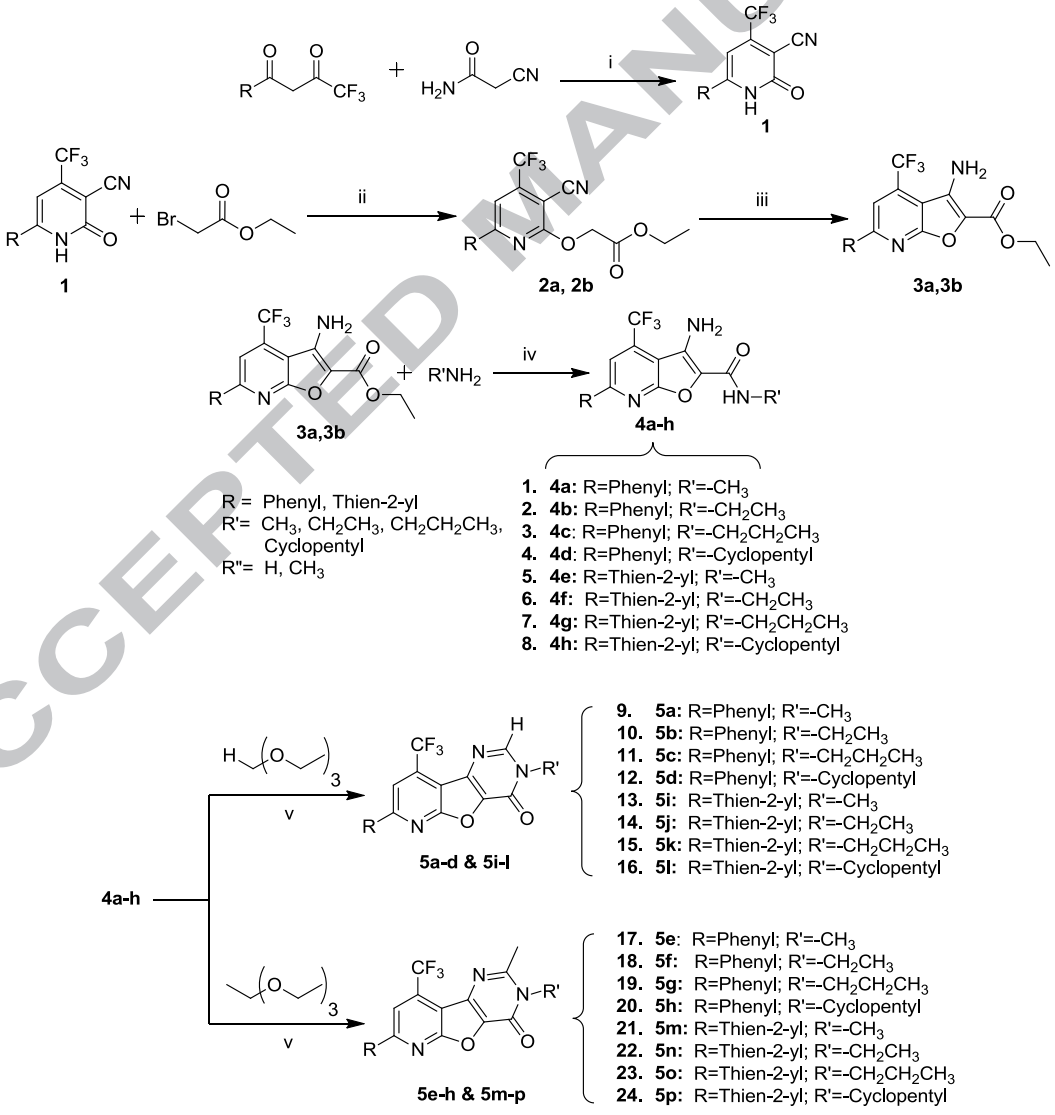
Fused pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy. Hydrazones have been demonstrated to possess antimicrobial, anticonvulsant, analgesic, anti-inflammatory, anti-platelet, anti-tubercular and anti-tumor activities. Hydrazide derivatives are not only intermediates, coupling products can be synthesized by using the active hydrogen component of –CONHN=CH– azo-methine group.[14] Similarly, thieno[2,3-b]quinoline-2-carboxamide derivatives are considered as anticancer agents.[15] Further, it was found that the fluorine[16] or trifluoromethyl[17,18] group at a strategic position of an organic molecule dramatically alters the properties of molecule in terms of lipid solubility, oxidative thermal stability thereby enhances the transport mechanism and bio-efficacy. However, no reports are available on synthesis of fluorinated furo [2,3-*b*] pyridine derivatives except our reports.[19,20] In continuation of our efforts, we designed and synthesized a series of novel trifluoromethyl substituted furo [2,3-*b*] pyridine-2-carbohydrazide Schiff's bases and pyrido [3',2':4,5] furo [3,2-*d*] pyrimidin-4(3*H*)-one derivatives and screened them for anticancer activity against four human cancer cell lines. Compounds **4e**, **4f**, **4g**, **5h**, **7c**, **7d**, **7l** and **7o** which showed promising anticancer activity have been identified.

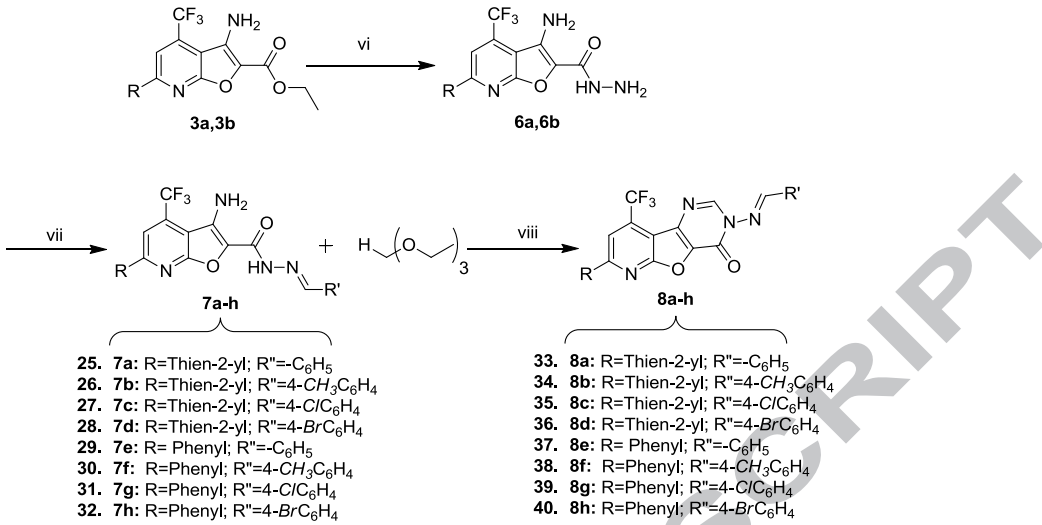
The 3-cyano-4-trifluoromethyl-6-substituted pyridine 2(1*H*) one **1** was reacted with 2-bromoethyl acetate under basic conditions and obtained selectively 2-O-ethylacetoxy-3-cyano-4-trifluoro methyl-6-substituted pyridine derivatives **2**. Compounds **2** were cyclized in DMF using potassium carbonate as base and obtained furo[2,3-*b*]pyridine derivatives **3**. The reaction sequence includes selective O-alkylation then abstraction of proton by base from an active methylene followed by cyclization onto nitrile carbon. This type of cyclization is also known as Thorpe-Ziegler cyclization. Compounds **3** were reacted with different substituted aliphatic primary amines to result in carboxamide derivatives **4** and were further independently reacted with triethyl orthoacetate and triethyl orthoformate to obtain pyrido furo pyrimidinone derivatives **5a-p**. The sequence of reactions outlined in Scheme 1.

