ORIGINAL RESEARCH

Comparative 3D QSAR study on β_1-, β_2-, and β_3-adrenoceptor agonists

P. Senthil Kumar · Prasad V. Bharatam

Abstract A quantitative structure–activity relationship study of tryptamine-based derivatives of β_1-, β_2-, and β_3-adrenoceptor agonists was conducted using comparative molecular field analysis (CoMFA). Correlation coefficients (cross-validated r^2) of 0.578, 0.595, and 0.558 were obtained for the three subtypes, respectively, in three different CoMFA models. All three CoMFA models have different steric and electrostatic contributions, implying different requirements inside the binding cavity. The CoMFA coefficient contour plots of the three models and comparisons among these plots provide clues regarding the main chemical features responsible for the biological activity variations and also result in predictions which correlate very well with the observed biological activity. Based on the analysis, a summary regeospecific description of the requirements for improving β-adrenoceptor subtype selectivity is given.

Keywords β-Adrenoceptor · G-protein-coupled receptor · 3D-QSAR · Comparative molecular field analysis · Structure–activity relationships · Antiobesity

Introduction

The β-adrenoceptor (β-AR), a member of the G-protein-coupled receptor (GPCR) family, has been the object of several studies aimed at understanding its physiological role and establishing structure–activity relationships for ligands which bind selectively to specific subtypes (Bikker *et al*., 1998; Lefkowitz, 1998; Wess, 1998; Schoneberg *et al*., 1999). β-ARs are widely distributed in the human

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body and are found, for example, in the lung, heart, and adipose tissue. The β-AR subtypes mediate several physiological processes including heart rate (Baker, 2005) (β-1), bronchodilatation (Waldeck, 2002; Sears, 2001) (β-2), and lipolysis (Weyer et al., 1999) (β-3). The β_3-AR is involved in various pharmacological and physiological effects including lipolysis, thermogenesis, and relaxation of intestinal smooth muscle (deSouza and Burkey, 2001; Dow, 1997; Igawa et al., 1999). Agents which selectively activate β_3-ARs were proposed to be useful in the treatment of obesity (Weyer et al., 1999), non-insulin-dependent diabetes mellitus, and frequent urination. β_3-AR agonists stimulate the intracellular signaling process to initiate the lipolysis of triglycerides in white adipose tissue. The resulting free fatty acids are processed by uncoupling protein, leading to thermogenesis in brown adipose tissue. The glucose-lowering effect of β_3-AR agonists is mediated through improved peripheral insulin sensitivity. The exact mechanism of the antidiabetic action of this class of compounds is not fully understood. Most of the previously developed β_3-AR compounds have suffered from one or more unacceptable pharmacokinetic or pharmacodynamic problems, including lack of β_3-AR selectivity, tissue specificity, full agonist activity, drug toxicity, and a short plasma half-life (Arch and Wilson, 1996; Himms-Hagen and Danforth, 1996; Danforth and Himms-Hagen, 1997), as a result of which no drug targeted to human the β_3-AR has reached the market so far. Hence attempts to identify clues for β_3-AR selectivity are an urgent requirement.

Many structural classes of β_3-adrenoceptor agonists have been developed; prominent among these classes are the derivatives of arylethanolamine and aryloxypropanolamine (Kordik and Reitz, 1999). The following are the important leads in these series: BRL-37344 (Arch et al., 1984), CL-316243 (Bloom et al., 1992), BMS-201620 (Washburn et al., 2004), and L-749372 (Naylor et al., 1998). BRL-37344 was reported to be a selective β_3-AR partial agonist (β_3 EC$_{50}$ = 450 nM, 23% activation) (Naylor et al., 1998). L-749372 is also a β_3-AR partial agonist (EC$_{50}$ = 3.6 nM, 33% activation), with 270- and 30-fold selectivity over binding to β_1- and β_2-ARs, respectively (Naylor et al., 1998). The 4-piperidino-benzoic acid derivative CL-316243 was found to be a modestly potent human β_3-AR agonist (EC$_{50}$ = 0.22 μM) (Sum et al., 1999), and N-(4-hydroxy-3-methylsulfonanilidoethanol)arylglycinamide (BMS-201620) a potent β_3 full agonist (k_i = 93 nM) (Washburn et al., 2004).
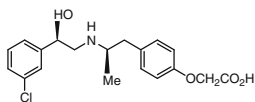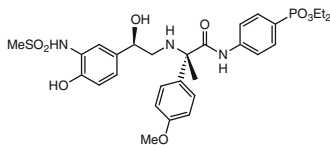

BRL-37344
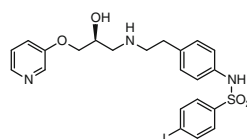
L-749372
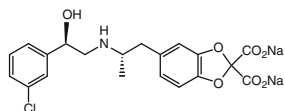
Cl-316243

