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3D-QSAR CoMFA study of benzoxazepine derivatives as mGluR$_5$ 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 pEC$_{50}$ 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 (mGluR$_5$) belongs to a family of G protein coupled receptors that play important roles in synaptic plasticity and other neuro-physiological and pathological processes.[1–3] Prevailing cellular studies increasingly demonstrate a positive association between *N*-methyl D-aspartate (NMDA) receptors and mGluR$_5$, suggesting that activation of mGluR$_5$ potentiates NMDA receptor function.[4–6] 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 mGluR$_5$, 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 mGluR$_5$ were cultured and plated as previously described.[8] The cells were loaded with a Ca^{2+}-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 EC$_{20}$ 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.

A comparative molecular field analysis (CoMFA)[9,10] was conducted on 118 benzoxazepine derivatives which act as positive allosteric modulators for mGluR$_5$ (Fig. 1a). 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 pEC$_{50}$ values were derived from the reported bioactivity values (pEC$_{50}$ = −log (EC$_{50}$)) 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

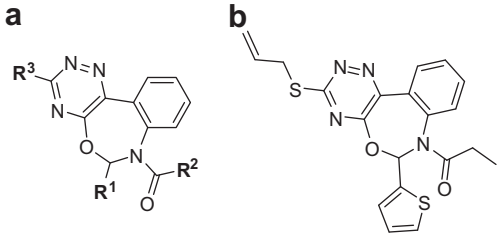

Figure 1. (a) Base structure of the benzoxazepine analogs examined. (R^1) predicted to prefer ambivalent electronegative and electropositive character with low steric bulk, (R^2) no signal, and (R^3) predicted to prefer high steric bulk.

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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 $mGluR_5$ 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 method.[13] Steric and electrostatic interactions were calculated using the Tripos force field[14] using a 2 Å grid spacing, while a sp^3 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 Coulomb-type potentials, a dielectric constant of $1/r$, and a cutoff of 30 kcal/mol. Regression analysis was executed using the leave one out (LOO) cross-validated partial least squares (PLS) method[15] 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 7.3[16] on a Dell Precision 380 workstation.

Initially, alignment of the compound set afforded a median q^2 of 0.45. Outliers based on residual value (molecules **45** and **78** in Supplementary data) were omitted and region focusing using determinant power as a weight was implemented to yield an improved q^2 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 analysis.[17] 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 molecules.[18] 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 Firefly.[19]

Table 1
Summary of PLS analyses of 3 CoMFA sets

	I[a]	II[b]	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

[a] No region focusing.
[b] Region focusing with weight by StDev*Coefficient.
[c] Region focusing with weight by discriminant power.
[d] Standard error of estimate.
[e] Fraction of explained variance.
[f] Optimum number of components.
[g] Fraction of steric field contribution.
[h] Fraction of electrostatic field contribution.

Usage of the resulting conformations yields an improved r^2 of 0.82 (Fig. 3) and q^2 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 q^2 of 0.58 and r^2 of 0.81, were retained. The graph of the actual pEC_{50} versus the predicted pEC_{50} values for this model demonstrates that these values

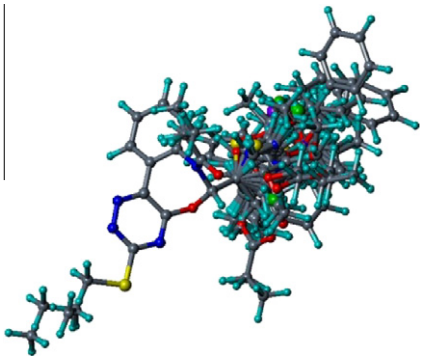

Figure 2. A plot of the 118 benzoxazepine derivatives after geometry optimization, overlapped for the CoMFA analysis to illustrate the variability at R^1. Oxygen is shown in red, carbon in silver, hydrogen in cyan, nitrogen in blue, chlorine in green, and sulfur in yellow.

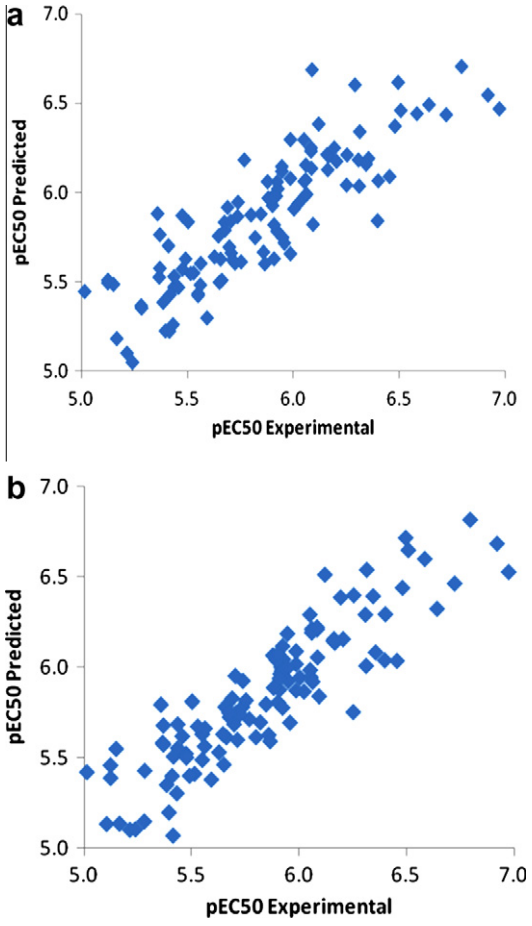

Figure 3. (a) Represents the predictive versus experimental pEC_{50} values for the CoMFA model, after geometry optimization, without region focusing. (b) Represents the predictive versus experimental pEC_{50} values for the best CoMFA model.