Scheme 1



Reagents and conditions (i) EtOH, Piperidine, reflux, 4-5 h (ii) K_2CO_3, NaI, Acetone, reflux, 6h (iii) DMF, K_2CO_3, 110 ºC, 6h. (iv) Neat, Δ, 6h (v) Acetic acid, 140 ºC, 3 h.

Scheme 2



Reagents and conditions: (vi) $N_2H_4.H_2O$, EtOH, 80 ºC, 4-6h. (vii) R′CHO, EtOH, cat. Piperidine, 80 ºC, 2h (viii) cat. Acetic acid, 120 ºC, 3h.

Compounds **3** were reacted with hydrazine hydrate to result carbohydrazide derivatives **6** which were further reacted with diverse substituted aromatic aldehydes to obtain furo[2,3-*b*]pyridine-2-carbohydrazide Schiff's bases **7**. Compounds **7** were reacted with TEOF (triethyl orthoformate) in the presence of catalytic amount of acetic acid and obtained the final products of furo pyrido pyrimidinone derivatives **8a-h**. The sequence of reactions outlined in Scheme 2.

Anticancer activity & structure-activity relationship

Compounds **4a-h** and **5a-p** were screened against four human cancer cell lines such as HeLa (cervical cancer, CCL-2), COLO-205 (colon cancer, CCL-222), HepG2 (liver cancer, HB-8065) and MCF7 (breast cancer, HTB-22) using MTT assay.[21] Among all the compounds screened, compounds **4e**, **4f**, **4g**, and **5h** showed promising activity at micro molar concentration. The structure-activity relationship studies revealed that the carboxamide derivatives **4a-h** showed more promising activity as compared to pyrimidinone derivatives **5a-p**. It was attributed to the presence of amide and primary amine group in compounds **4a-h** and was considered as polar groups. Among all the compounds screened in **4a-h** series, compounds **4e-g** showed promising activity. Similarly, in **5a-p** series, the presence of thiophenyl group in 6th position was crucial for activity and compound **5h** exhibited the promising activity. The activity data is shown in Table 1 & 2.

Table 1. *In vitro* cytotoxicity of compounds **4a-h.**

Compd.	IC_{50} values (in µM)				
	HeLa	**COLO205**	**HepG2**	**MCF7**	**HEK293**
4a	40.2 ± 0.22	36.8 ± 0.26	41.2 ± 0.28	37.4 ± 0.18	62 ± 0.26
4b	36.2 ± 0.21	29.2 ± 0.24	42.8 ± 0.14	54.0 ± 0.22	70 ± 0.44
4c	36.1 ± 0.32	38.9 ± 0.23	37.4 ± 0.27	35.6 ± 0.32	81 ± 0.51
4d	25.8 ± 0.28	51.1 ±0.41	32.4 ± 0.32	59.8 ± 0.28	85 ± 0.22
4e	15.6 ± 0.25	17.8 ± 0.24	12.3 ± 0.32	18.0 ± 0.18	75 ± 0.35
4f	14.2 ± 0.12	18.7 ± 0.28	12.1 ± 0.20	21.8 ± 0.22	62 ± 0.19
4g	18.9 ± 0.18	15.3 ± 0.31	17.2 ± 0.17	20.4 ± 0.20	64 ± 0.22
4h	21.8 ± 0.09	18.4 ± 0.29	20.8 ± 0.15	22.9 ± 0.19	51 ± 0.16
5-Fluorouracil (Std control)					
	1.8 ± 0.09	1.9 ± 0.11	1.7 ± 0.08	1.8 ± 0.07	19.6 ± 0.18

Table 2. In vitro cytotoxicity of compounds **5a-p.**

Compd.	IC_{50} values (in µM)				
	HeLa	**COLO205**	**HepG2**	**MCF7**	**HEK293**
5a	---	---	220.9 ± 0.41	---	---
5b	---	---	---	82.4 ± 0.52	121 ± 0.52
5c	---	---	---	85.5 ± 0.36	90 ± 0.48
5d	---	---	---	---	---
5e	---	---	---	---	---
5f	---	---	---	---	---
5g	---	---	---	116.8±0.38	52 ± 0.32
5h	18.2 ± 0.12	18.7 ± 0.28	17.1 ± 0.20	22.8 ± 0.22	96 ± 0.28
5i	---	---	---	---	---
5j	---	---	---	---	---
5k	---	---	---	---	---
5l	50.2 ± 0.42	42.5 ± 0.36	49.8 ± 0.23	62.7 ± 0.38	---
5m	---	---	---	---	---
5n	---	---	---	---	---
5o	28.5 ± 0.18	18.9 ± 0.18	22.8 ± 0.42	31.5 ± 0.42	68 ± 0.34
5p	---	---	---	---	124 ± 0.22
5-Fluorouracil (Std control)					
	1.8 ± 0.09	1.9 ± 0.11	1.7 ± 0.08	1.8 ± 0.07	19.6 ± 0.18

---indicates IC50 value > 220 µg/mL; Cell lines used: HeLa - Cervical cancer (CCL-2); COLO 205- Colon cancer (CCL-222); HepG2- Liver cancer (HB-8065); MCF7 - Breast cancer (HTB-22); HEK-293 –Human Embryonic Kidney cells (CRL-1573).

Compounds **7a-h** and **8a-h** were also screened against four human cancer cell lines such as HeLa, COLO-205, HepG2 and MCF7. Compounds **7a-h** are uncyclized Schiff's base products, while compounds **8a-h** were cyclized pyrimidinone products. The structure verses activity revealed that the presence of $-NH_2$ on furyl and amide linkage (–NHCO) are crucial for activity. Among all the compounds screened, compounds **7a-h** showed promising activity as compared to compounds **8a-h**. Compounds **7c**, **7d**, **7e** and **7f** were found to exhibit high activity. Among all the compounds **7c**, **7d**, **7e** and **7f** were found to be more potent with IC_{50} values of < 10 µg/mL on all the tested cancer cell lines. The activity data to this regard is tabulated in Table 3 & 4. Compounds **7c** and **7d** were considered as lead molecules for further optimization. The introduction of thiophenyl group at 6th position of furopyridine and chloro, bromo substituents at 4th position of phenyl ring enhanced the activity to a greater extent as compared to other compounds. However, the same substituents for compounds **8c** and **8d** could not show activity

due to non-availability of $–NH_2$ group and uncyclized amide linkage (–NHCO). Remaining compounds were not active upto the concentration of 60 µg/mL. The activity data is shown in Table 3.

