3D QSAR Study of 2-Methoxyphenylpiperazinylakanamides as 5-Hydroxytryptamine (Serotonin) Receptor 7 Antagonists

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Abstract

5-hydroxytryptamine (serotonin) receptor (5-HT$_7$R) is one of G-Protein coupled receptors, which is activated by the neurotransmitter Serotonin. After activation by serotonin, 5-HT$_7$R activates the production of the intracellular signaling molecule cyclic AMP. 5-HT$_7$R receptor has been found to be involved in the pathophysiology of various disorders. It is reported that 5-HT$_7$R receptor antagonists can be used as antidepressant agents. In this study, we report the important structural and chemical parameters for 2-methoxyphenylpiperazinylakanamides as 5-HT$_7$R inhibitors. A 3D QSAR study based on comparative molecular field analysis (CoMFA) was performed. The best predictions were obtained for the best CoMFA model with $q^2$ of 0.594 with 6 components, $r^2$ of 0.986, Fisher value as 60.607, and an estimated standard error of 0.043. The predictive ability of the test set was 0.602. Results obtained the CoMFA models suggest that the data are well fitted and have high predictive ability. The contour maps are generated and studied. The contour analyses may serve as tool in the future for designing of novel and more potent 5-HT$_7$R derivatives.

Keywords: 3D-QSAR, CoMFA, 5-HT$_7$R, Serotonin

1. Introduction

5-Hydroxytryptamine (serotonin) receptor (5-HT$_7$R) 7 belongs to the G-protein coupled receptor superfamily of cell surface receptors[1]. It is activated by the Serotonin, a neurotransmitter. Serotonin receptors have been divided into seven families, 5-HT$_1$R to 5-HT$_3$R. 5-HT$_7$R is the recently cloned subtype of serotonin receptor family. After activated by serotonin, 5-HT$_7$R leads to a cascade of events starting with release of the stimulatory G protein G$_s$ from the GPCR complex. This activates the production of cyclic AMP, an intracellular signaling molecule[2]. It is expressed in a variety of human tissues, including brain, the gastrointestinal tract, and in various blood vessels[3]. 5-HT7 receptor plays important role in smooth muscle relaxation within the vasculature and in the gastrointestinal tract[1]. It is involved in learning and memory, thermoregulation, circadian rhythm, and sleep. The 5-HT$_7$R receptor is encoded by the HTR7 gene. The HTR7 gene is transcribed into 4 different splice variants, designated h5-HT$_7$R(a), h5-HT$_7$R(b), h5-HT$_7$R(c) and h5-HT$_7$R(d)[4].

5-HT$_7$ receptor has been reported in the pathophysiology of various disorders. In recent times, 5-HT$_7$ receptor is in limelight because of its role in various neurological disorders including depression, sleep disorders, memory deficiency and neuropathic pain. They are also found to down regulate after antidepressant administration[5]. These findings suggest that the 5-HT$_7$ receptor antagonists can be used as antidepressant agents[6,7]. It is also reported that in animal models, 5-HT$_7$ receptor activation produced anti-hyperalgesic effects[8]. It is also speculated that this receptor may be involved in mood regulation. This suggests that 5-HT$_7$ receptor can be a useful target in the treatment of depression[9].

Various neutral antagonists and inverse agonists have been reported for 5-HT$_7$ receptor. Due to the differing levels of inverse agonist efficacy between receptor splice variants, differentiation between neutral antagonists and inverse agonists becomes complex. For example, mesulergine and metergoline, neutral antagonists of h5-HT$_7$R(a) and h5-HT$_7$R(b) receptor isoforms displays marked inverse agonist effects at h5-HT$_7$R splice variant[9].
Table 1. Structures and biological activities (pKᵢ) of 5-HT₇R inhibitors

