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AN INSIGHT TO STRUCTURAL ANALYSIS OF COUMARINS AS NON NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

Vikas Kumar*[1], Indra P. Pandey[1] and Jainendra Jain[2]

[1]DAV (PG) College Dehradun, Uttarakhand, India.

[2]Ram-Eesh Institute of Vocational and Technical Education, Noida, Uttar Pradesh, India.

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***Corresponding Author**
Vikas Kumar
DAV (PG) College Dehradun, Uttarakhand, India.

ABSTRACT

A series of eighty three compounds of Coumarins was evaluated for HIV inhibitory activity against HIV strain: HIV-1 (RF) cell type: CEM using 3HVT (Human Immunodeficiency Virus Type 1 reverse transcriptase heterodimer), 1IKW (Wild type HIV 1 reverse transcriptase), 1LW2 (T215Y mutant HIV-1 reverse transcriptase) macromolecules. Ligand and Macromolecule interaction studies were carried out using software VLife Molecular Design Suite 4.3.2 (VLife MDS) installed on Pentium based Windows workstation. The same series of molecules were also subjected to 3DQSAR studies to pursuit analog based drug design. Nevirapine, a known NNRTI anti HIV drug, in clinical use was used as a reference molecule. Binding studies were analyzed in terms of H-bonding and π-stacking interactions. The result obtained from the study has revealed that both H-bonding and pi-stacking interactions contributed significantly towards anti-HIV activity. Improved binding interactions were observed among few 2, 3 and 4-membered cyclic coumarins with macromolecules. The data has also been well supported by 3DQSAR studies (r^2 = 0.9597, q^2 = 0.9174 and Pred_r^2 = 0.9254). The study has indicated that coumarins could serve as a lead non nucleoside reverse transcriptase inhibitor in the treatment of HIV mediated manifestation.

KEYWORDS: Anti-HIV, coumarins, calanolide, docking, 3DQSAR.

INTRODUCTION

HIV I reverse transcriptase (HIV-I RT) has been one of the major therapeutic target in designing of novel anti HIV drugs.[1,2] Inhibition of reverse transcriptase enzyme permits replication of HIV-1. Two classes of RT inhibitors are widely used in the treatment for HIV-1 infected individuals.

A) Nucleoside / nucleotide RT inhibitors (NRTIs)

B) Non nucleoside RT inhibitors (NNRTIs)

Standard regimen of anti-retroviral therapy (ART) consists of two NRTIs plus one NNRTI or protease inhibitors (PI). NNRTI has been one of the key components against HIV-1 replication and infection. Nevirapine (NVP), Efavirenz (EFV) and Etravirine (ETR) are the most widely used NNRTI's available for the treatment of AIDS patients. However, side effects and low genetic barrier to drug resistance and cross resistance to these agents have shown poor results in HIV patient.[3,4] NVP was found to exhibit drug resistant mutations within one week of monotherapy.[5] K103N and Y181C mutations showed high degree resistance to both NVP & EFV.[6,7] A newly approved NNRTI drug, ETR retains high efficacy against NVP or EFV resistant virus both *in vitro* and *in vivo*[8,9,10]. ETR has not been reported to show serious side effects but it is administered twice a day which results in inconvenience and heavy burden on patients.[11]

In NVP binding pocket, significant number of residues were found to be in contact with NVP and sites of non nucleoside drug resistance mutations. Mutation to Tyr 181 and Tyr 188 are among the central mutations, responsible for drug resistance.[12,13,14,15] There has been proposed that one approach to minimize drug resistance might be to target interactions with the conserved part of the binding pocket i.e. PHE227, TRP229, LEU234, TYR319 and polypeptide backbone.[23]

In NVR binding pocket, mutations to TYR181 and TYR 188 were observed as central mutation responsible for drug resistance. This has been proposed to minimize drug resistance by targeting interaction with the converted part of binding pocket. Therefore discovery of new and novel NNRTIs remain an ongoing necessity to ensure effective treatment for HIV patients.

(+) Calanolide A (Fig 1), a natural product extracted from tropical rainforest tree *Calophyllum lanigerum* has been identified as an attractive NNRTI against HIV-1 despite virus strains

containing drug resistant K103N/Y181C mutations.[16,17,18,19] Chemically (+) Calanolide A contains coumarin ring as basic skeleton. It was reported to compete with dNTPs in binding to the HIV-1 RT active site.[20] Despite promising results, (+) Calanolide A is very difficult to purify and in addition, it possess low therapeutic index and non ideal antiviral activity.[21, 22]

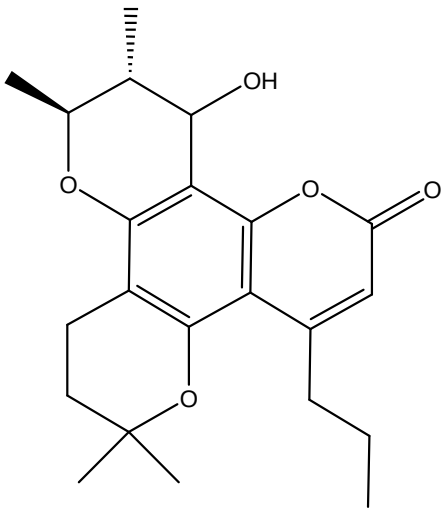


Figure 1: Chemical structure of Calanolide.

In the present paper, we are reporting molecular docking and interaction studies of 83 different derivatives of coumarin nucleus with structure of the binding pocket of non nucleoside reverse transcriptase inhibitor of HIV-1 (PDB ID: 3HVT, 1IKW, 1LW2). Data of chemical structures was obtained from website chemdb.niaid.nih.gov while molecular docking studies were carried out using software VLife Molecular Design Suite 4.3.0 (VLife MDS). VLife Engine and BioPredicta tools were most commonly used for analyzing interactions in the form of H-bonding and pi stacking.

EXPERIMENTAL

The data comprises of molecular modeling and docking interaction studies of previously reported 83 different coumarin derivatives. These compounds containing coumarin as the basic skeleton in the form of two, three and four membered ring structures were drawn and energetically minimized by Vlife MDS 4.3.0. Detailed structures are specified in Table 1**.** Molecular modeling and docking studies were carried out on binding pocket of NNRTI of HIV-1 virus (PDB ID: 3HVT, 1IKW, 1LW2) using VLife Molecular Design Suite (MDS) 4.3.0 obtained from VLife Sciences Technolgy Pvt. Ltd, Pune (India), running on Core 2 Duo processor. Docking interactions were analyzed in terms of H-bonding and pi stacking interactions.

Computational Studies (Ligand Preparation)

Structures of ligands were built in 2D Draw module of VLife MDS 4.3.0. Ligand geometry of all compounds was optimized by energy minimization using MMFF force field and Gasteiger Marsili charges for the atoms till a gradient of 0.001 kcal/mol/Aº was reached. The conformation search was carried out to identify the lowest energy conformation, using the systematic search of VLife MDS Engine. All lowest energy conformations were saved as .mol2 file.

