CoMFA study on selective human β_3-adrenoceptor agonists

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Dedicated to Dr. A.V. Rama Rao, on the occasion of his 70th birthday
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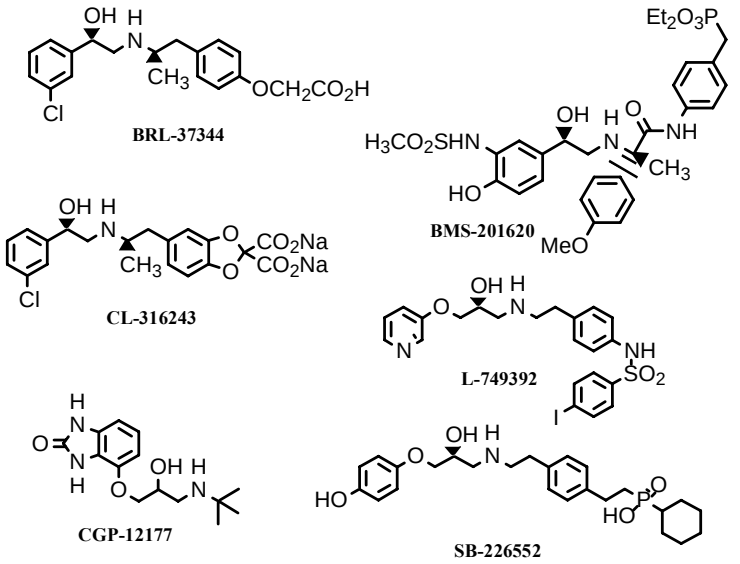
Abstract
Comparative molecular field analysis (CoMFA) was performed on a series of 47 compounds as potent selective human β_3-adrenoceptor (AR) agonists. Low energy conformation of the most active compound in the chosen series was found by Molecular dynamics simulated annealing method. The statistically significant model was established from 33 molecules, which were validated by evaluation of test set of 14 compounds. The fit atom based alignment yielded best predictive CoMFA model (r^2_{cv}=0.583, r^2_{cnv}=0.992, F Value=534.974, SEE=0.074, r^2_{pred}=0.743 with six components). The contour maps obtained from 3D-QSAR studies were appraised for the activity trends of the molecules analyzed. The results indicate that the steric, electrostatic substituents play significant role in β_3-AR activity and potency of these compounds. The data generated from the present study can be used as putative pharmacophore in the design of novel, potent, human β_3-adrenoceptor agonists as anti-obesity and anti-diabetic agents.

Keywords: β_3-Adrenoceptor agonists, anti-obesity agent, anti-diabetic agent, CoMFA; 3D-QSAR

Introduction

The β_3-adrenergic receptor (β_3-AR)[1] is a G-protein-coupled seven trans membrane domain receptor that is expressed mainly in adipose tissue where the excess fats are stored.[2] The β_3-AR plays a major role in mediating lipolysis in white adipocyte tissue (WAT) and thermogenesis (energy expenditure) in brown adipocyte tissue (BAT).[3] It was found that stimulation of β_3-AR by selective agonists produced remarkable anti-obesity effects.[3,4] β_3-AR agonists have also been found to cause insulin sensitisation.[5,6] A set of lead compounds identified for β_3-AR agonistic activity are given in scheme 1.[5-9] All the developed new chemical entities for β_3-AR produced significant effect in rodents but failed to produce similar effects in humans. This has been

attributed to the differences in the amino acid sequences and active sites of β_3-ARs of humans and animals.[10] The efficacy of these agents towards β_1 and β_2-ARs also has been found to be a liability. Current research in this area is mainly focused on developing selective human β_3-AR agonists for producing anti-obesity as well as anti-diabetic effects.[11]



Scheme 1

Three-dimensional structure of β_3-AR has not been resolved yet and the cause of their broad substrate specificities of β_3-AR agonists is not known. Therefore, we decided to use a ligand-based approach to extrapolate quantitative structure-activity relationships (QSARs)[12] for known β_3-AR agonists. CoMFA[13] method, which calculates steric and electrostatic properties according to Lennard-Jones and Coulomb potentials respectively, from the 3D structures of a series of compounds, has been employed in this study. CoMFA model can characterize the relative changes in magnitude of steric and electrostatic fields as a function of the sample chosen from the data set. It can account for the variance in measured biological activity, giving rise to the capacity to predict anti-obesity and anti-diabetic activities of new β_3-AR agonist analogous. As a result only agonists with high activities can be selected for syntheses through the analysis.

