QSAR studies on Withanolide analogues for Anticancer activity

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Introduction

Withanolides are group of pharmacologically active compounds present in most prodigal amounts in roots and leaves of *Withania somnifera* (Indian ginseng), one of the most important medicinal plant of Indian systems of medicine. Withanolides are basically steroidal lactones (highly oxygenated C-28 phytochemicals) and similar to ginsenosides activity. Some of the withanolides have been reported to possess immunomodulatory, anticancer & other activities. In the present investigation, a quantitative structure activity relationship (QSAR) model have been developed against the MCF7, MCF7/BUS, and SK-Br-3 human solid tumor breast cancer cell lines. Relationship correlation coefficient (r^2) and cross validation correlation coefficient (r^2CV) of QSAR model were 0.77 and 0.73 for MCF7, 0.91 and 0.85 for MCF7/BUS, 0.93 and 0.90 for SK-Br-3 respectively. Developed QSAR model was also evaluated for prediction accuracy through internal, external and randomization validation methods. QSAR results indicate that for MCF7 cancer cell line, atom count (all atoms), and connectivity index (order 2, standard) descriptors correlate well with the activity. Similarly, for , SK-Br-3 cancer cell line, Connectivity Index (order 0, standard), Dipole Vector X (debye), Molar Refractivity, and Shape Index (basic kappa, order 2) descriptors correlated well with activity. Lastly, for MCF7/BUS cancer cell line chemical 2D descriptors viz., Atom Count (all atoms), Dielectric Energy (kcal/mole), Total Energy (Hartree), and Heat of Formation (kcal/mole) correlate well with the breast cancer activity. Moreover, on the basis of screening for oral bioavailability, *in silico* ADME and toxicity risk assessment, we concluded that compounds Withn_1, Withn_3, Withn_4 and Withn_8 have higher activity. These results can offer useful references for directing the molecular design of lead compound with improved activity.

Method

Retrieval of Crystal structures

➢3D crystal structure of 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) protein (PDB: 3HB5) and chemical compounds were retrieved from PDB (www.rcsb.org) and PubChem databases respectively.

Cleaning, Optimization & molecular docking of Withanolide analogues

➢3D structure of Withanolide analogues were designed and optimized through ChemBioOffice software. Molecular structures of target protein 17beta-HSD1 and chemical molecules were prepared and docked through Scigress Explorer v7.7 (Fujitsu). Molecular docking was performed to find out the binding affinity or molecular interaction energy (kcal/mol) of docked compounds calculated by using PMF scoring scheme. Lowest energy of docked molecule indicates high binding affinity with the target protein. Binding pocket of docked Withanolide analogues were studied for selection radius 3Å.

Multiple linear regression QSAR modeling for anticancer activity targeting 17beta-HSD1

➢Calculation of 2D and 3D molecular chemical descriptors for QSAR modeling by using forward stepwise multiple linear regression method and drug likeness study (Lipinski et. al., 2001) was done through Scigress Explorer v7.7. The model was constructed with 22 compounds in the training set, which was validated by the remaining molecules in the test set. We employed 'leave-one-out' cross validation procedure.

Results

• MLR method was employed to generate a linear relationship that correlates changes in the computed steric and electrostatic potential fields with changes in the corresponding experimental values of the training compounds activity (GI_{50}).

• Docking results showed that all the active Withanolide analogues docked on 17beta-HSD1 with high binding affinity.

• 2D contour maps of Withanolide analogues were compared with the crystal structure of 17beta-HSD1 complex (PDB: 3HB5).

• Docking results of Withanolide analogues showed that the important residues of 17beta-HSD1 active pocket are hydrophilic and polar. Ale-191, Phe-192, Met-193, & Gly-198 are the key residues in the binding pockets responsible for molecular binding with Withanolide analogues .

• QSAR results indicate that for MCF7 cancer cell line, atom count (all atoms), and connectivity index (order 2, standard) descriptors correlate well with the activity. Similarly, for , SK-Br-3 cancer cell line, Connectivity Index (order 0, standard), Dipole Vector X (debye), Molar Refractivity, and Shape Index (basic kappa, order 2) descriptors correlated well with activity. Lastly, for MCF7/BUS cancer cell line chemical 2D descriptors viz., Atom Count (all atoms), Dielectric Energy (kcal/mole), Total Energy (Hartree), and Heat of Formation (kcal/mole) correlate well with the breast cancer activity .

Conclusion

• The docking results of 10 Withanolide analogues showed that the active residues of 17beta-HSD1 binding pocket are hydrophilic and polar in nature.

• There was a significant correlation between binding affinity (docking energy) and the experimental (growth inhibition) GI_{50}.

