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Three-Dimensional Quantitative Structure–Activity Relationship (3D-QSAR) Studies of Tricyclic Oxazolidinones as Antibacterial Agents†

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Abstract—Oxazolidinones exemplified by eprezolid and linezolid are a new class of antibacterials that are active against Gram positive and anaerobic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE) and vancomycin resistant enterococci (VRE). In an effort to have a better antibacterial agent in the oxazolidinone class, we have performed three-dimensional quantitative structure–activity relationship (3D-QSAR) studies for a series of tricyclic oxazolidinones. 3D-QSAR studies were performed using the Comparative Molecular Field Analysis (CoMFA) and Comparative Molecular Similarity Indices Analysis (CoMSIA) procedures. These studies were performed using 42 compounds; the QSAR model was developed using a training set of 33 compounds. The predictive ability of the QSAR model was assessed using a test set of 9 compounds. The predictive 3D-QSAR models have conventional r^2 values of 0.975 and 0.940 for CoMFA and CoMSIA respectively; similarly, cross-validated coefficient q^2 values of 0.523 and 0.557 for CoMFA and CoMSIA, respectively, were obtained. The CoMFA 3D-QSAR model performed better than the CoMSIA model.

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 lead to a search for novel antibacterial agents. The oxazolidinones exemplified by eprezolid and linezolid are one such class of antibacterial agents with potent activity against Gram-positive organisms including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE) and vancomycin resistant enterococci (VRE).[4] These compounds have been shown to inhibit translation at the initiation phase of protein synthesis in bacteria by binding to the 50S ribosomal subunit.[5,6] This paper describes the application of two three dimensional quantitative structure activity relationship (3D-QSAR) methods, comparative molecular field analysis (CoMFA)[7] and comparative molecular similarity indices analysis (CoMSIA)[8,9] for tricyclic oxazolidinones as antibacterial agents. The present study is aimed to gain insights into the steric, the electrostatic, the hydrophobic and the hydrogen-bonding properties of these molecules, their influence on the activity and to derive predictive 3D-QSAR models for design and prediction of the activities of new derivatives for this class of inhibitors.

Results and Discussion

The CoMFA and CoMSIA methods were employed for deriving 3D-QSAR models consisting for a training set

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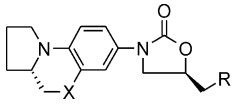
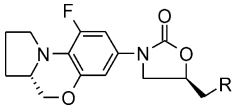
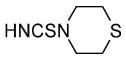
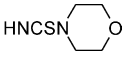
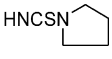
Table 1. Antibacterial activity (MIC) of selected training set and test set molecules



Compd	X	R	*S.a*[a]
1	O	$NHCOCH_3$	4
2	O	NHCHO	4
3	O	$NHCOCH=CH_2$	4
4	O	$NHCOCH(CH_3)_2$	8
5	O	$NHCOCH_2Cl$	2
6	O	$NHCOCHCl_2$	4
7	O	$NHCOCHF_2$	2
8	O	$NHCOCH_3$	2
9	O	$NHCOCH=CH_2$	2
10	O	$NHCOCH_2Cl$	2
11	S	$NHCOCH_3$	8



Compd	R	*S.a*[a]
12	NHCHO	4
13	$NHCOCH_3$	1
14	$NHCOCH_2CH_3$	2
15	$NHCOCH_2CH_2CH_3$	4
16	$NHCO(CH_2)_3CH_3$	8
17	$NHCO(CH_2)_4CH_3$	16
18	$NHCO(CH_2)_5CH_3$	32
19	$NHCOCH_2Cl$	1
20	$NHCOCH_2Br$	4
21	$NHCOCH_2OH$	4
22	$NHCOCH=CH_2$	4
23	$NHCOCH_2CF_3$	4
24	$NHCOCH(CH_3)_2$	8
25	NHCHS	1
26	NHCSMe	0.5
27	NHCSCH2CH3	0.5
28	$NHCSCH_2CH_2CH_3$	1
29	$NHCS(CH_2)_3CH_3$	2
30	$NHCO_2Me$	2
31	$NHCO_2Et$	4
32	$NHCS_2Me$	1
33	NHCSOMe	0.5
34	NHCSOEt	1
35	$NHCSOCH(CH_3)_2$	8
36	$NHCSNH_2$	0.5
37	NHCSNHMe	2
38	NHCSNH(CH2)3Me	16
39	NHCOPh	16
40		16
41		16
42		32

