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3D-QSAR CoMFA study of benzoxazepine derivatives as mGluR5 positive allosteric modulators

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Abstract

Positive allosteric modulation of the metabotropic glutamate receptor subtype 5 was studied by conducting a comparative molecular field analysis on 118 benzoxazepine derivatives. The model with the best predictive ability retained significant cross-validated correlation coefficients of $q^2=0.58$ ($r^2=0.81$) yielding a standard error of 0.20 in pEC₅₀ for this class of compounds. The subsequent contour maps highlight the structural features pertinent to the bioactivity values of benzoxazepines.

Metabotropic glutamate receptor subtype 5 (mGluR₅) belongs to a family of G protein coupled receptors that play important roles in synaptic plasticity and other neuro-physiological and pathological processes¹⁻³. Prevailing cellular studies increasingly demonstrate a positive association between N-methyl D-aspartate (NMDA) receptors and mGluR₅, suggesting that activation of mGluR₅ potentiates NMDA receptor function⁴⁻⁶. Given evidence which suggests the role of hypofunction of NMDA receptors in the manifestation of the positive, negative, and cognitive symptoms of schizophrenia, potentiation of mGluR₅, specifically through its allosteric binding site, is considered a possible avenue for ameliorating the symptoms of this debilitating disease^{5, 7}.

HEK293 cells expressing rat mGluR₅ were cultured and plated as previously described⁸. The cells were loaded with a Ca²⁺-sensitive fluorescent dye and the plates were washed and placed in the Functional Drug Screening System (Hamamatsu). Test compound was applied to cells 3 s after baseline readings were taken. Cells were incubated with the test compounds for 140 s then stimulated with an EC₂₀ concentration of glutamate and readings taken for an additional 60 s. Allosteric modulation by the compounds was measured by comparing the amplitude of the responses at the time of glutamate addition plus and minus test compound.

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Supplement: Three-dimensional structures for the benzoxazepine derivatives analyzed in this article along with the experimental pEC₅₀ values for allosteric potentiation of mGluR₅ can be found in the supplementary materials.

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A comparative molecular field analysis (CoMFA)^{9, 10} was conducted on 118 benzoxazepine derivatives which act as positive allosteric modulators for mGluR₅. Briefly, CoMFA correlates sterics and electrostatics to the bioactivity data establishing a three-dimensional quantitative structural activity relationship (3D QSAR).

The biological data for the 118 benzoxazepine derivatives investigated in the present study resulted from validated hits of a high-throughput screen (HTS)⁸⁸. Corresponding pEC₅₀ values were derived from the reported bioactivity values (pEC₅₀=-log(EC₅₀)) and used as the dependent variables in the CoMFA analysis. Geometries for the benzoxazepine derivatives were generated using the Corina software package^{11, 12}. Prior to CoMFA analysis, molecular alignment was achieved by the SYBYL routine database align method, whereby the superimposition of molecules was based on minimizing root-means-squares (rms) differences in the fitting of selected atoms with those of a template molecule. All molecules were aligned based on the assumption that they bind to the allosteric site of mGluR₅ in the same manner, wherein, all atoms of the benzoxazepine moiety were used as the structural element for superimposition. Partial atomic charges were calculated using the Gasteiger-Marsilli method¹³. Steric and electrostatic interactions were calculated using the Tripos force field¹⁴ using a 2 Å grid spacing, while a sp³ carbon atom and +1 charge were used as the steric and electrostatic probes, respectively.

All CoMFA calculations were performed using electrostatic fields with Lennard-Jones and Coulombtype potentials, a dielectric constant of 1/r, and a cutoff of 30 kcal mol⁻¹. Regression analysis was executed using the leave one out (LOO) cross-validated partial least squares (PLS) method¹⁵ with a minimum sigma of 2.0 kcal/mol to improve the signal to noise ratio. LOO-PLS was thus used to ascertain the predictive ability of the models and to determine the optimal number of components to be used in the final QSAR models, which were derived from the non cross-validated, conventional analysis. All calculations were performed using Sybyl 7.3¹⁶ on a Dell Precision 380 workstation.

Initially, alignment of the compound set afforded a median q² of 0.45. Outliers based on residual value (molecules 45 and 78 in supp. mat.) were omitted and region focusing using Determinant Power as a weight was implemented to yield an improved q² of 0.51.