• Moreover, Ale-191, Phe-192, Met-193 & Gly-198 are the key residues in the binding pockets responsible for molecular binding with Withanolide analogues /derivatives.

• QSAR model for Withanolide analogs against anticancer activity was successfully developed to understand the pharmacophoric factors governing its activity. Results indicate that developed QSAR model is robust and have good predictive accuracy for anticancer activity targeting MCF7, SK-Br-3 and MCF7/BUS breast cancer cell lines.

Table 1. Compliance of Withanolide analogues to computational parameters of pharmacokinetics (ADME).

Principal Descriptors:	Withn_1	Withn_2	Withn_3	Withn_4	Withn_5	Withn_6	Withn_7	Withn_8	Withn_9	Withn_10	Stand. Range
log S for aqueous solubility	-4.824	-4.670	-4.989	-4.684	-5.487	-5.377	-5.032	-5.228	-5.140	-5.220	(-6.5 / 0.5)
log Khsa Serum Protein Binding	-0.095	0.337	0.226	-0.063	0.457	-5.561	0.322	0.534	0.406	0.407	(-1.5 / 1.5)
log BB for brain/blood	-2.904	-1.120	-1.469	-2.052	-1.775	-0.948	-1.409	-0.938	-0.972	-1.511	(-3.0 / 1.2)
No. of Primary Metabolites	5	4	5	4	6	6	4	6	5	5	(1.0 / 8.0)
Predicted CNS Activity	-2	-2	-2	-2	-2	-1	-2	-1	-1	-2	–2 (inactive), +2 (active)
Log IC50 for HERG K+ Channel Blockage	-2.754	-4.309	-4.483	-2.673	-4.629	-4.343	--	-4.155	-4.311	-4.558	(concern below -5)
Apparent Caco-2 Permeability (nm/sec)	3	351	261	17	164	491	211	475	464	199	(<25 poor, >500 great)
Apparent MDCK Permeability (nm/sec)	1	159	116	8	74	229	92	221	216	86	(<25 poor, >500 great)
log Kp for skin permeability	-6.218	-3.493	-3.767	-4.880	-4.063	-3.392	-4.010	-3.464	-3.463	-3.983	(–8.0 to –1.0, Kp in cm/hr)
Jm, max transdermal transport rate	0.000	0.003	0.0001	0.000	0.000	0.001	0.000	0.001	0.001	0.000	(micrograms/cm^2-hr)
Lipinski Rule of 5 Violations	1	0	1	1	1	0	0	0	0	0	maximum is 4
Jorgensen Rule of 3 Violations	1	0	0	1	0	0	0	0	0	0	(maximum is 3)
% Human Oral Absorption in GI (+-20%)	37	91	73	53	73	95	86	95	93	86	(<25% is poor)
Qual. Model for Human Oral Absorption	Medium	HIGH	HIGH	Medium	Medium	HIGH	HIGH	HIGH	HIGH	HIGH	(>80% is high)

Table 2. Compliance of active Withanolide analogues against in silico toxicity risk parameters

Compound	Toxicity risk parameters				Drug likeness parameters (Osiris)				
	MUT	TUMO	IRRI	REP	CLP	S	MW	DL	DS
Withan_1	No risk	No risk	No risk	No risk	0.21	-4.31	570	1.97	0.51
Withan_2	Medium risk	No risk	High risk	No risk	1.89	-4.56	460	-2.37	0.17
Withan_3	No risk	No risk	No risk	No risk	1.49	-2.61	503	3.43	0.63
Withan_4	High risk	No risk	Medium risk	No risk	2.08	-4.56	528	-2.64	0.14
Withan_5	Medium risk	No risk	Medium risk	No risk	3.49	-5.01	502	1.45	0.29
Withan_6	High risk	No risk	High risk	No risk	3.26	-5.15	468	-1.13	0.12
Withan_7	Medium risk	No risk	Medium risk	No risk	2.55	-4.47	470	1.69	0.37
Withan_8	High risk	No risk	High risk	No risk	2.3	-4.53	470	-0.63	0.15
Withan_9	High risk	No risk	High risk	No risk	2.3	-4.53	470	0.14	0.17
Withan_10	Medium risk	No risk	Medium risk	No risk	2.38	-4.42	472	1.25	0.36

Figure 1. Multiple linear regression curve plot for MCF7 breast cancer cell line showing comparison of experimented log GI_{50} and predicted log GI_{50} (µM) (Growth inhibition).