[a]All MICs measured against *Staphylococcus aureus* ATCC 33591 (MRSA) strain.

of 33 oxazolidinone derivatives, (Table 1) keeping in vitro activity pMIC as a dependent variable. The conformations of the molecules used in the study were generated by systematic search method. The relative alignments of the molecules were then carried out using atom based fit (RMS fitting). The 3D-QSAR models were validated using a test set (Table 1) of 9 compounds, which were not included in the development of the models. Results of the Partial Least Square (PLS) analysis are shown in Table 2.

CoMFA and CoMSIA 3D-QSAR models

CoMFA models were developed using atom based alignment method. The cross-validated q^2 for atom-based alignment is 0.523, while the non-cross validated r^2 with six components is 0.975. The Predictive Residual Sum of Squares (PRESS) value is 1.376. The actual and predicted pMIC values of the training set are shown in Table 3. The CoMFA steric and electrostatic field contour plots obtained from atom-based alignment is shown in Figures 1 and 2. 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 indicate areas where electropositive groups enhance biological activity, while red regions represent areas where electronegative groups enhance activity. The 'R' groups of **5**, **7**, **8**, and **9** occupy the green contour similarly and hence exhibit similar activities. The slightly lower activity of **12** as compared to **5**, **7**, **8**, and **9** may be due to the fact that it only touches the green contour rather than occupying it. While **5** and **7** have electronegative groups attached to them, **8**, **9** and **14** have electropositive groups with no difference in activity: these molecules only touch the electrostatic contours while all of them fit well into the steric contours. The lower activity of **18** as compared to **8**, **9**, **14** and **16** is due to the fact that it occupies space near a yellow contour, which is sterically unfavourable. The higher activity of

Table 2. PLS statistics of CoMFA and CoMSIA 3D-QSAR models

PLS statistics	CoMFA	CoMSIA
q^{2}[a]	0.523	0.557
r^{2}[b]	0.975	0.940
N[c]	6	6
SEE[d]	0.096	0.147
F value[e]	167.436	68.240
Field Contribution[f]		
Steric	68.0%	10.2%
Electrostatic	32.0%	18.9%
Hydrophobic	—	27.9%
H-Bond Donor	—	27.9%
H-Bond Acceptor	—	15.1%
PRESS[g]	1.376	1.554

[a]Cross-validated correlation coefficient.
[b]Non cross-validated correlation coefficient.
[c]Optimum number of components obtained from cross-validated PLS analysis and same used in final non cross-validated analysis.
[d]Standard error of estimate.
[e]F-test value.
[f]Field contributions: Steric and electrostatic fields from CoMFA. Steric, Electrostatic, Hydrophobic, H-bond Donor and Acceptor fields from CoMSIA.
[g]Predictive residual sum of squares of the training set.

Table 3. Predicted activities and residuals of the training set molecules by the CoMFA and CoMSIA models