BMS-201620

A schematic diagram showing the important structural units considered for β_3-AR agonistic activity in recently reported molecules is given in Scheme 1. The chirality at the hydroxyl center shows that (R) isomers possess the most favorable β_3 potency and selectivity profile over (S) isomers (Washburn *et al.*, 2001). The aryl group attached to the ethanolamine substructure is important for the biological activity, which can be either phenyl (Nakajima *et al.*, 2005), pyridine (Naylor *et al.*, 1999; Parmee *et al.*, 1999), *N*-(2-hydroxy-phenyl)methanesulfonamide (Gavai *et al.*, 2001; Hu *et al.*, 2001c) or phenol (Parmee *et al.*, 1998; Weber *et al.*, 1998). β_3-AR agonist activity of 2,4-thiazolidinedione with several β-amino alcohols (R1) was reported by Hu *et al.* (2001b). The amine portion of the ethanolamine group was attached to the central aryl fragment by a two- or three-carbon atom spacer. The central aryl linker fragment was replaced by a benzene (Naylor *et al.*, 1998) or indole moiety (Harada *et al.*, 2003). The central aromatic region was linked to the sulfonamide group, which is understood to be essential for selectivity of β_3-AR agonistic activity (Uehling *et al.*, 2002). Various research groups introduced acidic functionality on R2 to increase the selectivity for β_3-AR activity. In addition, they suggested that the steric bulk of the R2 substituent also contributed to the potency and selectivity of β_3-AR agonists. However, it is thought that introduction of such
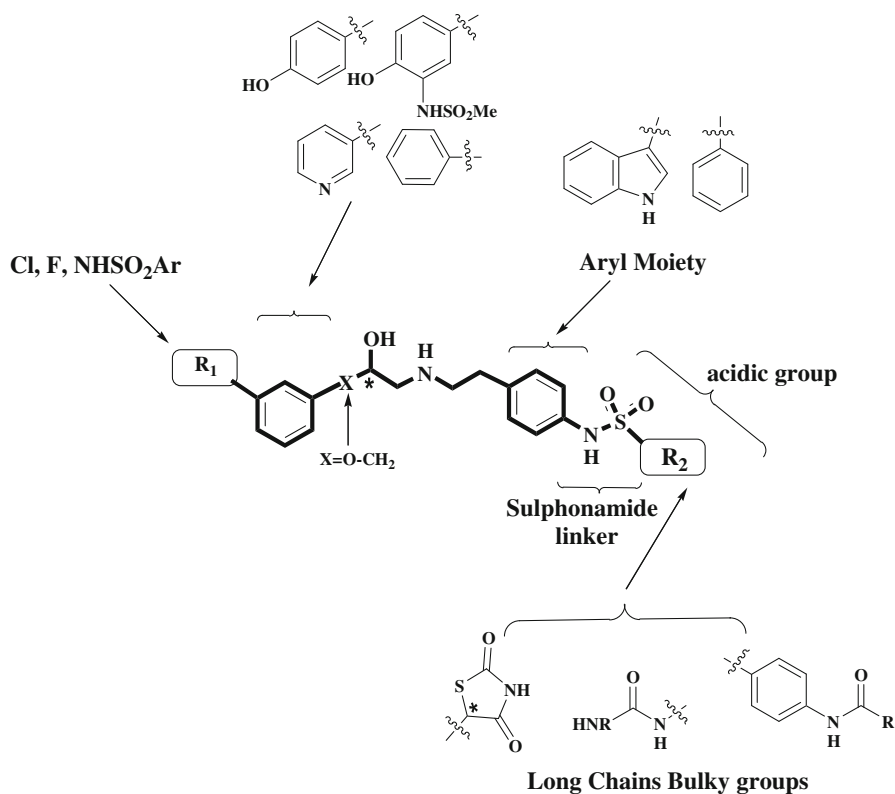
Scheme 1 Essential pharmacophore elements present in β_3-AR agonists, as identified from the reported β_3-selective arylethonolamine/aryloxypropanolamine derivatives

hydrophilic groups may generally cause low oral bioavailability, partly due to poor absorption (van de Waterbeemd *et al.*, 2001). Numbers of various bulky fragments attached to R2 have been reported. These fragments are long chains with oxadiazolidinedione (Hu *et al.*, 2001d), thiazolidinediones (Hu *et al.*, 2001a), urea (Ashwell *et al.*, 2001), triazole (Brockunier *et al.*, 2000), oxazole (Ok *et al.*, 2000), oxadiazole (Feng *et al.*, 2000; Biftu *et al.*, 2000), thiazole (Mathvink *et al.*, 2000), etc.
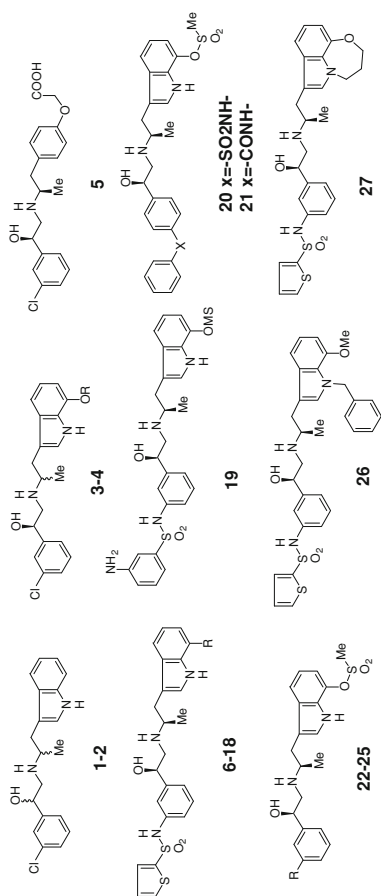
Molecular modeling studies offer several valuable tools for understanding the interactions of drugs and their receptors on a molecular level (Silverman, 2004). In the case of β-ARs very few molecular modeling studies have appeared to date. This is mainly due to the absence of three-dimensional (3D) information about these receptors. Some bold attempts have been made to computationally model the 3D structure of these targets. Lybrand *et al.* reported 3D models for agonist and antagonist complexes with β-adrenoceptors using computer modeling techniques (Kontoyianni *et al.*, 1996; Furse and Lybrand, 2003). Saxena and coworkers reported 3D quantitative structure–activity relationship (QSAR) studies on a cyclic ureidobenzenesulfonamides series of molecules using the Apex-3D method (Kashaw *et al.*, 2003; Prathipati and Saxena, 2005), and comparative molecular field analysis (CoMFA) and CoMSIA for different therapeutic areas (Gyanendra *et al.*, 2004; Stuti *et al.*, 2004). Recently, we reported CoMFA studies on a 4-aminomethylpiperidine series of β_3-AR agonists (Kumar and Bharatam, 2005). In this paper we report comparative studies on the molecular field requirements for a tryptamine based series of molecules toward β_1-, β_2-, and β_3-ARs. Kato and coworkers reported the relative biological activities of tryptamine-based agonists toward β_1-, β_2-, and β_3-ARs and pointed out that the compounds may be more specific to β_3-ARs (Mizuno *et al.*, 2004, 2005; Sawa *et al.*, 2004, 2005). A set of 27 molecules from these series was employed in this work to carry out CoMFA studies to identify relative steric and electronic requirements against these three receptors.