Receptor Preparation

The 3D crystal structures of 3HVT, 1IKW, 1LW2 (Figure 2) were downloaded from Brookhaven protein data bank (PDB, http://www.rcsb.org/pdb) and repaired with Swiss PDB viewer. The receptors were loaded to Biopredicta tools of VLife MDS. The non-bonded oxygen atoms of waters present in the crystal structure were removed. Non bonding chains were removed by using BioPredicta module. Co crystal ligands were also extracted from the receptor and both structures were saved saved separately as .pdb file. Missing H atoms were added to both structures and partial atomic charge was calculated using MMFF (Merck molecular force field). The receptor and NVP files were then saved as .mol2 file.

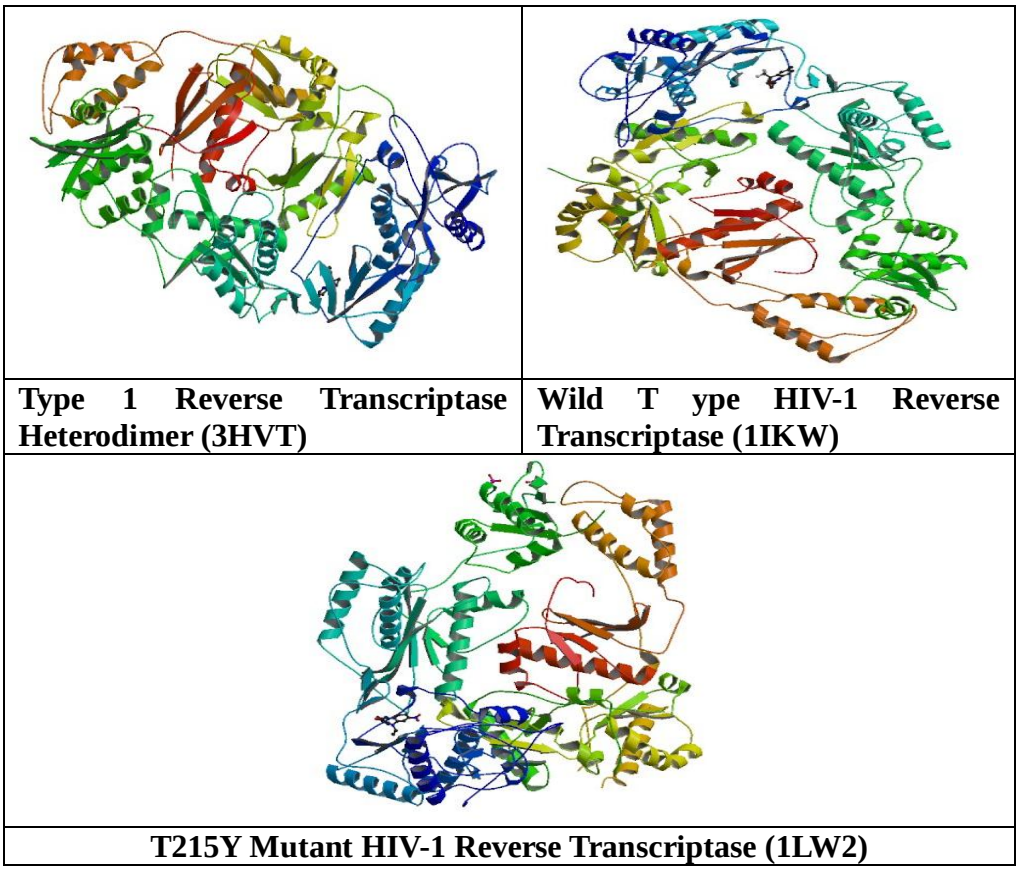
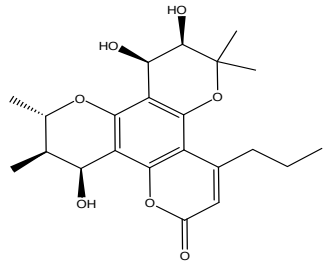
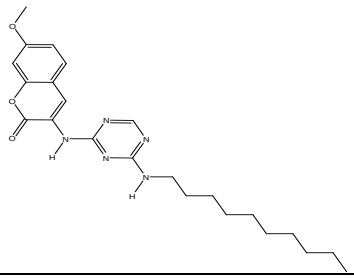
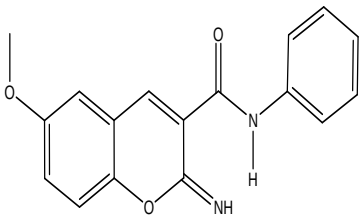
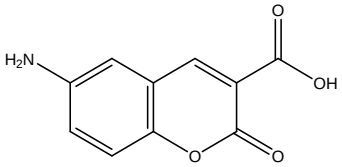
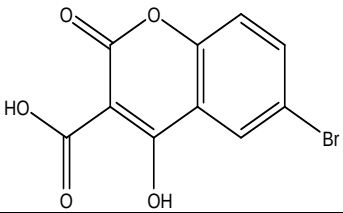
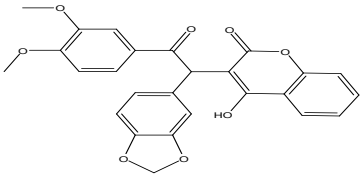
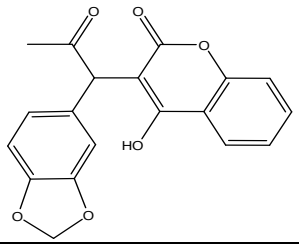
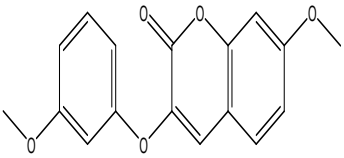
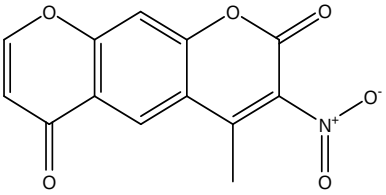
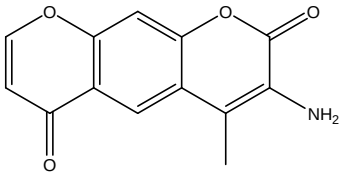
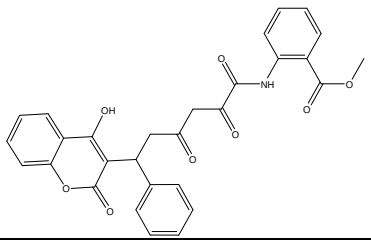
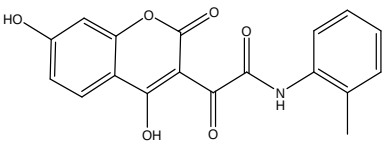
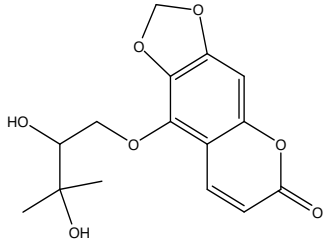
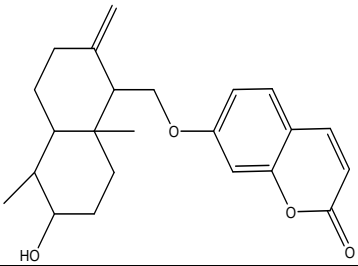
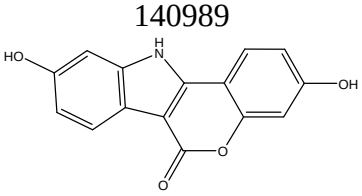


