

Quantitative Structure-activity relationships using Comparative Molecular Field Analysis Studies on 2-(*p*-Substituted benzyl)-5-(substituted carbonylamino)benzoxazoles as Antibacterial Agents against *Staphylococcus aureus*

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The 3D-QSAR studies using comparative molecular, field analysis (CoMFA) approach on a set of 2-(*p*-substituted benzyl)-5-(substituted carbonylamino)benzoxazoles as antibacterial agents against *Staphylococcus aureus* by considering the steric and electrostatic influences have been presented. The CoMFA analysis gave cross-validated r^2 value of 0.480 and non cross-validated $r^2 = 0.950$ with an optimized component of 4. The model deduced from this investigation provides underlying structural requirements and good predictive ability, which could aid the new antibacterial agents for *Staphylococcus aureus* prior to their synthesis.

Key Words: 3D-QSAR Comparative Molecular Field Analysis, Antibacterial activity, 2-Benzylbenzoxazole, *Staphylococcus aureus*.

INTRODUCTION

The number of life-threatening infections caused by multi-drug-resistant Gram-positive pathogens has reached an alarming level in hospitals and the community^{1–3}. Infections caused by these organisms pose a serious challenge to the scientific community and the need for an effective therapy has led to a search for novel antibacterial agents.

Substituted benzoxazole derivatives have been the aim of many researchers for many years because they constitute an important class of heterocyclic compounds^{4–21} with antibacterial and antifungal^{4–14}, topoisomerase inhibitors¹⁵ and antitumor activities^{16–21}. Moreover, structure-activity relationships of benzoxazole derivatives have revealed that the substitution of the 2nd position is decisive for the biological activity and position 5 is important for the intensity of the activity^{22–24}.

Quantitative structure-activity relationships (QSARs) are now acknowledged to be in the heart of the long-term task of systematical evaluation of existing chemicals²⁵. At present, the challenge is to improve the accuracy and predictability of

QSAR by taking into account, in a very detailed way, the structural and physico-chemical features of the tested compounds. The comparative molecular field analysis (CoMFA) program is in keeping with the general pattern of searching for these new descriptors, where steric and electrostatic fields of the molecule are mapped by a probe atom²⁶⁻²⁸. CoMFA, which is applied to a set of molecules exhibiting biological activity with a similar mechanism of action, was proposed by Cramer *et al.*²⁹ The advantages of CoMFA are the abilities to predict the biological activities of the molecules and to represent the relationships between steric/electrostatic property and biological activity in the form of contour maps giving the key features of not only the ligand-receptor interaction but also the topology of the receptor.

We present here the 3D-QSAR studies using CoMFA method on a set of 2-(*p*-substituted benzyl)-5-(substituted carbonylamino)benzoxazoles as anti-bacterial agents against *Staphylococcus aureus* by considering the steric and electrostatic influences. The model deduced from this investigation provides underlying structural requirements and good predictive ability, which could aid the new antibacterial agents prior to their synthesis.

EXPERIMENTAL

Compounds and biological data: Nineteen 2-(*p*-substituted benzyl)-5-(substituted carbonylamino)benzoxazole derivatives listed in Table-1 were synthesized and tested against Gram-positive bacterium *Staphylococcus aureus* ATCC 25923 strain by us³⁰. Their *in vitro* minimum inhibitory concentration (MIC) values were used for this study. The MIC values ($\mu\text{g/mL}$) were converted to pMIC according to the formula

$$\text{pMIC} = -\log \text{MIC}$$

pMIC values were used as dependent variables in the CoMFA analysis. The training set of 19 molecules with structures and their activities are shown in Table-1.

Computational methods

Molecular modeling: Molecular modeling studies were performed using the SYBYL 6.8 Software package, Silicon Graphic workstation. Molecular structures were built using the SKETCH option in SYBYL³¹. Geometry optimization was carried out using MAXIMIN molecular mechanics and Tripos force field supplied within SYBYL, with convergence criterion set at 0.05 kcal/(\AA mol). The alignment of the training set molecules was derived by using FlexS in SYBYL. One of the more active molecules **6** was used as the template for alignment by considering the heavy atoms of the 2-methylen-benzoxazole ring as shown in Fig. 1. All values were filled with valence and Gasteiger charges were calculated for each compound. The superimposition of all the molecules is shown in Fig. 2. CoMFA models were generated using 19 molecules (1-19, Table-2), with column filtering value (σ) of 2.0.