Computational details

All molecular modeling techniques and CoMFA studies were performed on a Silicon Graphics Octane2 (R12000) workstation with an IRIX6.5 operating system using the sybyl6.9 molecular modeling software package from Tripos, Inc. (St. Louis, MO, USA, 2002).

Data sets

CoMFA was performed on a series of 27 tryptamine derivatives for which biological activities (EC_{50} values) are reported with respect to β_1-, β_2-, and β_3-ARs (Harada *et al.*, 2003; Mizuno *et al.*, 2004, 2005; Sawa *et al.*, 2004, 2005). The structures and biological activity values of the 27 compounds forming the training set and test set are listed in Table 1; they were assayed in one research laboratory under the same experimental conditions. Only those compounds for which all three biological activities toward β-ARs were available (i.e., β_1, β_2, and β_3) were selected from the published data. The EC_{50} is the concentration at which half the maximal

Table 1 Structures of the 27 agonists in the training set and test set and their reported biological activity values

Molecule	Substituent R	β_1-AR EC$_{50}$ (nM)	β_2-AR EC$_{50}$ (nM)	β_3-AR EC$_{50}$ (nM)
1[a]	–	1.9	25	5.4
2[b]	–	47	330	220
3	Me	0.13	5.2	0.36
4	CH$_2$COOH	6.4	13	0.062
5	–	1700	290	21.0
6	H	21	66	0.88
7	OMe	6.6	29	0.55
8	OCH$_2$Ph	6.6	54	0.76
9	OCH$_2$CONEt$_2$	6.8	19	1.30
10	OCH$_2$COOH	19	180	1.70
11	OSO$_2$Me	18	44	0.21

Table 1 continued
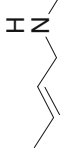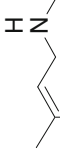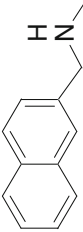
Molecule	Substituent R	β_1-AR EC$_{50}$ (nM)	β_2-AR EC$_{50}$ (nM)	β_3-AR EC$_{50}$ (nM)
12	OSO$_2$-n-butyl	7.3	26	0.59
13	OSO$_2$-n-octyl	5.6	20	0.28
14	OSO$_2$-iPr	6.2	40	0.51
15	OSO$_2$Ph	3.1	72	0.87
16	OSO$_2$-3-pyridyl	1.3	22	0.26
17	OSO$_2$-2-thienyl	1.2	49	0.64
18	OSO$_2$-2-CO$_2$Et	7.2	58	1.20
19	–	13	26	0.47
20	–	19	13	0.54
21	–	69	120	160
22	–	10	170	1.2
23		36	160	36
24		9.6	45	10

Table 1 continued

Molecule	Substituent R	β_1-AR EC$_{50}$ (nM)	β_2-AR EC$_{50}$ (nM)	β_3-AR EC$_{50}$ (nM)
25		7.6	44	2.9
26	–	22	32	4.4
27	–	44	53	1.0

^a Configuration **R** at hydroxyl and methyl center

^b Configuration **S** at hydroxyl and **R** at methyl center

response of the compound was observed. Biological activities are reported with EC_{50} values ranging from 0.13 to 1700, 5.2 to 330, and 0.062 to 220 nM for human β_1-, β_2-, and β_3-ARs, respectively. The biological activities in the training set were converted to pEC_{50} values of the agonists, which are the negative logarithms of the molar concentration value, and used as dependent variables in the CoMFA.

Structure generation and alignment

Compounds in the training set were generated from the x-ray crystal structures or by modification of the crystal structure of similar compounds using the SYBYL BUILD option (Tripos Inc. 2002). Conformation of compound **4** in the training set was taken from the x-ray crystal structure reported on the same molecule as given in the Cambridge Crystallographic Structural Database Centre (CCDC No. 203813) (Harada *et al.*, 2003). All remaining compounds were built from the crystal structure of compound **4**. Energy minimization was performed using the Tripos force field with a distance-dependent dielectric and conjugate gradient algorithm with a convergence criterion of 0.005 kcal/mol. Partial atomic charges were calculated using the Gasteiger–Huckel method (Gasteiger and Marsili, 1980). CoMFA studies require that the 3D structures of the molecules to be analyzed be aligned according to a suitable conformational template, which is assumed to be a "bioactive" conformation. Molecular alignment was carried out using the SYBYL "fit-atom" alignment function (Tripos Inc. 2002). The crystal structure of compound **4** was used as the alignment template. Figure 1 shows the 3D alignment of 27 molecules according to the alignment scheme in Fig. 2.

