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3D-QSAR studies on PU3 analogues by comparative molecular field analysis

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Abstract—A comparative molecular field analysis (CoMFA) of PU3 derivatives of Hsp90 (Heat shock protein 90) inhibitors has been performed to determine the factors contributing the corresponding activities. The energy minimized conformations were obtained by molecular mechanics using *SYBYL* package. The developed model, with r^2 value of 0.947, was verified by performing leave-one out (LOO) cross-validation, which showed q^2 value of 0.513. The calculated model not only elucidates the relationship between compound structures and biological activities but, more importantly, facilitates design of new Hsp90 inhibitors with calculated antiproliferative activity.

1. Introduction

Association of a molecular chaperone, such as Hsp90 is required for the stability and function of multiple mutated, chimeric and over-expression signaling proteins, for promoting growth and/or survival of cancer cell.[1–7] As a function of oncogenic transformation, Hsp90 has been shown to be over expressed in many human tumors.[8] Furthermore, Hsp90 is considered a novel molecular target for anticancer treatment because the cancer cells are especially prone to short-term pharmacological interference that will cause the degradation of mutated proteins, such as v-src, bcr-abl and p53.[9–11] Hsp90 possesses an ATP/ADP binding pocket at their N-terminus,[12] where geldanamycin (GM),[13] herbimycin(HA), 17-AAG[14] and radicicol (RD)[15] were found to bind to N-terminus pocket. Rosen et al. used the structural requirements for binding Hsp90 and designed a small inhibitor molecule PU3,[16] which displayed a marginal binding affinity of 15–20 μM and also exhibited very similar qualitative effects on cellular protein inhibition and morphologic changes induced by ansamycins. Although a large number of PU3 analogous have been synthesized and tested, however, relatively little is known about the nature of the forces involved in the PU3–Hsp90 interactions.[17] To explore this adequately, CoMFA method was used to study these interactions in a quantitative manner as CoMFA provides an easy interpretable coefficient contour maps identifying the compound moieties required for a specific biological property. In this study, we used CoMFA method, which calculates steric and electrostatic properties according to Lennard–Jones and Coulomb potentials respectively, to study the quantitative structure–activity relationships (QSAR) of PU3 inhibitors.[18] CoMFA model can characterize the relative change in magnitude of steric and electrostatic fields as a function of the sample chosen from the data set, and can account for the variance in measured biological activity, giving rise to the capacity to predict antiproliferative activities of new PU3 inhibitor analogues and as a result only inhibitors with high antiproliferative activities will be selected for syntheses through the analysis of contour map.

1.1. Data sets

Forty compounds, whose structures and associated biological activities are given in Figure 1 and Table 1,

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respectively, were taken from the published results,[16,17] and placed in the training set for the CoMFA study.

1.2. CoMFA study

The CoMFA studies for compounds selected from *SciFinder* database were performed on a Silicon Graphics workstation using *SYBYL*[19] molecular modeling software from Tripos Inc. (St. Louis, MO, USA). The structures of 40 compounds were subjected to full geometry optimization by molecular mechanics using standard Tripos force field (with 0.005 kcal/mol energy gradient convergence criterion); and their charge were calculated by the Gasteiger–Hückel method. The optimized structures were then superimposed onto a template—compound **39**, whose antiproliferative activity is the most potent, using *SYBYL*/database align fit, followed by using AOS-APS to get higher q^2. The aligned molecules were put into a 3D grid with a spacing of 2 Å, using four selected groups and atoms of the compounds designated in Figure 2 as the fitting centers. The steric and electrostatic fields were then calculated using a sp^3 C-atom with +1 charge with the default cutoff energy 30 kcal/mol. Partial Least Squares (PLS) was used to derive linear equations from the resulting field matrix. A stereo view of the superimposed complexes based on alignment is shown in Figure 3.

