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QSAR analysis of 5-aryl thiazolidine-2,4-diones as PPAR-α and PPAR-γ agonists

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A quantitative structure activity relationship (QSAR) study on a series of analogs of 5-arylthiazolidine-2, 4-diones with activity on PPAR-α and PPAR-γ has been made using combination of various thermodynamic, electronic and spatial descriptors. Several statistical regression expressions are obtained using multiple linear regression analysis. The best QSAR model is further validated by leave one out cross validation method. The studies reveal that for dual PPAR-α/γ activity modification at R_2 position in molecule is more favourable and also lower value of resultant dipole moment play a key role in activity. Thus, QSAR brings important structural insight to aid the design of dual PPAR-α/γ receptor agonist.

Keywords: QSAR analysis, thiazolidine-2,4-diones, peroxisome, proliferator activated receptor (PPAR-α/γ) agonist

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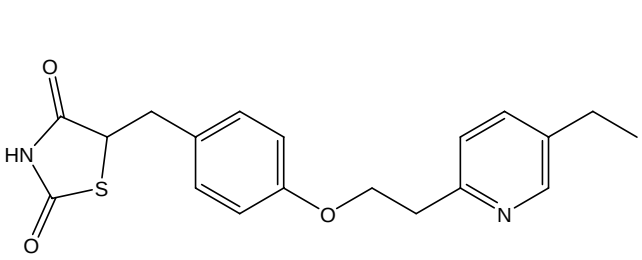
Resistance to the biological actions of insulin in its target tissues is a major feature of the patho-physiology in human obesity and in non-insulin dependent diabetes mellitus (NIDDM). The type-2 diabetes is characterized by decreased glucose transport and utilization at the level of muscle and adipose tissue and increased glucose production by the liver. It is the major cause of morbidity and mortality primarily through associated dyslipemias, atherosclerosis, hypertension, cardiovascular disease and renal dysfunction[1].

Therapeutic options for treating hyperglycaemia include sulphonylureas and other insulin secretagogues, biguanides, alpha-glucosidase inhibitors, thiazolidinediones (TZDs) and of course insulin[2]. Thiazolidinediones such as pioglitazone, rosiglitazone and troglitazone (**Figure 1**) target insulin resistance directly by stimulation of the nuclear transcription factor peroxisome proliferator activated receptor (PPAR) and thus tackle an underlying cause of the disease[3]. Three different isoforms PPAR-α, PPAR-δ, PPAR-γ of PPARs perform different physiological functions. PPAR-γ is mainly expressed in insulin sensitive tissues and hypothesized that their activation by TZDs affects the expression of number of genes involved in lipid and glucose metabolism and preadipocyte differentiation.

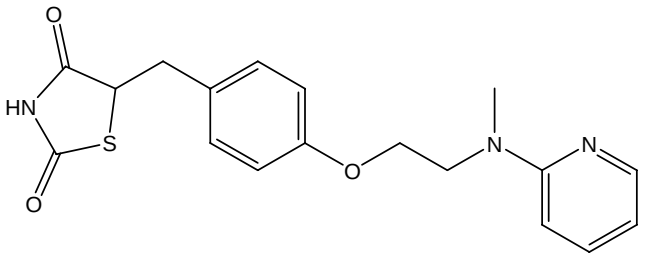
PPAR-α is found primarily in the liver and is the molecular target for the fibrate class of lipid lowering drugs[4]. Fibrates are effective at lowering serum triglycerides, raising high density lipoprotein (HDL) cholesterol level and also slow the progression of atherosclerosis and reduce the number of coronary events in patients with normal levels of low density lipoprotein (LDL) cholesterol and lately in diabetic patients. The recent identification of the dual PPAR-α and PPAR-γ agonist like KRP-297 and (-) DRF2725 (**Figure 2**) as being the targets for the normo-glycaemic thiazolidinediones and the lipid lowering fibrates lead to identify novel compounds for the treatment of type 2 diabetes[5-8]. Therefore, the combined profile of dual PPAR α/γ agonists would offer an attractive option for the management of hyperglycemia and hypertriglyceridemia. The aim of the present work is to study the QSAR of the dual acting receptor agonists and therefore to identify associated molecular properties and also optimize their agonist activity.