Table 3. In vitro cytotoxicity of compounds **7a-h** & **8a-h**.

Compd	IC_{50} Values in (µM)				
	HeLa	**COLO205**	**HepG2**	**MCF7**	**HEK293**
7a	11.8±0.21	9.7±0.11	9.9±0.22	12.1±0.21	72±0.36
7b	24.4±0.11	21.2±0.32	19.8±0.23	18.7±0.19	78±0.21
7c	8.0±0.19	7.8±0.48	8.1±0.46	7.4±0.52	83±0.22
7d	6.4±0.32	5.9±0.34	6.1±0.26	5.2±0.18	86±0.28
7e	13.4±0.33	12.2±0.28	13.1±0.31	14.2±0.48	70±0.16
7f	11.8±0.51	9.1±0.21	8.9±0.44	10.2±0.11	69±0.22
7g	4.1±0.15	11.1±0.46	12.1±0.14	11.9±0.09	81±0.52
7h	23.9±0.41	21.2±0.05	19.9±0.13	18.7±0.22	64±0.35
8a	42.3±0.13	---	---	---	90±0.41
8b	42.3±0.23	---	---	29.7±0.28	---
8c	---	51.2±0.31	---	42.1±0.22	102±0.34
8d	---	---	---	---	---
8e	---	---	---	---	---
8f	---	---	---	---	---
8g	---	43.6±0.4	---	56.8±0.25	---
8h	---	---	---	---	---
5-Fluorouracil (Std control)					
	1.8±0.09	1.9±0.11	1.7±0.08	1.8±0.07	19.6 ± 0.18

---indicates IC50 value > 220 µg/mL; Cell lines used: HeLa - Cervical cancer (CCL-2); COLO 205- Colon cancer (CCL-222); HepG2- Liver cancer (HB-8065); MCF7 - Breast cancer (HTB-22); HEK-293 –Human Embryonic Kidney cells (CRL-1573).

All the above compounds were also screened against human normal cell line (HEK-293, Human Embryonic Kidney cells, CRL-1573) and they were found to be not cytotoxic upto the concentration of 51 µg/mL to 124 µg/mL as compared to 5-Fluorouracil (19.6 µg/mL).

QSAR Introduction

In general, the 3D-QSAR techniques are valuable methods of ligand-based drug design by correlating physicochemical properties from a set of related compounds to their known molecular property or molecular activity values. Validation of QSAR models plays the vital role in defining the applicability of the QSAR model for the prediction of designed molecules. QSAR model is mostly used to correlate properties, *i.e*. biological activities with chemical structures, and also used to predict the biological activity of non-synthesized compounds, which are structurally related to training sets. The present investigation reports the first application of 3D-QSAR to study of furo [2,3-*b*] pyridine and pyrido [3',2':4,5] furo [3,2-*d*] pyrimidin-4(3*H*)-one derivatives as potent anticancer agents. We studied twenty one compounds for HeLa and COLO205 cell lines, twenty six compounds for MCF-7 cell line inhibitors as anticancer agents using CoMFA (comparative molecular field analysis)[22] and CoMSIA (comparative molecular similarity indices analysis).[23] Models obtained from 3D-QSAR studies provided a strong basis for future rational design of more active and selective HeLa, MCF-7 and COLO205 cell line inhibitors.

Results and discussion

CoMFA and CoMSIA

CoMFA and CoMSIA methods were applied to derive 3D-QSAR models for furo [2,3-*b*] pyridine and pyrido [3',2':4,5] furo [3,2-*d*] pyrimidin-4(3*H*)-one derivatives as potential anticancer inhibitors. The statistical results of CoMFA and CoMSIA analysis are summarized in Table 4. Best predictions were obtained with CoMFA standard model involving cross-validated coefficient (q^2) = 0.820, 0.876 and 0.853 for HeLa, MCF-7 and COLO205 cell lines, respectively, correlation coefficient (r^2)= 0.960, 0.959 and 0.978 for HeLa, MCF-7 and COLO205, respectively. Standard Error of Estimate (SEE) =0.084, 0.081 and 0.056 for HeLa, MCF-7 and COLO205, respectively. Cross Validation (cv) = 0.818, 0.867 and 0.841 and Fischer statistic (F-value) = 47.511, 75.303 and 87.829 for HeLa, MCF-7 and COLO205, respectively with number of components as 5 and column filtering 2.0 kcal/mol. In CoMSIA, the standard model predictions obtained are q^2 = 0.812, 0.824 and 0.885, r^2 = 0.946, 0.959 and 0.980, SEE =0.102, 0.084 and 0.056, CV = 0.804, 0.814 and 0.889 and F-value = 26.373, 59.015 and 72.306 respectively for HeLa, MCF-7 and COLO205 respectively, with number of components as 6 and column filtering 1.0 kcal/mol. Except for COLO205, CoMFA results show a higher predictive ability for furo [2,3-*b*] pyridine and pyrido [3',2':4,5] furo[3,2-*d*] pyrimidin-4(3*H*)-one against anticancer on comparison with the CoMSIA results.

Table 4. The statistical results of CoMFA and CoMSIA analysis

	HeLa				**MCF-7**				**COLO205**			
	CoMFA		**CoMSIA**		**CoMFA**		**CoMSIA**		**CoMFA**		**CoMSIA**	
q^2	0.820		0.812		0.876		0.824		0.853		0.885	
r^2	0.960		0.946		0.959		0.959		0.978		0.980	
SEE	0.084		0.102		0.081		0.084		0.056		0.056	
F-Value	47.511		26.373		75.303		59.015		87.829		72.306	
CV	0.818		0.804		0.867		0.814		0.841		0.889	
N	5		6		5		6		5		6	
Bootstrap												
	Mean	Std.dev	Mean	Stddev	Mean	Std.dev	Mean	Stddev	Mean	Std.dev	Mean	Stddev
SEE	0.055	0.051	0.052	0.059	0.064	0.042	0.067	0.045	0.034	0.026	0.030	0.026
r^2	0.983	0.015	0.984	0.023	0.973	0.012	0.973	0.010	0.991	0.005	0.994	0.004
Field Contribution (%)												
Steric	66.7		23.1		69.9		32.8		73.6		21.7	
Electrostatic	33.3		21.5		30.1		26.9		26.4		19.2	
Hydrophobic	-		34.0		-		07.8		-		30.8	
Donor	-		16.3		-		17.1		-		23.0	
Acceptor	-		5.1		-		15.4		-		05.3	

For COLO205, CoMSIA results are better predictive ability for these compounds. Bootstrapping method is used to evaluate the robustness and the statistical confidence of the QSAR model. It involves simulating a large number of datasets which are of the The table 4 shows the results of relative contributions for CoMFA and CoMSIA methods. For CoMFA, 66.7%, 69.9% and 73.6% field contribution was observed for steric, and 33.3%, 30.1% and 26.4% field contribution was observed for electrostatic, respectively, for HeLa, MCF-7 and COLO205 cell lines. For CoMSIA, the observed contributions for steric, electrostatic, hydrophobic, donor and acceptor properties are given in Table 4, respectively. Electrostatic property is the main contributor in CoMSIA analysis. The affinities between same size as original and are produced by randomly selecting samples from the original set. Biological activities, predicted and residual values of both training set and test set CoMFA and CoMSIA are shown in Table 4.

experimental and calculated values of the training set and the test set of CoMFA and CoMSIA models derived from non-cross-validated analysis of HeLa; MCF-7; COLO205 cell lines are plotted in Figure 3(a), 3(b); 3(c), 3(d); 3(e) 3(f), respectively. CoMSIA and the best CoMFA models were used to predict the inhibitory activities of the compounds in the test set.