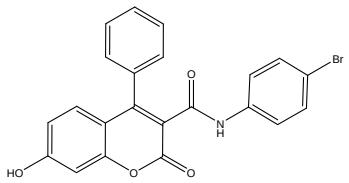
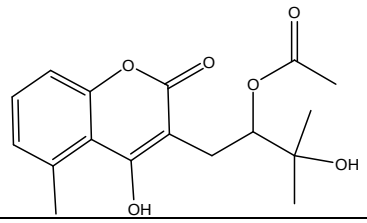
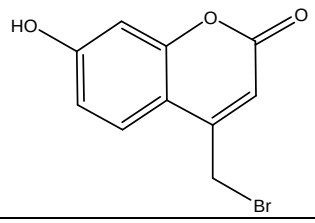
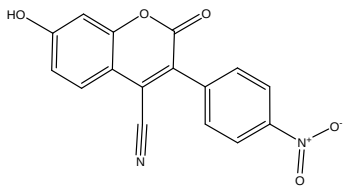
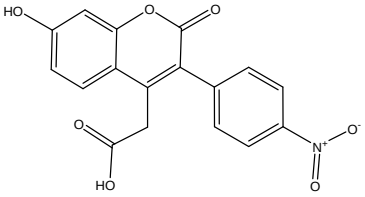
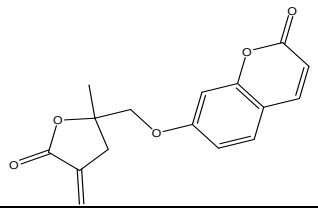
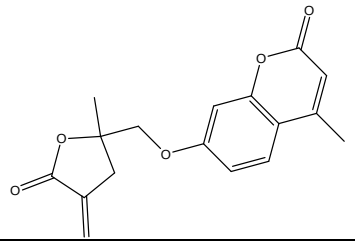
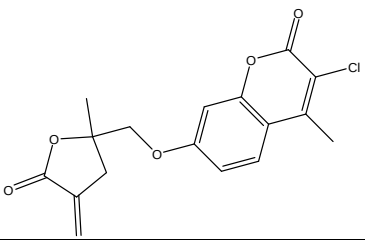
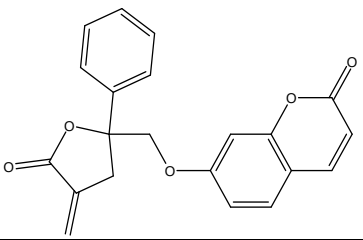
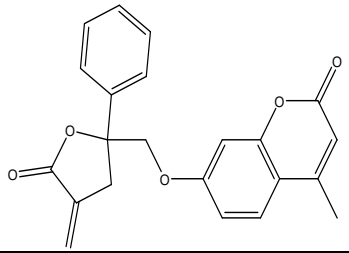
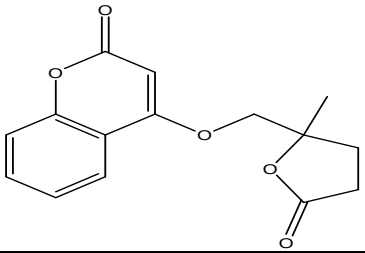
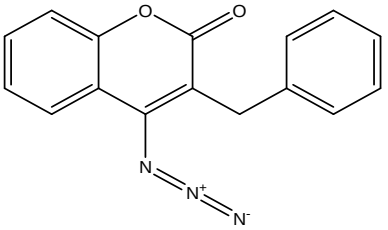
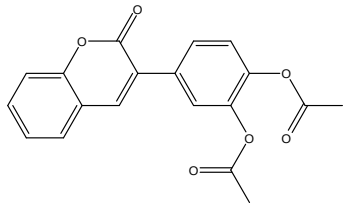
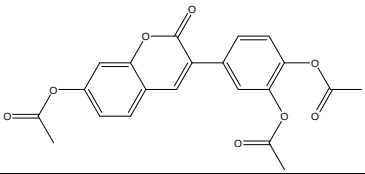
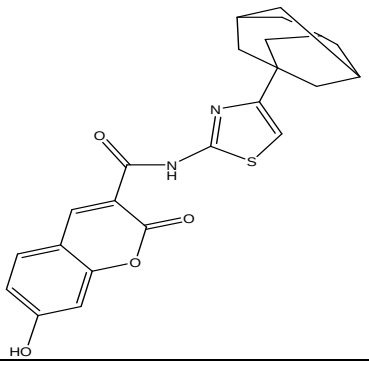
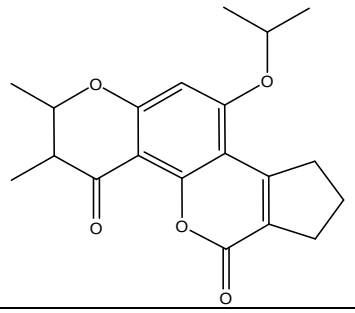
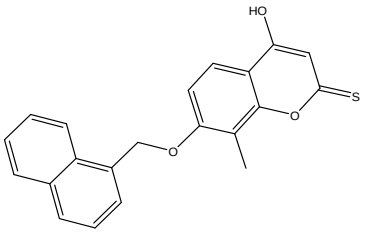
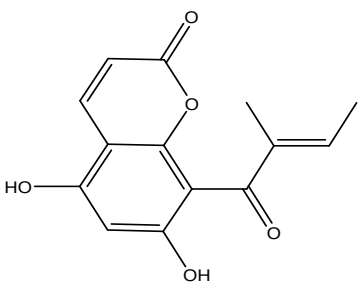
Figure 2: 3D structures of HIV receptors.