Method of calculations

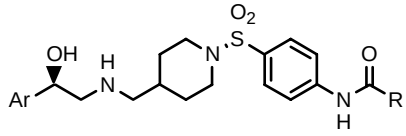
All molecular modeling techniques and CoMFA studies were performed on Silicon Graphics Fuel R14000 workstation with IRIX6.5 operating system using the SYBYL6.9 molecular modeling software package from Tripos, Inc., St.Louis, MO.[14]

Data set

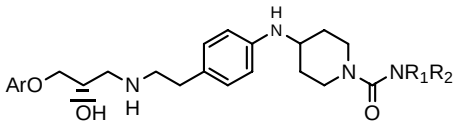
The structures of 47 agonists and their binding affinities (EC_{50}) to β_3-AR used in the study originate from same organization Wyeth Research USA.[15] All the compounds have been shown

to be selective agonists of β_3-AR. In the present study, a set of 33 compounds, served as training set, whose structures and associated biological activities are given in Table 1. Additional 14 compounds were used as test set to evaluate the predictive ability of the model obtained in this experiment. The structure and biological activities of test set compounds are also given in Table 1. The compounds selected in this study have been assayed by same experimental conditions, biological activities are (EC_{50}) expressed in nM concentration, and used as dependent variable in the correlation analysis. The compounds selected in this set have wide range of biological activities, ranging from 1 nM to 1020 nM. All the biological activities are converted into pEC_{50} ($-\log EC_{50} \times 10^9$) for CoMFA study.

Table 1. Structure and biological activity (EC_{50}) of compounds used in CoMFA study (Training set and test set)



1-6, 34



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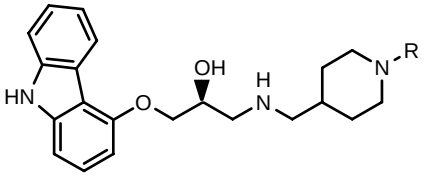
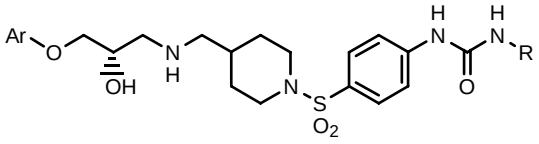


23-29, 37



30-33, 38-47

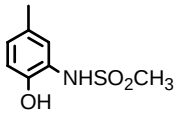
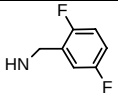
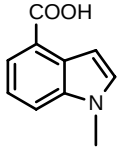
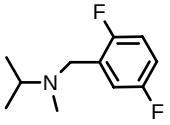
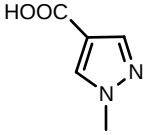
S. No	Ar	R	EC_{50} (nM)	pEC_{50}
1			5	8.301
2	″	Octyl	6	8.221
3	″		9	8.045
4	″		27	7.568
5	″		43	7.366
6	″	NH(C=O)Ph	31	7.508
7	4-OHC_6H_4	R_1=Me R_2=H	47	7.327
8	4-OHC_6H_4	R_1=H R_2=H	30	7.522