Region focusing corresponds to rotating the model components through a high-order space, and thus refining the model by increasing the weight for the lattice points which are most pertinent to the model. This enhances the resolution and predictive power of a corresponding PLS analysis¹⁷. Thereby the cross-correlated “brown” noise in the data matrix is reduced.

This procedure was accomplished through SYBYL PLS region focusing which selectively re-weights the grid points in a region to create a new CoMFA model. Two flavors of region focusing were tested: 1) Weights were derived for each lattice point according to the fraction of the variation of the model's components attributable to each lattice point (menu option “Discriminate Power”). 2) Weights were assigned to each lattice point according to the product of the variation at each lattice point and the lattice point's regression coefficient from the model (menu option “Stdev*Coefficients”). The accuracy of prediction of CoMFA models has been shown to depend strongly on the geometry and structural alignment of molecules¹⁸. To test influence of conformation and superimposition on the present model, the 118 benzoxazepine structures were geometry optimized at the HF 6-31G* level of theory using Firefly¹⁹

Usage of the resulting conformations yields an improved q² of 0.53 with no observed outliers (Table 1). Ultimately, the model with the highest predictive power was obtained using the CoMFA standard model with region focusing. Variance×coefficients were assigned as weight values, through which significant cross-validated correlation coefficients, a q² of 0.58 and r² of 0.81, were retained. The graph of the actual pEC₅₀ versus the predicted pEC₅₀ values for

this model demonstrates that these values are in good agreement, and compared to the model without region focusing, shows an improvement.

Most of the variation in the data set resides at the R¹ position (Figure 2). Thus, a model was constructed with the R¹ groups aligned in an attempt to reduce noise in the model. The best model with this alignment, q² of 0.37 and r² of 0.78, was achieved with region focusing with weight by “Discriminant Power.”

The CoMFA contours of the best model (Figure 4) reveal that primarily bulky substituents are required at the meta position of the 1,2,4-triazine ring (R³). With most analogs containing a ring-substitution at the R¹ position, the contour maps reflect the preference for electropositive character proximal to the acyl carbonyl oxygen at the R² position of the oxazepine moiety. The thiophene group at the R¹ position of the most potent mGluR₅ potentiator (Figure 1b) reflects the ambivalent electrostatic character with electropositivity found along the aromatic carbons and electronegativity due to the sulfur on the opposite side of the ring.

To better assess the predictive power of the models constructed, an independent data set was created containing 18 molecules from the original training set. A new model was constructed using the parameters of the best model (StDev*Coefficient) with the remaining 100 molecules as a training set. The non cross-validated QSAR model was then used to predict the activities of the independent test set. The model produced a good predictive r² value of 0.88 for this validation test set.

In conclusion, we present here the first example in which a comparative molecular field analysis (CoMFA) based on 3D-QSAR was conducted on 118 benzoxazepine derivatives shown to positively modulate mGluR₅ allosterically. The final CoMFA model, derived with region focusing, where variance×coefficients were assigned as weight values, suggests the potential, statistical significance of this class of compounds. The resulting contour maps afford insight into the features vital for the design of efficacious benzoxazepine-based analogs as positive allosteric modulators for mGluR₅. Specifically, the analysis suggests bulky substituents at the meta position of the 1,2,4-triazine ring (R³), and substituents at the 2 position of the oxazepine moiety (R¹) with both an electropositive and an electronegative functional group are preferred.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol* 1997;37:205. [PubMed: 9131252]
2. Anwyl R. Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. *Brain Res Brain Res Rev* 1999;29(1):83. [PubMed: 9974152]
3. Coutinho V, Knopfel T. Metabotropic glutamate receptors: electrical and chemical signaling properties. *Neuroscientist* 2002;8(6):551. [PubMed: 12467377]
4. Coyle JT, Tsai G, Goff DC. Ionotropic glutamate receptors as therapeutic targets in schizophrenia. *Curr Drug Targets CNS Neurol Disord* 2002;1(2):183. [PubMed: 12769626]