CoMFA study

The CoMFA descriptors were used as independent variables, and pEC_{50} values where used as dependent variables, in partial least squares (PLS) (Wold *et al.*, 1984) regression analysis to derive 3D QSAR models. The steric (Lennard-Jones) and electrostatic (Coulomb) CoMFA fields were calculated using an sp^3 carbon as the steric probe atom and a +1 charge for the electrostatic probe. A grid spacing of 2 Å and a distance-dependent dielectric constant were chosen. The cutoff value for both steric and electrostatic interactions was set to 30 kcal/mol.
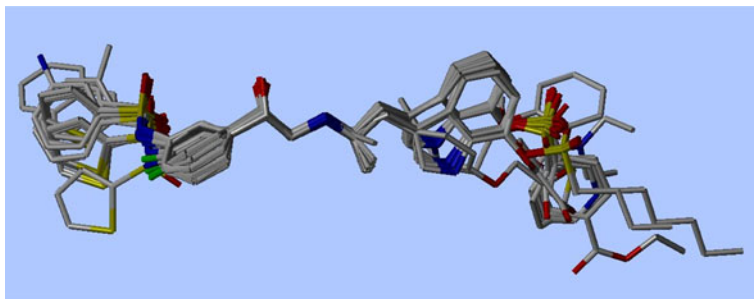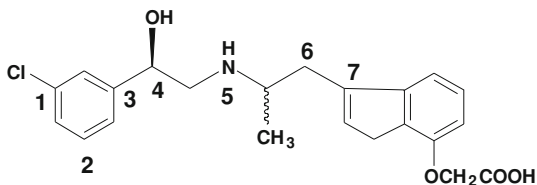

Fig. 1 The 3D alignment of the 27 molecules is shown by capped sticks without hydrogens

Fig. 2 Molecule **4** with atoms used for superimposition are named 1 to 7

Partial least squares analysis

PLS regression analyses were performed using cross-validation to evaluate the predictive ability of the CoMFA models. Initial PLS regression analyses were performed in conjunction with the cross-validation (leave-one-out method) option to obtain the optimal number of components to be used in the subsequent analysis of the dataset. All the leave-one-out cross-validated PLS analyses were performed with a column filter value of 2.0 kcal/mol to improve the signal-to-noise ratio by omitting those lattice points whose energy variation was below this threshold value. The final PLS regression analysis with 10 bootstrap groups and the optimal number of components was performed on the complete dataset. The optimal number of components was determined by selecting the smallest PRESS value. Usually this value corresponds to the highest cross-validated r^2 $\left(r_{cv}^2\right)$ value. The r_{cv}^2 was calculated using the formula

$$r_{cv}^2 = 1 - \frac{\sum \left(Y_{predicted} - Y_{observed}\right)^2}{\sum \left(Y_{observed} - Y_{mean}\right)^2}$$

where $Y_{predicted}$, $Y_{observed}$, and Y_{mean} are the predicted, actual, and mean values of the target property (pEC_{50}), respectively. The number of components obtained from the cross-validated analysis was subsequently used to derive the final QSAR models. In addition to r_{cv}^2, the corresponding PRESS [PRESS $= \sum(Y_{predicted} - Y_{observed})^2$], the number of components, the nonconventional correlation coefficient r_{ncv}^2, and its standard errors were also computed.

Test sets

To test the predictive power of the CoMFA model, seven agonists from the β_1- and β_2-AR subtypes and five agonists from the β_3-AR subtype were selected as the test set (Table 2). The agonists in the test set were chosen by random sampling of biological activity. The conformation, minimization, and alignment of these agonists in the test set were obtained by the same protocol as that described for the agonists in the training set used. All predicted activities for test-set molecules were calculated using the optimized CoMFA model. The results of the non-cross-validated calibration model on the test sets are summarized in Table 3.

Table 2 Experimental (exp) and predicted (pre) biological activities along with estimated residual values (res) of training- and test-set molecules (log $1/EC_{50}$) $\times$ 10^{-9} associated with the three CoMFA models β_1, β_2, and β_3

Molecule	β_1			β_2			β_3		
	Exp	Pre	Res	Exp	Pre	Res	Exp	Pre	Res
1	8.72	7.66	1.06	7.60	6.38	1.22	8.26	6.99	1.27
2	7.32	7.26	0.06	6.48	6.42	0.06	6.65	6.61	0.04
3	9.88	7.69	2.19	8.28	6.64	1.64	9.44	6.91	2.53
4	8.19	8.17	0.02	7.88	6.59	1.29	10.20	7.27	2.93
5	5.76	7.76	−2.0	6.53	6.59	−0.06	7.67	7.28	−0.01
6	7.67	7.68	−0.01	7.18	7.20	−0.02	9.05	9.12	−0.07
7	8.18	8.18	0.00	7.53	7.44	0.09	9.25	9.21	0.04
8	8.18	8.24	−0.06	7.26	7.29	−0.03	9.11	9.06	0.05
9	8.16	8.69	−0.53	7.72	7.76	−0.04	8.88	8.93	−0.05
10	7.72	7.90	−0.18	6.74	7.33	−0.59	8.76	8.70	0.06
11	7.74	8.05	−0.31	7.35	7.36	−0.01	9.67	9.59	0.08
12	8.13	8.19	−0.06	7.58	7.37	0.21	9.22	9.24	−0.02
13	8.25	8.18	0.07	7.69	7.69	0.0	9.55	9.53	0.02
14	8.20	8.26	−0.06	7.39	7.42	0.03	9.29	9.33	−0.04
15	8.50	8.64	−0.14	7.14	7.24	−0.10	9.06	9.15	−0.09
16	8.88	8.87	0.01	7.65	7.24	0.41	9.58	9.39	0.19
17	8.92	8.88	0.04	7.30	7.31	−0.01	9.19	9.21	−0.02
18	8.14	8.39	−0.25	7.23	7.20	0.03	8.92	8.95	−0.03
19	7.88	7.82	0.06	7.58	7.66	−0.08	9.32	9.34	−0.02
20	7.72	7.71	0.01	7.88	7.73	0.15	9.26	9.19	0.07
21	7.16	7.19	−0.03	6.92	7.70	−0.78	6.79	9.19	−2.4
22	8.00	8.03	−0.03	6.76	6.79	−0.03	8.92	7.67	1.25
23	8.00	8.08	−0.08	6.79	6.72	0.07	7.44	7.41	0.03
24	8.01	7.16	0.85	7.34	7.34	0.0	8.00	8.00	0.0
25	8.11	8.11	0.00	7.35	7.36	−0.01	8.53	8.54	−0.01
26	7.65	7.66	−0.01	7.49	7.50	−0.01	8.35	8.38	−0.03
27	7.35	7.36	−0.01	7.29	7.34	−0.05	9.00	9.07	−0.07

