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3D–QSAR analysis of 5–cyano–6–aryl–2–thiouracil as inhibitors of Hepatitis C viral NS5B RNA–dependent RNA polymerase

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ABSTRACT

A series of structurally similar 5–cyano–6–aryl–2–thiouracil derivatives as Hepatitis C viral NS5B RNA-dependent RNA polymerase (HCV NS5B RdRp) inhibitors have been subjected for QSAR analysis using VLife MDS 3.5 software. The compounds were divided into training and test set. Best QSAR model was selected on the basis of various statistical parameters like square correlation coefficient (r^2), cross validated square correlation coefficient (q^2) and sequential Fischer test (F). 3D– QSAR study reveals that electronegative and slightly less bulky substitution at sulfur atom, the bulky group on the carbonyl oxygen, reduction in electronegativity of the cyano group and introduction of bulkier group at C–6 position of the 5–cyano–6–aryl–2–thiouracil improve the inhibition of HCV NS5B RdRp.

Keywords: 3D–QSAR, 5–cyano–6–aryl–2–thiouracil, hepatitis C viral NS5B RNA–dependent RNA polymerase inhibitors, 3D Descriptors.

INTRODUCTION

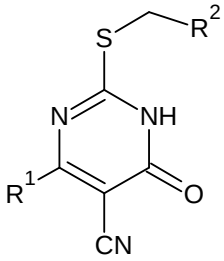
The chronic viral infection caused by Hepatitis C virus (HCV) has been recognized as one of the leading cause of liver impairment such as cirrhosis and hepatocellular carcinoma. HCV is a 9.6 kb positive strand RNA virus of the flaviviride, genus Hepacivirus. It contains a single open reading frame coding for a ~ 3000 amino acid polyproteins, which is further processed into various structural and non-structural viral proteins by host and viral protease [1]. The NS5B RNA–dependent RNA polymerase (RdRp) is the central enzyme that is responsible for replication of the viral genome and has become a target of choice for screening and designing of small molecular inhibitors which in the principle, should interfere with the viral replication [2].

The 5–cyano–6–aryl–2–thiouracil is the inhibitors of HCV NS5B RdRp used in the treatment of cirrhosis and hepatocellular carcinoma [3]. So there is a need to correlate HCV NS5B RdRp inhibitor activity and the physicochemical parameters of the compound by QSAR methods for increasing the potency of the molecules.

MATERIALS AND METHODS

Biological activity data

The 48 compounds of 5–cyano–6–aryl–2–thiouracil having HCV NS5B RdRp inhibitor activity, were selected from literature [3] and their experimental biological activity is in the form of IC_{50} were converted into pIC_{50} and used as dependent variable to develop the 3D–QSAR model. Table 1 shows the structure of 48 compounds along with their biological activity.



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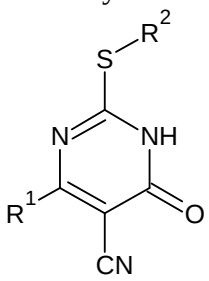
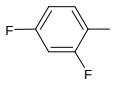
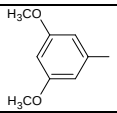
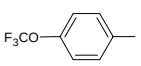
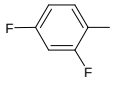
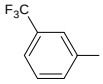
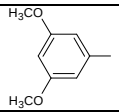
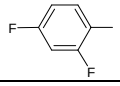
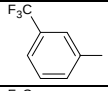
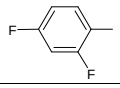
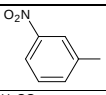
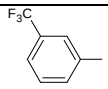
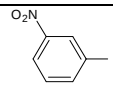
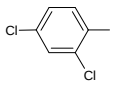
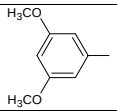
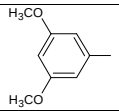
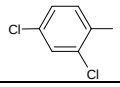
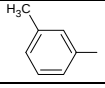
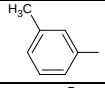
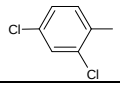
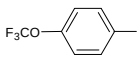
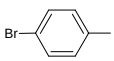
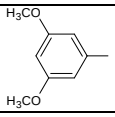
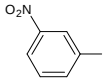
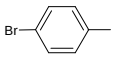
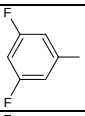
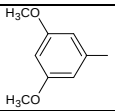
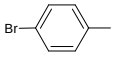
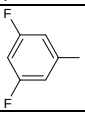
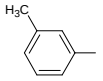
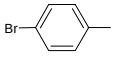
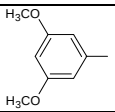
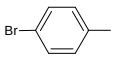
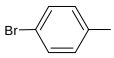
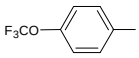
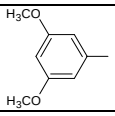
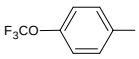
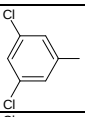
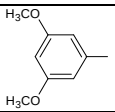
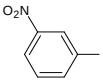
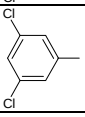
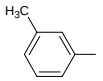
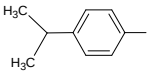
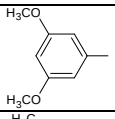
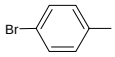
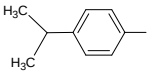