The 2-methoxyphenylpiperazinylakanamides inhibitor scaffold

| Compound | n  | pKᵢ values |
|----------|----|-------------|
| 1*       | 2  | 6.729321    |
| 2        | 3  | 7.19044     |
| 3        | 4  | 7.421361    |
| 4        | 5  | 7.359519    |

b) Compound 5-17

| Compound | R          | pKᵢ values |
|----------|------------|-------------|
| 5*       | 2'- F      | 7.437707    |
| 6*       | 3'- F      | 7.459671    |
| 7*       | 4'- F      | 7.459671    |
| 8        | 2'- Cl     | 8.06098     |
| 9        | 3'- Cl     | 6.392223    |
| 10       | 4'- Cl     | 7.5867      |
| 11       | 2'- OMe    | 7.50307     |
| 12       | 3'- OMe    | 7.657577    |
| 13*      | 4'- OMe    | 7.359519    |
| 14       | 2',6'- diOMe | 7.489455 |
| 15       | 2'- Me     | 7.583359    |
| 16       | 3'- Me     | 7.322393    |
| 17       | 4'- Me     | 7.179799    |
It becomes apparent that there is a need for discovery of structurally diverse 5-HT$_7$ receptor antagonists for the treatment of various neurological disorders. Here, in this study, we report on the 3D-Quantitative structural activity relationship study on 2-methoxyphenylpiperazinyllakanamides derivatives as 5-hydroxytryptamine (serotonin) receptor 7 antagonists.

2. Computational Methods

2.1. Data Set

The structures of the 2-methoxyphenylpiperazinyllakanamides derivatives and the biological activities of 19 compounds were taken from the literature[11]. $K_i$ values of each inhibitor were converted into $pK_i$ in order to use the data as dependent variable in CoMFA model. $pK_i$ value is the negative logarithm of a $K_i$ value. The test set molecules were selected which is the representative molecule for training set molecules. The test set molecules were selected manually so as to cover all the biological activity which is similar to the training set molecule. Compound 9 was omitted during the study as it was found to be an outlier. The total set of compounds was divided into a training set consist of 13 compounds and test set consist of 6 compounds. The structures and their activity values are displayed in Table 1.

2.2. Ligand-based Alignment Method

For each compound, the partial atomic charges were assigned by utilizing Gasteiger Hückel method available in SYBYLX 2.1 package (Tripos Inc., St. Louis, MO, USA). All rotatable bonds were searched with incremental dihedral angle from 120° by using systematic search conformation method. Conformational energies were computed with electrostatic term, and the lowest energy conformer was selected as template molecule. Then the template was modified for other ligands of the series. The common scaffold was constraint for each molecule and only the varying parts were energy minimized by Tripos force field with Gasteiger-Huckel charge by using conjugate gradient method, and convergence criterion was 0.05 kcal/mol at 10,000 iteration. Using the atom fit method; the minimized structures were aligned over the template (Fig. 1). Subsequently this alignment is used for 3D QSAR study based on Comparative molecular field analysis (CoMFA).

2.3. CoMFA Field Generation

SYBYLX 2.1 (Tripos Inc., St. Louis, MO, USA) package molecular modeling package was used for the 3D QSAR studies based on CoMFA. Generally used steric and electrostatic fields were used for this study. CoMFA studies helps in deriving a relation between the biological activities and three dimensional structures of the set of molecules of the dataset. The molecular alignment was placed in a 3D grid and the molecular field values of each conformation of a molecule are calculated. 2Å lattice spacing was used. The CoMFA method was performed using steric and electrostatic fields with standard ±30 kcal/mol cutoffs. CoMFA calculated steric and electrostatic field values.

2.4. Partial Least Square (PLS) Analysis

PLS algorithm quantifies the relationship between the structural parameters and the biological activities[12,13]. CoMFA descriptors used as independent variables and
pKᵢ values used as dependent variables in PLS analysis for the generation of 3D-QSAR models. Leave-one-out (LOO) cross-validation procedures were used to obtain the cross-validated correlation coefficient (q²), non-cross-validated correlation coefficient (r²), standard error estimate (SEE) and Fisher’s values (F).[14,15] A non-cross-validated analysis was carried out without column filtering was then followed. The cross-validated correlation coefficient (q²) was calculated using the following equation:

\[ q^2 = 1 - \frac{\sum (\hat{\gamma_{\text{pred}}}-\gamma_{\text{actual}})^2}{\sum (\gamma_{\text{actual}}-\gamma_{\text{mean}})^2} \]

where \( \gamma_{\text{pred}} \), \( \gamma_{\text{actual}} \), and \( \gamma_{\text{mean}} \) are the predicted, actual, and mean values of the target property (pKᵢ), respectively.

The predictive power of CoMFA models were determined from the set of seven test molecules which was excluded during model development. The predictive correlation coefficient (r² pred) based on the test set molecules, is defined as:

\[ r^2_{\text{pred}} = \frac{(SD-PRESS)}{SD} \]

where PRESS is the sum of the squared deviation between the predicted and actual activity of the test set molecules, and SD is defined as the sum of the square deviation between the biological activity of the test set compounds and the mean activity of the training set molecules.