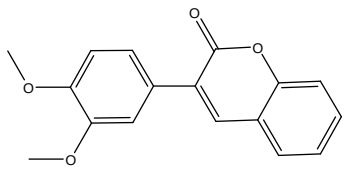
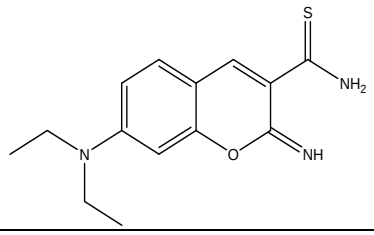
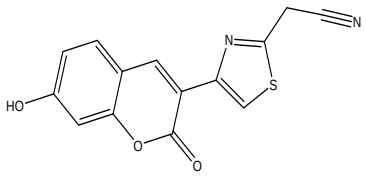
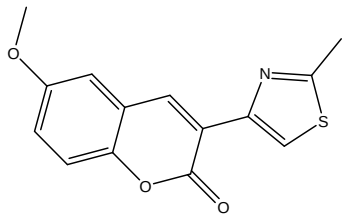
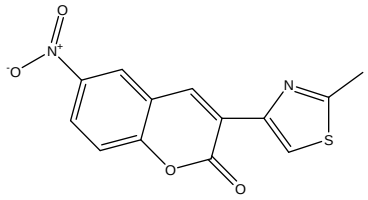
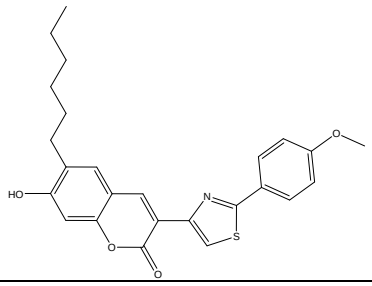
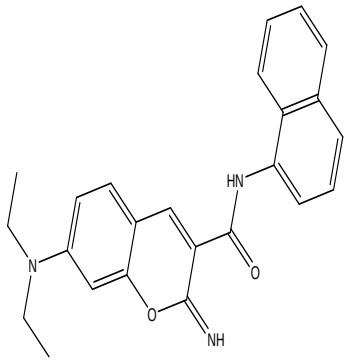
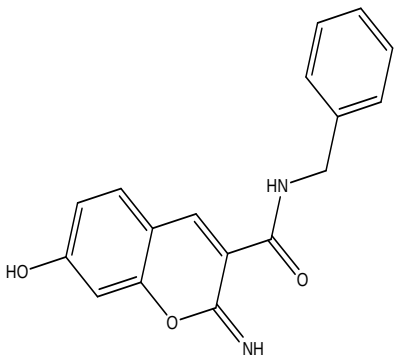
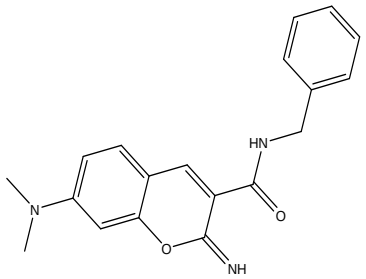
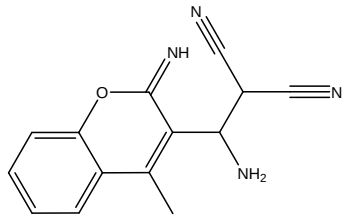
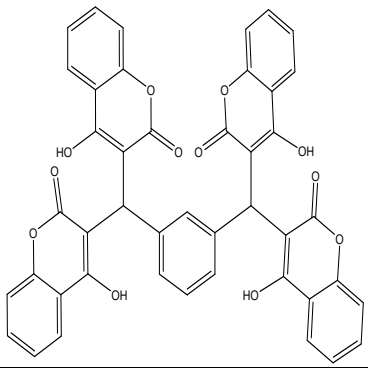
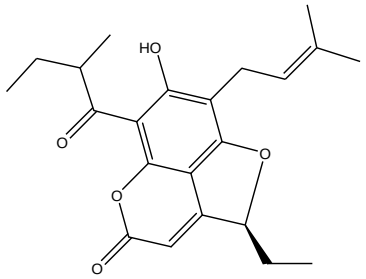
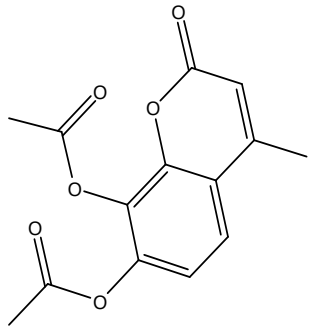
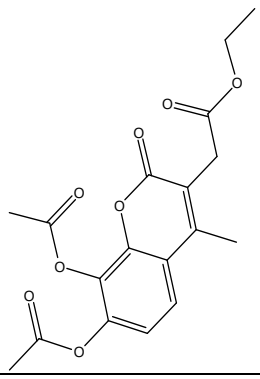
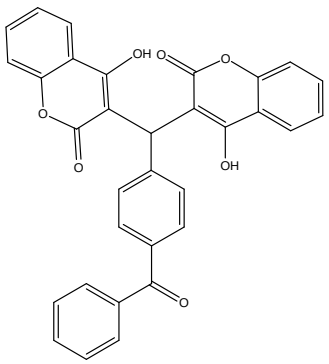
Docking Studies

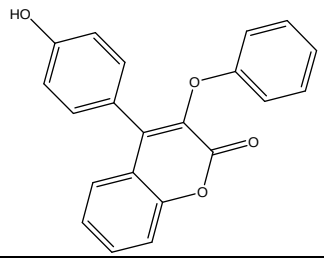
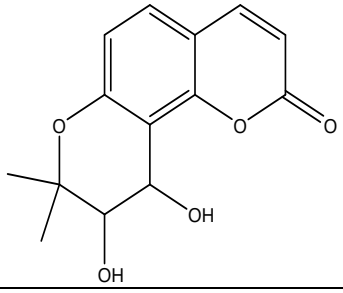
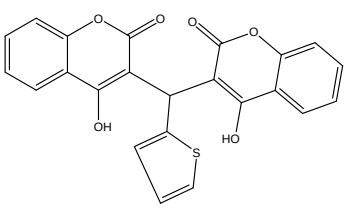
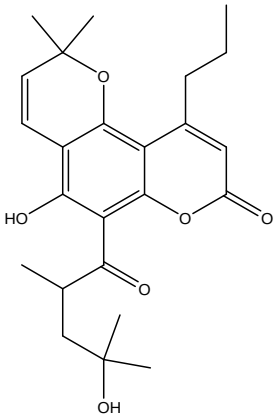
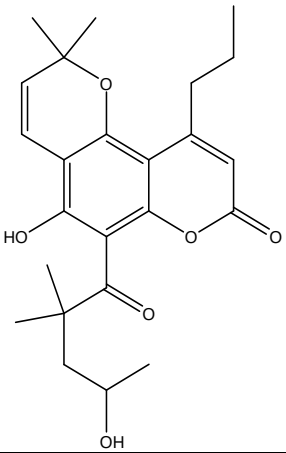
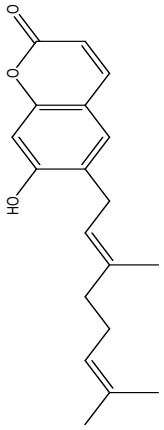
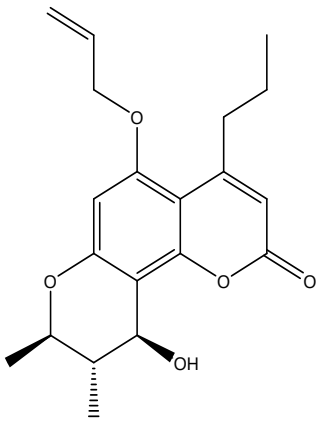
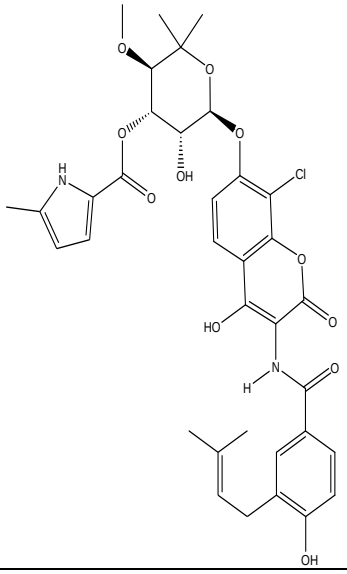
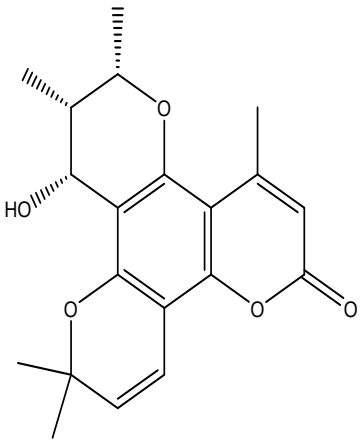
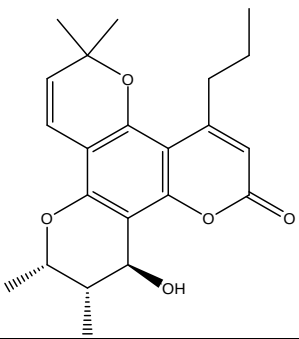
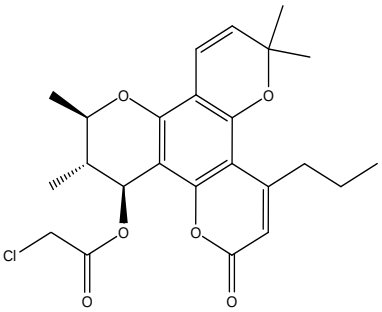
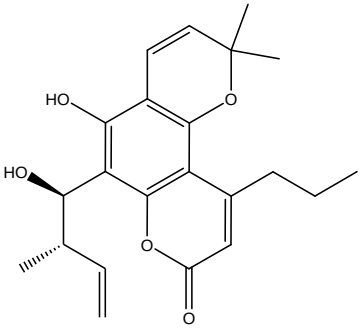
Chemical structures of all energy minimized coumarin derivatives were docked into crystal structures of 3HVT, 1IKW, 1LW2 by using Biopridica module of Vlife MDS 4.3.0. A systematic conformational search was performed to obtain the low energy conformations of the receptor and Nevirapine, Efavirenz, 1051U91 respectively. The low energy conformations, thus obtained were optimized till they reached rms gradient energy of 0.001 kcal/mol/Aº.

