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Comparative Molecular Field Analysis of Dioxins and Dioxin-like Compounds

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Abstract

Because of their widespread occurrence and substantial biological activity, halogenated aromatic hydrocarbons are one of the important classes of contaminants in the environment. We have performed comparative molecular field analysis (CoMFA) on structurally diverse ligands of Ah (dioxin) receptor to explore the physico-chemical requirements for binding. All CoMFA models have given q^2 value of more than 0.5 and r^2 value of more than 0.83. The predictive ability of the models was validated by an external test set, which gave satisfactory predictive r^2 values. Best predictions were obtained with CoMFA model of combined modified training set ($q^2 = 0.631$, $r^2 = 0.900$), giving predictive residual value = 0.002 log unit for the test compound. We have suggested a model comprises of four structurally different compounds, which offers a good predictability for various ligands. Our QSAR model is consistent with all previously established QSAR models with less structurally diverse ligands. The implications of the CoMFA/QSAR model presented herein are explored with respect to quantitative hazard identification of potential toxicants.

Keywords: QSAR, CoMFA, molecular modeling

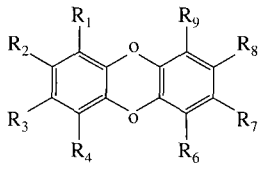
Halogenated aromatic hydrocarbons, typified by the polychlorinated dibenzo-p-dioxins (PCDDs), dibenzo-furans (PCDFs), and biphenyls (PCBs), have been identified in almost every compartment of the global ecosystem. Because of their lipophilic nature, these compounds have also been detected in fish, wildlife, and various human body fluids and tissues. There is considerable public and regulatory concern over the potential adverse human health effects and environmental damage associated with exposure to these chemicals. The prototypical halogenated aromatic hydrocarbon, 2, 3, 7, 8 tetrachlorodibenzo-p-dioxin (TCDD or dioxin) and related compounds elicit similar biochemical and toxic responses in humans, laboratory animals, and mammalian cells in culture. There is a substantial body of evidence that many, if not all, of their important biological responses are mediated by a common (aryl hydrocarbon or dioxin) receptor mechanism of action. The activity of the different halogenated aromatics is structure dependent, and a number of studies have delineated the various structure-activity relationships (SARS)[1-4].

A key step in predicting the toxic effects of polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-p-dioxins (PCDDs) is the estimation of their binding to a common intracellular cytosolic protein called the aryl hydrocarbon receptor (AhR)[1,5]. This receptor controls the induction of hepatic cytochrome P4501A1 and associated aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin-O-diethylase (EROD) activities[4,6,7]. Moreover, the relative affinity of individual PCBs, PCDFs, and PCDDs for the receptor has been correlated with many toxic responses such as thymic atrophy, body weight loss, immunotoxicity, and acute lethality[4-10].

Several structure-activity relationship (SAR) studies have been reported for dibenzo-p-dioxins, dibenzofurans, and biphenyls by using comparative molecular field analysis (CoMFA), a three-dimensional quantitative structure-activity relationship (3D QSAR) paradigm. While the results from that study were positive, it was believed that further validation of the model was necessary. In doing so, the original training set was expanded to include a wider variety of chemical classes. (Naphthalenes and indol 3, 2-bicarbazoles)[11].

In this study we have performed CoMFA to build the 3D QSAR models. We included the four classes of compounds to build an extensive training set. It was of interest to see if all four classes of compounds could be combined into a single predictive model since various members of all four classes have been shown to produce qualitatively similar toxicities. Such a model might have value in extending efforts to develop toxic equivalency factors (TEFs) for these