Table 1. Continued

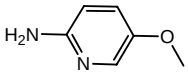
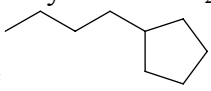
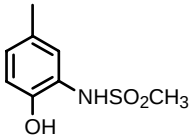
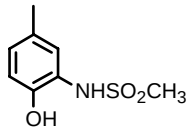
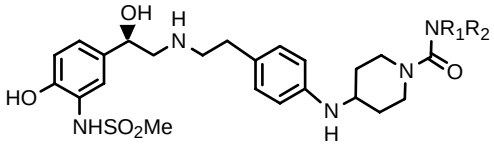
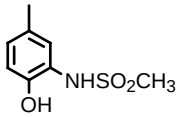
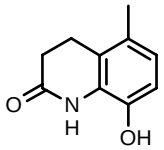
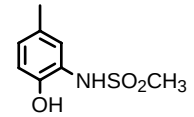
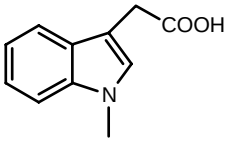
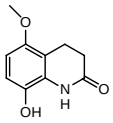
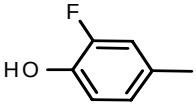
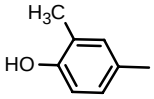
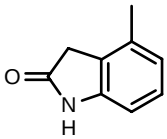
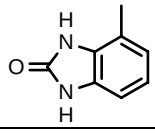
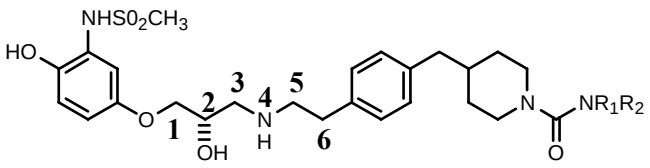
9	4-OHC_6H_4	R_1=iPr	R_2=H	90	7.045
10	4-OHC_6H_4	R_1=Et	R_2=Et	36	7.443
11		R_1=Octyl	R_2=H	290	6.537
12	3-ClPh	R_1=Octyl	R_2=H	1020	5.991
13	4-OHC_6H_4	R_1= 	R_2=H	50	7.301
14	4-OHC_6H_4	R_1=4-Fbenzyl	R_2=H	41	7.387
15	4-OHC_6H_4	R_1=2,4-diFbenzyl	R_2=H	30	7.522
16	4-OHC_6H_4	R_1=2,4-diClbenzyl	R_2=H	250	6.602
17	4-OHC_6H_4	R_1=2-Fbenzyl	R_2=H	37	7.431
18	4-OHC_6H_4	R_1=2,6-diFbenzyl	R_2=H	32	7.494
19	4-OHC_6H_4	R_1=2,5-diFbenzyl	R_2=H	23	7.638
20		R_1=Octyl	R_2=H	200	6.698
21		R_1=2,5-diFbenzyl	R_2=H	1	9.0
22		R_1= 2,5-diFbenzyl	R_2=H	5	8.301
23	-	3,3,3-trifluoropropyl		187	6.725
24	-	iPr		290	6.537
25	-	Pentyl		319	6.496
26	-	cHexyl		665	6.177
27	-	4-Hexylureido-$C_6H_4SO_2$-		48	7.318
28	-	2-Naphthyl-SO_2-		380	6.420
29	-	3-(HOOC)-C_6H_4NHCO-		70	7.154
30	4-OHC_6H_4	Isobutyl		126	6.899
31	4-OHC_6H_4	2,5-diFbenzyl		29	7.537
32		2,5-diFbenzyl		1	9.0
33		Octyl		5	8.301

Table 1. Continued

		Test set			
34				11	7.958
35	4-OHC_6H_4	R_1=cHexyl	R_2=H	80	7.096
36		R_1=Octyl	R_2=H	66	7.180
37	-	Propyl		870	6.060
38	4-OHC_6H_4	Hexyl		58	7.236
39	4-OHC_6H_4	Octyl		49	7.309
40	4-OHC_6H_4	Ph		135	6.869
41	4-OHC_6H_4	cHexyl		60	7.221
42	4-OHC_6H_4	3-(2-Thienyl)propyl-		20	7.698
43	4-OHC_6H_4	2-Pyridyl-		26	7.585
44		2,5-diFbenzyl		45	7.346
45		Octyl		306	6.514
46		Octyl		55	7.259
47		Hexyl		10	8.0