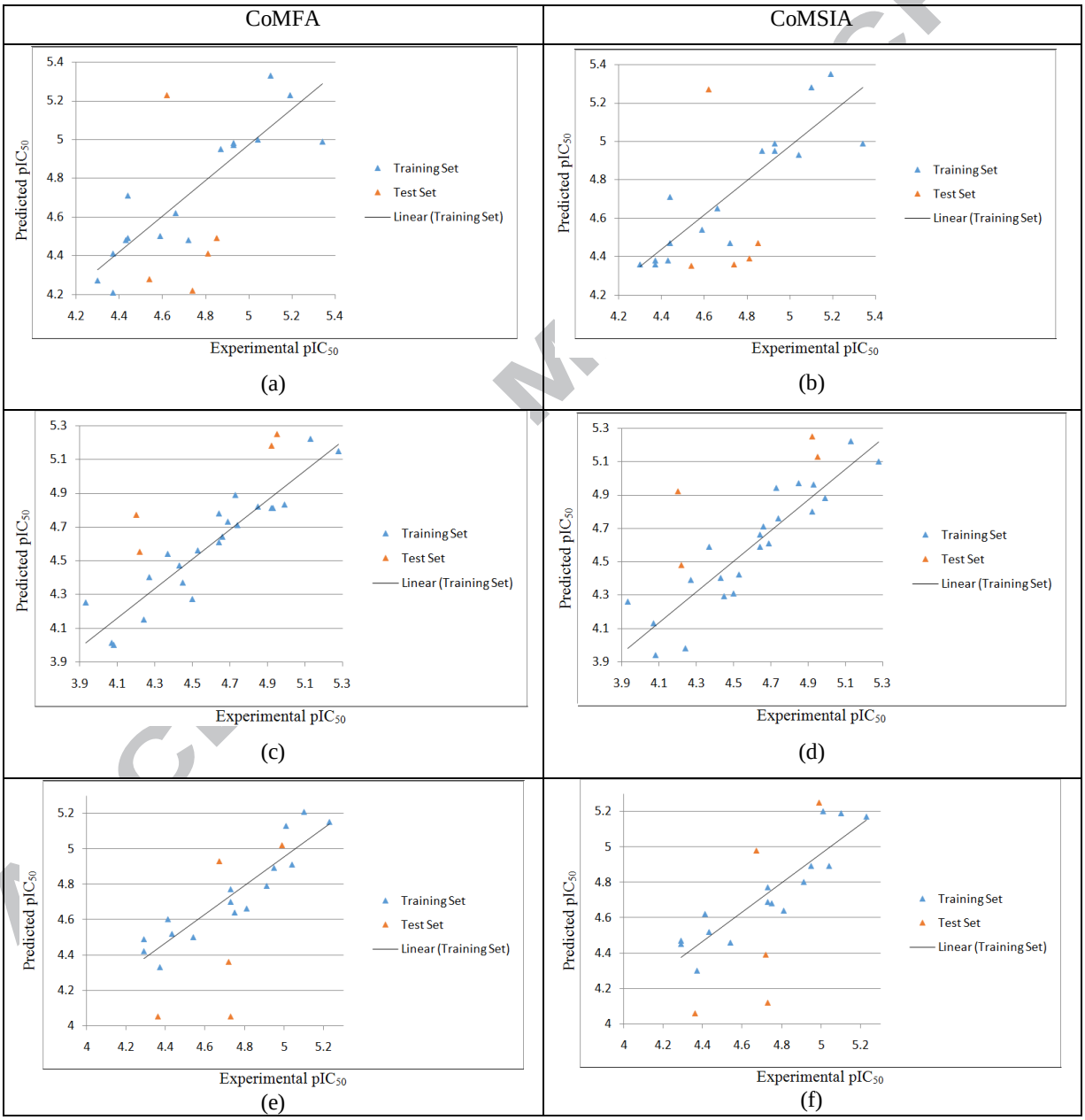


Figure 3. Calculated pIC_{50} versus experimental pIC_{50} values for the molecules of the training set (Blue color) and test set (red color) obtained by PLS analysis using CoMFA and CoMSIA models for (a), (b); (c), (d); (e) (f)

Table 5. Experimental and predicted activities (pIC_{50}) of the training set molecules for furo[2,3-b]pyridine and pyrido[3',2':4,5]furo[3,2-*d*]pyrimidin-4(3*H*)-one derivatives as HeLa Cell line inhibitors.

HeLa - Training Set					
C.No	pIC_{50}	CoMFA		CoMSIA	
		Predicted	Residual	Predicted	Residual
4a	4.34	4.48	-0.05	4.38	0.05
4b	4.44	4.49	-0.05	4.47	-0.03
4c	4.44	4.71	-0.27	4.71	-0.27
4d	4.59	4.50	0.09	4.54	0.05
4g	4.72	4.48	0.24	4.47	0.25
4h	4.66	4.62	0.04	4.65	0.01
5i	4.30	4.27	0.03	4.36	-0.06
7a	4.93	4.97	-0.04	4.95	-0.02
7b	5.04	5.00	0.04	4.93	0.11
7c	5.10	5.33	-0.23	5.28	-0.18
7d	5.19	5.23	-0.24	5.35	-0.16
7e	4.87	4.95	-0.08	4.95	-0.08
7f	4.93	4.98	-0.05	4.99	-0.06
7g	5.39	4.99	0.35	4.99	0.35
8a	4.37	4.21	0.16	4.36	0.01
8b	4.37	4.41	-0.04	4.38	-0.01

Table 6. Experimental and predicted activities (pIC_{50}) of the test set molecules for furo[2,3-*b*]pyridine and pyrido [3',2':4,5] furo[3,2-*d*]pyrimidin-4(3*H*)-one derivatives as HeLa Cell line inhibitors

HeLa - Test Set					
C.No	pIC_{50}	CoMFA		CoMSIA	
		Predicted	Residual	Predicted	Residual
4e	4.81	4.41	0.40	4.39	0.42
4f	4.85	4.49	0.36	4.47	0.38
5h	4.74	4.22	0.52	4.36	0.38
5o	4.54	4.28	0.26	4.35	0.19
7h	4.62	5.23	-0.61	5.27	-0.65

