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Understanding allosteric modulation in PPARγ receptors by comparative QSAR analysis

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Abstract

*Quantitative structure activity relationship (QSAR) analysis was carried out on different series of 2-alkoxydihydrocinnamates(eq.1), azaindole-α-alkyloxyphenyl propionic acids(eq.2), combined series of oxime ethers of α-acyl-β-henylpropanoic acids(**I**) and N-(2-Benzoylphenyl)-L-tyrosine derivatives(**II**) (eq.3) as PPARγ agonists. A range of electronic, steric and lipophilic parameters were tried. These results indicate the importance of Vw (van der Waals radius) and CMR. The combined model of (**I**) and (**II**) was found to be of interest in deriving new analogues with better activity as it indicated a positive parabolic relationship of biological activity (EC_{50}) with CMR (eq3).*

$-logEC_{50}(M)=2.053(0.899)R_1Vw-1.921(0.625)R_2Vw+6.476(0.375)$
$r=0.950, R^2=0.900, Q^2=0.800, s=0.177, CMR_{range}=11.04-13.59$ (1)
$-logEC_{50}(M)=-0.953(0.472)RVw+0.847(0.344)I_1+0.495(0.249)I_2+6.476(0.379)$
$r=0.935, R^2=0.875, Q^2=0.785, s=0.186, CMR_{range}=9.72-12.4$ (2)
$-logEC_{50}(M)=8.404(2.509)CMR-0.293(0.089)CMR^2-1.757(0.475)In-50.286(17.487)$
$r=0.917, R^2=0.841, Q^2=0.754, s=0.380, CMR_{range}=14.2-17.03, CMR_{optim}=14.3$ (3)

*Where In=1, for the presence of NH fragments in the subsitutents. New substituents were proposed in (**I**) which bring the CMR value of the molecules in the optimum range. It is tempting to speculate that the binding mechanism of **(I)** with PPARγ is different than 2-alkoxydihydrocinnamates and azaindole-α-alkyloxyphenylpropionic acids as the relationship of CMR (range:9-14.3) of **(I)** with activity is opposite to cinnamates and phenylpropionic acids. The downward slope of CMR in eq.(3) and modified forms(models with parameter CMR) of*

equation (1) and (2) were similar(0.293,0.247 and 0.329) which laterally authenticates the credibility of the proposed models.

Key words: QSAR, PPARγ, CMR, Type-2 Diabetes.

INTRODUCTION

Peroxisome Proliferator-Activated Receptors (**PPARS**)

The peroxisome proliferator-activated receptor **γ (PPARγ**) has been the focus of intense research during the past decade because ligands for this receptor have emerged as potent **insulin sensitizers used in the treatment of type 2 diabetes**. Recent advances include the discovery of novel genes that are regulated by PPARγ which helps explain how activation of this adipocyte predominant transcription factor regulates glucose and lipid homeostasis. Increased levels of circulating free fatty acids and lipid accumulation in non-adipose tissue have been implicated in the development of **insulin resistance**. This situation is improved by PPARγ ligands, which promote fatty acid storage in fat depots and regulate the expression of adipocyte-secreted hormones that impact on glucose homeostasis. The net result of the pleiotropic effects of PPARγ ligands is improvement of insulin sensitivity.[1]

Physiological Function of PPARγ:

The knowledge of molecular pharmacology of PPARγ receptor, may lead to improved hypoglycemic activity devoid of weight gain liabilities of earlier compounds. Thus, target selectivity is essential for the development of safe and effective therapy.

a) Adipocyte factors that contribute to insulin resistance in obesity via an endocrine mechanism. Increased levels of free fatty acids (FFAs) inhibit insulin signaling in muscle and liver, where increased lipid accumulation might also contribute to insulin resistance.
b) Regulation of gene expression by PPARγ ligands results in insulin sensitization via:

(**I)** Retention of fatty acids in adipose tissue through activation of fatty acid transporters (FATP1 and CD36), phosphoenol pyruvate carboxykinase (PEPCK) and glycerol kinase (GyK);

(II) Regulation of adipocyte hormone gene expression [adiponectin expression is increased, whereas the production of leptin, resistin, tumor necrosis factor a (TNF-**α,** interleukin 6 (IL-6), plasminogen activator inhibitor 1 (PAI-1) and cortisol via 11-b-hydroxysteroid dehydrogenase 1 (11b-HSD1) is decreased] by thiazolidinedione treatment.

c) Direct enhancement of adipocyte glucose disposal by induction of c-Cbl associating protein (CAP) and the glucose transporter GLUT4.