Note: β_1-AR: training set, **2**, **4**, **6–8**, **12–14**, **16**, **17**, **19–22**, **25–27**; test set **9–11**, **15**, **18**, **23**, **24**; outliers, **1**, **3**, **5**. β_2-AR: training set, **2**, **5–9**, **11**, **13**, **15**, **18–20**, **22–26**; test set **10**, **12**, **14**, **16**, **17**, **21**, **27**; outliers, **1**, **3**, **4**. β_3-AR: training set, **2**, **5–7**, **9**, **11–13**, **15–17**, **19**, **23–27**; test set **8**, **10**, **14**, **18**, **20**; outliers, **1**, **3**, **4**, **21**, **22**

Predictive r^2 values

The predictive r^2 $\left(r^2_{\mathrm{pre}} \right)$ was based only on molecules not included in the training set and is defined as explained by Marshall and co-workers (Oprea *et al.*, 1994; Waller *et al.*, 1993).

Table 3 Statistical parameters associated with the three CoMFA models β_1, β_2, and β_3

Parameter	β_1	β_2	β_3
N	17	17	17
No. components	5	5	6
r^2_{cv}	0.578	0.595	0.558
r^2_{ncv}	0.993	0.976	0.995
F_{test}	305.3	90.5	310.7
r^2_{bs}	0.996	0.997	0.999
r^2_{pre}	0.847	0.607	0.758
Std dev (bs)	0.003	0.003	0.001
SEE	0.027	0.023	0.033
Steric field contribution	0.558	0.394	0.401
Electrostatic field contribution	0.442	0.606	0.599

Note: N, number of compounds; r^2_{cv}, cross-validation by leave-one-out method; r^2_{ncv}, non-cross-validation; r^2_{bs}, 10 bootstrapping runs; r^2_{pre}, predictive r^2; SEE, standard error of estimate

$$r^2_{pre} = (SD - PRESS)/SD$$

where SD is the sum of the squared deviations between the biological activities of molecules in the test set and the mean activity of the training-set molecules, and PRESS is the sum of the squared deviations between predicted and actual biological activity values for every molecule in the test set. This is analogous to Cramer's definition: whenever PRESS is larger than SD, this results in a negative value reflecting complete lack of predictive ability of the training set for the molecules included in the test set (Cramer *et al.*, 1988).

Results

CoMFA of the β_1-adrenoceptor

PLS analysis was used in combination with cross-validation to obtain the optimal number of components to be used in the subsequent non-cross-validation analysis. PLS analysis based on least squares fit gave a correlation with a cross-validated r^2_{cv} of 0.578, with the maximum number of components set equal to five. The non-cross-validated PLS analysis was repeated with the five components, giving an r^2_{ncv} of 0.993. To obtain statistical confidence limits, the non-cross-validated analysis was repeated with 10 bootstrap groups, which yielded an r^2 of 0.996 (five components, SEE = 0.027, std dev = 0.003, steric contribution = 0.558, and electrostatic contribution = 0.442). These parameters are listed in Table 3. The above satisfactory cross-validated correlation coefficient indicates that the CoMFA model is highly reliable. The high bootstrapped r^2 value and low standard deviation suggest a high degree of confidence in the analysis. The calculated biological activities obtained from the analysis are plotted versus the actual values in Fig. 3a. Compounds **9**, **10**, **11**, **15**, **18**, **23**, and **24** (test set) were used to evaluate the