Experimental Section

Analogs of 5-arylthiazolidine-2,4-diones (**Table I**), as dual PPAR α/γ agonists were taken from the reported work of Desai *et al.*[9], excluding compounds with biological activities numerically not well defined (NNWD)/not reported (NR). The binding affinity data IC_{50} values (50 per cent binding affinity in μM) was converted to negative logarithmic dose (pIC_{50}) for QSAR analysis. The correlations were sought



Pioglitazone



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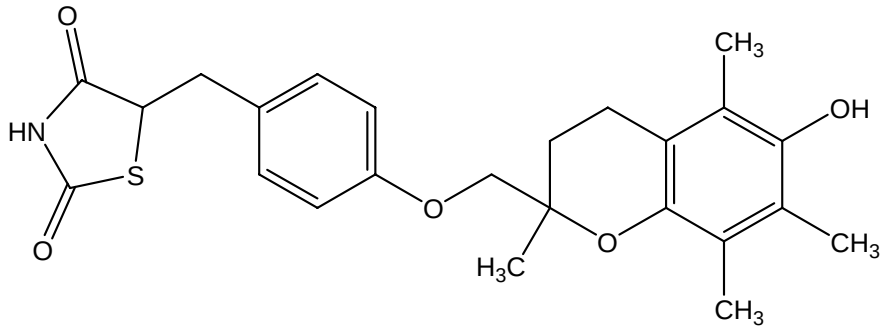
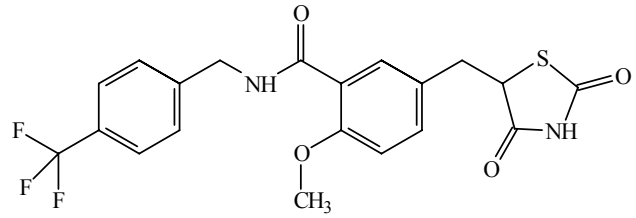
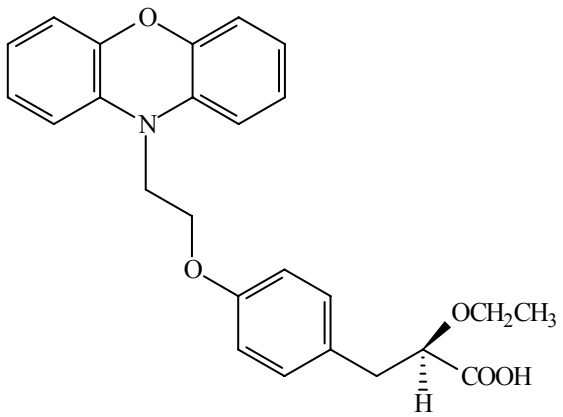