Table 1. Structure and Biological activities of Dibenzo-*p*-dioxins



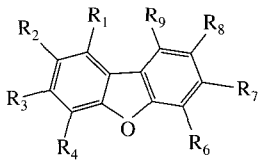
ID	Chemical name	$_pEC_{50}$
1	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	8.00
2	1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	7.10
3	2,3,6,7-Tetrachlorodibenzo-*p*-dioxin	6.80
4	2,3,6-Trichlorodibenzo-*p*-dioxin	6.66
5	1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	6.55
6	1,3,7,8-Tetrachlorodibenzo-*p*-dioxin	6.10
7	1,2,4,7,8-Pentachlorodibenzo-*p*-dioxin	5.96
8	1,2,3,4-Tetrachlorodibenzo-*p*-dioxin	5.89
9	2,3,7-Trichlorodibenzo-*p*-dioxin	7.15
10	2,8-Dichlorodibenzo-*p*-dioxin	5.50
11	1,2,3,4,7-Pentachlorodibenzo-*p*-dioxin	5.19
12	1,2,4-Trichlorodibenzo-*p*-dioxin	4.89
13	1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin	5.00
14	1-Chlorodibenzo-*p*-dioxin	4.00
15	2,3,7,8-TetraBromodibenzo-*p*-dioxin	8.82
16	2,3-Di Bromo-7,8-Dichlorodibenzo-*p*-dioxin	8.83
17	2,8-Di Bromo-3,7-Dichlorodibenzo-*p*-dioxin	9.35
18	2-Bromo-3,7,8-Trichlorodibenzo-*p*-dioxin	7.94
19	1,3,7,8,9-PentaBromodibenzo-*p*-dioxin	7.03
20	1,3,7,8-TetraBromodibenzo-*p*-dioxin	8.70
21	1,2,4,7,8-PentaBromodibenzo-*p*-dioxin	7.77
22	1,2,3,7,8-PentaBromodibenzo-*p*-dioxin	8.18
23	2,3,7-TriBromodibenzo-*p*-dioxin	8.93
24	2,7-Dibromodibenzo-*p*-dioxin	7.81
25	2-Bromodibenzo-*p*-dioxin	6.53

compounds for possible use in hazard and risk assessment.

Training Set: Dibenzo-p-dioxins and Dibenzo-furans

The results of all CoMFA/QSAR analyses are presented in Table 5. In 1992, Waller and McKinney[13] published the results of a CoMFA/QSAR study, which accurately correlated variations in the three dimensional structural features of a series of polyhalogenated dibenzo-p-dioxins and dibenzofurans with variations in their relative binding affinities for the Ah receptor. These results were reconfirmed in the present study. The training set (Tables 1 and 2) yielded a model with a $q^2 = 0.742$ using 10 PCs with an SEP of 0.843. The conventional statistical results for the ten-component model were $r^2 = 0.883$ and SEE = 0.568. These results indicate that this ten-component model is internally consistent (or internally predictive), and it was selected on this basis for additional validation. The external predictive ability

Table 2. Structure and Biological activities of Dibenzofurans



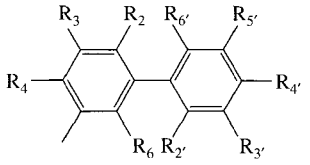
ID	Chemical name	$_pEC_{50}$
26	2-Chlorodibenzofuran	3.55
27	3-Chlorodibenzofuran	4.38
28	4-Chlorodibenzofuran	3.00
29	2,3-Dichlorodibenzofuran	5.33
30	2,6-Dichlorodibenzofuran	3.61
31	2,8-DiChlorodibenzofuran	3.59
32	1,3,6-Trichlorodibenzofuran	5.36
33	1,3,8-Trichlorodibenzofuran	4.07
34	2,3,4-Trichlorodibenzofuran	4.72
35	2,3,8-Trichlorodibenzofuran	6.00
36	2,6,7-Trichlorodibenzofuran	6.35
37	2,3,4,6-Tetrachlorodibenzofuran	6.46
38	2,3,4,8-Tetrachlorodibenzofuran	6.70
39	1,3,6,8-Tetrachlorodibenzofuran	6.66
40	2,3,7,8-Tetrachlorodibenzofuran	7.39
41	1,2,4,8-Tetrachlorodibenzofuran	5.00
42	1,2,4,6,7-Pentachlorodibenzofuran	7.17
43	1,2,4,7,9-Pentachlorodibenzofuran	4.70
44	1,2,3,4,8-Pentachlorodibenzofuran	6.92
45	1,2,3,7,8-Pentachlorodibenzofuran	7.13
46	1,2,4,7,8-Pentachlorodibenzofuran	5.89
47	2,3,4,7,8-Pentachlorodibenzofuran	7.82
48	1,2,3,4,7,8-Hexachlorodibenzofuran	6.64
49	1,2,3,6,7,8- Hexachlorodibenzofuran	6.57
50	1,2,4,6,7,8- Hexachlorodibenzofuran	5.08
51	2,3,4,6,7,8- Hexachlorodibenzofuran	7.33
52	2,3,6,8-Tetrachlorodibenzofuran	6.66
53	1,2,3,6-Tetrachlorodibenzofuran	6.46
54	1,2,3,7-Tetrachlorodibenzofuran	6.96
55	1,3,4,7,8-Pentachlorodibenzofuran	6.70
56	2,3,4,7,9-Pentachlorodibenzofuran	6.70
57	1,2,3,7,9-Pentachlorodibenzofuran	6.40
58	Dibenzofuran	3.00
59	2,3,4,7-Tetrachlorodibenzofuran	7.60
60	1,2,4,6,8-Pentachlorodibenzofuran	5.51

of this model is examined in detail below.