Fig. 3 A graph of experimental vs. predicted activities of the training-set and test-set molecules as β_1-AR (a), β_2-AR (b), and β_3-AR (c) agonists. (◆) Training set; (▲) test set
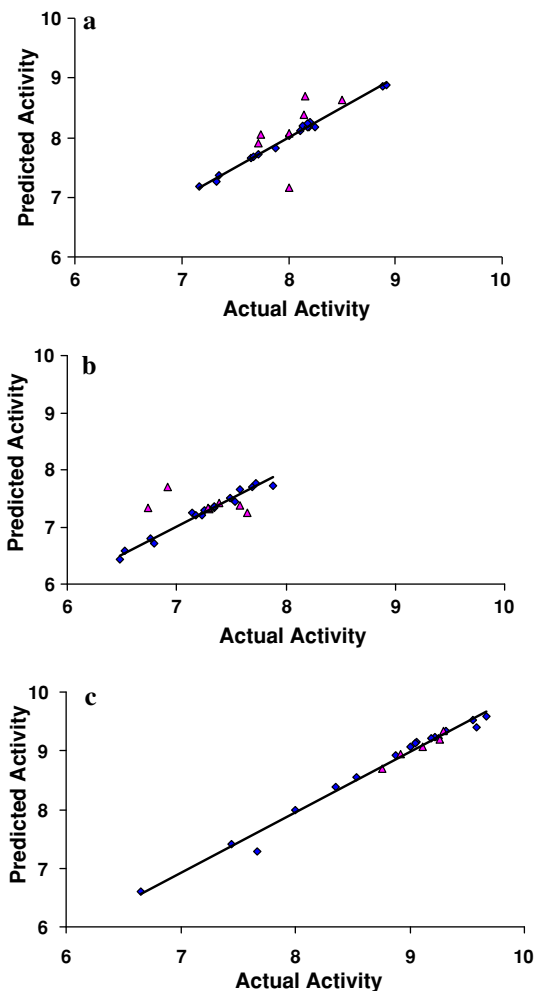
predictive power of this CoMFA model. As in the calibration step, a good predictive ability, with an $r^2_{\mathrm{pre}} = 0.847$, for the compounds in the test set was obtained. Table 2 reports that the predicted values fall close to the observed biological activity value, deviating by less than one logarithmic unit.

The β_1 CoMFA steric and electrostatic fields from the final non-cross-validated analysis are plotted as three-dimensional color contour maps in Figs. 4a and 5a, respectively, along with the reference compound, **16**. These contour maps indicate the regions where differences in molecular fields are associated with differences in biological activity. Green contours indicate regions in which increasing steric bulk is tolerable, and yellow contours indicate regions in which the steric bulk decreases the activity. In the β_1 model the steric contours show that the substituents attached to the ring of the arylethanolamine group are placed in sterically unfavorable regions. Of the four yellow contours near the arylethanolamine group three of them
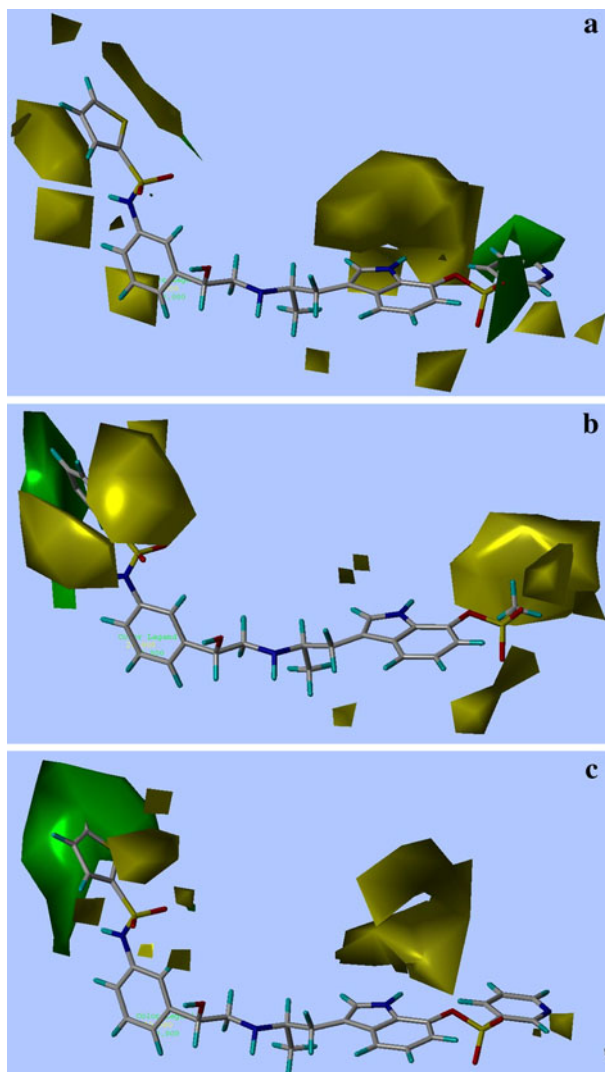
Fig. 4 CoMFA steric STDEV*COEFF contour plots of the tryptamine-based derivative training set generated for the β_1 (**a**), β_2 (**b**), and β_3 (**c**) models. Compounds **16** (**a**, **c**) and **20** (**b**) are shown inside the field

are below the local plane of the reference compound and one is above the five-membered ring of the reference compound. These yellow regions indicate that additional steric interactions in these regions would lead to decreased biological activity. The above observations indicate that for good β_1-agonistic activity there should be only very small groups or no substituents on the aryl ring of arylethanolamine. These can account for a limiting size and shape for the substituents that would be effective for tight binding to the receptor. A big yellow contour above the indole ring indicates that any substituents on the nitrogen of the