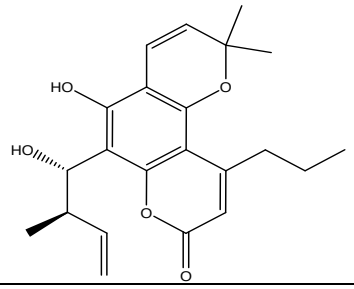
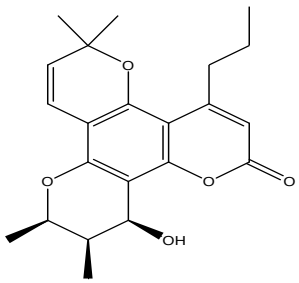
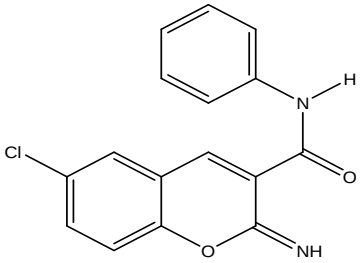
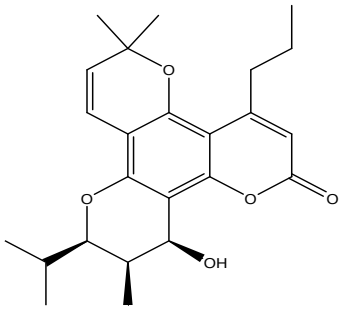
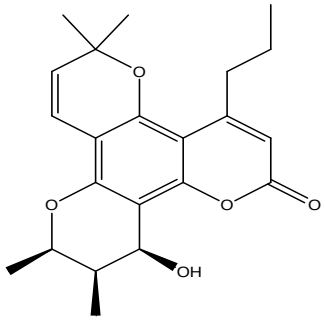
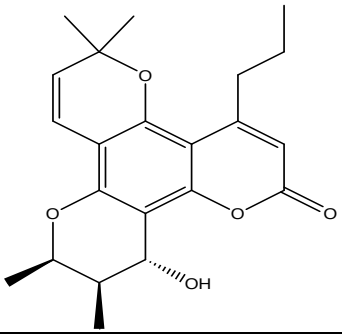
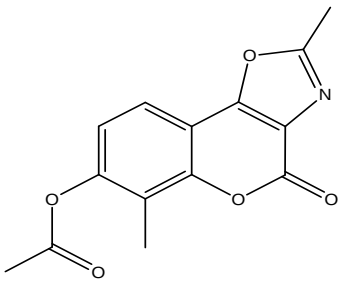
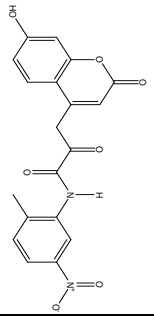
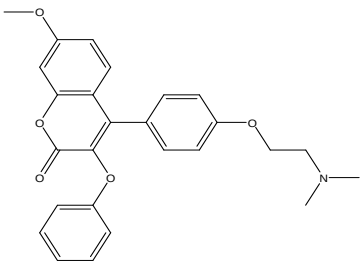
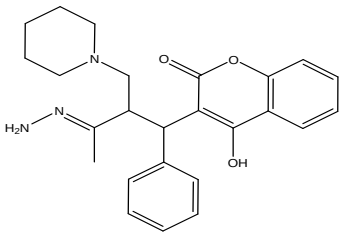
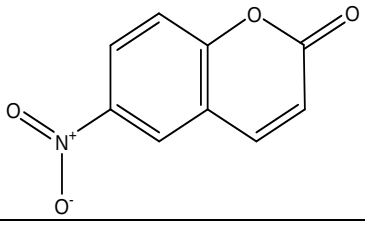
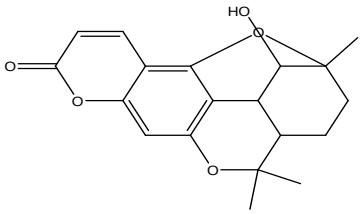
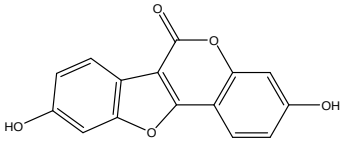
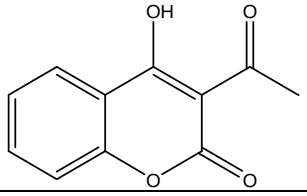
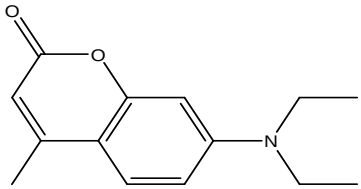
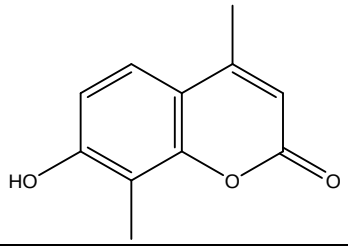
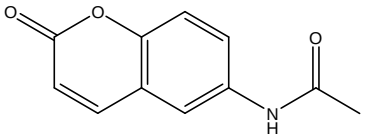
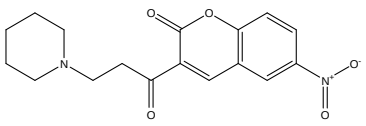
Table 1: Structures of coumarin derivatives (C_1-C_{83}).