Table 7. Experimental and predicted activities (pIC_{50}) of the training set molecules for furo[2,3-b]pyridine and pyrido [3',2':4,5] furo[3,2-*d*]pyrimidin-4(3*H*)-one derivatives as MCF-7 Cell line inhibitors

MCF-7 - Training Set					
C.No	pIC_{50}	CoMFA		CoMSIA	
		Predicted	Residual	Predicted	Residual
4a	4.43	4.47	-0.04	4.40	0.03
4b	4.27	4.40	-0.13	4.39	-0.12
4c	4.45	4.37	0.08	4.29	0.16
4e	4.74	4.71	0.03	4.76	-0.02
4f	4.66	4.64	0.02	4.71	-0.05
4g	4.69	4.73	-0.04	4.61	0.08
4h	4.64	4.78	-0.14	4.66	-0.02
5b	4.08	4.00	0.08	3.94	0.14
5c	4.07	4.01	0.06	4.13	-0.06
5g	3.93	4.25	-0.32	4.26	-0.33
5h	4.64	4.61	0.03	4.59	0.05
5o	4.50	4.27	0.23	4.31	0.19
5p	4.93	4.81	0.12	4.96	-0.03
7c	5.13	5.22	-0.09	5.22	-0.09
7d	5.28	5.15	0.13	5.10	0.18
7e	4.85	4.82	0.03	4.97	-0.12
7f	4.99	4.83	0.16	4.88	0.11
7g	4.92	4.81	0.11	4.80	0.12
7h	4.73	4.89	-0.16	4.94	-0.21
8b	4.53	4.56	-0.03	4.42	0.11
8c	4.37	4.54	-0.17	4.59	-0.22
8g	4.24	4.15	0.09	3.98	0.26

Table 8. Experimental and predicted activities (pIC_{50}) of the test set molecules for furo[2,3-*b*]pyridine and pyrido [3',2':4,5] furo[3,2-*d*]pyrimidin-4(3*H*)-one derivatives as MCF-7 Cell line inhibitors

MCF-7 - Test Set					
C.No	pIC_{50}	CoMFA		CoMSIA	
		Predicted	Residual	Predicted	Residual
4d	4.22	4.55	-0.33	4.48	-0.26
5l	4.20	4.77	-0.57	4.92	-0.72
7a	4.92	5.18	-0.26	5.25	-0.33
7b	4.95	5.25	-0.30	5.31	-0.18

Table 9. Experimental and predicted activities (pIC_{50}) of the training set molecules for furo [2,3-*b*] pyridine and pyrido [3',2':4,5] furo [3,2-*d*] pyrimidin-4(3*H*)-one derivatives as COLO205 Cell line inhibitors

COLO205 - Training Set					
C.No	pIC_{50}	CoMFA		CoMSIA	
		Predicted	Residual	Predicted	Residual
4a	4.43	4.52	-0.09	4.52	-0.09
4b	4.54	4.50	0.04	4.46	0.08
4c	4.41	4.60	-0.19	4.62	-0.21
4d	4.29	4.42	-0.13	4.45	-0.16
4e	4.75	4.64	0.11	4.68	0.07
4f	4.73	4.77	-0.04	4.77	-0.04
4g	4.81	4.66	0.15	4.64	0.17
4h	4.73	4.70	0.03	4.69	0.04
5l	4.37	4.33	0.04	4.30	0.07
5a	5.01	5.13	-0.12	5.20	-0.19
7c	5.10	5.21	-0.11	5.19	-0.09
7d	5.23	5.15	0.08	5.17	0.06
7e	4.91	4.79	0.12	4.80	0.11
7f	5.04	4.91	0.13	4.89	0.15
7g	4.95	4.89	0.06	4.89	0.06
8c	4.29	4.49	-0.20	4.47	-0.18

Table 10. Experimental and predicted activities (pIC_{50}) of the test set molecules for furo [2,3-*b*] pyridine and pyrido [3',2':4,5] furo[3,2-*d*]pyrimidin-4(3*H*)-one derivatives as COLO205 Cell line inhibitors

COLO205 - Test Set					
C.No	pIC_{50}	CoMFA		CoMSIA	
		Predicted	Residual	Predicted	Residual
5h	4.73	4.05	0.68	4.12	0.61
5o	4.72	4.36	0.36	4.39	0.33
7b	4.99	5.02	-0.03	5.25	-0.26
7h	4.67	4.93	-0.26	4.98	-0.31
8g	4.36	4.05	0.31	4.06	0.30

Contour analysis

CoMFA and CoMSIA steric contour plots

For HeLa, the CoMFA model was used to generate the three dimensional contour maps to represent the quantitative structure activity result. A single green polyhedron present above Br at 4th position of C_6H_4. It indicates that the position contains more steric hindrance. The compound shows more yellow contours at thien-2-yl and 4-BrC_6H_4 positions, which indicates that steric hindrance is less. In CoMSIA a very large green contour present above the C_6H_4 ring, which increases the steric hindrance and a large yellow contour present at C_6H_4. Adding bulk groups at this ring decreases the biological activity. In MCF-7 CoMFA, a medium and a small green contours present around C_6H_4 and

two small contours present around thien-2-yl position which indicated that bulky groups had favorable steric interactions. The compound shows two yellow contours one at thien-2-yl and another at second position of C_6H_4 ring, it explains bulky groups had unfavourable steric interactions. In CoMSIA, a medium green contour above 2nd position of C_6H_4 ring. While adding the bulk groups at this position increases the biological activity and a very large yellow contour present above 2nd position of the C_6H_4 ring which indicates decrease in the activity. In CoMFA, COLO205 the most active compound shows a large green polyhedron at 4-BrC_6H_4, and two small contours around thien-2-yl ring indicating steric favourable region where biological activity increases by adding bulky groups. Two medium sized yellow contours present at 2nd position of C_6H_4 ring and at thien-2-yl ring where bulky groups decreases the activity of the compound. In CoMSIA, the most active compound shows a large green contour present above Br of 4th position of C_6H_4 where activity increases, and a large yellow contour present above 3rd position of C_6H_4 which decreases the activity by adding bulk groups. CoMFA and CoMSIA steric contour plots are shown in figure 4.

