Quantitative Structure Activity Analysis of 2-Alkoxydihydrocinnamates as PPARα/γ Dual Agonist

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Abstract: To optimize the physiochemical properties of 2-alkoxydihydrocinnamates as PPARα/γ dual agonist, a quantitative structure activity relationship, Hansch approach was made using combination of various thermodynamic, electronic and spatial descriptors. Several regression expressions are obtained using multiple linear regression analysis. The best QSAR model is further validated by leave-one-out cross validation method. Analyses of results from the present QSAR study suggest that for favorable dual PPARα/γ agonist activity electronic property of the substituents in hydrophobic tail phenyl ring plays a key role. The contribution of Hammett constant and dipole moment in the models deduced the importance of electron withdrawing substituents for dual activity. Additionally the study also indicates that bulky substituents in head acid moiety not confer selectivity towards the PPAR activity. Thus the QSAR study brings important structural insight to aid the design of dual PPARα/γ receptor agonist.

Key Words: QSAR, diabetes, hansch analysis, PPARα, PPARγ, hammett constant, dipole moment, heat of formation.

INTRODUCTION

Type II diabetes is characterized by decreased glucose transport and utilization at the level of muscle and adipose tissue and increased glucose production by the liver [1]. Hyperglycemia in type II diabetes is caused by resistance to the biological action of insulin in its target tissues and impaired insulin secretion from the pancreas [2]. It causes subsequent chronic complications leading to renal failure, blindness and coronary artery disease [3]. Peroxisome-proliferator activated receptor (PPAR)s are pharmaceutical target of great importance with their different isoforms PPARα, PPARγ, PPARδ, which differs by their target tissue and physiological functions [4,5]. The nuclear hormone receptors PPARα and PPARγ are important regulators of lipid and carbohydrate metabolism and its agonist action benefit for the treatment of diabetes and dyslipedimia [6-9]. The PPAR gamma modulators demonstrated clinical success in the treatment of type 2 diabetes [10-12] accompanied with a body weight gain [13]. PPAR alpha found primarily in the liver and is the molecular target for the fibrate class of lipid lowering drugs [14]. 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 patient with normal levels of low density lipoprotein (LDL) cholesterol and lately in diabetic patients [15,16]. The combined treatment with PPAR gamma and PPAR alpha agonist has been well suited for the treatment of patient with type 2 diabetes [17,18].

QSAR studies represent an attempt to correlate structural or property descriptors of compounds with activities. It explains the reasons of observed variations caused by the change of the substituents. Thus QSAR studies have predictive ability and simultaneously provide deeper insight into the mechanism of drug-receptor interactions even before their synthesis. We therefore, report here a QSAR study on thirteen molecules of 2-alkoxydihydrocinnamates for their PPARα/γ dual agonist activity.

MATERIALS AND METHODS

In the present work, even if the 2-alkoxydihydrocinnamate derivatives (Fig. (**1**)) as PPARα and PPARγ dual agonists reported in the literature [19] have structural similarity, narrow range of biological activity, we have tried to identify the associated molecular properties and exploited them to optimize PPAR dual agonist activity by using QSAR Hansch approach and it may be helpful in designing more potent PPAR dual agonists. The transactivation data (EC_{50} values) were converted to negative logarithmic dose, thus correlating the data linear to free energy change and reducing the skewness of the data set (Table **1**). The correlations were sought between PPARα/γ agonist activity and substituents constant at position R^1 and indicator variable for R^2 of molecules. The structural indicator variable I_1expresses 1 for

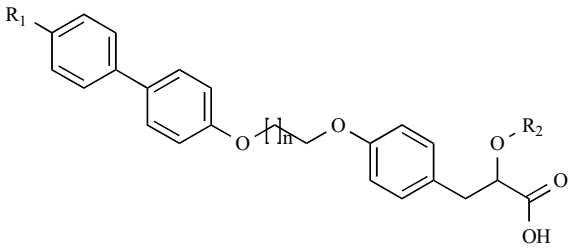

Fig. (1). Basic structure of 2-alkoxydihydrocinnamates.