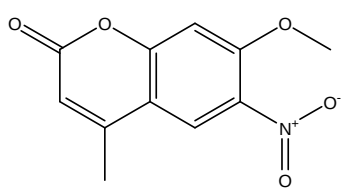
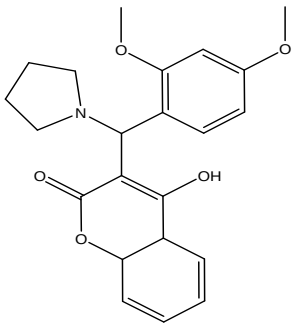
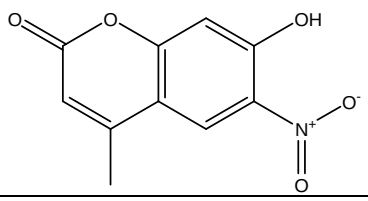
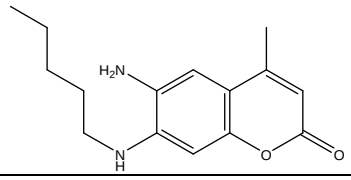
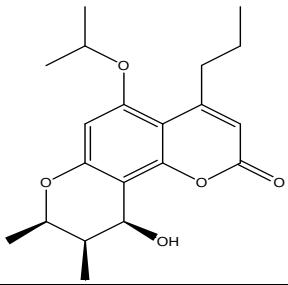
054231 	054759 	056458 
125724 	126547 	127986 
128116 	128249 	130823 
130825 	139017 	139031 
139640 	140974 	140989 

143061 	143062 	144483 
144489 	144510 	144822 
144823 	144824 	144826 
144827 	144830 	145069 
146374 	146375 	146946 
148330 	148515 	148811 

149013 	149710 	149717 
149727 	149728 	149733 
149736 	149739 	149740 
149873 	150115 	150252 
150751 	150761 	150908 

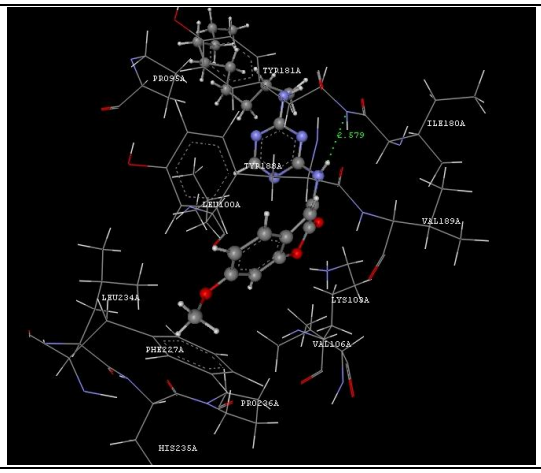
153238 	006522 	006523 
058229 	058230 	073140 
088859 	032096 	051447 
051442 	051576 	051611 

051614 	051617 	056465 
058245 	071808 	071809 
073082 	108833 	153244 
157682 	159909 	011388 
011954 	124082 	125105 
125112 	125475 	`125516 

128830 	130007 	130188 
130387 	161017 	

In order to define ligand-receptor interactions, docking of all the low energy conformations within a range of 5 kcal/mol/Aº from the lowest energy conformation of each molecule, into the cavity of 3HVT, 1IKW, 1LW2 was done by positioning the molecule appropriately into the active site of chain reference ligands Nevirapine, Efavirenz, 1051U91 using GRIP docking of BioPredicta module of VLife MDS 4.3.0. Docking score of all eighty three analogs were compared with standard inhibitors. Docking interactions were calculated in terms of H-bonding and Pi-stacking interactions. Details of docking score and molecular interactions of all eighty three analogs are given in Table 2. Docking interactions of various ligand poses displaying H-bonding and pi stacking with various residues and pharmacophoric features are shown in Fig. 3. and Fig 4.

3D QSAR Studies: *In vitro* inhibitory activity data (IC_{50}: µM) of the coumarin derivatives was taken for study. Three dimensional structures were drawn for each molecule and the molecular geometries optimized using Monte Carlo conformational search, MMF fields and charges. Optimized molecules were aligned by template based method using the most active molecule as reference. Optimal training and test set were generated using the random selection algorithm. This algorithm allows the construction of training set covering the descriptor space occupied by representative points. A training set of 70% molecules, a test set of 20% molecules and validation set of 10% were generated. Multiple regression with stepwise forward, variable selection method was employed for selection of variables to obtain the QSAR model. (r^2 = 0.9597, q^2 = 0.9174 and Pred_r^2 = 0.9254)



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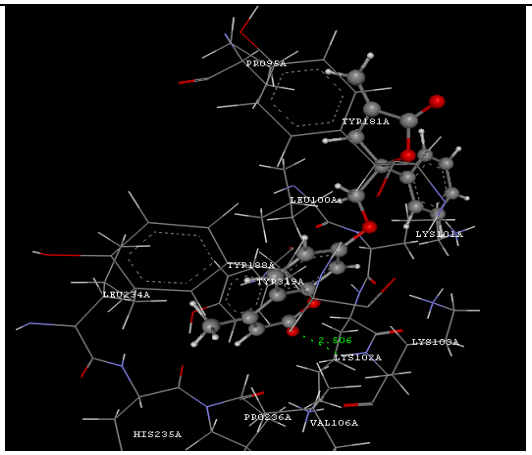

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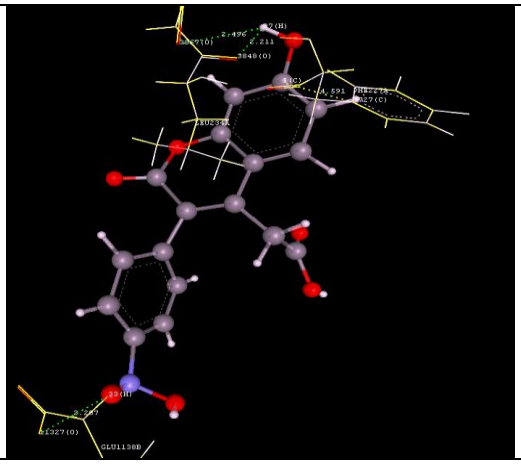

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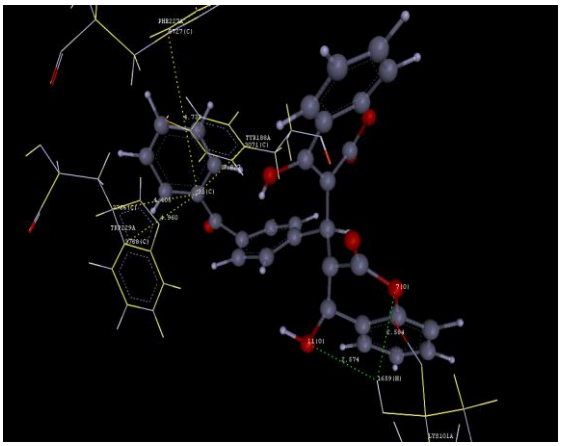

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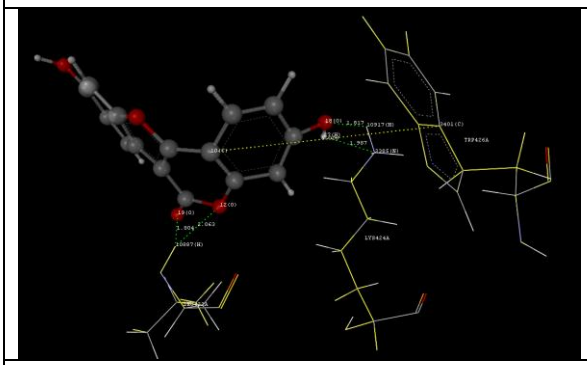