Test Set Predictions: Biphenyls

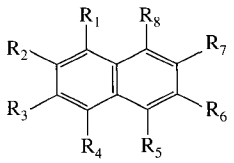
As originally published[13], the biphenyl congeners (Table 3) were an integral part of the CoMFA/QSAR model. For the purposes of exploring the predictive ability of a limited training set, these molecules were removed from the training set to comprise the first external test set. The model proved to be capable of making such predictions, yielding an r^2_{pred} of 0.779. The inclusion of the molecules comprising the biphenyl test set into the training set, thereby constructing a model based on dibenzo-p-dioxin, dibenzofuran, and

Table 3. Structure and Biological activities of Biphenyls



ID	Chemical name	pEC_{50}
61	3, 3′, 4, 4′-Tetrachlorobiphenyl	6.15
62	2, 3, 4, 4′-Tetrachlorobiphenyl	4.55
63	3, 3′, 4, 4′, 5-Pentachlorobiphenyl	6.89
64	2′, 3, 4, 4′, 5-Pentachlorobiphenyl	4.85
65	2, 3, 3′, 4, 4′-Pentachlorobiphenyl	5.37
66	2, 3′, 4, 4′, 5-Pentachlorobiphenyl	5.04
67	2, 3, 4, 4′, 5-Pentachlorobiphenyl	5.39
68	2, 3, 3′, 4, 4′, 5-Hexachlorobiphenyl	5.15
69	2, 3′, 4, 4′, 5, 5′-Hexachlorobiphenyl	4.80
70	2, 3, 3′, 4, 4′, 5-Hexachlorobiphenyl	5.33
71	2, 2′, 4, 4′-Tetrachlorobiphenyl	3.89
72	2, 2′, 4, 4′, 5, 5′-Hexachlorobiphenyl	4.10
73	2, 3, 4, 5-Tetrachlorobiphenyl	3.85
74	2, 3′, 4, 4′, 5′ 6-Hexachlorobiphenyl	4.00
75	4′-Hydroxy-2, 3, 4, 5-Tetrachlorobiphenyl	4.05
76	4′-Methyl-2, 3, 4, 5-Tetrachlorobiphenyl	4.51
77	4′-Fluoro-2, 3, 4, 5-Tetrachlorobiphenyl	4.60
78	4′-Methoxy-2, 3, 4, 5-Tetrachlorobiphenyl	4.80
79	4′-Acetyl-2, 3, 4, 5-Tetrachlorobiphenyl	5.17
80	4′-Cyano-2, 3, 4, 5-Tetrachlorobiphenyl	5.27
81	4′-Ethyl-2, 3, 4, 5-Tetrachlorobiphenyl	5.46
82	4′-Bromo-2, 3, 4, 5-Tetrachlorobiphenyl	5.60
83	4′-Iodo-2, 3, 4, 5-Tetrachlorobiphenyl	5.82
84	4′-Isopropyl-2, 3, 4, 5-Tetrachlorobiphenyl	5.89
85	4′-Trifluromethyl-2, 3, 4, 5-Tetrachlorobiphenyl	6.43
86	3′-Nitro-2, 3, 4, 5-Tetrachlorobiphenyl	4.85
87	4′-*N*-Acetylamino-2, 3, 4, 5-Tetrachlorobiphenyl	5.09
88	4′-Phenyl-2, 3, 4, 5-Tetrachlorobiphenyl	5.18
89	4′-*t*-Butyl-2, 3, 4, 5-Tetrachlorobiphenyl	5.17
90	4′-*n*-Butyl-2, 3, 4, 5-Tetrachlorobiphenyl	5.13