Quantitative Structure Activity Relationship (QSAR)

QSAR attempts to correlate structural molecular properties (descriptors) with functions (i.e. biological activities, toxicity, etc.) for a set of similar compounds by means of statistical methods, as a result, a simple mathematical relationship is established[2].

Function = f (structural molecular or fragment properties)

Approaches in QSAR Studies [3, 4]
There are different widely used approaches in QSAR studies. Following are the commonly used once
1-Hansch analysis (linear free energy relationship or extra thermodynamic approach)
2- Free and Wilson analysis.
3- Pattern recognisation.
4 - Quantum mechanical methods.

1-Hansch analysis-
The first application of QSAR came from Hansch in 1962 when they correlated the plant growth regulatory activity of phenoxyacetic acids to Hammett constants and partition coefficients. It is widely used approach, where the variation in biological activity is explained as a function of certain physicochemical and structural properties of molecules which include electronic characteristics, steric factors, hydrophobic effects and topological indices. The major advance in QSAR occurred later in 1964, when Hansch, showed that the biological activity could be correlated linearly by free-energy related terms (different physicochemical parameters). This approach was originally coined as Linear Free Energy Relationships (LFER) and later changed, more appropriately, to extra thermodynamic approach and expressed by the following equation.

$$log \quad 1/C = a \log P + b(\log P)^2 + c$$

Where a and b are the coefficients of the *log P* and $(log P)^2$ terms, respectively, and c is a constant term. The hypothesis was that substituent on a parent molecules have quantitative relationship with biological activity.

2- Free and Wilson analysis
Related biological activity to the presence/absence of a specific functional group at a specific location on the parent molecules.

$$\text{Activity} = \sum (a_1, I_1) + \mu$$

Where,
I_1 = the substituents
a_1 = the contribution of substituents.
μ = the contribution of parent structure.

3- Pattern recognisation-
They are similar to classical QSAR methodology. Only the number of variable is much higher than hansch analysis [5,6]

Intent of QSAR Study
1- Refinement of synthetic target.
2- Reduction and replacement of animals.
3- Diagnosis of mechanism.
4- To develop new model for a biological system.
5- Optimization of new lead molecule.

6- QSAR models act virtual screening tools for predicting ADME and toxicity studies.
7- To elucidate the phenomena and nature of drugs and receptor interactions.

RESULTS AND DISCUSSION

We correlated the activity of oxime ethers of α-acyl-β-phenylpropanoic acid derivatives (Table 1) and *N-(*2-Benzoylphenyl)-*L*-tyrosine derivative (Table 2) with various physicochemical, electronic and steric parameters. After many trial Equation 1 was found to be promising.

-log EC_{50} (M) = 4.907 (4.012) *CMR* -0.167 (0.141) CMR^2 -1.884 (0.750) In –26.438(28.020)
n = 31 r = 0.763 s = 0.674 F = 12.579…….1
In= 1, For the presence of NH group
0, For its absence

Then standard deviation was multiplied by factor 2, It was observed that amongst 31 data points, only three points having calculated residual value which were outside 2*s* range, this indicates that three **(10, 25, 31)** outliers are present in the models. After removing them a new equation was generated with the same parameters as in equation 1. The resultant equation 2 is as follows.