Compd	Actual pMIC	Predicted pMIC (CoMFA)	Residual	Predicted pMIC (CoMSIA)	Residual
1	5.358	5.484	−0.127	5.604	−0.246
4	5.057	4.928	0.129	5.124	−0.067
5	5.659	5.549	0.110	5.388	0.270
7	5.659	5.689	−0.030	5.672	−0.013
8	5.659	5.598	0.061	5.606	0.053
9	5.659	5.651	0.008	5.669	−0.010
12	5.358	5.361	−0.003	5.358	0.000
13	6.000	5.987	0.013	5.831	0.169
14	5.659	5.549	0.110	5.590	0.069
16	5.057	5.038	0.110	5.083	−0.026
17	4.756	4.869	−0.113	4.836	−0.080
18	4.455	4.495	−0.040	4.238	0.217
20	5.358	5.484	−0.126	5.358	0.000
21	5.358	5.376	−0.018	5.454	−0.096
22	5.358	5.429	−0.017	5.264	0.094
23	5.358	5.458	−0.100	5.406	−0.048
24	5.057	4.938	0.118	5.156	−0.099
25	6.000	5.999	0.001	6.140	−0.140
26	6.361	6.302	−0.001	6.312	0.049
27	6.361	6.348	−0.047	6.262	0.099
28	6.000	6.039	−0.039	6.043	−0.043
29	5.659	5.648	0.011	5.645	0.014
31	5.358	5.244	0.114	5.469	−0.111
33	6.361	6.136	0.165	5.973	0.388
35	5.057	5.046	0.011	5.015	0.042
36	6.361	6.284	0.017	6.349	0.012
37	5.659	5.833	−0.174	5.665	−0.006
38	4.756	4.810	−0.054	4.739	0.017
39	4.756	4.712	0.044	4.816	−0.060
40	4.756	4.830	−0.074	4.648	0.108
41	4.756	4.612	0.143	4.705	0.051
42	6.000	5.959	0.041	5.992	0.008
42	4.455	4.553	−0.098	4.830	−0.375

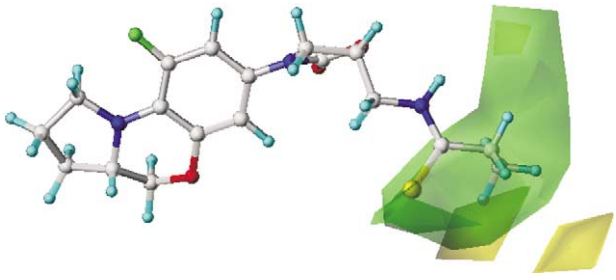


Figure 1. CoMFA S.D.*coeff. Steric contour plots; green contours indicate regions where bulky groups increase activity, whereas yellow contours indicate regions where bulky groups decrease activity.

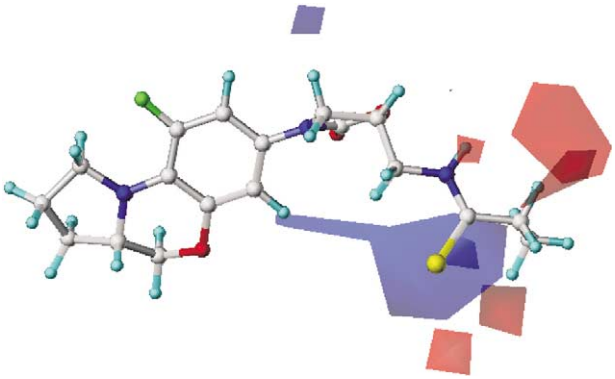


Figure 2. CoMFA S.D.*coeff. Electrostatic Contour plots; blue contours indicate regions where electropositive groups increase activity, whereas red contours indicate regions where electronegative groups may increase activity.

Table 4. Predicted activities and residuals of the test set by the CoMFA and CoMSIA models

Compd	Actual pMIC	Predicted pMIC (CoMFA)	Residual	Predicted pMIC (CoMSIA)	Residual
2	5.358	5.789	−0.431	5.596	−0.238
3	5.358	5.543	−0.185	5.609	−0.251
6	5.358	5.678	−0.320	5.719	−0.361
10	5.659	5.565	0.094	5.392	0.267
11	5.057	5.761	−0.704	5.795	−0.738
15	5.358	5.031	0.327	5.729	−0.371
19	6.000	5.539	0.461	5.413	0.587
30	5.659	5.576	0.083	5.418	0.241
32	6.000	5.528	0.472	6.385	−0.385

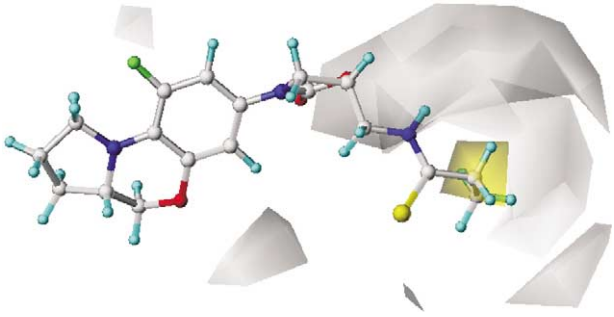


Figure 3. CoMSIA S.D.*coeff. Hydrophobic contour plots; yellow contours indicate regions where hydrophobic groups increase activity, whereas grey contours indicate regions where hydrophobic groups decrease activity.