Troglitazone

Figure 1 — Structures of some PPAR-γ inhibitors



KRP-297

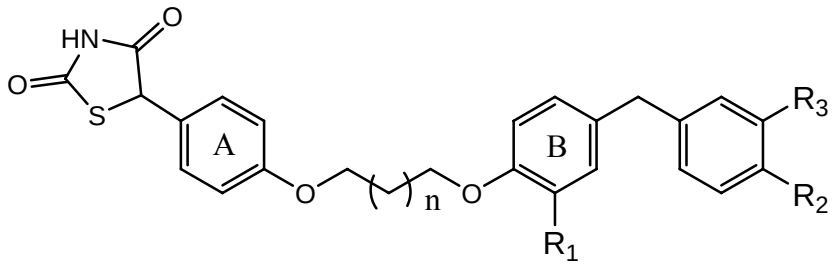


(-) DRF2725

Figure 2 — Structures of some PPAR-α and PPAR-γ dual inhibitors

between PPAR α/γ agonists activity and various substituents constants at positions R_1, R_2 and R_3 of molecule. The values of substituents constants like hydrophobic (π), steric (molar refractivity or MR), hydrogen acceptor (HA), hydrogen donor (HD) and electronic (field effect or $\mathcal{F}$, resonance effect or $\mathcal{R}$ and Hammett's constant or σ), taking into account from the literature, reported by Hansch *et al.*[10] The series was further subjected to molecular modeling and 3D-QSAR studies using CS Chem-Office Software version 6.0 (Cambridge soft)[11] running on a P-III processor. Structures of all the compounds (**Table I**) were sketched using builder module of the program. Then, structure was subjected to energy minimization using molecular mechanics (MM2) until the root mean square (RMS) gradient value becomes smaller than 0.1kcal/mol. Å. Minimized molecule was subjected to re-optimization via Austin model-1 (AM1) method until the root mean square (RMS) gradient attains a value smaller than 0.0001 kcal/mol. Å using MOPAC. The geometry optimization of the lowest energy structure was

Table I — Analogs of 5-aryl thiazolidine-2, 4-diones and their PPAR-α and PPAR-γ activities



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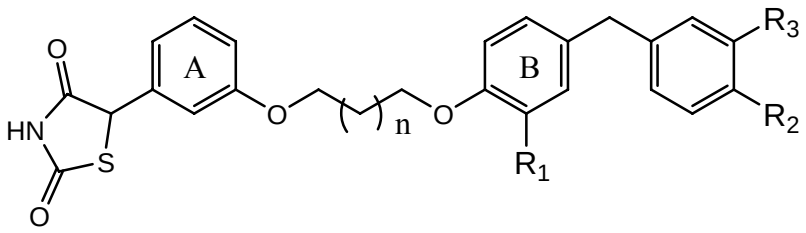

2-16

Compd	N	R_1	R_2	R_3	Binding IC_{50}[a]		Transactivation
					PPAR-α	PPAR-γ	EC_{50}[b]
1	1	C_3H_7	H	H	NNWD	0.180	0.300
2	1	C_3H_7	H	H	0.028	0.057	0.014
3	1	H	H	H	0.047	0.076	NR
4	2	C_3H_7	H	H	NNWD	0.195	NR
5	1	C_3H_7	$(CH_3)_2CH$	H	2.100	0.170	0.167
6	1	C_3H_7	$(CH_3)_3C$	H	NNWD	0.335	NR
7	1	C_3H_7	$(CH_3)_3C$	H	NNWD	0.291	NR
8	1	C_3H_7	$(CH_2)_5$	H	2.000	0.330	0.123
9	1	C_3H_7	C_6H_5	H	NNWD	0.226	0.353
10	1	C_3H_7	Cl	H	0.100	0.073	0.165
11	1	C_3H_7	F	H	0.028	0.077	0.085
12	1	C_3H_7	OCH_3	H	2.550	0.300	0.069
13	1	C_3H_7	OH	H	0.950	0.030	0.023
14	1	C_3H_7	Cl	Cl	0.068	0.064	0.047
15	1	C_3H_7	Cl	CH_3	0.162	0.056	0.027
16	1	C_3H_7	F	CH_3	0.112	0.078	0.106

[a] concentration of 50 per cent binding affinity in μM

[b] concentration of 50 per cent **t**ransactivation of PPAR-γ agonists activity in μM

carried out using Eigenvector following (EF) routine. The descriptor values for all the molecules were calculated using "compute properties module" of program.

Calculated thermodynamic descriptors included critical temperature (T_c), ideal gas thermal capacity (C_p), critical pressure (P_c), boiling point (BP), Henry's law constant (H), bend energy (E_b) and logP.

Steric descriptors derived were Connolly accessible area (CAA), Connolly molecular area (CMA), Connolly solvent excluded volume (CSEV), exact mass (EM), molecular weight (MW), principal moments of inertia-X component (PMIX), principal moments of inertia-Y component (PMIY) and principal moments of inertia-Z component (PMIZ), molar refractivity (MR) and ovality (OVAL).