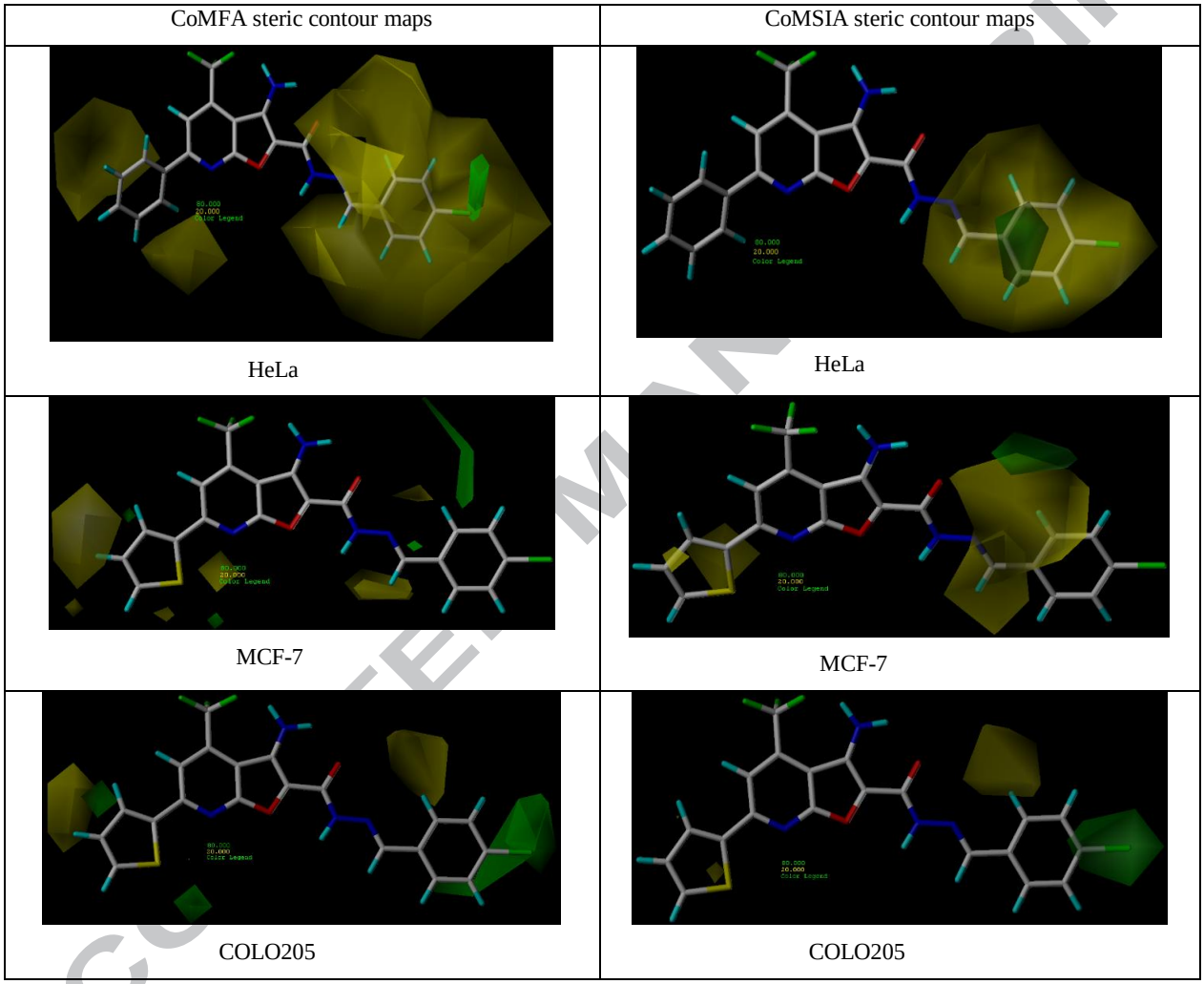


Figure 4. CoMFA and CoMSIA steric contour plots

CoMFA electrostatics contours plots

For HeLa, the electrostatic contours maps of CoMFA are shown in blue and red colour. Two medium sized blue contours present near O-NH-N position indicating addition of positive charge groups may increase the biological activity of a compound. The blue contours are in favorable region and red contours in disfavorable region. There is a large red contour present around the O-NH-N, at this position electron density is estimated to increase the biological activity. By adding electronegative groups may enhance the activity. In MCF-7 only blue color contours are present at thien-2-yl position and near the NH-N position which increases the biological activity. Whereas in COLO205 a small blue color contour present at NH-N position and two medium sized red colored contours present above oxygen of O-NH position and at N position. Where addition of negative groups enhance the biological activity. Figure 5 shows contour maps of CoMFA and CoMSIA electrostatics contour maps.