33, **34** and **37** may be due to the fact that the –C=S group is near to the blue contour while the corresponding analogues **35** and **38** are less active as they occupy neither steric nor electrostatic contours. Although **40**, **41** and **42** occupy green contours, they are less active than other compounds because they occupy a yellow contour, which is sterically unfavourable.

CoMSIA is a relatively new alternative molecular field analysis method to CoMFA. It is touted to be less affected by changes in molecular alignment and provides more smooth and interpretable contour maps as a result of employing Gaussian type distance dependence with the molecular similarity indices it uses.[8] Furthermore, in addition to the steric and electrostatic fields of CoMFA, CoMSIA defines explicit hydrophobic and hydrogen bond donor and acceptor descriptor fields, which are not available with standard CoMFA. In most instances CoMSIA performs similar to CoMFA with regard to predictive ability, sometimes slightly better and other times slightly worse than CoMFA. The q^2 for atom-based alignment is 0.557, while the non-cross validated r^2 with six components is 0.940. The Predictive Residual Sum of Squares (PRESS) value is 1.554. The actual and predicted pMIC value of the training set is shown in Table 4. The CoMSIA steric and electrostatic contours are similarly placed as those of CoMFA. The additional hydrophobic, hydrogen bond donor and acceptor

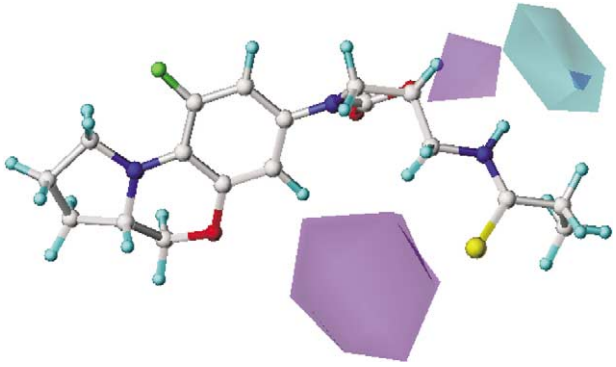


Figure 4. CoMSIA S.D.*coeff H-bond donor plots: cyan contours indicate regions where H-bond donor group increases activity, whereas purple contours indicate regions where H-bond donor group decreases activity.

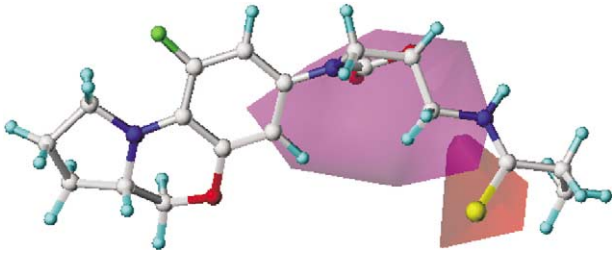


Figure 5. CoMSIA S.D.*coeff H-bond acceptor plots: magenta contour indicates regions where H-bond acceptor group increases activity, whereas red contour indicates regions where H-bond acceptor group decreases activity.

contours are displayed in Figures 3, 4 and 5, respectively. The hydrophobic fields (yellow, hydrophobic group favoured; grey, hydrophobic disfavoured), H-bond donor (cyan, favoured; purple, disfavoured) and the H-bond acceptor (magenta, favoured; red, disfavoured) fields indicate areas around the molecules where changes either increase or decrease activity.

Based on the PLS statistics of both CoMFA and CoMSIA (Table 3) 3D-QSAR models, it is clear that CoMFA is better than CoMSIA; this is further validated by the residual values of the test set (Table 4). Figure 6 represents graph of the actual versus predicted pMIC values of the training and test set molecules for CoMFA and CoMSIA 3D-QSAR models using atom-based alignment.