(38-48)

Table1. List of the compounds with their IC_{50}.

Compound	R_1	R_2	IC_{50} (µM)	Compound	R_1	R_2	IC_{50} (µM)
1			19	**17**			8.6
2			57	**18**			11
3			13	**19**			14
4			32	**20**			13
5			34	**21**			25
6			33	**22**			14
7			11	**23**			32

8			13	**24**			37
9			11	**25**			15
10			10	**26**			27
11			14	**27**			12
12			7.1	**28**			14
13			8.7	**29**			21
14			30	**30**			9.2
15			22	**31**			34
16			20	**32**			29
33			25	**41**		$CH_3(CH_2)_4$	11
34			32	**42**		$PhCO_2(CH_2)_2$	30
35			3.8	**43**		$CH_3(CH_2)_4$	18
36			10	**44**		$CH_3(CH_2)_4$	12
37			9.9	**45**		$PhCO_2(CH_2)_2$	13
38		$CH_3(CH_2)_4$	12	**46**		$CH_3(CH_2)_4$	18
39		$CH_3(CH_2)_4$	17	**47**		$CH_3(CH_2)_4$	20
40		$Ph(CH_2)_3$	12	**48**		$CH_3(CH_2)_4$	12

Geometry optimization

3D–QSAR studies of 5–cyano–6–aryl–2–thiouracil were carried out by using Molecular Design Suit software version 3.5 [4]. 3D structures of all compounds have been constructed using MDS 3.5 and their geometries were subsequently optimized to make the conformation having least potential energy. Energy minimization was performed using Merck Molecular Force Field (MMFF) and MMFF charge for an atom followed by distance dependent dielectric constant of 1.0 and convergence criteria (rms gradient) of 0.01kcal/mol [5].