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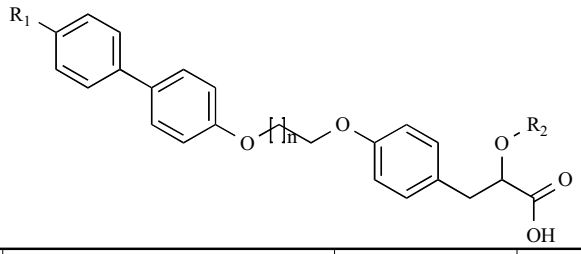
Table 1. Structure of Compounds Selected for QSAR Study



S.No	R^1	R^2	n	PPARγ (EC50μM)	PPARα (EC50μM)
1	H	Me	2	0.89	0.58
2	H	Et	2	2.57	1.77
3	H	Cyclopentyl	2	6.31	6.3
4	F	Me	2	0.3	0.05
5	F	Et	2	1.23	0.12
6	F	Cyclopentyl	2	6.46	0.5
7	Et	Me	2	0.14	0.1
8	Et	Et	2	0.15	0.4
9	Et	Cyclopentyl	2	2.24	1.12
10	H	Et	3	1.58	5.88
11	H	Cyclopentyl	3	3.8	3.8
12	Br	Me	2	1.69	3.24
13	Br	Et	2	0.76	2.19

presence of two carbon spacer between biphenyloxy and the central ring, I_2 expresses 1 for presence of methyl and ethyl group at R^2 and 0 for its absence. 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_p) taken into account from the literature, reported by Hansch [20]. The series was further subjected to molecular modeling and 3D-QSAR studies using CS Chem-Office [21] software version 6.0 (Cambridge soft). Structures of all the compounds were sketched using builder module of the program. The sketched structures were subjected to energy minimization using molecular mechanics (MM2) until the root mean square (RMS) gradient value becomes smaller than 0.1 kcal/mol. Å. Each energy minimized molecule was subjected to re-optimization *via* Austin model-1 (AM1) [22] 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 carried out using Eigenvector following (EF) routine. The descriptor values for all the molecules were calculated using compute properties module of the program.

Calculated thermodynamic descriptors included Critical temperature (T_c), ideal gas thermal capacity (IGTC), critical pressure (P_c), boiling point (BP), heat of formation (HOF), Henry's law constant (HL), bend energy (BE), and logarithmic partition coefficient (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 moment of inertia X-axis (PMIX), principal moment of inertia Y-axis (PMIY), principal moment of inertia Z-axis (PMIZ), molar refractivity (MR), and Ovality (OVAL). Electronic descriptors such as Electronic energy (EE), highest occupied molecular orbital energy (HOMO), lowest unoccupied molecular orbital energy (LUMO), dipole moment of X-axis (D1), dipole moment of Y-axis (D2), dipole moment of Z-axis (D3), resultant dipole (D4), repulsion energy (RE), VDW-1, 4-energy (E14), Non-1, 4-VDW energy (EV) and total energy (TE) were calculated.

Sequential multiple regression analysis method was used to perform QSAR analysis employing in-house VALSTAT [23] program. The ± data within the parentheses are associated with t-value at 95%confidence interval of coefficient of the descriptors in regression equation. The equations were selected on the basis of various statistical parameters such as correlation coefficient (r), standard error of estimate (s), sequential Fisher 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 (LOO) validated square correlation coefficient (r^2_{cv}) using cross validation method [24-25], boot strapping square correlation coefficient (r^2_{bs}) randomize biological activity data (chance) and test for outliers (z-score value). Use of more than one variable in the multivariate equation was justified by autocorrelation study.

RESULTS AND DISCUSSION

When a data set of thirteen compounds of 2-alkoxydihydrocinnamates were subjected to sequential multiple regression analysis, in order to develop 2D QSAR between transactivaion value at PPARα or PPARγ receptor as dependent variables and substituents constant as independent variables, several equations were obtained. The statistically significant equation (Eq.1) with coefficient of correlation (r) = 0.896 was considered as best model for PPAR-gamma agonist after removal of the outlier. The reason for its outlier is not immediately apparent. The model showed overall internal statistical significance level better than 99% as it exceeded the tabulated $F_{(2,9\ \alpha\ 0.01)}$ =8.02 .The inter-correlation within the parameter (ICWP) is significantly low (less than 0.15) suggested the non dependence of the parameters on each other.