QSAR model for MCF7 breast cancer cell line :

Predicted log GI50(µM) = 0.230892 x Atom Count (all atoms)
-0.297816 x Connectivity Index (order 2, standard)
-0.0275772 x Molecular Weight
+2.92179
[r^2CV=0.733719 r^2=0.771875]

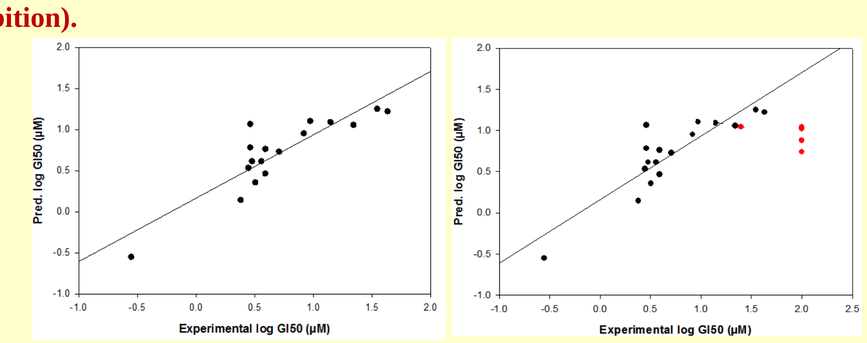


Figure 2. Multiple linear regression curve plot for SK-Br-3 breast cancer cell line showing comparison of experimented log GI_{50} and predicted log GI_{50} (µM) (Growth inhibition).

QSAR model for SK-Br-3 breast cancer cell line:

Predicted log GI50 (µM) = -0.847554 x Connectivity Index
-0.105787 x Dipole Vector X (debye)
+0.247149 x Molar Refractivity
-1.1174 x Shape Index (basic kappa, order 2)
-2.22636
[r^2CV=0.903815 r^2=0.934013]

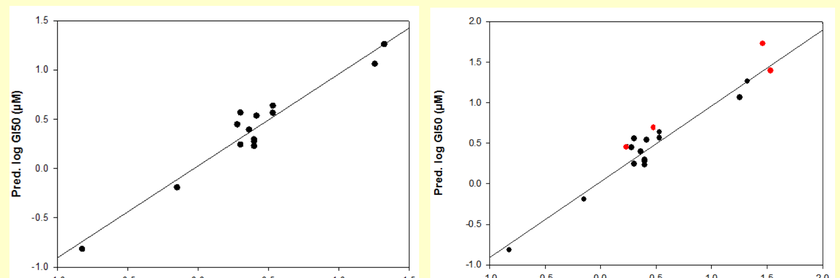


Figure 3. Multiple linear regression curve plot for MCF7/BUS breast cancer cell line showing comparison of experimented log GI_{50} and predicted log GI_{50} (µM) (Growth inhibition).

QSAR model for MCF7 / BUS breast cancer cell line:

Predicted log GI50 (µM) = 0.23502 x Atom Count (all atoms)
+1.84326 x Dielectric Energy (kcal/mole)
+0.0797426 x Total Energy (Hartree)
-0.00518695 x Heat of Formation (kcal/mole)
+5.58755
[r^2CV =0.856145 r^2=0.914576

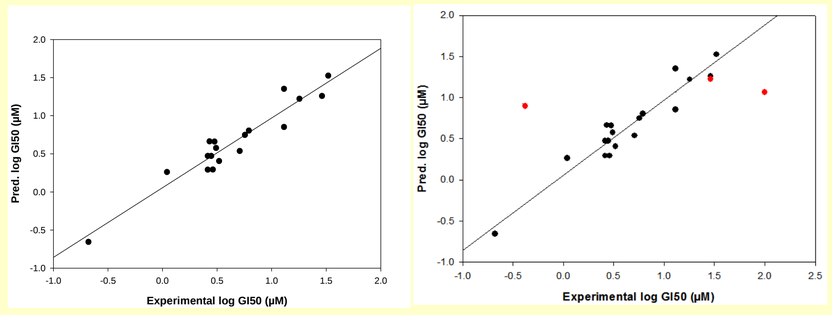


Figure 4. Superimposition of Withanolides analogues and E2B showing complementary structural conformation.