When no structural information is available, methods that investigate conformational space (e.g., using simulated annealing[16] and cluster analysis) may find the best match between various ligands. The fragment libraries in SYBYL database were used as building blocks for the construction of most active molecule **21** in the training set. A preliminary minimization was performed to remove close atom contacts by 1000 cycles of minimization using standard Tripos force field[17] (with 0.005 kcal/mol energy gradient convergence criterion). The structure was next subjected to molecular dynamic simulation to heat the molecule at 700k for 1000 fs followed by anneal the molecule to 200k for 1000 fs. All the remaining molecules were constructed using **21** as template and subjected to simple minimization and filled with Gasteiger-Huckel charges.[18] The minimized molecules were superimposed by the atom-fit method choosing the atoms 1-6 as shown Scheme 2. The superimposed structures are shown in Figure 1.



Scheme 2

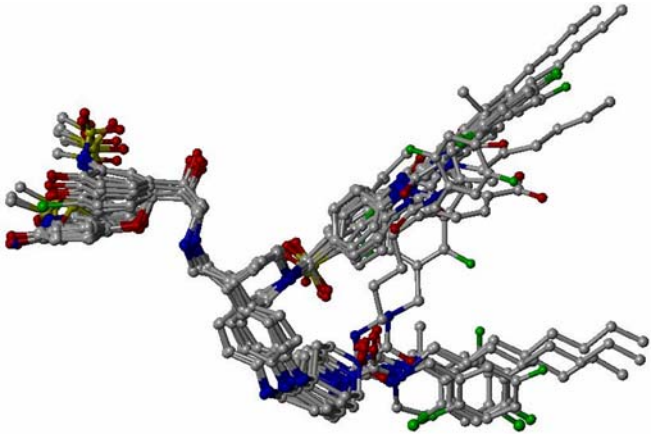


Figure 1. The superimposed structure of all compounds used in the CoMFA.

CoMFA study

Conventional CoMFA was performed with the QSAR option on SYBYL software. The training set of 33 aligned molecules was put into a 3D grid with a spacing of 2.0 Å. The steric and electrostatic fields were then calculated using an sp^3 C-atom with +1 charge and the default cutoff energy was set to 30 kcal/mol. The CoMFA QSAR equations were developed with the Partial Least Square (PLS) algorithm.[19] The cross validation of the model was performed using Leave One Out (LOO). The final non-cross-validated model was developed using optimal number of components that had both the highest r^2_{ncv} value and the smallest value of standard error predictions. To improve the signal-to-noise ratio, all leave-one-out calculations were performed with column filter value which was set to 2. The predictive r^2 was used to evaluate the predictive power of the CoMFA model, and was based only on molecules from the test set. Several CoMFA models were built by considering permutations of molecules between training and test sets. The best model amongst them was chosen on the basis of high r^2_{cv}, r^2_{ncv} values and small Standard Error of Estimate (SEE) value, reasonable r^2_{pred} value.

Results and Discussion

The two models derived by fit-atom based alignment using steric and electrostatic fields produced r^2_{cv} 0.670 and 0.583, respectively. Later various CoMFA models were generated using different combination of training set and test set, however none of these models lead to significant improvement in the r^2_{cv} and r^2 values of the models.

Statistical analysis

The CoMFA model-I developed using 30 compounds in the training set, which exhibited to highest r^2_{cv} value of 0.670 and non-cross validated r^2_{ncv} value of 0.993 with minimum standard error (0.069) and optimum number of component (6). It led to the development of a new CoMFA model with steric (0.540) and electrostatic (0.460) contributions exhibited superior statistical parameters are shown in the equation. The estimated predictive ability (r^2_{pred}) of the model was 0.462.