pEC_{50}= [-0.811(± 0.353)] +σ_p [1.637(± 1.064)] +I_2 [0.949(± 0.412)] (1)

n=12, r=0.896, r^2 =0.803, SEE=0.290, F=18.338, Q^2= 0.705, SPRESS=0.354, SDEP=0.307

While for PPAR-alpha agonist activity, the Eq.2 was considered as best model, which showed good correlation coefficient value (0.812). The model showed overall internal statistical significance level better than 99% as it exceeded the tabulated $F_{(2,10\ \alpha\ 0.01)}$ = 7.56.The inter-correlation within the parameter is less than 0.05.

pEC_{50}= [-0.502(± 0.409)] + σ_p [1.855(± 1.588)] + $\mathcal{R}$ [-3.366(± 2.129)] (2)

n=13, r=0.812, r^2=0.660, SEE =0.444, F=9.698, Q^2=0.405, SPRESS = 0.587, SDEP=0.515

Eq. 1 indicates that electronic effect (Hammett's constant or σ_p) at R^1 substitution position contributed positively and indicator variable (I_2) for R^2 substitution position contributed positively to PPARγ agonist activity. Similarly for PPARα agonist activity (Eq.2) Hammett's constant or σ_p (electronic property) contributed positively, while resonance effect or $\mathcal{R}$ at R^1 substituted position contributed negatively.

Sigma is a descriptor of the substituent. The magnitude of σ gives the relative strength of the electron withdrawing or donating properties of the substituent. The positive contribution of sigma-para constant (σ_p) in both PPARα and PPARγ inferred that for dual PPARα/γ agonist activity, the R^1 substitution of tail phenyl ring could be substituted with electron withdrawing groups or atoms, which increase the receptor activation.

The positive contribution of indicator variable (I_2) showed, the bulky substituent at R^2 position of head acid group is not favorable for PPARγ agonist activity. This is in accordance with the reported work of Ying, which states that bulky substituent conveys unfavorable effect on the activities.

The analogs of 2-alkoxydihydrocinnamates were also subjected to 3D QSAR analysis; the descriptor values for the molecules calculated from the program were considered as independent variables and transactivaion value (pEC_{50}) for PPARα/γ agonist activity was taken as dependent variables. Sequential multiple linear regression analysis method was employed to develop multi-variant relationship between transactivation value and descriptors. Amongst them, several statistical significant equations were obtained. For PPARγ agonist activity

pEC_{50}= [-0.363(± 0.221)] +D1 [-0.349 (± 0.137)] +D3 [-0.160(± 0.138)] (3)

n=13, r=0.914, r^2=0.835, SEE=0.272, F=25.336, Q^2= 0.755, SPRESS=0.331, SDEP=0.291

The Equation 3 having better statistical significance is considered as the model for the PPARγ agonist activity. The model has good correlation coefficient value (r≥0.914) and significantly low standard error of estimation (SEE=0.272). The data showed overall better statistical significance >99.9% with $F_{(2,10)}$ = 25.336 against tabulated value for sequential fisher test at 99.9% significance ($F_{2,10\ \alpha\ 0.001}$=14.91). The inter-correlation of the descriptors in the model is insignificant indicating that all the descriptors in the model were contributing independently (less than 0.25) to the biological activity.

The model was subjected to leave one out (LOO) cross validation method, the value of Q^2≥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 coefficient (Q^2 = 0.755), predictive residual sum of square (SPRESS = 0.331) and standard error of predictivity (SDEP = 0.291) suggested good predictive ability (Table **2**) of the biological activity of diversified structure with low SDEP. The r^2_{bs} = 0.841 is at par with 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.

For PPARα agonist activity Eq.5 was obtained as statistical significant, which explains for more than 87.3 % of the variance in the biological activity with low inter-correlation within the parameter (0.218).

pEC_{50}= [-4.640(± 1.450)] +HOF [-0.006(± 0.002)] +D1 [-0.347(± 0.136)] (4)

n=13, r=0.934, r^2=0.873, SEE=0.271, F=34.415, Q^2=0.776, SPRESS=0.360, SDEP=0.316

Model (eq.4) for PPARα agonist exhibit better correlation coefficient value (r=0.934) and significantly low standard error of estimate (SEE= 0.271). The data showed better statistical significance >99.9 with $F_{(2,10)}$ = 34.415 against tabulated value for sequential Fisher test at 99.9% significant ($F_{2,10\ \alpha\ 0.001}$= 14.91). The model was further subjected for leave one out cross validation method, the value of Q^2=