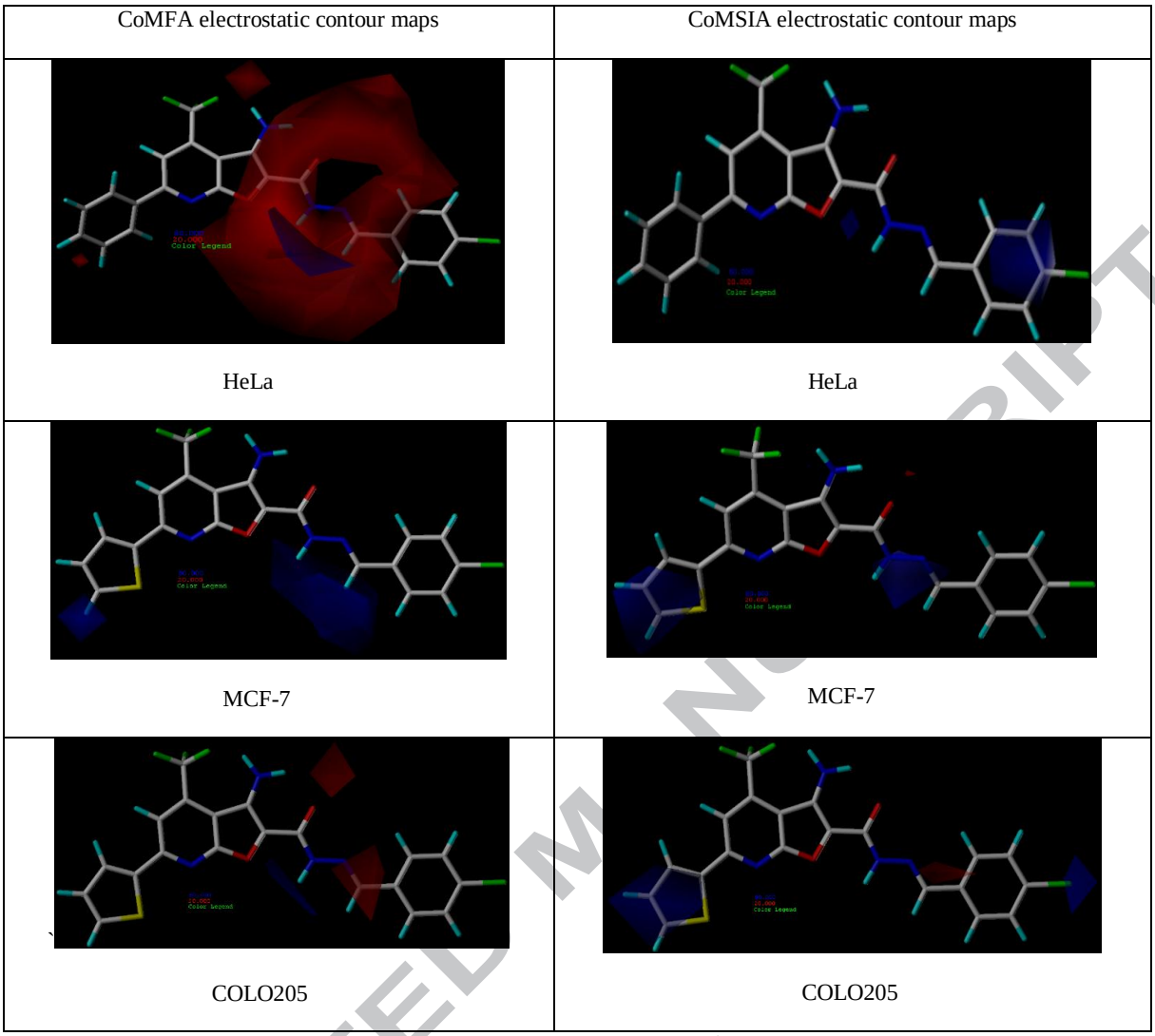
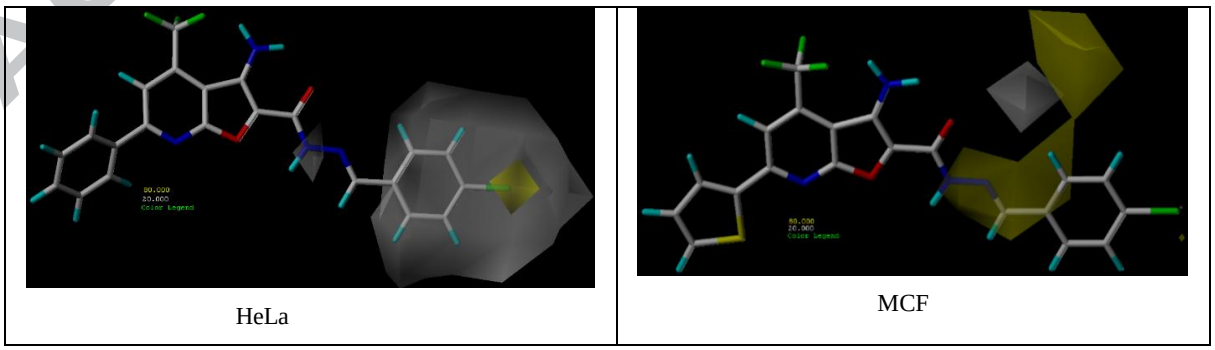
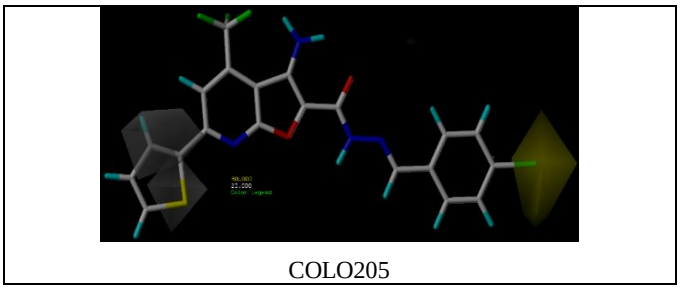


Figure 5. CoMSIA steric and electrostatic contour maps for highly active compound

CoMSIA Hydrophobic contour analysis

CoMSIA hydrophobic contour maps are shown in white and yellow. A small to medium sized yellow contours present above Br of the C_6H_4 ring for both HeLa and COLO205 targets and a large yellow contour present at N-NH position for MCF-7 indicates favorable for hydrophobic substitution increase the activity. A large white contour present around C_6H_6 ring for HeLa, a medium sized white contour present above N-NH position indicates disfavored conformation for hydrophobic substitution for MCF-7. A medium sized white contour present above thien-2-yl ring indicating disfavored region. CoMSIA hydrophobic contour plots are shown in figure 6.



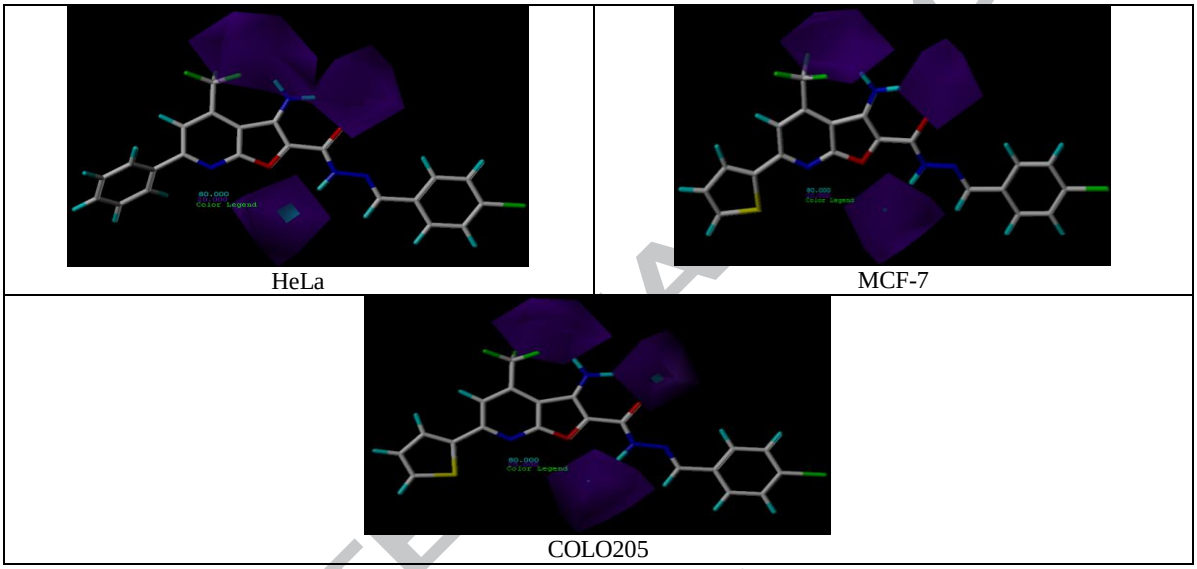


COLO205

Figure 6. CoMSIA hydrophobic contour maps for highly active compound

CoMSIA hydrogen bond donor contour analysis

The graphical representation of the field contributions of the hydrogen bond donor is shown in Figure 7. Donor contour maps are shown in cyan and purple color. Cyan contour maps explains the position of hydrogen bond donor groups which increases biological activity, while purple contours are biologically unfavored. For HeLa, MCF-7, COLO205 the most active compound shows a small cyan isopleth which enhance the biological activity, and the presence of three large purple isopleths reduces the biological activity.