The CoMFA model-II selected for the analysis employed 33 compounds in the training set with a r^2_{cv} value of 0.583 and non-cross validated r^2_{ncv} value of 0.993, with minimum standard error (0.074) and optimum number of components (6). In this model, the steric (0.544) and electrostatic (0.456) fields have been found to be almost equally important. From the test set analysis the estimated predictive ability of the model was (r^2_{pred}) 0.743.

Model I

$$-\log EC_{50}\,(pEC_{50}) = 0.540\ (\text{steric}) + 0.460\ (\text{electronic})$$

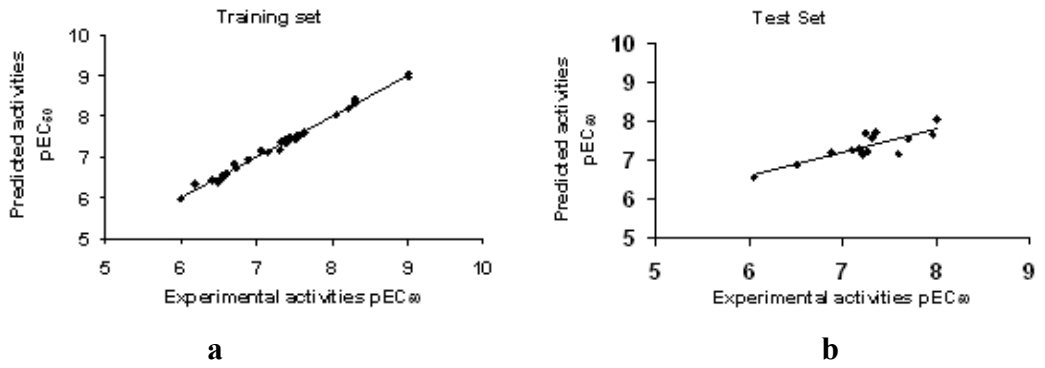
$N=30$; $r^2_{cv}=0.670$; $r^2_{ncv}=0.993$; $r^2_{pred}=0.462$; $F=575.25$; $SEE=0.069$; $ONC=6$; $SD=4.76$; $PRESS=2.56$

Model II

$$-\log EC_{50}\,(pEC_{50}) = 0.544\ (\text{steric}) + 0.456\ (\text{electronic})$$

$N=33$; $r^2_{cv}=0.583$; $r^2_{ncv}=0.992$; $r^2_{pred}=0.743$; $F=534.97$; $SEE=0.074$; $ONC=6$; $SD=5.98$; $PRESS=1.18$

The experimental, calculated activities and residual values for training set as well as test set are given in Table 2. A plot of experimental versus calculated β_3-AR agonists activities is illustrated in Figure 2. The best 3D-QSAR equation derived from the above analysis is, N=Number of compounds; r^2_{cv}=cross-validated correlation coefficient; r^2_{ncv}=non-cross validated correlation coefficient; r^2_{pred}=predicted cross-validated correlation coefficient; SEE=Standard Error of Estimate; PRESS=Predicted Residual Sum of Squares of test set molecules; ONC=Optimum Number of Components; SD= Standard Deviation for the test set molecules.



a **b**

Figure 2. (a) Predicted verses actual values for compounds used to build the CoMFA model, (b) predicted verses actual values for compounds used to test the CoMFA model.

Table 2. Actual activities vs Predictive activities by CoMFA for β_3-AR agonists activities (training set)

S. No	Actual pEC_{50}	CoMFA	
		Predicted	Residuals
1	8.30	8.42	-0.12
2	8.22	8.21	0.01
3	7.57	7.54	0.03
4	8.05	8.02	0.02
5	7.37	7.43	-0.06
6	7.51	7.44	0.07
7	7.33	7.40	-0.07
8	7.52	7.52	0.0
9	7.05	7.14	-0.10
10	7.44	7.44	0.0
11	6.54	6.55	-0.02
12	5.99	5.96	0.03
13	7.30	7.18	0.12
14	7.39	7.35	0.04
15	7.52	7.42	0.10
16	6.60	6.59	0.01
17	7.43	7.49	-0.05
18	7.49	7.45	0.04
19	7.64	7.60	0.04
20	6.70	6.84	-0.14
21	9.00	9.02	-0.02
22	8.30	8.31	-0.01