Electronic descriptors such as electronic energy (ElcE), highest occupied molecular orbital energy (HOMO), lowest unoccupied molecular orbital energy (LUMO), dipole moment of X-component (DPL_1),

Table II — Observed (obs.) and calculated (cal.) pIC_{50} values of PPAR-α and PPAR-γ using 2D-QSAR model

Compd	PPAR-α			PPAR-γ		
	Obs. pIC_{50}[a]	Cal. pIC_{50}[b]	Residual	Obs. pIC_{50}[c]	Cal. pIC_{50}[d]	Residual
2	1.553	1.631	-0.078	1.244	1.017	0.227
3	1.328	1.631	-0.303	1.119	1.017	0.102
4	-	-	-	0.710	1.017	-0.307
5	-0.322	0.165	-0.487	0.770	0.693	0.077
6	-	-	-	0.475	0.583	-0.108
7	-	-	-	0.536	0.552	-0.016
8	-0.301	-0.632	0.331	0.481	0.488	-0.007
9	-	-	-	0.646	0.535	0.111
10	1.000	0.867	0.133	1.137	1.114	0.023
11	1.553	0.931	0.622	1.114	1.233	-0.119
12	-0.407	-0.051	-0.356	0.523	1.000	-0.477
13	0.022	0.130	-0.108	1.523	1.122	0.401
14	1.167	0.867	0.300	1.194	1.114	0.08
15	0.790	0.867	-0.077	1.252	1.114	0.138
16	0.951	0.930	0.021	1.108	1.233	-0.125

[a] observed negative logarithm of concentration of 50 per cent binding affinity to PPAR-α in μM
[b] calculated negative logarithm of concentration of 50 per cent binding affinity to PPAR-α in μM using equation 2.
[c] observed negative logarithm of concentration of 50 per cent binding affinity to PPAR-γ in μM
[d] calculated negative logarithm of concentration of 50 per cent binding affinity to PPAR-γ in μM using Equation 1.

dipole moment of Y-component (DPL_2), dipole moment of Z-component (DPL_3), resultant dipole (DPL_4), repulsion energy (NRE), VDW-1,4-energy (E14), Non-1, 4-VDW energy (E_v) and total energy were calculated.

Stepwise multiple linear regression analysis method was used to perform QSAR analysis employing in-house VALSTAT[12] program. The ± data within the parentheses are associated with t-value at 95% confidence interval of coefficient of the descriptors in regression equations. The equations were selected on the basis of various statistical parameters such as correlation coefficient (r), standard error of estimation (SE), sequential Fischer test (F). The robustness and applicability of QSAR equation as best model, on the structural analogs was further confirmed, using various QSAR validation technique like leave one out cross validated square correlation coefficient (Q^2) using cross validation method[13], boot-strapping square correlation coefficient (r^2_{bs}), randomize biological activity data test (chance) and test for outliers (Z-score value).

Results and Discussion

When data set was subjected to stepwise multiple linear regression analysis, in order to develop 2D-QSAR between binding affinity at PPAR-α or PPAR-γ receptor as dependent variables and substituents constants as independent variables, several equations were obtained. The statistically significant equation with coefficient of correlation (r) =0.786 was considered as model for PPAR-γ agonist (**Table II, Figure 3**). The model showed overall internal statistical significance level better than 99% as it exceeded the tabulated $F_{(2,12\ \alpha\ 0.01)} = 8.51$. The inter-correlation within the parameter (ICWP) is significantly low (less than 0.55) suggested the non-dependency of the parameters on each other.

$pIC_{50} = 0.498 * \mathcal{F}_2 - 0.021 * MR_2 + 1.039$
n=15, r=0.786, r^2=0.618, SE=0.229, F=9.706, ICWP<0.55 … (1)

While for PPAR-α the Eq. 2 was considered as model, which showed good correlation coefficient value (0.904) with low standard error of estimation (**Table II, Figure 4**). The model showed overall internal statistical significance level better than 99.0% as it exceeded the tabulated $F_{(2,8\ \alpha\ 0.01)} = 11.00$. The inter-correlation within the parameters is less than 0.23.