indole ring would greatly reduce the biological activity, suggesting limited bulk tolerance. The small green region at the C7 position of the indole nucleus indicates that increases in the steric bulk at this position are marginally favorable for β_1-AR activity. The electrostatic contour map (Fig. 5a) of the CoMFA model shows a small blue contour near the SO_2 group attached to arylethanolamine and red contours near the C7 substituents on the indole ring. This indicates that a reduction in the electronegativity near the SO_2 group and increasing electronegativity at the C7 position of indole should lead to increased β_1 activity.
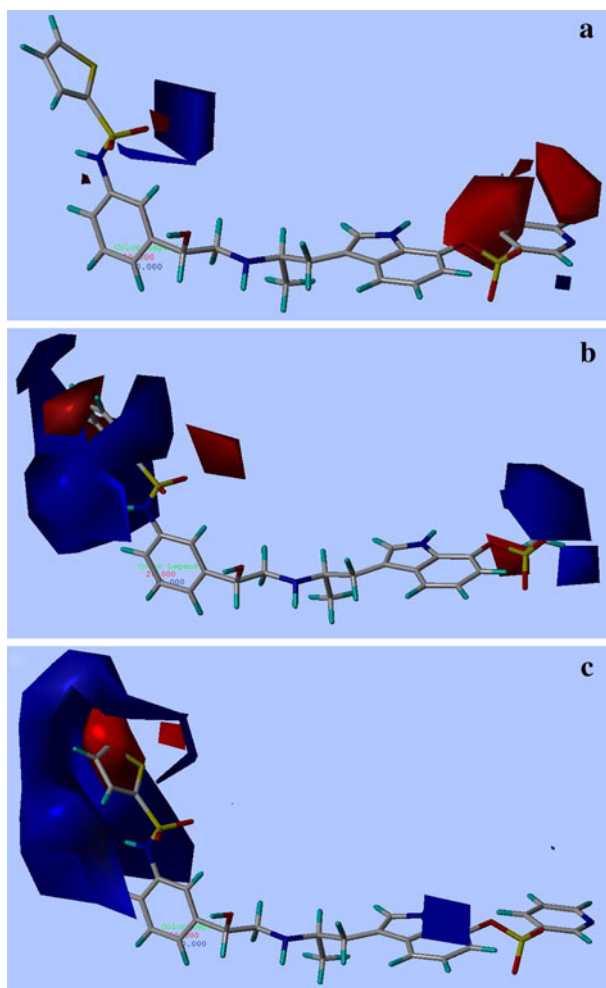

Fig. 5 CoMFA electrostatic STDEV*COEFF contour plots of the tryptamine-based derivative training set generated for the β_1 (**a**), β_2 (**b**), and β_3 (**c**) models. Compounds **16** (**a**, **c**) and **20** (**b**) are shown inside the field

CoMFA of the β_2-adrenoceptor

The β_2 CoMFA analysis based on the fit atom alignment yielded good cross-validated ($r^2_{cv} = 0.595$) and conventional r^2($r^2 = 0.976$. $F-$ test value $= 90.518$), with the optional number of components found to be five. The steric and electrostatic fields contribute to the QSAR equation by 39.4% and 60.6%, respectively. A high bootstrapped (10 sampling) r^2_{bs} value of 0.997 (SEE = 0.023, std dev = 0.003) was found. A plot of actual versus calculated biological activity obtained from the analysis is given in Fig. 3b. Compounds **10**, **12**, **14**, **16**, **17**, **21**, and **27** (test set) were used to evaluate the predictive power of this CoMFA model. A good predictive ability, with an $r^2_{pre} = 0.607$, for the compounds in the test set was obtained in this calibration step. Table 2 reports that the predicted values fall close to the observed biological activity value, deviating by less than one logarithmic unit.

The β_2 CoMFA steric and electrostatic fields from the final non-cross-validated analysis are plotted in Figs. 4b and 5b respectively. The most active compound, **20**, was treated as the reference molecule. The graphical interpretation of the field contribution of the steric contour map is shown in Fig. 4b. The steric contour map shows three yellow regions surrounding the phenyl unit in the $NHSO_2Ph$ group, and a small green at the para position on the same ring. This indicates that it is preferable to reduce the steric bulk due to the Ph group. The presence of a simple thiophen ring, as in many other molecules in this series, is preferable for β_2 activity. A very large yellow contour is noted near the C7 of the indole ring in Fig. 4b, indicating that the steric bulk should be reduced for improved β_2 activity. The CoMFA electrostatic contour map displays a large blue region surrounding the SO_2Ph group and two small red regions in close proximity, suggesting that a strong reduction in the electronegative groups is preferred in this region. There are two small blue regions and one small red region at the C7 of the indole ring of the reference compound. The distribution range of blue is higher than that of red, indicating that electropositive groups in this region are very important for the β_2 biological activity.
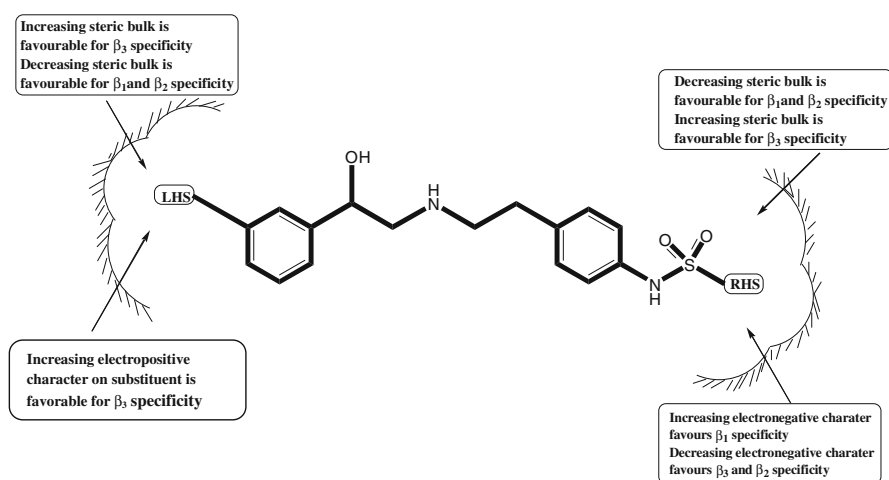
CoMFA of the β_3-adrenoceptor