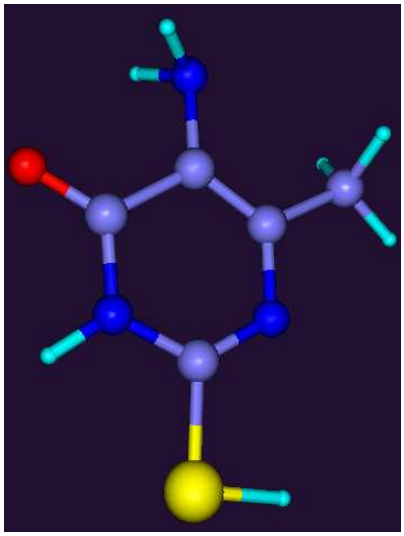


Figure 1: Template

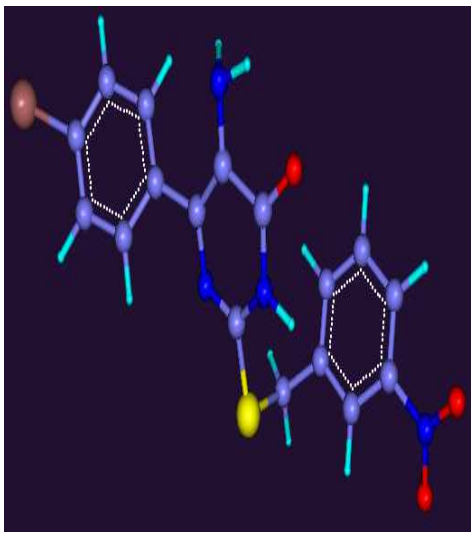


Figure 2: Reference Molecule

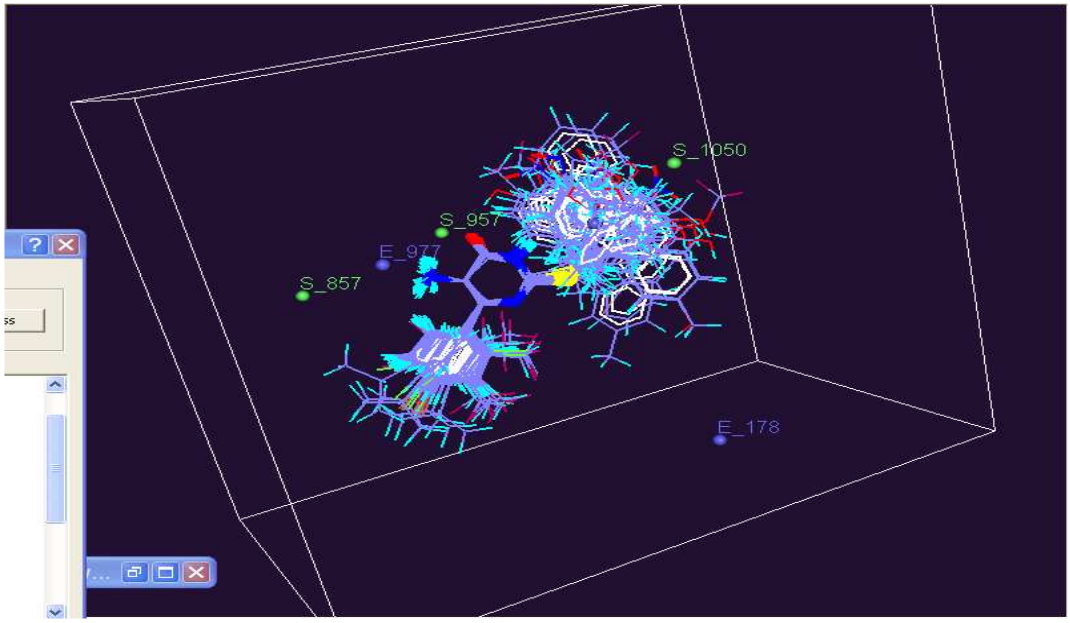


Figure 3: Distribution of chosen points in template based alignment.

Alignment of molecules

All molecules were aligned by template-based methods where template is built by considering the common structure in the series. The structure of 5–cyano–6–aryl–2–thiouracil template is

shown in Figure 1. Highly bioactive energetically stable conformation in this series of compound is chosen as reference molecule (Figure 2) on which other molecules in the series are aligned, considering the template as a basis for alignment (Figure 3). The 3D descriptors for each optimized molecule were calculated by "compute descriptors" module of the software and selected descriptors are shown in Table 2.

Activity prediction

The predictability of the QSAR model would be good if the biological activity predicted by QSAR model do not appreciably differ from the observed. The model selected on the basis of r^2, q^2 and F-test. QSAR model was evaluated using statistical measures such as n represents number of observations, df is degree of freedom, r is square root of multiple R-square for regression, q is cross validated r^2 and F is F - statistic for the regression model.

Computational details

3D–QSAR

Several 3D–QSAR techniques such as comparative molecular field analysis (COMFA), comparative molecular similarity analysis (COMSIA) and *k*-nearest neighbor (*k*NN) are being used in modern QSAR research [6]. In the present study, molecular field analysis coupled with partial least squares (PLS) was applied to obtain a 3D–QSAR model, PLS is frequently used as the regression method in 3D–QSAR. The calculated steric and electrostatic field descriptors were used as independent variables and pIC_{50} values were used as dependent variables in PLS regression analysis to derive the 3D–QSAR model.

RESULTS AND DISCUSSION

The model 1 describes the optimum structural features for HCV NS5B RdRp inhibitor activity. The training set of 38 molecules and test set of 10 molecules was used. S_857, S_957, E_178, E_977,E_931, S_1050 and E_942 are the steric and electrostatic field energy interaction between methyl probe and compounds at there corresponding spatial grid points of 857, 957, 178, 977, 931, 1050, 942. The model 1 is validated by predicting the biological activities of the molecules as indicated in the Table 3.