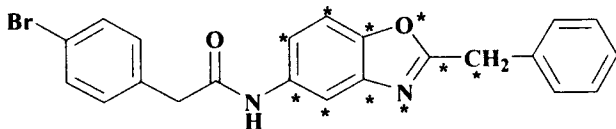
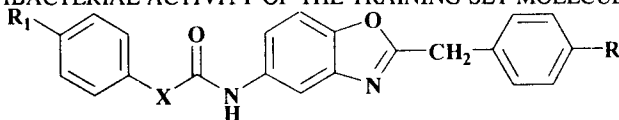


Fig. 1. Molecule 6 with atoms used for superimposition are marked.

TABLE-1
ANTIBACTERIAL ACTIVITY OF THE TRAINING SET MOLECULES



Compounds	X	R	R ₁	Sa*
1	—	H	H	200
2	—	H	C ₂ H ₅	50
3	—	H	NO ₂	50
4	—	H	Br	50
5	—	H	F	50
6	CH ₂	H	Br	25
7	CH ₂	H	F	50
8	CH ₂	H	H	25
9	CH ₂	H	CH ₃	25
10	—	Cl	H	200
11	—	Cl	C ₂ H ₅	50
12	—	Cl	NO ₂	100
13	—	Cl	C(CH ₃) ₃	25
14	—	Cl	Br	50
15	—	Cl	F	50
16	CH ₂	Cl	Br	50
17	CH ₂	Cl	H	50
18	CH ₂	Cl	Cl	50
19	CH ₂	Cl	NO ₂	50

*All MICs (µg/mL) measured against *Staphylococcus aureus* ATCC 25923 strain.

TABLE-2
PLS STATISTICS OF CoMFA 3D-QSAR MODEL

PLS statistics	CoMFA
q ² (Leave-one-out cross-validated predicted power of model)	0.480
r ² (Correlation coefficient squared of PLS analysis)	0.950
N (Optimum number of components obtained from cross-validated PLS analysis and the same used in final non cross-validated analysis)	4
X (Number of descriptors go into the PLS after column filtering is 2.0 kcal/mol)	185
SEE (Standard error of estimate)	0.068
F value (F-test value)	67.118
Field contribution (Steric and electrostatic fields from CoMFA)	
Steric	48.6%
Electrostatic	51.4%

CoMFA analysis: CoMFA was performed using the QSAR module of SYBYL 6.8. Comparative molecular field analysis²⁹ of these molecules was carried out on the steric and electrostatic fields using the default values. A three-dimensional cubic lattice, with a 2 Å grid spacing, was generated automatically around these molecules in order to ensure that the grid extended the molecular dimensions by 4 Å in all directions. Threshold column filtering of 2.0 kcal/mol was set to hasten the analysis and reduce the amount of noise. The steric and electrostatic fields were calculated separately for each molecule using sp^3 carbon atom probe with a charge of 1 (default probe atom in SYBYL) and energy cut-off values of 30 kcal/mol for both steric and electrostatic fields. The probe atom was placed at each lattice point and their steric and electrostatic interactions with each atom in the molecule were computed using CoMFA standard scaling.

PLS analysis: Initial PLS analysis was carried out using Leave-One-Out option (cross-validated) to obtain the optimal number of components to be used in the subsequent final analysis. A subset of CoMFA field sample points falling within a standard deviation of ≤ 2.0 kcal/mol was used to run PLS regression analysis. Finally, noncross-validated analysis was performed using the optimal number of previously identified components and was employed to analyze the result of CoMFA.