Table 4. Structure and Biological activities of Naphthalene



ID	Chemical name	pEC_{50}
91	2, 3-Dibromonaphthalene	5.50
92	2, 3, 6, 7-Tetrabromonaphthalene	7.50
93	1, 2, 4, 6, 7-Pentabromonaphthalene	7.30
94	1, 2, 3, 4, 6, 7-Hexabromonapthalene	7.44
95	1, 2, 3, 5, 6, 7-Hexabromonapthalene	7.82

biphenyl molecules, showed insignificant changes with respect to the internal consistency or statistical

Table 5. Summary of CoMFA results

	Dioxins and furans	Dioxins furans, and biphenyls	Dioxins furans, and napthalenes	All	Combined model
q^2	0.742	0.624	0.710	0.574	0.631
SEP	0.843	0.903	0.855	0.983	0.883
r^2	0.883	0.838	0.879	0.831	0.900
SEE	0.568	0.608	0.563	0.620	0.475
F	39.359	40.825	39.241	41.161	67.447
r^2_{bs}	0.894	0.889	0.916	0.895	0.931
SD	0.019	0.030	0.032	0.023	0.015
			Relative contribution		
STE	71	64	65	59	58
ELE	29	36	35	41	42

q^2-LOO cross validated correlation coefficient, r^2-non cross validated correlation coefficient, SEE-standard error of estimate, F-F-test value, SEP-standard error of prediction, q^2bs-mean r^2 of boot strapping analysis (10 runs), SD-standard deviation, STE-steric field, ELE-electrical field.

robustness in the overall model as compared to the original training set model using only dibenzo-p-dioxin and dibenzofuran molecules ($q^2 = 0.624$ using 10 PCs; SEP = 0.903; $r^2 = 0.838$; SEE = 0.608).

Test Set Predictions: Naphthalenes

The predictions for the external test set of naphthalene derivatives (Table 4) the model proved to be capable of making such predictions, yielding an r^2_{pred} of 0.926. The inclusion of the naphthalene test set into the dibenzo-p-dioxin and dibenzofuran training set resulted in a statistically unaltered model ($q^2 = 0.710$ using 8 PCs, SEP = 0.855 $r^2 = 0.879$ SEE = 0.563)

Combined Model

The analysis including all original training and external test set molecules including dibenzo-p-dioxins, dibenzofurans, biphenyls, naphthalenes in a combined CoMFA/QSAR yield a satisfied result (q^2= 0.574 SEP = 0.983; $r^2 = 0.831$; SEE = 0.620). The external predictive ability of this combined model was assessed using 3-methylcholanthrene (MC) as a single external test molecule ($pEC_{50} = 7.77$). The predictive value obtain from the combined model was 7.73. An analysis of the cross-validated residuals from the combined CoMFA/QSAR model revealed nine compounds (4, 9, 11, 30, 31, 33, 34, 42, 50) as the largest outliers (the molecules most poorly predicted during the LOO cross validation routine). That being the case, it was decided to remove these compounds from the combined training set. Removing these compounds from the training set resulted in a much improved ($q^2 = 0.631$ using 9 PCs; SEP = 0.883; r^2 = 0.900; SEE = 0. 475) yet a somewhat less structurally

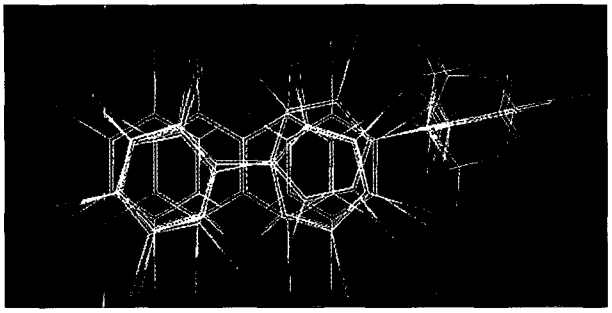


Fig. 1. Alignment of all molecules.

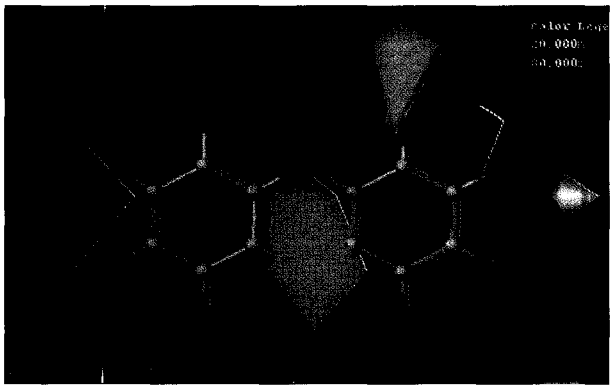


Fig. 2. CoMFA steric contour plots; green contours indicate regions where bulky groups increase activity, whereas yellow contours indicate regions where bulky groups decrease activity.

diverse model. This training set is termed as modified combined training set. The external predictive ability for this modified model using the external test set molecule 3 MC was comparable to the larger model as indicated by the predicted pEC_{50} value for MC of 7.75 (predictive residual value = 0.02 log unit). Figure 4 shows plots of observed vs. predicted activities of modified combined model for CoMFA.