Table 2. Observed (Obs.) and Predicted (Pred.) pEC_{50} Values of PPARγ and PPARα Using 2D and 3D QSAR Model

S.No	PPARγ			PPARα		
	Obs.pEC_{50}[a]	Pred.pEC_{50}[b]	Pred.pEC_{50}[c]	Obs.pEC_{50}[d]	Pred.pEC_{50}[e]	Pred.pEC_{50}[f]
1	0.506	0.1376	0.5837	0.2366	-0.502	0.1157
2	-0.4099	0.1376	-0.3193	-0.248	-0.502	-0.0849
3	-0.8	-0.811	-0.6854	-0.7993	-0.502	-0.8168
4	0.4202	0.2358	0.2008	1.301	0.7536	1.2151
5	-0.8991	----------	-0.9328	0.9208	0.7536	0.7317
6	-0.8102	-0.7128	-0.5211	0.301	0.7536	0.6964
7	0.8539	0.7433	0.6249	1	0.521	0.5193
8	0.8239	0.7433	0.6094	0.3979	0.521	0.6598
9	-0.3503	-0.2054	-0.5229	-0.0492	0.521	-0.3252
10	-0.1987	0.1376	-0.6009	-0.7694	-0.502	-0.7102
11	-0.5798	-0.811	-0.5354	-0.5798	-0.502	-0.7224
12	-0.2279	-0.1243	0.2827	-0.5106	-0.2267	-0.2752
13	0.1191	-0.1243	0.2696	-0.3404	-0.2267	-0.1425

[a] Observed pEC_{50} of PPARγ in μM

[b] Predicted pEC_{50} of PPARγin μM using 2D QSAR (Eq.1) by leave one out method

[c] Predicted pEC_{50} of PPARγ in μM using 3D QSAR (Eq.3) by leave one out method

[d] Observed pEC_{50} of PPARα in μM

[e] Predicted pEC_{50} of PPARα in μM using 2D QSAR (Eq.2) by leave one out method

[f] Predicted pEC_{50} of PPARαin μM using 3D QSAR (Eq.5) by leave one out method

0.776, SPRESS=0.360 and SDEP=0.316 suggested good predictive ability of the biological activity (Table **2**). The r^2_{bs} =0.880 is at par with the conventional squared correlation coefficient (r^2). Randomize biological activity data test is less than 0.001. No compound was found to be an outlier in this model.

Predicted values usually have the measure of statistical significance associated with the experimental values. With the statistical validation the generated correlations were also tested for the ability to reproduce the –logEc50 of compound in the series and a comparison was made with observed value (Table **2**). From the Table **2** it was observed that QSAR could not replace experimental values but have reasonable predictive values to show the statistical significance.

The 3D QSAR study reveals that for PPARγ agonist activity, dipole moment at X-axis and dipole moment at Z-axis contributed negatively, while for PPARα agonist activity dipole moment at X-axis and heat of formation contributed negatively.

Dipole moment indicates the strength and orientation behavior of a molecule in an electrostatic field. Dipole properties have been correlated to long-range ligand-receptor recognition and subsequent binding. The electron density in the enzyme cavity is related with this electronic property. The negative contribution of dipole moment at X-axis and dipole moment at Z-axis suggests that the compounds having orientation towards X-axis and Z-axis may show less PPARγ activity as well as the compound having dipole moment at X-axis shows less PPARα activity. This showed the importance of ionizable electron withdrawing functional group and their orientation in determining the dual PPARα/γ agonist activity. Our previous work on PPAR agonist activity also provides the evidence for contribution of dipole moment in their activity [26, 27].

Heat of formation is change of enthalpy that accompanies the formation of one mole of a compound in its standard state from its constituent element in their standard states. The number of atoms, number of bonds and order of bonds and number of non-organic elements directly affect the heat of formation. The negative contribution of heat of formation indicates that for PPARα agonist activity it can be substituted with molecule or atom, which decreases the heat of formation of that compound.