RESULTS AND DISCUSSION

The CoMFA method was employed for deriving 3D-QSAR model consisting of 19 training sets of 2-(*p*-substituted benzyl)-5-(substituted carbonylamino)-benzoxazole derivatives (Table-1) keeping *in vitro* activity pMIC as a dependent variable. The statistical parameters of CoMFA analysis of 19 compounds are summarized in Table-2. The CoMFA analysis using steric and electrostatic fields, gave cross-validated r^2 value of 0.480 and non cross-validated $r^2 = 0.950$ with an optimized components of 4, which is relatively acceptable. The actual and predicted pMIC values of the training set are shown in Table-3. Observed and predicted biological activities of the training set are plotted in Fig. 3. The CoMFA steric and electrostatic field contour plots obtained from atom-based alignment is shown in Figs. 4 and 5, respectively. The *green regions* indicate areas where steric bulk enhances biological activity, while the *yellow contours* indicate regions where steric bulk is detrimental to biological activity. Blue coloured regions show areas where electropositive groups enhance biological activity, while red regions represent areas where electronegative groups enhance activity against Gram-positive bacterium *S. aureus*.

TABLE 3
OBSERVED ACTIVITIES AND RESIDUALS OF THE TRAINING SET MOLECULES
BY THE CoMFA MODEL

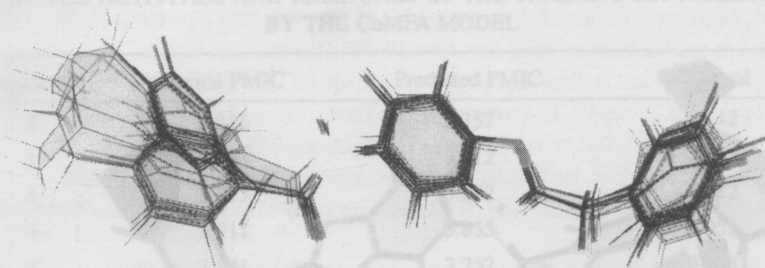
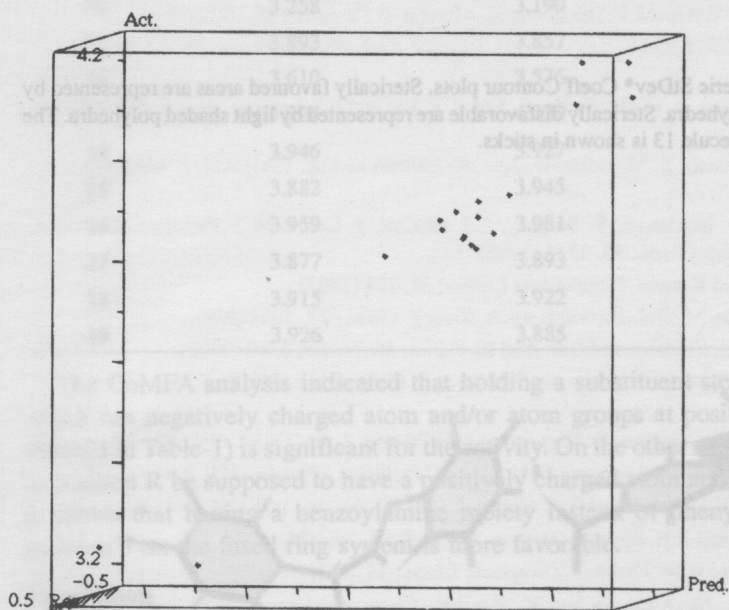


Fig. 2. Superimposition of the training set molecules using FlexS in Sybyl.



Residuals from FLEXS

3.2

4.2

Fig. 3. Graph of observed activity versus predicted activities of training set molecules from CoMFA analysis, activity expressed as pMIC.

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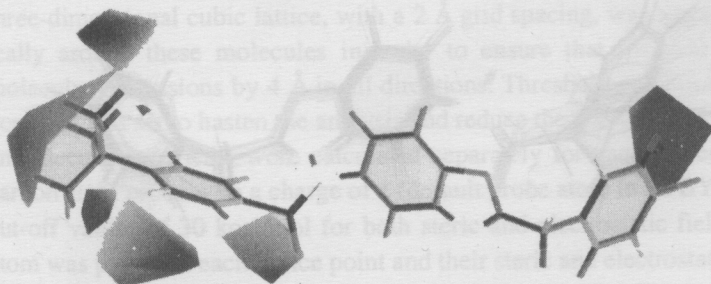


Fig. 4. CoMFA steric StDev*Coeff Contour plots. Sterically favoured areas are represented by shaded polyhedra. Sterically disfavored are represented by light shaded polyhedra. The active molecule 13 is shown in sticks.