Table 2. Continued

23	6.73	6.73	0.0
24	6.54	6.50	0.04
25	6.50	6.37	0.12
26	6.18	6.33	-0.15
27	7.32	7.36	-0.04
28	6.42	6.43	-0.01
29	7.15	7.12	0.03
30	6.90	6.93	-0.03
31	7.54	7.50	0.04
32	9.00	8.96	0.04
33	8.30	8.30	0.0
		Predictive Data set	
34	7.96	7.66	0.30
35	7.10	7.24	-0.14
36	7.18	7.28	-0.10
37	6.06	6.58	-0.52
38	7.24	7.68	-0.44
39	7.31	7.57	0.30
40	6.87	7.20	-0.33
41	7.22	7.14	0.08
42	7.70	7.55	0.15
43	7.59	7.16	0.43
44	7.35	7.71	-0.36
45	6.51	6.89	-0.38
46	7.26	7.22	0.04
47	8.00	8.06	-0.06

Contour map analysis

In the present study out of the two CoMFA models developed initially only the CoMFA model-II was taken up for contour map analysis. The derived CoMFA model-I relatively poor external test set prediction value of 0.462.

The results of a CoMFA are best interpreted as CoMFA electrostatic and steric field graphs. These graphs show regions in the space around the molecules as solid contoured volumes, where specific steric or electronic interactions favorable or unfavourable for biological activity. In general, "Bio-Activity Measurement" is correlated with: more bulk near green; less bulk near yellow; more positive charge near blue and more negative charge near red. The CoMFA coefficient contour maps of steric and electrostatic potentials are displayed in Figures 3 and 4, respectively, along with the most active compound **21** as the reference.

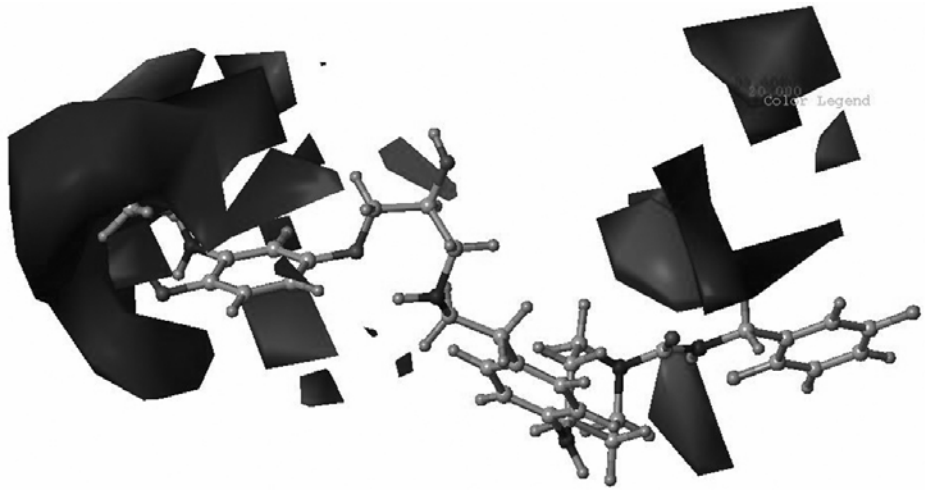


Figure 3. CoMFA electronic contour maps depicted around highest active molecule 21. Red and blue regions show where fields are favorable or unfavorable.

The steric contour map shows a green region at substituents on aryl ring in aryloxypropanolamine, indicating more bulky substituent is preferred at meta position on the aryl ring to result in higher bioactivity. This is also consistent with the fact that molecules **21,** and **32** with $-NHSO_2CH_3$ substituents at meta position show high activity (EC_{50} 1 nM) than the others. On the other hand derivatives bearing less bulky or no substituents on aryl ring at meta position show low bioactivity, which is consistent with the fact that molecule **12** has very poor activity (EC_{50} 1020 nM). This indicates that bulky substituents at meta position on aryl ring on arylethanolamine or aryloxy propanolamine give higher affinity towards β_3-AR agonists activity.