CoMFA Contour Plots

It has been previously demonstrated that for a diverse training set of molecules in their "active conformations"(i.e., derived from crystallized ligand receptor complexes) superimposed in a reasonable manner (i.e., superposition of the atomic coordinates of the receptors), a CoMFA model which is internally consistent, statistically robust, and externally predictive may be generated[23,24]. It was subsequently demonstrated that the graphical results of this model (i.e., CoMFA steric and electrostatic contour plots) were consistent with the known characteristics of the molecular binding domain of the receptor[25]. In the

Fig. 3. CoMFA electrostatic contour plots; blue contour indicate regions where positive groups increase activity, whereas red contours indicate regions where negative charge increases activity.

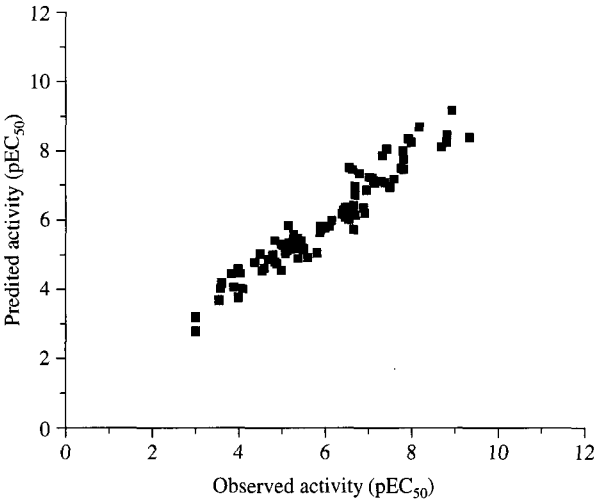


Fig. 4. Plot of predicted vs observed values of CoMFA std model.

present study, the diverse structural nature of the combined training set is apparent. And although the "active conformations" of the molecules were not determined from crystal coordinates, the relative conformational rigidity of all the training set molecules assures reasonable molecular geometries. While the alignment rule was not derived from crystallized complexes, every effort has been made to provide for maximal superposition with respect to molecular similarity.

Fig. 2 depicts steric contour maps of CoMFA of modified combined model. Sterically favored green regions are found near 2, 3, 7, 8 positions of TCDD

and DBF; 3, 4, 3′, 4′ of biphenyl and 2, 3, 6, 7 of Naphthalene. Where as sterically disfavored yellow regions are found in the medial positions. So simply we can tell any substitution in lateral position evoke the greater binding energy relative to medial substitutions.

Fig. 3 shows CoMFA electrostatic contour maps. Negative charge favored red regions were found near 2, 3 position for TCDD, DBF and naphthalene; 3, 4 position for biphenyl. This can be explained by the fact that compound 14 having halogen R_1 substituent in the electrostatically disfavored region is less toxic than the compound 17 which has R_2 and R_3 halogen substituent in the favored region. Positive charge favored or negative charge unfavored blue region is found on the medial position of the ring.

Conclusion

CoMFA method was applied in our study to build 3D QSAR models that can accurately predict the binding affinity. The 3D QSAR studies led to the identification of the regions important for steric and electronic interactions and the derived models well explain the observed variance in the activity. We have successfully generated predictive model by CoMFA with structurally diverse ligands. But CoMFA alone is not enough for predicting all the interactions. To consider other interaction such as hydrophobic, hydrogen bonding we can apply CoMSIA along with CoMFA. Thus we may get a more efficient model to predict the structure activity relationship.

Methods

Molecular Modeling

All Molecular modeling calculations were performed using SYBYL program[12], package version 6.9 on Linux operating system. Energy minimizations were performed using Tripos force field[12] and Gasteiger Huckel charge with distance dependent dielectric and conjugate gradient method with convergence criterion of 0.01 kcal/mol.