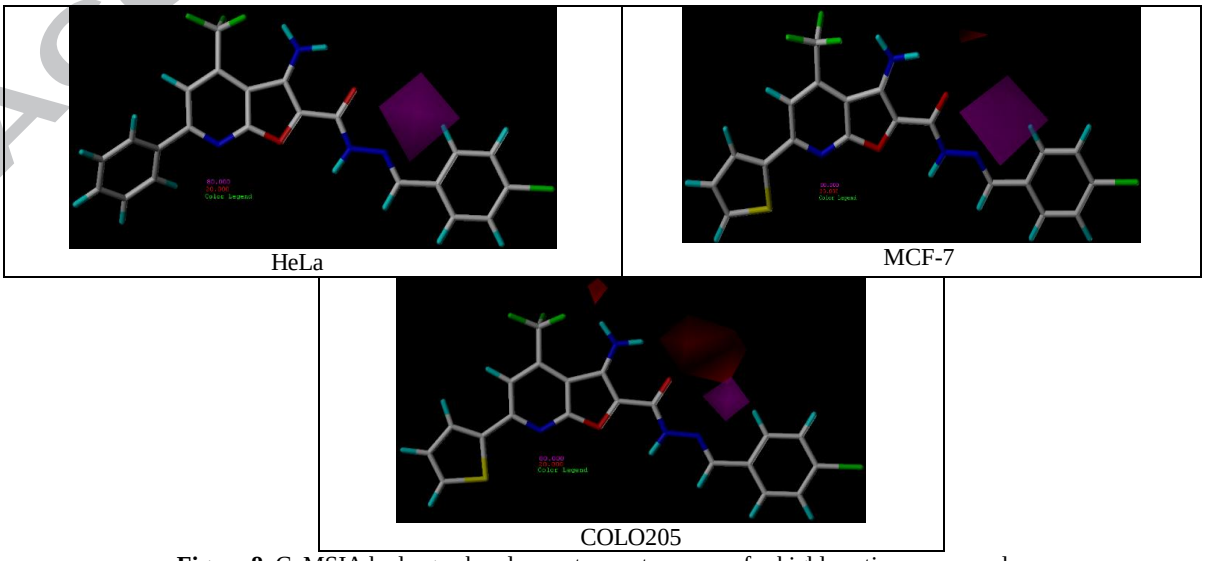
HeLa MCF-7

COLO205

Figure 7. CoMSIA hydrogen bond donor contour maps for highly active compound

CoMSIA hydrogen bond acceptor contour analysis

Hydrogen bond acceptor contours are represented in magenta and red in color. Magenta color indicates favorable region and red color indicates unfavorable region of biological activity. In HeLa, the most active compound shows a large magenta isopleth only. In MCF-7, the compound exhibit a large magenta and a small red contours. Whereas in COLO205, the most active compound shows a medium sized magenta and a large, a small red contours present are shown in figure 8.



HeLa MCF-7

COLO205

Figure 8. CoMSIA hydrogen bond acceptor contour maps for highly active compound

In conclusion, a series of novel furo [2,3-*b*] pyridine carboxamide and pyrido [3',2':4,5] furo [3,2-*d*] pyrimidinone derivatives were prepared and evaluated for cytotoxicity against four human cancer cell lines and one normal (non-tumor) cell line. Among all the compounds screened, the compounds **4e, 4f, 4g,** and **5h** showed significant activity against all cell lines at micro molar concentration. A series of novel furo [2,3-*b*] pyridine-2-carbohydrazide Schiff's base & pyrido [3',2':4,5] furo [3,2-*d*] pyrimidin-4(3*H*)-one derivatives were also evaluated for anticancer activity. The activity results showed that Schiff's base derivatives **7a-h** showed promising cytotoxic activity as compared to pyrido furo pyrimidin-4(3*H*)-one derivatives **8a-h**. Among the promising ones, compounds **7c**, **7d**, **7e** and **7f** exhibited potent anticancer activity against five human cancer cell lines and all compounds screened against human normal cell line were found to be non-cytotoxic to normal cell line, HEK-293. 3D-QSAR CoMFA, CoMSIA studies have been applied to a set of furo [2,3-*b*] pyridine carboxamide and pyrido [3',2':4,5] furo[3,2-*d*] pyrimidinone derivatives. Both these CoMFA and CoMSIA models for HeLa, MFC-7 and COLO205 inhibitors generated have confirmed to be statistically precise with higher q^2 and r^2. The information achieved from CoMFA and CoMSIA models could lead to a better design of novel selective and higher potent furo[2,3-*b*] pyridine carboxamide and pyrido [3',2':4,5] furo [3,2-*d*] pyrimidinone derivatives as cancer cell line inhibitors

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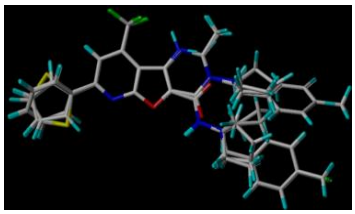
Research Highlights

- Novel hetero ring fused pyridine derivatives.
- All products screened against four human cancer cell lines and one normal cell line.
- Compounds **4e, 4f, 4g, 5h, 7c, 7d, 7e** and **7f** showed promising anticancer activity.
- All compounds found to be non-toxic to normal cell line.

Graphical Abstract

Synthesis of novel hetero ring fused pyridine derivatives; their anticancer activity, CoMFA and CoMSIA studies

G. Santhosh Kumar, Y. Poornachandra, Shravan Kumar Gunda, K. Ratnakar Reddy, Jaheer Mohmed, Kamal Shaik, C. Ganesh Kumar and B. Narsaiah*



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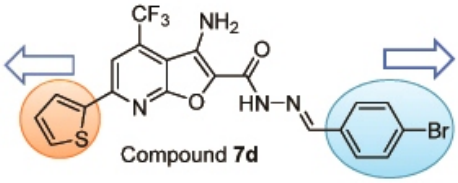
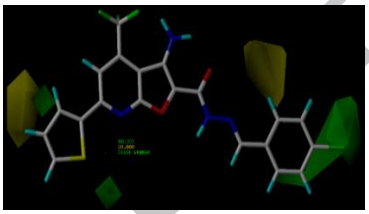


IC_{50} (μM) = 5.2 ± 0.18
Steric Contribution = 73.6%



CoMFA steric contour map

A series of novel furo[2,3-*b*]pyridine-2-carboxamide **4a-h**/pyrido[3',2':4,5]furo[3,2-d] pyrimidin-4(3*H*)-one **5a-p**, furo [2,3-*b*] pyridine-2-carbohydrazide Schiff's base **7a-h** and pyrido [3',2':4,5] furo[3,2-*d*] pyrimidin-4(3*H*)-one derivatives **8a-h** were prepared. All the final products **4a-h**, **5a-p, 7a-h** and **8a-h** were screened against four human cancer cell lines (HeLa, COLO205, HepG2 and MCF7) and one normal cell line (HEK293). Compounds **4e**, **4f**, **4g, 5h**, **7c**, **7d**, **7e** and **7f** showed significant anticancer activity against all the cell lines at micro molar concentration and found to be non-toxic to normal cell line. Further studied for HeLa, COLO205 and MCF-7 using CoMFA and CoMSIA. Models obtained from 3D-QSAR studies provided a strong basis for future rational design of more active and selective HeLa, COLO205 and MCF-7 cell line inhibitors.