$pIC_{50} = 2.089 * \mathcal{R}_2 - 0.090 * MR_2 + 1.724$
n=11, r=0.904, r^2=0.818, SE=0.368, F=17.936, ICWP<0.23 … (2)

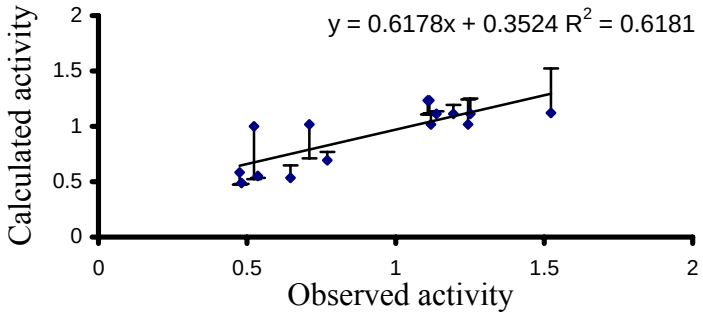

Figure 3 — A plot of observed Vs calculated pIC_{50} values of PPAR-γ activity with residual presentation using 2D-QSAR model

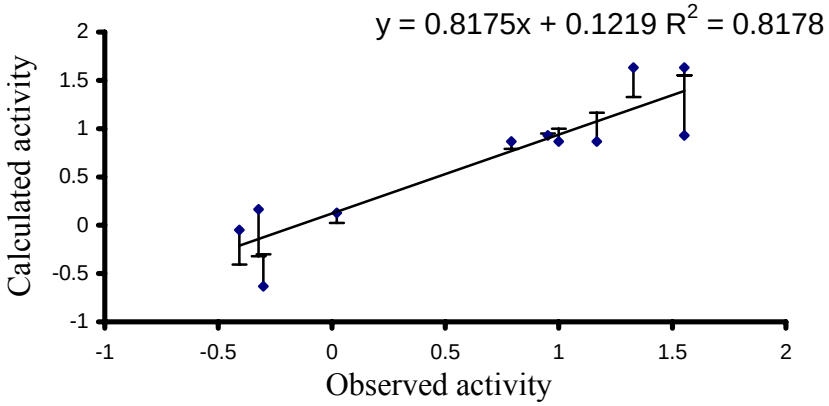

Figure 4 — A plot of observed Vs calculated pIC_{50} values of PPAR-α activity with residual presentation using 2D-QSAR model

Eq. 1 indicates that electronic effect (($\mathcal{F}_2$) contributed positively while steric effect (MR_2) at R_2 substitution position contributed negatively to PPAR-γ agonist activity. Similarly, for PPAR-α agonist activity (Eq. 2) electronic effect (resonance effect or $\mathcal{R}_2$) contributed positively while steric effect (MR_2) at R2 substitution position contributed negatively. The study suggested that R_2 position is more important as compared to other substituted positions like R1, R3 and change in chain length between the phenyl ring A and B (**Table I**) for modulation of PPAR α/γ agonists activity reveals that modification in electronic effect (increase in resonance and field effect) and decrease in molar refractivity at R2 position is favourable for both PPAR α/γ agonists activities.