Conclusion

We have developed predictive CoMFA and CoMSIA 3D-QSAR models for tricyclic oxazolidinones as antibacterial agents. The CoMFA 3D-QSAR model performs better than the CoMSIA model. The contour diagrams obtained for the various CoMFA and CoMSIA 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.

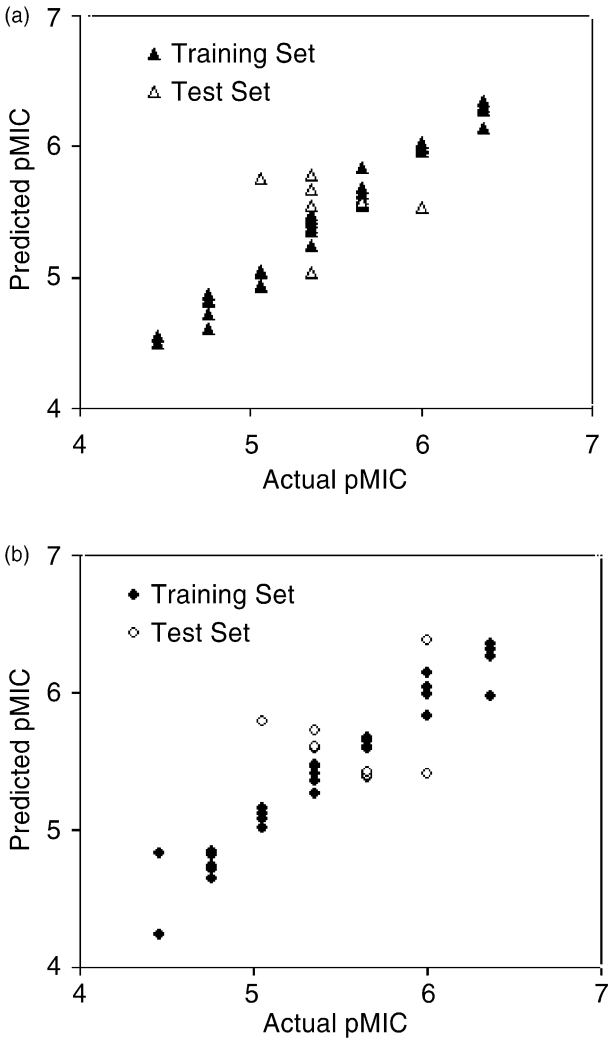


Figure 6. Graphs of actual vs predicted pMIC of training and test set molecules for (a) CoMFA and (b) for CoMSIA 3D QSAR models.

Methods

Data sets and biological activity

Recently Selvakumar et al.[10] from our laboratory have reported tricyclic oxazolidinone derivatives as potent and specific antibacterial agents. The in vitro Minimum Inhibitory Concentration (MIC) data against *Staphylococcus aureus* ATCC 33591 (MRSA) was used for the study. The MIC values (μg/mL) were converted to pMIC according to the formula.

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

pMIC values were used as dependent variables in the CoMFA and CoMSIA analysis. The training set of 33 molecules with structures and their activities are shown in Table 1. They are selected based on (a) structural diversity available on the oxazolidinone ring and (b) the range of biological activities. Molecules of the test set were selected similarly. The mean biological activity (pMIC) of the chosen training set and test set molecules were 5.13 and 5.44, respectively. The predictive power of the models was assessed using a test set of nine

compounds whose structures and activities are shown in Table 1.

Molecular modelling and alignment

Three-dimensional structure building and molecular modelling studies were performed using SYBYL 6.8[11] installed on a Silicon Graphics workstation with IRIX 6.5 operating system. Energy minimisations were performed using Tripos force field and Gasteiger Hückel charge with distance dependent dielectric and conjugate gradient method with a convergence criterion of 0.01 *k*cal/mol(∗A).