3. Results and Discussion

3.1. CoMFA Analysis

CoMFA models were derived with the combination of steric and electrostatic field contributions and

Fig. 1. (a) Maximum common substructure present in all molecules. (b) Alignment of molecules based on systematic search conformation of highly active compound 8.
Different combinations of training and test compounds were used for model generation. Based on the reliable $q^2$ and $r^2$ values, 5 best models were selected out of the number of models created. The statistical values of the 5 models are tabulated in Table 2. Leave one out (LOO) analysis gave a cross-validated $q^2$ of 0.594 with 6 components and non cross-validated PLS analysis resulted in a correlation coefficient $r^2$ of 0.986, Fisher value as 60.607, and an estimated standard error of 0.043. The predictive ability of the developed CoMFA model was assessed by the test set predictions, which were excluded during model generation. The predictive ability of the test set was 0.602.

Predicted and experimental activities and their residual values of all inhibitors are shown in Table 3, and the corresponding scatter plot is depicted in Fig. 2.

### 3.2. CoMFA Contour Map

Contour maps were generated based on the CoMFA analyses. The maps are represented as regions in 3D space. In these maps, the changes in the steric and electrostatic fields of a compound correlate strongly with changes in its biological activity. A scalar product of Gasteiger-Hückel charge method with 2.0 Å grid space. Different combinations of training and test compounds were used for model generation. Based on the reliable $q^2$ and $r^2_{pred}$ values, 5 best models were selected out of the number of models created. The statistical values of the 5 models are tabulated in Table 2. Leave one out (LOO) analysis gave a cross-validated $q^2$ of 0.594 with 6 components and non cross-validated PLS analysis resulted in a correlation coefficient $r^2$ of 0.986, Fisher value as 60.607, and an estimated standard error of 0.043. The predictive ability of the developed CoMFA model was assessed by the test set predictions, which were excluded during model generation. The predictive ability of the test set was 0.602. Predicted and experimental activities and their residual values of all inhibitors are shown in Table 3, and the corresponding scatter plot is depicted in Fig. 2.

### Table 2. Statistical results of CoMFA models obtained from systematic search conformation based alignment

| PLS statistics | Ligand-based CoMFA model (Systematic search conformation based alignment) |
|----------------|-------------------------------------------------------------------------|
|                | Model 1 | Model 2 | Model 3 | Model 4 | Model 5 |
| $q^2$          | 0.568   | 0.564   | 0.594   | 0.492   | 0.528   |
| N              | 6       | 6       | 6       | 6       | 6       |
| $r^2$          | 0.985   | 0.984   | 0.986   | 0.968   | 0.986   |
| SEE            | 0.046   | 0.0047  | 0.043   | 0.067   | 0.044   |
| F-value        | 55.085  | 50.348  | 60.607  | 25.353  | 59.326  |
| $r^2_{pred}$   | 0.512   | 0.507   | 0.602   | 0.452   | 0.484   |

Field contribution
- Steric: 0.486 | 0.459 | 0.469 | 0.451 | 0.476
- Electrostatic: 0.514 | 0.541 | 0.531 | 0.549 | 0.524

$q^2 =$ cross-validated correlation coefficient; $N =$ number of statistical components; $r^2 =$ non-cross validated correlation coefficient; $SEE =$ standard estimated error; $F =$ Fisher value; $r^2_{pred} =$ predictive correlation coefficient for test set.

The model chosen for analysis is highlighted in bold fonts.

**Test set compounds**
- Model 1- compound no 1, 5, 6, 7, 15, 18
- Model 2- compound no 1, 5, 6, 7, 12, 18
- Model 3- compound no 1, 5, 6, 7, 13, 18
- Model 4- compound no 1, 5, 6, 10, 18
- Model 5- compound no 1, 5, 6, 10, 15, 18