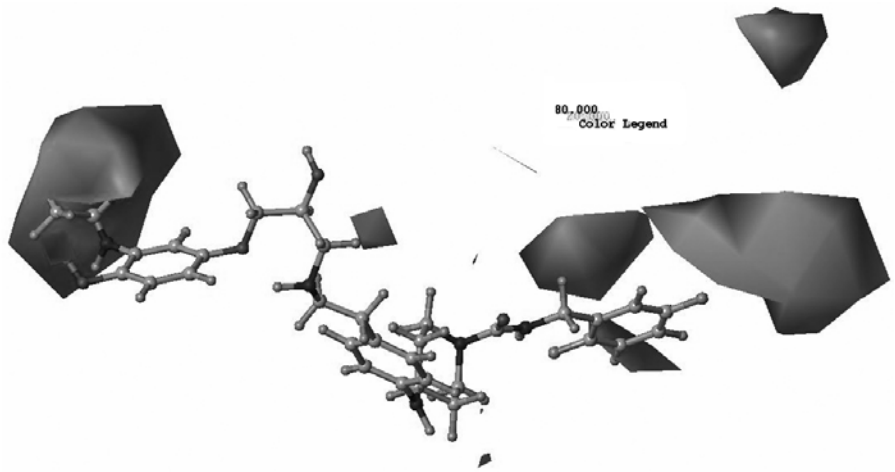


Figure 4. CoMFA Steric contour maps depicted around highest active molecule 21. Green and yellow regions show where fields are favorable or unfavorable.

In addition, the blue regions near aryl nucleus of the aryloxypropanolamine portion in the electrostatic contour map suggest that substituting a group or atom with a less electronegative group at meta position on an aryl ring system would yield a higher activity. It was found that molecules **21** and **32**, which have less electronegative group like $–NHSO_2CH_3$, show higher activities than the others. On the other hand high electronegative group or atom on an aryl ring system would yield a very poor activity as in **12**, which has chlorine atom.

A small red region inside the blue contour on the para position of the aryl ring of the aryloxypropanolamine section suggests that an electronegative group or atom is essential for biological activity. All the molecules have hydroxyl group on the para position of the aryl ring indicating that the hydroxyl group is essential for the biological activity. This hydroxyl group is important for making a hydrogen bond with an amino acid residue present in transmembrane 5 domain (TM5) of β_3-AR reported by Strosberg.[20] The compound **23, 24, 25, 26** and **28** are poor β_3-AR agonists (EC_{50} in the range 187-665 nM) due to electronegative atom nitrogen and also bulky indole ring substituted on aryl ring. In addition this molecule does not possess the essential hydroxyl group on the para position of the aryl ring.

The compound **1, 2** and **33** are fairly potent β_3-agonists (EC_{50} in the range 5-6 nM) because of bulky and less electronegative substituents on aryl ring. Compounds **5, 9** and **13** are better active towards β_3-AR (EC_{50} in the range 43-90 nM) due to hydroxyl group substituted on aryl ring. Compounds **9, 10, 14, 15, 16, 17** and **18** (EC_{50} in the range 30-250 nM) enter into the yellow contours, which indicate that a sterically less long chain is necessary for the biological activity. The three-dimensional contour map does not show any other steric and electronic fields major correlation with β_3-AR agonists activity.

Conclusions

The 3D QSAR study carried out using CoMFA has led to the identification of the regions of importance for steric and electronic interactions. The derived models well explain the observed variance in the activity and also provide important insight into structural variations that can lead to the design of novel and highly potent β_3-AR agonists. The contour map analysis indicates that the bulky and less electronegative substituents on aryl ring are favorable, unsubstitued and electronegative atoms such as chlorine, fluorine etc. in the aryl ring of the arylethanolamine are unfavorable for β_3-AR agonistic activity.

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