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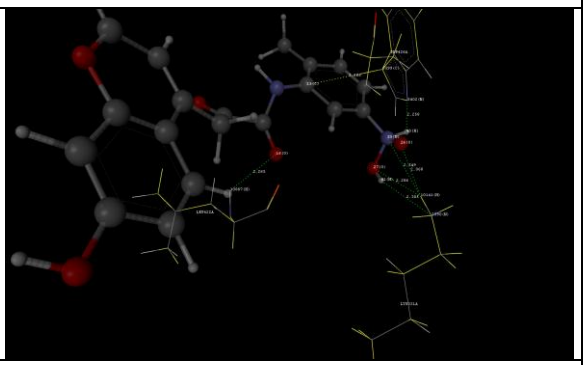

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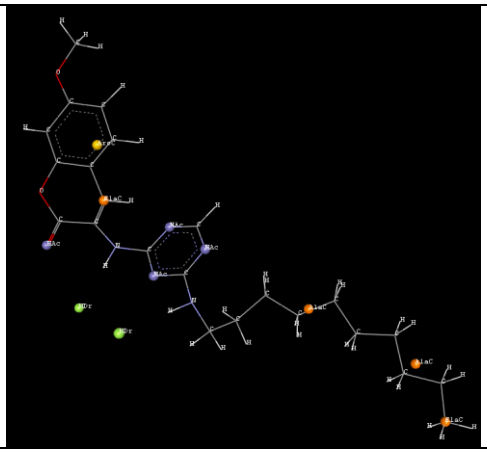
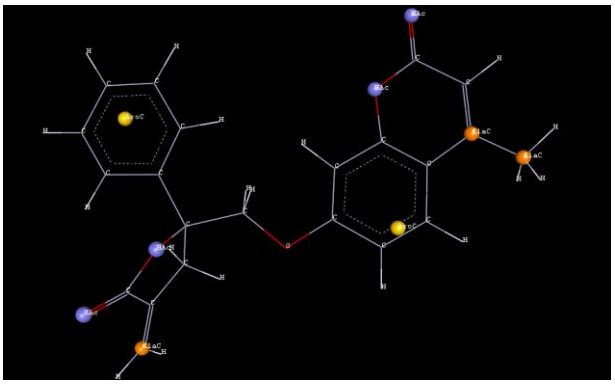
Figure 3: Docking interactions of coumarin derivatives.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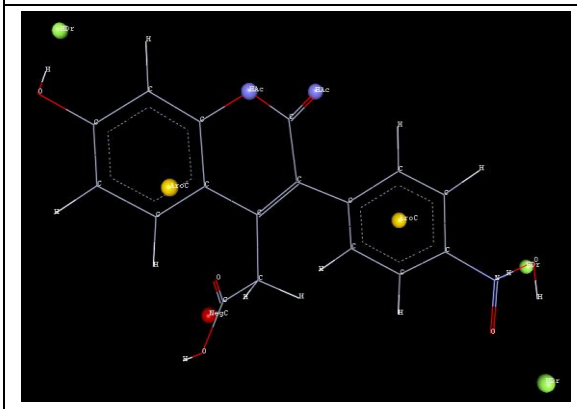	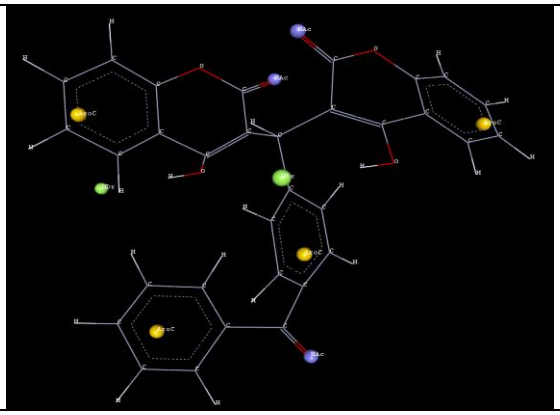
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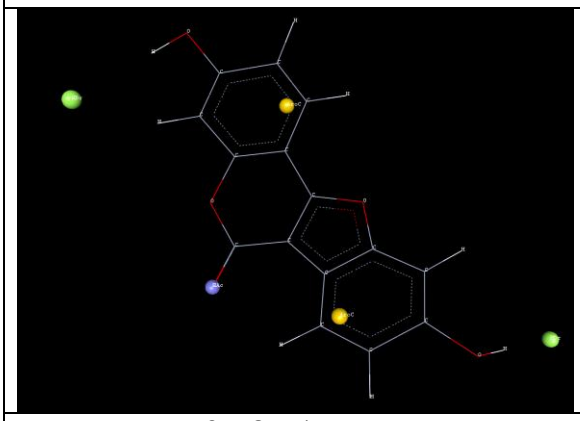	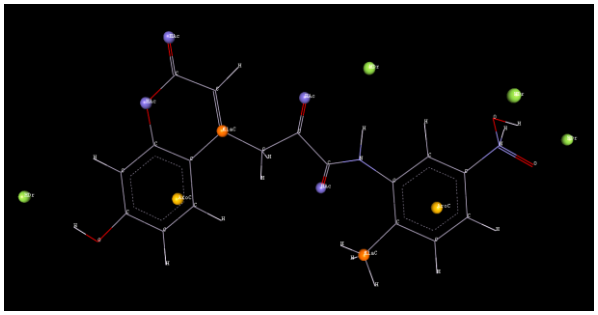
011954/1LW2	108833/1LW2

Figure 4: Pharmacophoric features.

Table 2: Details of docking score and molecular interactions.

S. No.	IC_{50} (µM)	Receptor	Molecular Weight	Docking Score	H-Bonding Interactions				Pi stacking interaction			
					Ligand Pose	H-Bond value (Å)	Interacting residue	Interacting ligand atom	Ligand Pose	pi-stacking value (Å)	Interacting residue	Interacting ligand atom
054759	7.11	3HVT	425.52	-74.93	LP11	2.579	Tyr181A	32H	LP9	4.786	Tyr181A	1C
144827	0.607	3HVT	362.38	-89.72	LP20	2.506	Lys103A	11O	LP7	4.655	Tyr181A	19C
144510	12.1	1IKW	294.3	-79.96	LP1	2.287	Glu130B	33H	LP1	4.591	Phe227A	1C
150908	72.1	1IKW	205.17	-58.67	LP6	2.584	Lys101A	7O	LP6	4.738	Phe227A	33C
011954	0.161	1LW2	268.22	-85.47	LP3	1.804	Leu422A	19O	LP3	5.028	Trp426A	10C
108833	372	1LW2	382.08	-79.80	LP10	2.266	Leu422A	16O	LP10	3.666	Trp426A	15C

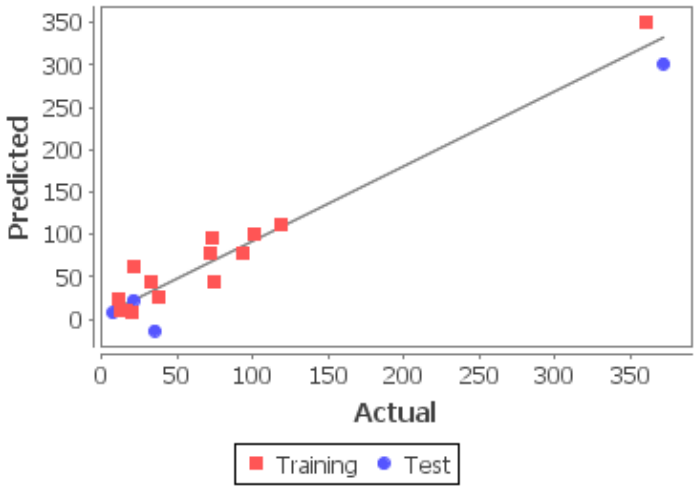

Figure 5: Fitness Plot of Calanolide Derivatives.