-logEC_{50} = 8.404(2.509) *CMR* -0.293(0.089) CMR^2 -1.757(0.475) In -50.286(17.487)

n = 28 r = 0.917 s = 0.380 F = 42.370 ……..2

Q^2 = 0.754 R^2 =0.841 R^2adj 0.706 RMSE 0.394

Interpretations
In equation 2, the positive contribution of steric parameter (*CMR*) indicates the presence of bulkier group is involved in the steric interaction with the receptors. Thus, the new substituent should be bulky with high *CMR*. So when graph is plotted between *CMR* and EC_{50} a closed parabolic graph was obtained as in figure 3.

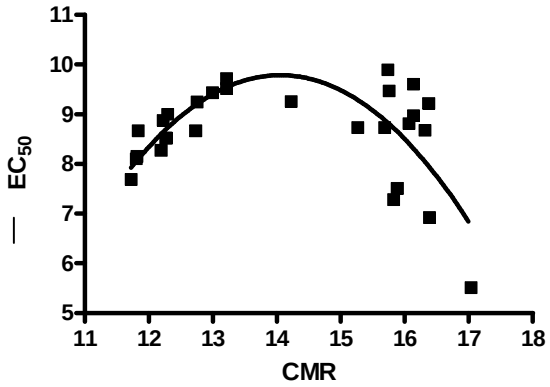


Figure 3: Graph between *CMR* and -EC_{50}

On the basis of above graph it was concluded that as the value of *CMR* increases up to certain limits the activity increases. The optimum range of *CMR* for the molecule should be 14.3(14.2-14.5) for which it gives the highest activity.

Based on the result of this QSAR equation, following compounds (Table 3) was proposed for the syntheses which suppose to have a good activity.

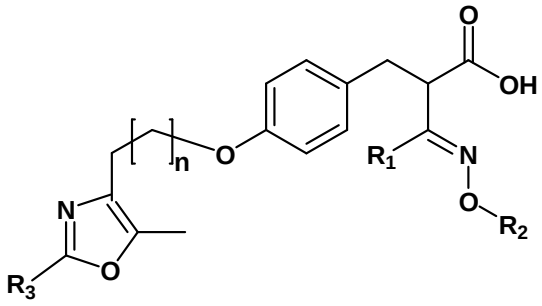


Figure 4: Lead molecule

Proposed Molecules

Table 3: list of substituent's proposed for introduction in lead molecule

S.NO.	R_1	R_2	R_3	*CMR*	Lipinski rule
1P	Pr	n-Bu	C_6H_5	14.064	Pass
2P	Bu	n-Bu	C_6H_5	14.524	Fail
3P	Me	n-Bu	C_6H_5SH	13.952	Pass
4P	Et	n-Bu	$C_6H_5NH_2$	14.074	Pass
5P	Pr	n-Bu	$C_6H_5NH_2$	14.534	Fail
6P	Me	n-Bu	C_6H_5OH	14.213	Pass
7P	Me	n-Bu	$C_6H_5CONH_2$	14.92	Fail

MATERIALS AND METHODS

The combined series of compounds which was subjected to QSAR analysis is oxime ethers of α-acyl-β-phenylpropanoic acid [7] (Fig: 1), the series is listed in Table: 1 and *N-(*2-Benzoylphenyl)-*L*-tyrosine [8] (Fig: 2) which is listed in Table:2 In these tables, EC_{50} (M) refers to the concentration of compounds required to activate 50% of the receptors.

During QSAR study, the value of EC_{50} (µM) was converted to –log EC_{50} (M) in order to bring out better linear correlations and reduce clustering of compounds during the generation of regression lines