The model 1 suggests that, the steric descriptors S_857 and S_957 with positive coefficient represents more bulky substitutions and the steric descriptor S_1050 with negative coefficient represents that slightly less bulky substitution is favorable in this region. Electrostatic field descriptor E_178 and E_931 with positive coefficient indicate that the electropositive (electron deficient or electron withdrawing) groups and the electrostatic field descriptors E_977, E_942 with negative coefficient indicate that the electronegative (electron rich or electron donating) groups are favorable in this region. 3D–QSAR study reveals that electronegative and slightly less bulky substitution at sulfur atom, the bulky group on the carbonyl oxygen, reduction in electronegativity of the cyano group and introduction of bulkier group at C–6 position of the 5–cyano–6–aryl–2–thiouracil improve the inhibition of HCV NS5B RdRp.

Table 2. Selected Molecular 3D Descriptor

Compound	S_857	S_957	E_178	E_977	E_931	S_1050	E_942
1	-0.07325	0.268635	0.187713	10	-10	2.311237	5.023768
2	-0.06773	4.206648	-0.31114	10	-10	-0.17695	9.380913
3	-0.06821	-0.65353	-0.41165	-1.24333	-10	-0.26098	3.838103
4	-0.05661	-0.28464	-0.01888	8.633759	10	-0.46862	-10
5	-0.06627	0.559499	0.319268	8.259135	2.144875	-0.34331	10
6	-0.06199	-0.39483	0.054413	3.275301	1.209678	30	-10
7	-0.07334	-0.38699	-0.1355	10	2.420452	30	-10
8	-0.07697	0.028029	0.107491	7.955014	-10	2.463244	3.862022
9	-0.05898	-0.12033	0.104962	4.428021	4.764601	-0.37451	-10
10	-0.07225	-0.34365	-0.25822	10	10	-0.18266	7.276153
11	-0.07678	1.465482	0.000185	-3.24009	10	4.16293	9.107298
12	-0.06332	-0.2522	-0.36272	10	-10	1.282975	3.785134
13	-0.06247	-0.44353	-0.23145	10	-10	11.96469	0.722657
14	-0.06513	-0.33482	0.169582	3.62678	-0.64988	1.101767	-10
15	-0.05935	-0.08789	0.087322	3.014501	2.560071	30	-10
16	-0.05546	-0.25033	-0.00228	6.644732	-2.0176	24.96487	-10
17	-0.06776	-0.32651	-0.01096	-5.61924	-10	-0.28893	4.924615
18	-0.07659	5.936151	-0.14201	1.215013	-10	-0.61093	5.642722
19	-0.0639	-0.18643	-0.39525	-4.90595	-10	15.26301	-1.43577
20	-0.07357	-0.24193	-0.22379	3.544274	-8.30386	-0.22355	5.323419
21	-0.068	-0.12657	0.123776	3.700995	-10	0.054393	-1.47723
22	-0.07935	-0.80473	0.010327	-1.26241	-10	-0.28238	2.801792
23	0.05391	-0.54726	-0.14273	10	0.750437	-0.27056	-10
24	-0.06409	-0.18736	-0.03495	6.509739	6.767677	30	-10
25	-0.08049	0.001188	0.000417	5.299512	-10	1.089282	5.759638
26	-0.06722	0.845938	-0.08233	9.328294	3.446274	-0.29523	10
27	-0.06401	-0.08709	-0.10804	5.432664	-4.44243	30	7.797345
28	-0.06558	-0.71977	-0.31526	10	4.161304	-0.04493	4.544717
29	-0.06219	-0.7964	-0.0412	10	-3.30499	-0.06423	1.176874
30	-0.06887	-0.2987	-0.45596	10	2.441002	12.69802	10
31	-0.06328	-0.31602	0.12191	2.433655	-3.18875	30	3.653196
32	-0.04536	-0.38971	0.036665	9.416364	-0.419	30	-10
33	-0.05099	-0.58599	0.394045	10	-0.54578	30	10
34	-0.08788	-0.41604	-0.1985	-6.0735	-10	7.439965	2.541755
35	-0.09717	-0.19644	-0.14924	9.660554	-10	30	9.111039
36	-0.09412	-0.41245	-0.05731	-6.61666	-10	1.476716	6.162975
37	-0.08144	0.050526	0.056858	10	-10	-0.17418	3.705192
38	-0.0566	-0.34586	-0.33694	10	7.156908	0.25711	9.84505
39	-0.07684	0.293647	-0.32924	4.582527	4.192975	9.487267	10
40	-0.06898	-0.04442	-0.26727	6.794829	1.907259	24.5174	4.386247
41	-0.08055	0.357272	-0.26141	0.532011	7.212506	27.0711	9.034264
42	-0.05481	-0.04346	-0.15583	10	10	6.119741	10
43	-0.07351	0.690163	-0.17738	4.03129	5.325516	30	10
44	-0.05969	0.004049	-0.4704	-4.71616	6.881743	5.953335	9.992202
45	-0.06515	0.491485	-0.44915	3.912676	4.406794	30	4.703073
46	-0.05225	-0.47379	-0.14103	10	3.332563	-0.02722	9.369979
47	0.06065	-0.22731	-0.46496	-2.73098	4.765503	30	10
48	-0.08922	0.389578	-0.25339	4.451279	4.805274	30	10