5. Marino MJ, Conn PJ. Direct and indirect modulation of the N-methyl D-aspartate receptor. *Curr Drug Targets CNS Neurol Disord* 2002;1(1):1. [PubMed: 12769631]
6. Tsai G, Coyle JT. Glutamatergic mechanisms in schizophrenia. *Annu Rev Pharmacol Toxicol* 2002;42:165. [PubMed: 11807169]
7. Moghaddam B. Targeting metabotropic glutamate receptors for treatment of the cognitive symptoms of schizophrenia. *Psychopharmacology (Berl)* 2004;174(1):39. [PubMed: 15205877]
8. Mueller R, Rodriguez AL, Dawson ES, Butkiewicz M, Nguyen TT, Oleszkiewicz S, Bleckmann A, Weaver CD, Lindsley CW, Conn PJ, Meiler J. Identification of Metabotropic Glutamate Receptor Subtype 5 Potentiators Using Virtual High-Throughput Screening. *ACS Chem Neurosci*.
9. Cramer RD 3rd, Patterson DE, Bunce JD. Recent advances in comparative molecular field analysis (CoMFA). *Prog Clin Biol Res* 1989;291:161. [PubMed: 2726839]
10. Cramer RD, Patterson DE, Bunce JD. Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins. *J Am Chem Soc* 1988;110(18):5959.
11. Gasteiger J, Rudolph C, Sadowski J. Automatic generation of 3D atomic coordinates for organic molecules. *Tetrahedron Comput Method* 1990;3(6c):537.
12. Gasteiger J, Rudolph C, Sadowski J. Automatic Generation of 3D-Atomic Coordinates for Organic Molecules. *Tetrahedron Comput Method* 1992;3:537.
13. Gasteiger J, Marsili M. Iterative Partial Equalization of Orbital Electronegativity - A Rapid Access to Atomic Charges. *Tetrahedron Lett* 1980;36:3219.
14. Matthew C, Richard DC III, Nicole Van O. Validation of the general purpose tripos 5.2 force field. *J Comput Chem* 1989;10(8):982.
15. Bush BL, Nachbar RB Jr. Sample-distance partial least squares: PLS optimized for many variables, with application to CoMFA. *J Comput Aided Mol Des* 1993;7(5):587. [PubMed: 8294948]
16. Tripos SYBYL, SYBYL 8.0. Tripos International; St. Louis, Missouri, USA: 2008.
17. Sulea T, Oprea TI, Muresan S, Chan SL. A Different Method for Steric Field Evaluation in CoMFA Improves Model Robustness. *J Chem Inf Comput Sci* 1997;37(6):1162.
18. Cho SJ, Tropsha A. Cross-validated R²-guided region selection for comparative molecular field analysis: a simple method to achieve consistent results. *J Med Chem* 1995;38(7):1060. [PubMed: 7707309]
19. Granovsky, AA. Firefly version 7.1.F.

Figure 1. (a) Base structure of the benzoxazepine analogs examined. (R¹) predicted to prefer ambivalent electronegative and electropositive character with low steric bulk, (R²) no signal, and (R³) predicted to prefer high steric bulk

Figure 2. A plot of the 118 benzoxazepine derivatives after geometry optimization, overlapped for the CoMFA analysis to illustrate the variability at R^1 . Oxygen is shown in red, carbon in silver, hydrogen in cyan, nitrogen in blue, chlorine in green, and sulfur in yellow

Figure 3. (a) Represents the predictive versus experimental pEC₅₀ values for the CoMFA model, after geometry optimization, without region focusing. (b) Represents the predictive versus experimental pEC₅₀ values for the best CoMFA model

Figure 4. Electrostatic (a) and steric (b) contour plots of the best CoMFA model, respectively. Areas favoring steric bulk are indicated by green polyhedra (contribution level 80%), while areas where steric bulk is unfavorable are represented by yellow polyhedra (contribution level 20%). Areas favoring electropositive values are indicated by blue polyhedra (contribution level 80%) while areas where electronegative values are favored are represented by red polyhedra (contribution level 20%). Contours were generated based on the results of the final, non cross-validated PLS analysis

Table 1

Summary of PLS analyses of 3 CoMFA sets

	$\mathbf{I}^a$	$\mathbf{II}^b$	$\mathbf{III}^c$
q^2	0.53	0.58	0.51
r^2	0.82	0.81	0.80
SEE ^d	0.20	0.20	0.20
F ^e	83	79	73
ONC ^f	6	6	6
SF ^g /EF ^h	0.61/0.39	0.55/0.45	0.55/0.45

^aNo region focusing.^bRegion focusing with weight by StDev*Coefficient.^cRegion focusing with weight by Discriminant Power.^dStandard Error of Estimate.^eFraction of explained variance.^fOptimum Number of components.^gFraction of steric field contribution.^hFraction of electrostatic field contribution.