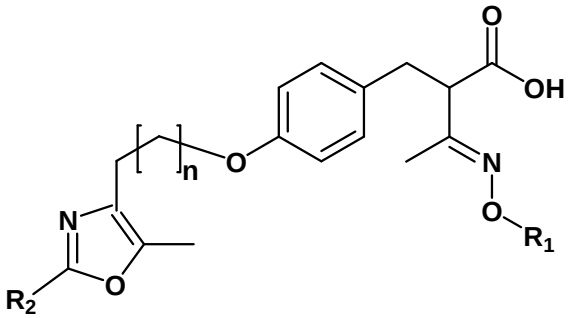


Figure 1: Oxime ethers of α-acyl-β-phenylpropanoic acid derivatives

Table 1: SAR of Oxime ethers of α-acyl-β-phenylpropanoic acid

Comp.	N	R_1	R_2	EC_{50} (nM)
1	2	Me	Ph	2.1
2	2	Et	Ph	1.0
3	2	n-pr	Ph	0.55
4	2	CH_2CHMe_2	Ph	0.3
5	2	n-Bu	Ph	0.19
6	2	CH_2 - cyclopropyl	Ph	0.36
7	2	4-*F*-phenylCH_2	Ph	0.54
8	1	Pr	Ph	13
9	1	Et	4-*F*- Phenyl	6.8
10	1	n-pr	4-*F*- Phenyl	28
11	1	2-*F*-Ethyl	4-*F*- Phenyl	7.7
12	2	Et	4-*F*- Phenyl	3.0
13	2	Pr	4-*F*- Phenyl	2.1
14	2	2-*F*-Ethyl	4-*F*- Phenyl	2.9
15	2	Et	i-Propyl	20
16	2	Pr	i-propyl	5.2

Multiple linear regression (MLR) analysis was adopted for QSAR study using Hansch approach. A self-generated software, kindly gifted by prof. S.P. Gupta (Chemistry group, BITS, Pilani) was utilized for generating QSAR equation, which provides correlation coefficient**(*r*)**, standard deviation **(*s*),** and ratio between the variance of calculated and observed activities **(*F*)**. The figures in the parentheses are 95% confidence interval and ***n*** is the number of data points. The software also gives intercorrelation matrix among the descriptors.

Figure 2: *N*-(2-Benzoylphenyl)-L-tyrosine derivatives

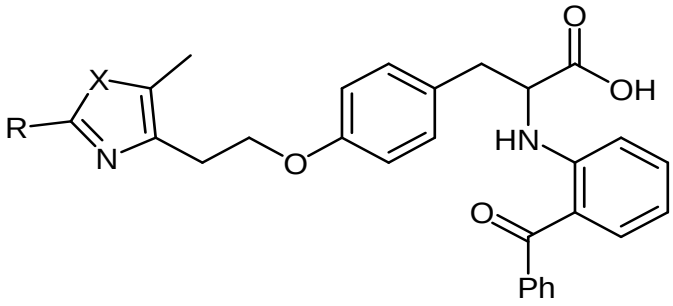
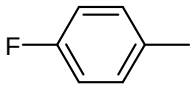
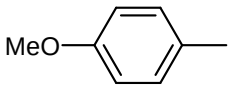
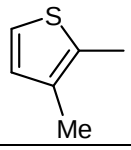
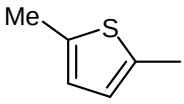
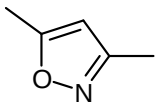
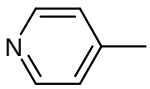
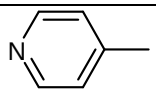
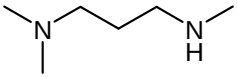
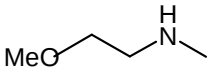
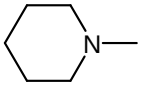
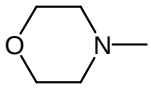
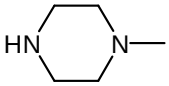
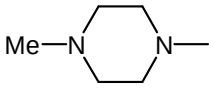


Table 2: SAR of *N*-(2-Benzoylphenyl)-*L*-tyrosine

S. NO.	R	X	EC_{50}
17		O	9.47 ± 0.44
18		O	9.90 ± 0.47
19		O	9.22 ± 0.27
20		O	8.98 ± 0.15
21		O	9.61 ± 0.17
22		S	8.82 ± 0.33
23		O	8.74 ± 0.23

24		S	8.68 ± 0.07
25		S	5.91 ± 0.20
26		S	5.52 ± 0.24
27		S	7.51 ± 0.33
28		S	7.29 ± 0.13
29		S	8.74 ± 0.19
30		S	6.93± 0.22
31		S	8.89 ± 0.04

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