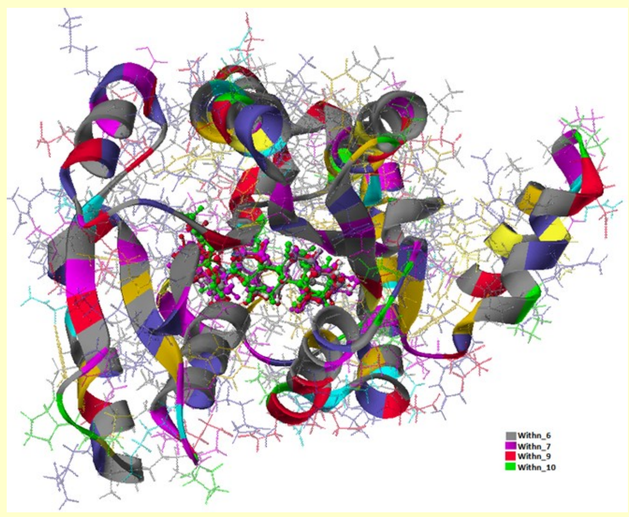


Figure 5. Docking score of Withanolide analogues against human anticancer target 17beta-HSD1

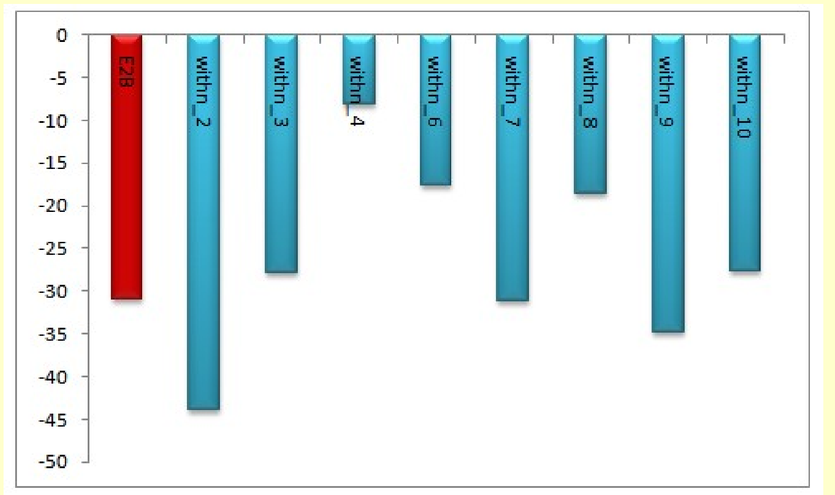
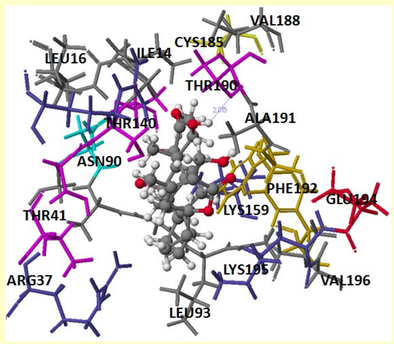
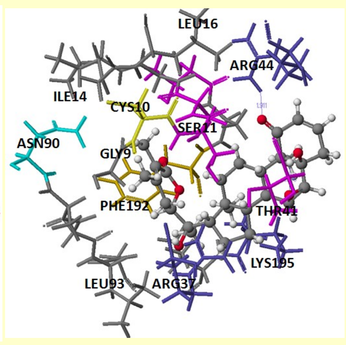


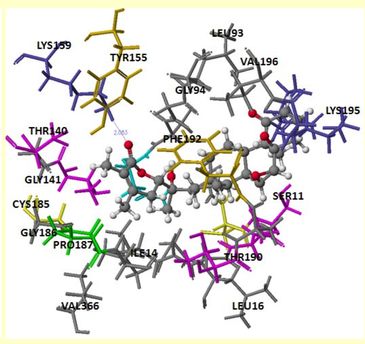
Figure 6. Molecular insight of Withanolide analogues docked on human anticancer target 17beta-HSD1 showing conserved pocket residues of binding site.



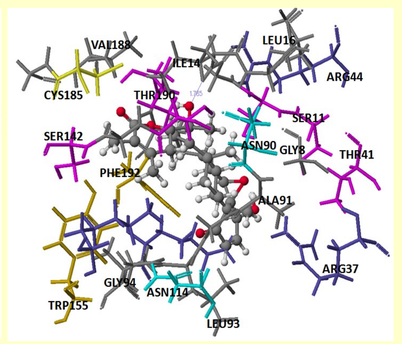
Withn_6 docked on 17beta-HSD1 anticancer receptor with docking score -17.647 kcal/mol.



Withn_7 docked on 17beta-HSD1 anticancer receptor with docking score -31.161 kcal/mol.



Withn_9 docked on 17beta-HSD1 anticancer receptor with docking score -34.901 kcal/mol.



Withn_10 docked on 17beta-HSD1 anticancer receptor with docking score -27.682 kcal/mol.

Acknowledgment

We acknowledge the Council of Scientific & Industrial Research (CSIR), New Delhi for financial support at Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India.

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