CONCLUSION

Quantitative structure-activity relationship (QSAR) models for PPARα/γ agonist activity of 2-alkoxydihydrocinnamates were established with CS Chem-office program. The QSAR models were generated by robust statistical techniques coupled with the use of combination of various descriptors. The selected QSAR models from the generated equations were analyzed for their statistical significance and predictive ability. It was observed from the selected models

that dual PPARα/γ agonist activity of 2-alkoxydihydrocinnamate derivatives is governed by electronic property. The findings of 2D QSAR and 3D QSAR study for dual PPARα/γ receptor agonist activity of alkoxydihydrocinnamates showed that the electron withdrawing substituted hydrophobic biphenyl tailpiece was proved to be effective scaffold. Though the acid head group is less contributing to dual activity, the bulkier substitution is not favorable for PPAR activity. The model also provides valuable insight into the mechanism of binding of these compounds to their receptor and it is helpful in designing more potent PPAR dual agonist molecules.

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REFERENCES

[1] Hotamisligil, G.S.; Spiegelman, B.M. *Diabetes*, **1994**, *43*, 1272.
[2] Shinkai, H. *Exp. Opin. Ther. Patents*, **2000**, *10*, 59.
[3] John, M.N.; Wagman, A.S. *Annu. Rep. Med .Chem*., **2000**, *35*, 211.
[4] Desvergne, B.; Wahli, W. *Endocr. Rev*., **1999**, *20*, 649.
[5] Lee, C.H.; Olson, P.; Evans, R.M. *Endocrinology*, **2003**, *144*, 2201.
[6] Akiyama, T.E.; Meinke, P.T.; Berger, J.P. *Curr. Diabetes Rep*., 2005, *5*, 45.
[7] Wilson, T.M.; Brown, P.J.; Sternbach, D.D.; Henke, B.R. *J. Med. Chem*., **2000**, 4, 527.
[8] Verges, B. *Diabetes Metab*., **2004**, *30*, 7.
[9] Shearer, B.G.; Hoekstra, W.J. *Curr. Med. Chem*., **2003**, *10*, 267.
[10] Campbell, I.W. *Curr. Mol. Med*., **2005**, *5*, 349.
[11] Zang, L.; Chawla, A. *Trends Endocrinol. Metab*., **2004**, *10*, 500.
[12] Walczak, R.; Tontonoz, P. *J. Lipid Res*., **2002**, *43*, 177.
[13] Methisen, A.L.; Brockley, M.R. *Diabetes*, **2000**, 49(1), A117.
[14] Noumura, M.; Kinoshita, S.; Satoh, H.; Maeda, T.; Murakami, K. *Bioorg. Med. Chem. Lett*., **1999**, *9*, 533.
[15] Torra, I.P.; Gervois, P.; Staels, B. *Curr. Opin. Lipidol*., **1999**, *10*, 151.
[16] Staels, B.; Dallongeville, J.; Auwerx, J.; Schoonjans, K.; Leitersdorf, E.; Fruchart, J.C. *Circulation*, **1998**, *98*, 2088.
[17] Pegorier, J.P. *Annu. Endocrinol*., **2005**, *66*, IS10.
[18] Henke, B.R. *J. Med. Chem*., **2004**, *47*, 4118.
[19] Ying Lu, Zongru, G.; Yanshen, G.; Jun, F.; Fengming, C. *Bioorg. Med. Chem. Lett*., **2006**, *16*, 915.
[20] Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley Intersceince, New York, **1979**, pp: 48.
[21] CS Chem Office, Version 6.0,Cambridge Soft Corporation, Software Publishers Association, 1730 M Street, NW, Suite 700, Washington, D.C. 20036.
[22] Keir, L.B. *Molecular Orbital Theory in Drug Research*, Academic Press, New York, **1971**, pp. 62.
[23] Gupta, A.K.; Babu, M.A.; Kaskhedikar, S.G. *Indian J. Pharm. Sci.*, **2004**, *66*, 396.
[24] Schaper, K.J. *Quant. Struct. Act. Relat*., **1999**, *18*, 354.
[25] Wold, S.; Eriksson, L. In *Chemometric Methods in Molecular Design*, Ed.; Van De Waterbeemd H. VCH: Weinheim, **1995**, pp. 321.
[26] Hemalatha, R.; Soni, L.K.; Gupta, A.K.; Kaskhedikar, S.G. *Indian J. Chem.,* **2006**, 45(B), 1247.
[27] Hemalatha, R.; Soni, L.K.; Gupta, A.K.; Kaskhedikar, S.G. *E-. J. Chem.,* **2004**, 01(5), 243.

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