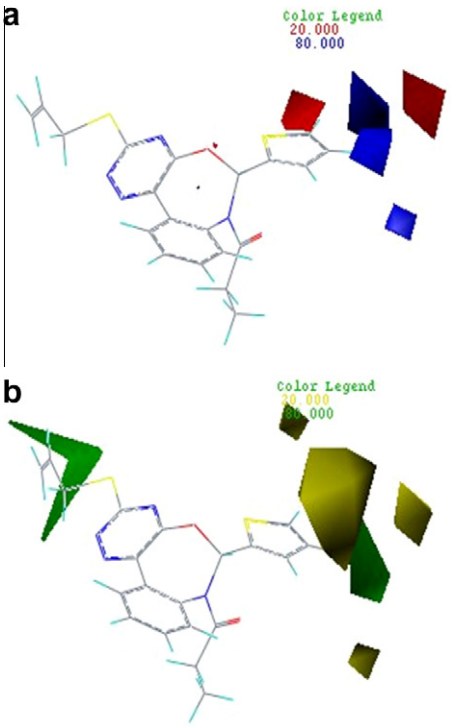

Figure 4. Electrostatic (a) and steric (b) contour plots of the best CoMFA model, respectively. Areas favoring steric bulk are indicated by green polyhedra (contribution level 80%), while areas where steric bulk is unfavorable are represented by yellow polyhedra (contribution level 20%). Areas favoring 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.

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 R^1 position (Fig. 2). Thus, a model was constructed with the R^1 groups aligned in an attempt to reduce noise in the model. The best model with this alignment, q^2 of 0.37 and r^2 of 0.78, was achieved with region focusing with weight by 'Discriminant Power.'

The CoMFA contours of the best model (Fig. 4) reveal that primarily bulky substituents are required at the *meta* position of the 1,2,4-triazine ring (R^3). With most analogs containing a ring-substitution at the R^1 position, the contour maps reflect the preference for electropositive character proximal to the acyl carbonyl oxygen at the R^2 position of the oxazepine moiety. The thiophene group at the R^1 position of the most potent $mGluR_5$ potentiator (Fig. 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 r^2 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 $mGluR_5$ 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 $mGluR_5$. Specifically, the analysis suggests bulky substituents at the *meta* position of the 1,2,4-triazine ring (R^3), and substituents at the 2-position of the oxazepine moiety (R^1) with both an electropositive and an electronegative functional group are preferred.

Acknowledgments

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Supplementary data

Three-dimensional structures for the benzoxazepine derivatives analyzed in this article along with the experimental pEC_{50} values for allosteric potentiation of $mGluR_5$ can be found in the Supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.061.

References and notes

1. Conn, P. J.; Pin, J. P. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205.
2. Anwyl, R. *Brain Res. Brain Res. Rev.* **1999**, *29*, 83.
3. Coutinho, V.; Knopfel, T. *Neuroscientist* **2002**, *8*, 551.
4. Coyle, J. T.; Tsai, G.; Goff, D. C. *Curr. Drug Targets: CNS Neurol. Disord.* **2002**, *1*, 183.
5. Marino, M. J.; Conn, P. J. *Curr. Drug Targets: CNS Neurol. Disord.* **2002**, *1*, 1.
6. Tsai, G.; Coyle, J. T. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 165.
7. Moghaddam, B. *Psychopharmacology (Berl)* **2004**, *174*, 39.
8. Mueller, R.; Rodriguez, A. L.; Dawson, E. S.; Butkiewicz, M.; Nguyen, T. T.; Oleszkiewicz, S.; Bleckmann, A.; Weaver, C. D.; Lindsley, C. W.; Conn, P. J.; Meiler, J. *ACS Chem. Neurosci.*
9. Cramer, R. D., 3rd; Patterson, D. E.; Bunce, J. D. *Prog. Clin. Biol. Res.* **1989**, *291*, 161.
10. Cramer, R. D.; Patterson, D. E.; Bunce, J. D. *J. Am. Chem. Soc.* **1988**, *110*, 5959.
11. Gasteiger, J.; Rudolph, C.; Sadowski, J. *Tetrahedron Comput. Methods* **1990**, *3*, 537.
12. Gasteiger, J.; Rudolph, C.; Sadowski, J. *Tetrahedron Comput. Methods* **1992**, *3*, 537.
13. Gasteiger, J.; Marsili, M. *Tetrahedron Lett.* **1980**, *36*, 3219.
14. Matthew, C.; Richard, D. C., III; Nicole Van, O. *J. Comput. Chem.* **1989**, *10*, 982.
15. Bush, B. L.; Nachbar, R. B., Jr. *J. Comput. Aided Mol. Des.* **1993**, *7*, 587.
16. Tripos *SYBYL*, SYBYL 8.0; Tripos International: St. Louis, Missouri, USA, 2008.
17. Sulea, T.; Oprea, T. I.; Muresan, S.; Chan, S. L. *J. Chem. Inf. Comput. Sci.* **1997**, *37*, 1162.
18. Cho, S. J.; Tropsha, A. *J. Med. Chem.* **1995**, *38*, 1060.
19. Granovsky, A. A. Firefly version 7.1.F.