RESULTS AND DISCUSSION

The CoMFA method was employed for defining 3D-QSAR models regarding of 19 training set of 2-(*p*-substituted benzyl)-5-(substituted benzylamino)-benzoxazole derivatives (Table-1) keeping *in vitro* activity pMIC as dependent variable. The statistical parameters of CoMFA analysis of 19 compounds are summarized in Table-2. The CoMFA analysis using steric and electrostatic fields gave cross-validated r^2 value of 0.59 and non cross-validated $r^2 = 0.70$ with an optimum number of components 4, 4, 4, 4. The steric field is the most important and the electrostatic field is the second most important. The observed and predicted activity of the training set molecules are shown in Table-3. The steric and electrostatic fields are shown in Figs. 4 and 5, and 6 and 7 respectively. The steric field shows that the steric bulk is detrimental to biological activity, whereas electrostatic positive charge enhances activity. The electrostatic field shows that positive charge favored areas are represented by light shaded polyhedra. Negative charge favored areas are represented by dark polyhedra. The active molecule 13 is shown in sticks.

Fig. 5. CoMFA electrostatic StDev*Coeff. Contour plots. Positive charge favoured areas are represented by light shaded polyhedra. Negative charge favored areas are represented by dark polyhedra. The active molecule 13 is shown in sticks.

TABLE-3
 PREDICTED ACTIVITIES AND RESIDUALS OF THE TRAINING SET MOLECULES
 BY THE CoMFA MODEL

Compounds	Actual PMIC	Predicted PMIC	Residual
1	3.215	3.357	-0.142
2	3.853	3.912	0.062
3	3.873	3.892	-0.022
4	3.911	3.855	0.055
5	3.841	3.752	0.088
6	4.226	4.138	0.088
7	3.857	3.904	-0.047
8	4.136	4.119	0.021
9	4.154	4.218	-0.068
10	3.258	3.190	0.068
11	3.893	3.857	0.033
12	3.610	3.576	0.034
13	4.224	4.220	0.004
14	3.946	3.927	0.019
15	3.882	3.945	-0.065
16	3.959	3.981	-0.022
17	3.877	3.893	-0.016
18	3.915	3.922	-0.007
19	3.926	3.885	0.041

The CoMFA analysis indicated that holding a substituent sterically favoured which has negatively charged atom and/or atom groups at position R₁ (general formula in Table-1) is significant for the activity. On the other side, the substituent at position R be supposed to have a positively charged atom and/or atom groups. It shows that having a benzoylamine moiety instead of phenylacetylamine at position 5 on the fused ring system is more favorable.

Conclusion

We have developed predictive CoMFA 3D-QSAR models for 2-(*p*-substitutedbenzyl)-5-(substitutedcarbonylamino)benzoxazoles as antibacterial agents against *Staphylococcus aureus*. The contour diagrams obtained for the various CoMFA field contributions can be mapped back onto structural features relating to the trends in activities of the molecules. On the basis of the spatial arrangement of the various field contributions, novel molecules are being designed with improved activity.