The series was also subjected to molecular modeling using 3D-QSAR, all the descriptor values for the molecules, calculated from the program were considered as independent variables and binding affinity (pIC_{50}) for PPAR α/γ agonists activity was taken as dependent variables. Stepwise multiple linear regression analysis method was used to develop multi-variant relationship between binding affinity and descriptors. Amongst them, the several statistically significant equations were obtained. For PPAR-γ agonists activity (see Eqs 3 and 4)

pIC_{50}= 6.187e-005(±3.824e-005)*PMI-Y - 0.167(±0.068)*E14 -0.088(±0.091)*DPL_1 +2.673
n=16, r=0.880, r^2=0.775, std=0.177, F = 13.792, ICWP<0.78 ... (3)

pIC_{50}= 0.005(±0.003)* MP -0.128(±0.051)*E14 - 0.162(± 0.140)*DPL_4 -0.909
n=16, r=0.873, r^2=0.763, std=0.182, F=12.870, ICWP<0.53 ... (4)

Both the equations explain for more than 76% of the variance in the binding affinity but Eq. 3 having high inter-correlation within the parameters that suggested dependency of the descriptors on each other while Eq. 4 suggested low/insignificant dependency of the parameters. Therefore, Eq. 4 is considered as model for the PPAR-γ agonists activity (**Table III**). The model has good correlation coefficient value ($r \geq 0.873$) and significantly low standard error of estimation (SE = 0.182). The data

Table III — Inter correlation matrix of parameters used in 3D-QSAR equations for PPAR-γ activity

	PMI-Y	E14	MP	DPL1	DPL4
PMI-Y	1.000				
E14	0.781	1.000			
MP	0.697	0.528	1.000		
DPL1	0.279	0.314	0.304	1.000	
DPL4	0.176	0.272	0.452	0.840	1.000

Table IV — Calculated (cal.) and predicted (Pred.) pIC_{50} values with Z-value of PPAR-α and PPAR-γ using 3D-QSAR model

Compd	PPAR-α			PPAR-γ		
	Cal. pIC_{50}[a]	Z-value	Pred. PIC_{50}[b]	Cal. pIC_{50}[c]	Z-value	Pred. pIC_{50}[d]
1	-	-	-	0.765	-0.124	0.768
2	1.058	1.694	0.981	0.942	1.852	0.897
3	1.684	-1.218	2.072	1.137	-0.089	1.144
4	-	-	-	0.961	-1.540	0.995
5	-0.458	0.466	-0.532	0.508	1.607	0.403
6	-	-	-	0.493	-0.113	0.500
7	-	-	-	0.653	-0.716	0.669
8	-0.183	-0.402	-0.087	0.413	0.419	0.366
9	-	-	-	0.809	-1.001	0.856
10	1.165	-0.566	1.199	1.199	-0.384	1.213
11	1.328	0.768	1.280	1.127	-0.083	1.129
12	0.143	-1.881	0.240	0.814	-1.789	0.842
13	-0.108	0.447	-0.254	1.457	0.406	1.212
14	1.161	0.023	1.159	1.079	0.704	1.001
15	0.562	0.781	0.537	1.1	0.931	1.080
16	0.984	-0.112	0.991	1.121	-0.083	1.124

[a] observed negative logarithm of concentration of 50 per cent binding affinity to PPAR-α in μM
[b] predicted negative logarithm of concentration of 50 per cent binding affinity to PPAR-α in μM using leave one out method.
[c] observed negative logarithm of concentration of 50 per cent binding affinity to PPAR-γ in μM
[d] predicted negative logarithm of concentration of 50 per cent binding affinity to PPAR-γ in μM using leave one out method.

showed overall better statistical significance >99.9% with $F_{(3,12)}$ = 12.870 against the tabulated value for sequential Fischer test at 99.9% significant ($F_{3,12\ \alpha\ 0.001}$=12.7). The inter-correlations of the descriptor in the model are insignificant indicating that all the descriptors in the model were contributing independently to the biological activity. The model was subjected for leave one out (LOO) cross validation method (**Table IV, Figure 5**), the value of $Q^2 \geq 0.3$ in cross validation method corresponds to a confidence limit greater than 95%, which minimized the risk of finding significant explanatory equation for the biological activity just by mere opportunity. The value of cross-validated squared correlation co-efficient (Q^2=0.593), predictive residual sum of square (S_{PRESS}=0.239) and standard error of predictivity (S_{DEP}=0.207) suggested good predictive ability of the biological activity of diversified structure with low S_{DEP}. The r^2_{bs}=0.751 is at par with