The β_3 CoMFA analysis based on the fit atom alignment yielded acceptable cross-validated ($r^2_{cv} = 0.558$) and conventional results ($r^2 = 0.995$, $F-$ test value $= 310.717$), with the optimal number of components found to be six. In this model, steric and electrostatic fields contribute to the QSAR equation by 40.1% and 59.9%, respectively. The high bootstrapped (10 sampling) r^2_{bs} value of 0.999 (SEE = 0.033, std dev = 0.001) was found. Compounds **8**, **10**, **14**, **18**, and **20** (test set) were used to evaluate the predictive power of this CoMFA model. The predicted versus the actual values of biological activities obtained from the analysis are plotted in Fig. 3c. The β_3 CoMFA model shows a very good predictive ability, with $r^2_{pre} = 0.758$ for the compounds in the test set, as obtained for the calibration steps. Table 2 shows that the predicted values fall close to the observed biological activity value, deviating by less than one logarithmic unit.

The steric and electrostatic contour maps obtained from the β_3 CoMFA model are shown in Figs. 4c and 5c, respectively, along with compound **16**. In Fig. 4c, the steric contour map shows a large green region around the substituent on the aryl ring, indicating the presence of a β_3-AR binding pocket which can accommodate bulky substituents of large size, such as isopropyl and t-butyl. The presence of a bulky substituent at the indole ring decreases activity because of steric hindrance, as indicated by the yellow contour. Figure 5c shows a huge blue contour near the thiophen ring, indicating that decreasing electronegative character/increasing electropositive character is an important consideration in this region for improved β_3-agonistic activity.

Discussion

Three different 3D QSAR models have been developed using the CoMFA methodology for tryptamine-based analogues of β-AR agonists. This is a first attempt to describe quantitatively the hypothetical receptor binding site of multiple subtypes of β-ARs. Comparison of the three CoMFA models helps in understanding β-AR selectivity. The main steric and electrostatic interactions on the binding cavity of β_1-, β_2-, and β_3-ARs are demonstrated in Scheme 2. The 3D QSAR CoMFA of these β-AR subtypes led to the following considerations:

The β_2 and β_3 CoMFA models show similarities in their overall steric and electrostatic contributions. In the β_1 CoMFA model the steric contribution is larger, whereas in the β_2 and β_3 CoMFA models the electrostatic contribution is larger (see Table 3). Detailed CoMFA contour map analysis shows that decreasing steric bulk is preferable for increased β_1 and β_2 activity near the sulfonamide unit. On the other hand, increasing steric bulk is preferable for the β_3-AR activity near the phenyl

Increasing steric bulk is favourable for β_3 specificity
Decreasing steric bulk is favourable for $\beta_1$and β_2 specificity

Decreasing steric bulk is favourable for $\beta_1$and β_2 specificity
Increasing steric bulk is favourable for β_3 specificity

LHS

RHS

Increasing electropositive character on substituent is favorable for β_3 specificity

Increasing electronegative charater favours β_1 specificity
Decreasing electronegative charater favours β_3 and β_2 specificity

Scheme 2 Proposed hypothetical receptor model of β-Ars binding site

sulfonamide unit. Strong yellow contours are observed near the C7 unit of the indole ring in all three CoMFA models, indicating that smaller functional units are preferable in this region. From this information, it may be inferred that the active site of β_3-AR can accommodate large substituents on the left-hand side for tight binding. Thus, β_3-selectivity of this series of compounds can be brought about by employing large groups on the phenyl unit of phenyl sulfonamide in **16**. It is preferable to reduce the steric effects on the C7 of the indole ring in **16** for all (β_1, β_2, β_3) activities. Figure 5 shows that there are distinguishable differences in the electrostatic fields of β_1, β_2, and β_3 CoMFA models. In all the models, increasing electropositive character is preferred near the SO_2Ar unit in **16**, the influence of which increased in the order $\beta_1 < \beta_2 < \beta_3$. This requirement is very strong for $\beta3$-agonistic activity. Thus, large substituents with a strong electropositive character on the Ar unit of SO_2Ar are required for β_3 specificity. On the other hand, electrostatic factors appear to be optimum for β_3 activity on the right-hand side of Scheme 2. However, increasing electronegative substituents are favorable for β_1 activity and increasing electropositive character is favorable for β_2 selectivity. These factors are summarized in Scheme 2. In summary, the absence of information on the experimental binding mode of these agonists toward their β-ARs, the binding mode information obtained for the comparative 3D QSAR studies shall be helpful in modulating the tryptamine series of molecules for selectivity against β_1, β_2, and β_3 activities.

Conclusion

In this paper, we have established CoMFA models for a series of tryptamine-based analogues for various subtypes of β-AR agonists, i.e., β_1-, β_2-, and β_3-AR agonists. Three different 3D QSAR models have been established for β_1-AR, β_2-AR, and β_3-AR agonistic activities in a series of tryptamine molecules using the CoMFA method. All three models show satisfactory statistical significance values r^2_{cv} (0.578, 0.575, 0.558), SEE (0.027, 0.023, 0.033), etc. Comparative study of the steric and electrostatic contour maps provided clues to the chemical modulations required for improving specificity. For β_3-specificity, for example, increased steric bulk and increased electropositive character are required on the aryl group of the SO_2Ar unit in this series of molecules. Based on the present 3D QSAR CoMFA studies, a hypothetical receptor model of these agonists with the β_3-AR is proposed (see Scheme 2). Since information related to the 3D structure of the active site of the three β-ARs is not available, information provided in this article in the form of molecular field requirement shall be of help in designing selective β_3-AR agonists.

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