Alignment Rule: General Comments

The relative success or failure of any 3D QSAR model is dependent on the procedure adopted for the alignment or superposition of molecules in the data set. We have adopted a set of rules, which provide a consistent and repeatable process for aligning these molecules of diverse structural type. The working hypothesis is that there may be a stacking interaction[3] operating in the molecular recognition event in which the most highly halogenated ring would be considered to be the preferred stacking plane for asymmetrically substituted molecules. The IUPAC numbering scheme adopted in the original model[13] and described in more detail for each chemical class below simply presented a rational and repeatable alignment technique.

Alignment Rule: Dibenzo-p-dioxins and Dibenzofurans

The conformations and alignment rule for all dibenzo-p-dioxins and dibenzofurans[14] (Tables 1 and 2) were taken as used in the original paper[13] and were not altered. Specifically, using TCDD as a template, all dibenzo-p-dioxins and dibenzofurans were superimposed via atom-to-atom fit of their lateral carbons (i.e., carbons 2, 3, 7, and 8).

Alignment Rule: Biphenyls

All biphenyls[15] (Table 3) were initially aligned by atom-to-atom fit method to TCDD, considering the 3, 4, 3′, and 4′ carbons to be lateral.

Alignment Rule: Naphthalenes

In accordance with the alignment rules for the dibenzo-p-dioxins and dibenzofurans, the naphthalenes[16] (Table 4) were aligned using atom to atom fit of their lateral (positions 2, 3, 6, and 7) carbons to the lateral (2, 3, 7, and 8) carbons of TCDD.

Biological Data (Dependent Variable) Calculations

All previously determined biological activity data[14-17] were utilized as the negative of the log of the molar concentration of chemical necessary to displace 50% of radio labeled TCDD from the Ah receptor.

CoMFA 3D QSAR Mdels

The steric and electrostatic potential fields for CoMFA were calculated at each lattice intersection of a regularly spaced grid of 2.0 Å. The lattice was defined automatically, and is extended 4 Å units past Van-der-waals volume of all molecules in X, Y, and Z directions. The Van der Waals potential (Lennard-Jones 6-12) and columbic term, which represent steric and electrostatic fields respectively, were calculated using Tripos force field[18]. A distance dependent dielectric expression $\varepsilon = \varepsilon_0$. Rij with $\varepsilon_0 = 1.0$ was used. An sp3 carbon atom with van-der-waals radius of 1.52 Å and +1.0 charge was served as the probe atom to calculate steric and electrostatic fields. The steric and elec-

trostatic contributions were truncated to ± 30 kcal/mol and electrostatic contributions were ignored at lattice intersections with maximum steric interactions. The CoMFA steric and electrostatic fields generated were scaled by CoMFA standard option given in SYBYL.

PLS Calculations and Validations

Partial least-square (PLS)[19,20] methodology was used for all 3D QSAR analyses. Column filtering was set to 1.0 kcal/mol to speed up the analysis and reduce the noise. The CoMFA descriptors were used as independent variables, and pEC_{50} values were used as dependent variables in partial least-squares regression analyses 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[21,22]. The cross-validated coefficient, q^2, was calculated using eq 1

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

Where $Y_{predicted}$, $Y_{observed}$ and Y_{mean} are predicted, actual, and mean values of the target property (pEC_{50}), respectively. $\Sigma(Y_{predicted} - Y_{observed})^2$ is the predictive sum of squares (PRESS). To maintain the optimum number of PLS components and minimize the tendency to over fit the data, the number of components corresponding to the lowest PRESS value was used for deriving the final PLS regression models. In addition to the q^2 and number of components, the conventional correlation coefficient r^2 and its standard errors (SEE) were also computed. To further assess the robustness and statistical confidence of the derived models, bootstrapping analysis[20] (10 runs) were performed and the mean r^2 is given as q^2 bootstrap.

Predictive r Squared (r^2 pred)

To validate the derived CoMFA models, biological activities of external test set were predicted using models derived from the training set. The predictive ability of the models is expressed by predictive r^2 value, which is analogous to crossvalidated r^2 (q^2) and is calculated by using eq 2

$$r^2_{pred} = \frac{SD - PRESS}{SD} \quad (2)$$

Where SD is the sum of squared deviation between the biological activities of the test set molecule and the mean activity of the training set molecules and PRESS is the sum of squared deviations between the observed and the predicted activities of the test molecules.

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