3D QSAR

pIC_{50} = 2.2386 + 10.8884(±1.3673) S_857 + 0.1818(±0.0057) S_957 + 0.4964(±0.0668) E_178 - 0.0198(±0.0000) E_977 + 0.0115(±0.0000) E_931 - 0.0043(±0.0000) S_1050 - 0.0073(±0.0000) E_942.

n = 38, Degree of freedom = 30, r = 0.9295, r^2 = 0.8640, q^2 = 0.7807, F test = 27.2333
Model 1

Table 3. The actual, predicted and residual pIC_{50} values for 3D–QSAR model

Compound	Actual	Predicted	Residue	Compound	Actual	Predicted	Residue
1	1.278754	1.224337	0.054417	**22**	1.146128	1.124576	0.021552
2	1.755875	1.73181	0.024065	**23**	1.50515	1.366043	0.139107
3	1.113943	1.055887	0.058056	**24**	1.568202	1.38138	0.186822
4	1.50515	1.579852	-0.0747	**25**	1.176091	1.096758	0.079333
5	1.531479	1.567579	-0.0361	**26**	1.431364	1.403502	0.027862
6	1.518514	1.411036	0.107478	**27**	1.079181	1.127787	-0.04861
7	1.041393	1.075608	-0.03422	**28**	1.146128	1.054664	0.091464
8	1.113943	1.148438	-0.0345	**29**	1.322219	1.152548	0.169671
9	41.04139	1.66791	-0.62652	**30**	0.963788	0.911146	0.052642
10	1	1.126364	-0.12636	**31**	1.531479	1.311938	0.219541
11	1.146128	1.763678	-0.61755	**32**	1.462398	1.444201	0.018197
12	0.851258	0.978038	-0.12678	**33**	1.39794	1.366549	0.031391
13	0.939519	0.993734	-0.05422	**34**	1.50515	1.062221	0.442929
14	1.477121	1.541439	-0.06432	**35**	0.579784	0.569553	0.010231
15	1.342423	1.532523	-0.1901	**36**	1	1.075257	-0.07526
16	1.30103	1.398414	-0.09738	**37**	0.995635	1.050861	-0.05523
17	0.934498	1.397814	-0.46332	**38**	1.079181	1.204155	-0.12497
18	1.041393	2.236119	-1.19473	**39**	1.230449	1.135937	0.094512
19	1.146128	1.239378	-0.09325	**40**	1.079181	1.096662	-0.01748
20	1.113943	1.079593	0.03435	**41**	1.041393	1.186458	-0.14507
21	1.39794	1.359254	0.038686	**42**	1.477121	1.374686	0.102435
43	1.255273	1.254873	0.0004	**46**	1.255273	1.286347	-0.03107
44	1.079181	1.42968	-0.3505	**47**	1.30103	1.212542	0.088488
45	1.113943	1.205142	-0.0912	**48**	1.079181	0.977242	0.101939

CONCLUSION

Novel antiviral might be generated from the series 5–cyano–6–aryl–2–thiouracil with committed improvement in inhibition of Hepatitis C viral NS5B RdRp by electronegative and slightly less bulky substitution at sulfur atom, the bulky group on the carbonyl oxygen, lowering in electronegativity of the cyano group and introduction of bulkier group at C–6 position.

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