The most important requirement for 3D-QSAR techniques (CoMFA and CoMSIA) is that the 3D structures of the molecules to be analysed be aligned according to a suitable conformational template, which is assumed to adopt a 'bioactive conformation'. In the present study, the structural information on these inhibitor-protein complexes is not available; therefore, the conformation of the molecules was obtained from systematic conformational search procedures. Atom fit molecular alignment methods were employed in the present study. This method involves atom based fitting (RMS fitting) of the ligands. The compounds were fitted to the template molecule **27**, one of the most active molecules (Fig. 7) and all the aligned molecules of the training set are shown in Figure 8.

CoMFA and CoMSIA 3D-QSAR models

In deriving the CoMFA and CoMSIA descriptor fields a 3D cubic lattice with grid spacing of 2.0 Å in x, y and z directions was created to encompass the aligned molecules. CoMFA descriptors were calculated using an sp^3 carbon probe atom carrying +1 charge to generate steric (Lennard-Jones 6–12 potential) filed energies and

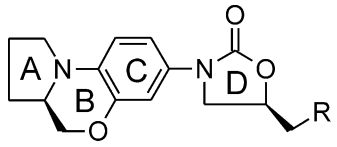


Figure 7. Atoms of rings A, B, C and D are used for molecular alignment.

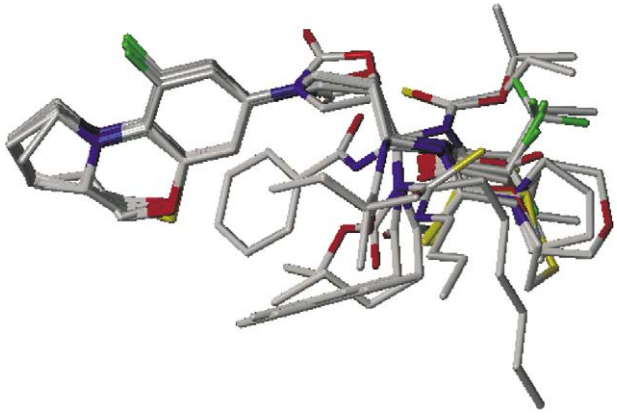


Figure 8. All the molecules used in the QSAR modelling aligned by the atom-based method.

electrostatic (Coulomb potential) fields with a distance dependent dielectric at each lattice points. Values of steric and electrostatic energy were truncated at 30 kcal/mol. The CoMFA steric and electrostatic fields generated were scaled by the CoMFA-STD method in SYBYL.

CoMSIA similarity indices descriptors were derived according to Klebe et al.[8] in the same manner as for the CoMFA calculations. CoMSIA similarity indices (A_F) for a molecule j with atoms i at a grid point q are calculated by eq 1 as follows:

$$A^{q}_{F,K}(j) = -\Sigma\omega_{probe,k}\omega_{ik}e^{-\alpha,r}_{iq2} \quad (1)$$

Five physicochemical properties k (steric, electrostatic, hydrophobic, hydrogen bond donor, and hydrogen bond acceptor) were evaluated using the probe atom. A Gaussian type dielectric dependence was used between the grid point q and each atom i of the molecule. The default value of 0.3 was used as the attenuation factor (α). In CoMSIA, the steric indices are related to the third power of the atomic radii, the electrostatic descriptors are derived from partial atomic charges, the hydrophobic fields are derived from atom based parameters[12] and the hydrogen bond donor and acceptor indices are obtained by a rule based method derived from experimental values.[13]

The CoMFA and CoMSIA descriptors were used as independent variables and pMIC values were used as dependent variables in partial least squares analysis to derive 3D-QSAR models using the standard implementation in the SYBYL package. The predictive value of the models was evaluated first by leave-one-out (LOO) cross-validation. The cross-validated q^2 was calculated using equation 2.

$$q^2 = 1 - \frac{\Sigma(Y_{predicted} - Y_{observed})^2}{\Sigma(Y_{observed} - Y_{mean})^2} \quad (2)$$

Where, $Y_{predicted}$, $Y_{observed}$ and Y_{mean} are predicted, actual and mean values of the target property (pMIC) respectively. $\Sigma(Y_{predicted} - Y_{observed})^2$ are the predictive residual sum of squares (PRESS). The optimum number of components was chosen which gave less standard error of prediction and more q^2. In addition to the q^2 and number of components the conventional correlation coefficient r^2 and its standard error were also computed.

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