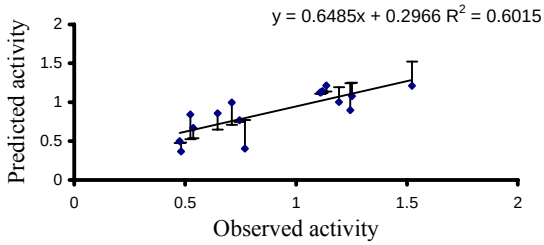

Figure 5 — A plot of observed Vs predicted pIC_{50} values of PPAR-γ activity with residual presentation using 3D-QSAR model

the conventional squared correlation coefficient (r^2), indicating that no single compound much more/less contributed to the model. Randomize biological activity data test (Chance < 0.001) revealed that the result was not based on chance correlation. The model was further tested for outlier by Z-score method. No compound was found to be outlier, suggested that the model is able to explain the structurally diversified analogs, which is helpful in designing of more potent compounds using physiochemical parameters. The correlation was also established between transactivation data (EC_{50}) of twelve compounds of the series although somehow poor correlation equation was obtained (Eq. 5).

EC_{50}= 0.100(±0.073)* H -88.227e-005(±61.961e-005) PMI-X +1.302

n=12, r=0.789, r^2=0.622, SE=0.300, F=7.408 … (5)

For PPAR-α agonists activity Eq. 6 was obtained as statistical significant, which explains for more than 85.6% of the variance in the binding affinity with low inter-correlation within the parameters (0.187).

pIC_{50}=-13.347(±8.976)*OVAL -0.645(±0.294)*DPL_4 +25.580
n=11, r=0.925, r^2=0.856, std=0.327, F=23.857,

ICWP<0.20 ... (6)

Model (Eq. 6) for PPAR-α agonist activity exhibits better correlation coefficient value ($r \geq 0.925$) and significantly low standard error of estimation (SE = 0.327). The data showed better statistical significance >99.9% with $F_{(2,8)}$ = 23.857 against the tabulated value for sequential Fischer test at 99.9% significant ($F_{2,8\ \alpha\ 0.001}$=22.7). The model was further subjected for leave one out cross validation method, the value of (Q^2=0.724), (S_{PRESS}=0.453) and (S_{DEP}=0.387) suggested good predictive ability of the biological activity (**Table IV, Figure 6**). The r^2_{bs}=0.877 is at par with the conventional squared correlation coefficient (r^2). Randomize biological activity data test is less than 0.001. The model also shows that no compound is outlier.

The study revealed that for PPAR-γ binding affinity, melting point of the compound contributed positively and the dipole moment and van der Waals - 1,4-energy contributed negatively while PPAR-α binding affinity, dipole moment and ovality contributed negatively. The models suggested that for dual activity, dipole moment is an essential parameter, which is contributing negatively to both PPAR-α/γ agonist activities. This suggests that modification at R_2 position (which is supported by 2D-QSAR analysis) occurs in such a way that resultant dipole of the overall molecule would be reduced and will be helpful for designing of more potent PPAR-α/γ receptor agonist.

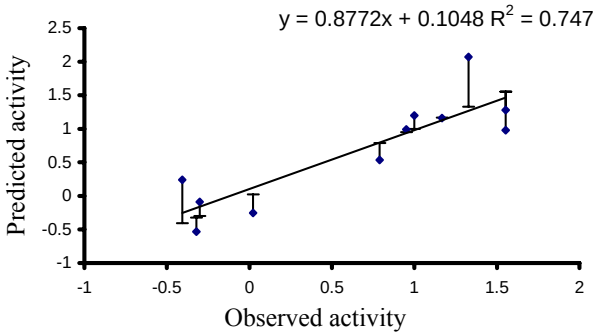

Figure 6 — A plot of observed Vs predicted pIC_{50} values of PPAR-α activity with residual presentation using 3D-QSAR model

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