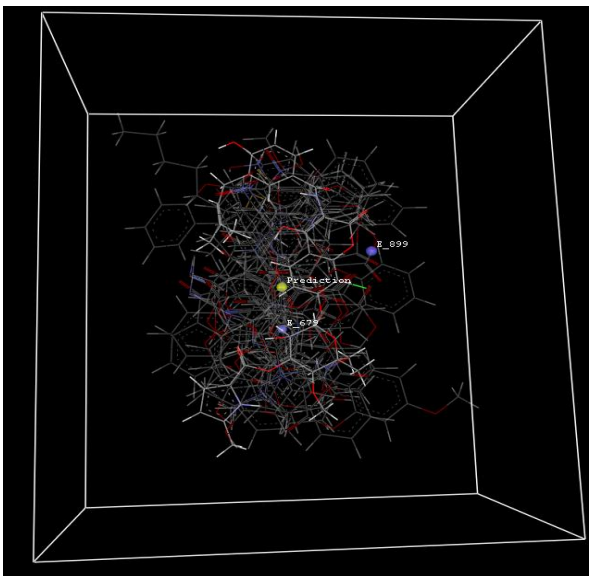

Figure 6: 3QSAR Model of Calanolide Derivatives.

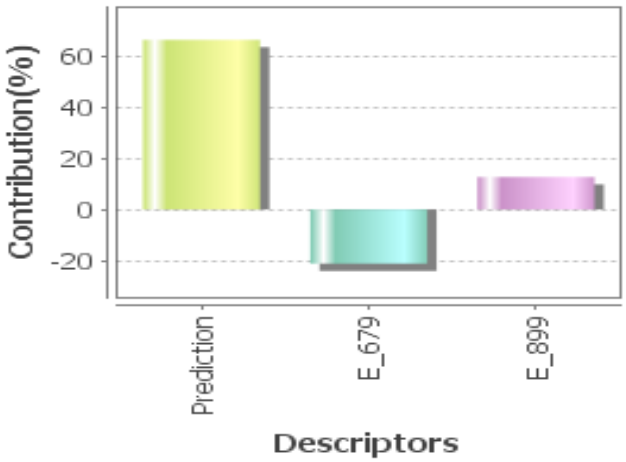

Figure 7: Contribution plot.

RESULT AND DISCUSSION

All the Compounds were studied for molecular docking interactions with non- nucleoside reverse transcriptase HIV-1 virus (3HVT, IKW, 1LW2), complexed with standard inhibitor; nevirapine, Efavirenz, 1051U91. Interactions were studied in terms of H-bonding and pi stacking.

As shown in table 2, all these calanolide derivatives have shown binding similarities as Nevirapine, Efavirenz, 1051U91. In the present paper, H bond value of ≤ 2Å was considered since it has indicated a strong H-bond in drug action.

Out of 83 compounds, 054759, 144827, 144510, 150908, 011954 and 108833 have displayed strong H-bonding interactions with 3HVT, IKW, 1LW2 respectively indicating potent inhibition of HIV-1 virus. Compound 011954 was the most potent analogs displaying H-Bonding of 1.804Å. This value was found to be in accordance with the corresponding IC50 values of 0.161 µM indicating a direct correlation between H-bond value and HIV-1 inhibition. All these compounds have displayed interactions with Tyr-181A, Lys-103A, Glu-130B, Lys-101A and Leu-422A residues. The structural component of various calanolide derivatives involved in H-bonding were 32H, 11O, 33H, 7O, 19O and 16O possibly indicating the active involvement of these oxygen atoms in H-bonding.

Coumarins nucleus with benzo-furan ring (compound 011954) was observed as the most potent analogues from the series. This was also supported by strong H-bond value, indicating that bicyclic coumaring ring system with benzo-furan moiety as the lead compounds from the series. Benzyl furan (144827) and triazidine with aliphatic side chain (054579) have shown comparatively less strong H-bonding interactions with 3HVT. This was also supported by their greater IC50 values.

As shown in table 2, many caumarins derivatives showed derivatives pi stacking interaction. In this paper, pi-stacking value ≤ 5 Å was considered. Above said compounds displayed interactions with Tyr-181A, Phe227A & Trp-426A residues of 3HVT, IKW, 1LW2 respectively. The structural component involved in pi stacking were 1C, 19C, 1C, 33C, 10C & 15C.

On comparison between H-bonding and pi-stacking of above coumarins, analogs with strong H bond value (≤ 2 Å) was involved less strongly with pi stacking interactions. Vice-versa was

also true. So this was considered that pi-stacking was another interacting force participating in anti-HIV-1 activity when H-bonding interaction was not in function.

When we focused at most potent molecules, this was clear that a bicyclic ring of coumarins attached with a substituent Bebzo-furanone ring at C-3 and C-4 on ring A was able to generate lead via pi stacking interactions.

Since the calculation of the pairwise molecular similarities and hence the prediction was based upon current training set, the r^2 value obtained (0.9597) is the indicative power of the current model. The predictive power of the current model for external test set was found q^2 = 0.9174 (Internal validation) and Predicted r^2 = 0.9254 (external validation) (Figure 5). As a result, electronic descriptors E_679 and E_899 (-0.1853, -0.1852), were used to build the model and to generate equation (Figure 6). The statistical significance of the model was evaluated as contribution plot (Figure 7). The robustness of the QSAR model for experimental training sets was examined by comparing this model to those derived for random dataset. The QSAR model was evaluated using the following statistical measures; numbers of observations, i.e numbers of descriptors (v_n=2); regression coefficient r^2 = 0.9597, cross validated r^2 (q^2 = 0.9174); predicted r^2 for the external test set (Pred_ r^2 = 0.9254); standard error of r^2 (r^2 _se =20.5718); standard error of cross validated r^2 (Pred_ r^2 se =49.2628).

The result obtained from the study has revealed that both H-bonding and pi-stacking interactions contributed significantly towards anti-HIV activity. Improved binding interactions were observed among few 2, 3 and 4-membered cyclic coumarins with macromolecules. The data has also been well supported by 3DQSAR studies (r^2 = 0.9597, q^2 = 0.9174 and Pred_r^2 = 0.9254).

The study has indicated that coumarins could serve as a lead non nucleoside reverse transcriptase inhibitor in the treatment of HIV mediated manifestation.

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