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REFERENCES

1. L.A. Mitscher, S.P. Pillai, E.J. Gentry and D.M. Shankel, *Med. Res. Rev.*, **19**, 477 (1999).
2. V.J. Lee and S.J. Hecker, *Med. Res. Rev.*, **19**, 521 (1999).
3. D.H. Williams and B. Bardsley, *Angew. Chem. Int. Ed.*, **38**, 1173 (1999).
4. T. Hisano, M. Ichikawa, K. Tsumoto and M. Tasaki, *Chem. Pharm. Bull.*, **30**, 2996 (1982).
5. M. Prudhomme, J. Guyot and G. Jeminet, *J. Antibiotics*, **39**, 934 (1986).
6. S. Ersan, S. Nacak, R. Berkem and T. Özden, *Arzneim. Forsch.*, **47**, 963 (1997).
7. H.M. El-Shaer, S.A. Abdel-Aziz, H.A. Allimony and R.M. Abdel-Rahman, *Pharmazie*, **52**, 585 (1997).
8. E. Sener, I. Yalcin and E. Sungur, *Quant. Struc. Act. Relat.*, **10**, 223 (1991).
9. I. Yalcin, I. Oren, E. Sener, A. Akin and N. Ucarturk, *Eur. J. Med. Chem.*, **27**, 401 (1992).
10. E. Sener, I. Yalcin, O. Temiz, I. Oren, A. Akin and N. Ucarturk, *Farmaco*, **52**, 99 (1996).
11. I. Oren, O. Temiz, I. Yalcin, E. Sener, A. Akin and N. Ucarturk, *Arzneim. Forsch.*, **47**, 1393 (1997).
12. O. Temiz, I. Oren, E. Sener, I. Yalcin and N. Ucarturk, *Farmaco*, **53**, 337 (1998).
13. E.A. Sener, O.T. Arpaci, I. Yalcin and N. Altanlar, *Il Farmaco*, **55**, 397 (2000).
14. O.T. Arpaci, E.A. Sener, I. Yalcin and N. Altanlar, *Arch. Pharm. Pharm. Med. Chem.*, **6**, 283 (2002).
15. J.S. Kim, Q. Sun, B. Gatto, C. Yu, A. Liu, L.F. Liu and E.J. La Voie, *Bioorg. Med. Chem.*, **4**, 621 (1996).
16. M. Ueki, K. Ueno, S. Miyadoh, K. Abe, K. Shibata and M. Taniguchi, *J. Antihiotics*, **46**, 1089 (1993).
17. D.F. Shi, T.D. Bradshaw, S. Wrigley, C.J. McCall, P. Lelieveld, I. Fichtner and M.F.G. Stevens, *J. Med. Chem.*, **39**, 3375 (1996).
18. M. DeLuca and Kerwin, *Tetrahedron Letters*, **38**, 199 (1997).
19. M.B. Reynolds, M. DeLuca and Kerwin, *Bioorg. Chem.*, **27**, 326 (1999).
20. Z.M. Nofal, M. El-Zahar and S.S. Abd El-Karim, *Molecules*, **5**, 99 (2000).
21. S. Sato, T. Kajiura, M. Noguchi, K. Takehana, T. Kobayashi and T. Tsuji, *J. Antibiotics*, **54**, 102 (2001).
22. By Water; W.R. Coleman, O. Kamm and H.H. Merrit, *J. Am. Chem. Soc.*, **87**, 905 (1945).
23. C.H. Cashin, W.W. Dawson and E.A. Kitchen, *J. Pharm. Pharmacol.*, **29**, 330 (1977).
24. D. Evans, D.W. Dunwell and T.A. Hicks, *J. Med. Chem.*, **16**, 1158 (1975).
25. D.J.W. Blum and R.E. Speece, *Environ. Sci. Technol.*, **24**, 284 (1990).
26. A. Agarwal and E.W. Taylor, *J. Computat. Chem.*, **14**, 237 (1993).
27. K. Hasegawa, M. Arakawa and K. Funatsu, *Chemometrics and Intelligent Laboratory Systems*, **50**, 253 (1999).
28. H. Liu, M. Ji, H.L. Jiang, *Bioorg. Med. Chem. Lett.*, **10**, 2153 (2000).
29. D.R. Cramer, D.E. Paterson, J.D. Bunce, *J. Am. Chem. Soc.*, **110**, 5959 (1988).
30. B. Tekiner-Gulbas, I. Yalcin, I. Yildiz-Oren, O. Temiz-Arpaci, E. Aki-Sener and N. Altanlar, 7th International Symposium on Pharmaceutical Sciences, June 24–27, 2003, Ankara, Turkey. pp. 187, 321.
31. Sybyl 6.8, Tripos Inc., St. Louis, USA.