### Table 3. Predicted activities and experimental pKi values obtained from CoMFA models

| Compound | Actual pKi | Predicted | Residual |
|----------|------------|-----------|----------|
| 1*       | 6.729321   | 7.345     | -0.6157  |
| 2        | 7.19044    | 7.188     | 0.0024   |
| 3        | 7.421361   | 7.505     | -0.0836  |
| 4        | 7.359519   | 7.332     | 0.0275   |
| 5*       | 7.437707   | 8.211     | -0.7733  |
| 6*       | 7.459671   | 8.048     | -0.5883  |
| 7*       | 7.459671   | 7.747     | -0.2873  |
| 8        | 8.06098    | 8.025     | 0.0360   |
| 10       | 7.5867     | 7.598     | -0.0113  |
| 11       | 7.50307    | 7.502     | 0.0011   |
| 12       | 7.657577   | 7.649     | 0.0086   |
| 13*      | 7.359519   | 7.351     | 0.0085   |
| 14       | 7.489455   | 7.468     | 0.0215   |
| 15       | 7.583359   | 7.401     | 0.1824   |
| 16       | 7.322393   | 7.31      | 0.0124   |
| 17       | 7.179799   | 7.177     | 0.0028   |
| 18*      | 7.331614   | 7.68      | -0.3484  |
| 19       | 7.798603   | 7.824     | -0.0254  |

*Test set compounds

Gasteiger-Hückel charge method with 2.0 Å grid space. Different combinations of training and test compounds were used for model generation. Based on the reliable $q^2$ and $r^2_{pred}$ values, 5 best models were selected out of the number of models created. The statistical values of the 5 models are tabulated in Table 2. Leave one out (LOO) analysis gave a cross-validated $q^2$ of 0.594 with 6 components and non cross-validated PLS analysis resulted in a correlation coefficient $r^2$ of 0.986, Fisher value as 60.607, and an estimated standard error of 0.043. The predictive ability of the developed CoMFA model was assessed by the test set predictions, which were excluded during model generation. The predictive ability of the test set was 0.602. Predicted and experimental activities and their residual values of all inhibitors are shown in Table 3, and the corresponding scatter plot is depicted in Fig. 2.

3.2. CoMFA Contour Map

Contour maps were generated based on the CoMFA analyses. The maps are represented as regions in 3D space. In these maps, the changes in the steric and electrostatic fields of a compound correlate strongly with changes in its biological activity. A scalar product of
coefficients and standard deviation (SD*Coeff) associated with each column were generated as contour maps. Favored levels were fixed at 70% and disfavored levels were fixed at 30%.

Based on the ligand-based (atom-by atom matching) alignment method, CoMFA contour maps were generated. The result is represented as a 3D ‘coefficient contour’ map. The steric contour map is represented in Fig. 3. Here, green color in the steric contour maps depicts the more bulk molecules favored region whether yellow color region represent the less bulk molecules favored in the region. The green steric contour near the R position of the phenyl ring indicates that substitution of bulky group is preferred at this position. Compounds 8, 15, 18 and 19 have bulkier substituents at this position, hence they are more active. There was a yellow contour region near to the green contour map; which indicates that substitution of bulkier groups would decrease the activity. This may be the reason why the compounds 13 and 17 having bulkier substitution shows less activity.
Fig. 4. CoMFA electrostatic contour map with highly active compound 8 for systematic search based alignment. Blue contour indicates regions where electropositive groups increases activity and red contours indicates regions where electronegative groups increases activity.

Fig. 4 represents the electrostatic contour map. In electrostatic field contours, red regions represent electronegative substituents favored regions and blue regions represent electropositive substituents favored regions. The plot shows a blue colored region situated surrounding the R positions of 7, 10, 13 and 17. Here the electropositive charges in these regions are very important for ligand binding, and electropositive group linked to this position will enhance the biological activity. A red contour region is present at the R position of 5, 8, 11, 14 and 15. In these positions the electronegative groups increases activity.

4. Conclusion

A reliable 3D-QSAR model from 2-methoxyphe- nylpiperazinylakanamides derivatives as 5-hydroxytryptamine (serotonin) receptor 7 antagonists was developed using CoMFA method based on atom-by-atom matching alignment. Important steric and electrostatic sites that can influence the bioactivities of the compounds were represented using the CoMFA contour maps. From steric contour maps, it is found that the substitution of bulkier groups in the R position enhances the biological activity. Yellow contour region indicated that the substitution of bulkier groups in the R position would decrease the activity. The electrostatic contour map showed that the substitution of electron donating group in R position improves the biological activity. These results can enlighten the important structural and chemical features in developing new potent and novel inhibitors for 5-hydroxytryptamine (serotonin) receptor 7.

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