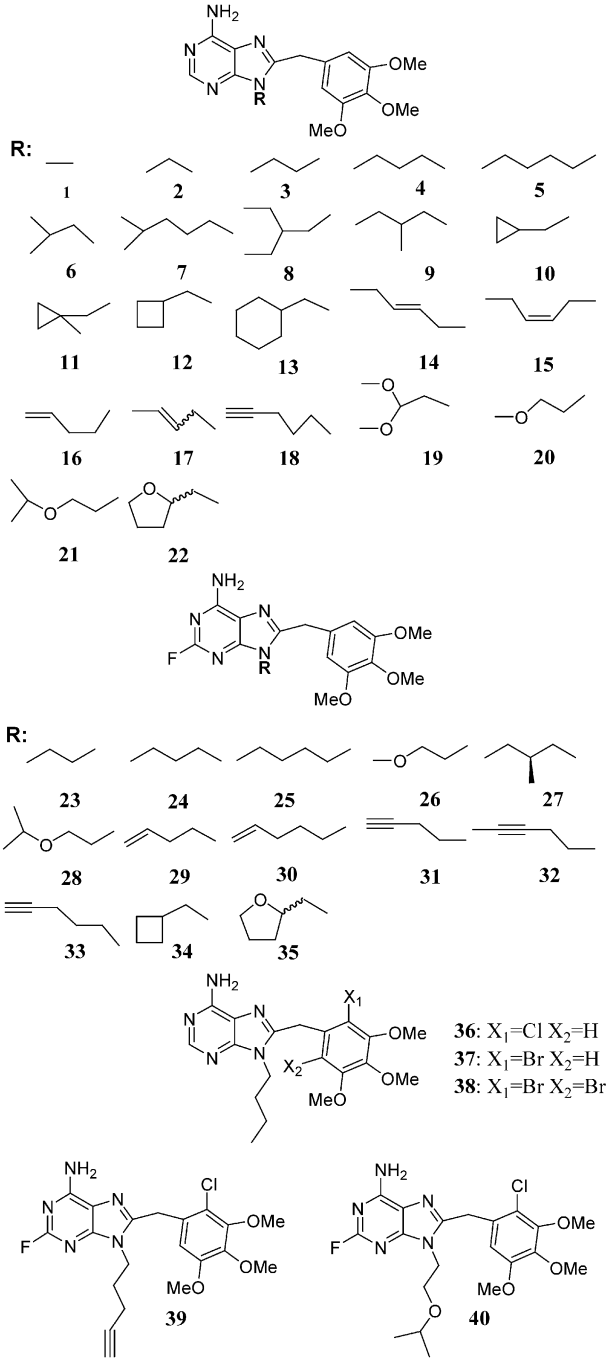

Figure 1. Compounds used in CoMFA training set.

2. Results and discussion

The CoMFA analyses were performed using a training set of 40 compounds aggregate to determine the factors required for the antiproliferative activities, which, measured by pIC_{50}, are a function of independent variables, namely steric and electrostatic fields, according to the PLS methodology (see Table 2). Table 2 shows the calculated CoMFA results. The leave-one-out (LOO) cross-validated PLS analysis of the best model gave rise to a q^2 value of 0.513, suggesting that the model is a useful tool for predicting antiproliferative activities.[20,21] The correlation coefficient between the calculated and experimental activities r^2 value of 0.947 with standard error 0.087 indicates that the fitness of analyzed results is 94.7% compared to experimental results. The respective relative contributions of steric and electrostatic fields were 79.1 and 20.9%, indicating that steric field is more predominant. The experimental and calculated activities of all compounds by the best model are given in Table 1, a plot of experimental versus calculated antiproliferative activities is illustrated in Figure 4.

Graphical representations of CoMFA results for Hsp90 inhibitors are shown in Figures 5 and 6, using compound **39** as reference structure. The steric contour map shows a green region at C-2 position of the benzene ring, indicating more bulky substituent is preferred at C-2 position to produce higher inhibition activity, which is consistent with the fact that molecules **36**, **37**, **38**, **39**, and **40** have higher inhibition activities ($IC_{50} < 30\mu M$) than the others. The observation of both yellow and green regions around the R group in the steric contour map (shown in Fig. 5) suggests that the substitution effect of the alkyl group is complex. R group can be separated to close and far regions; the former, shown by yellow region, indicate that the less bulky substituent is preferred and the later, mixed with yellow and green parts, suggest that more bulky substituent will lead to higher inhibition activity. This observation can be manifested by the fact that molecules **28** and **40** with 2-isopropoxyethyl chain or molecules **33** and **39** with pent-4-ynyl chain have higher inhibition activity ($IC_{50} < 20$ μM).[17] In addition, the red regions near the C-2 and C-6 positions of the phenyl ring in the electrostatic contour map suggested that substituting a group with an increase in the negative charge on a ring system

Table 1. Experimental and CoMFA calculated pIC_{50} values for Hsp90 inhibitors

No.	IC MCF (μm)	$logIC_{50}$ (obsd)	logIC (calcd)	Residue	No.	IC MCF (μm)	logIC (obsd)	$logIC_{50}$ (calcd)	Residue
1	70	4.15	4.06	0.09	**21**	39	4.41	4.485	−0.075
2	80	4.1	4.244	−0.144	**22**	92	4.04	4.153	−0.113
3	47	4.33	4.366	−0.036	**23**	25	4.6	4.619	−0.019
4	50	4.3	4.324	−0.024	**24**	24	4.62	4.693	−0.073
5	62	4.21	4.184	0.026	**25**	36	4.44	4.294	0.146
6	98	4.01	4.067	−0.057	**26**	64	4.19	4.159	0.031
7	69	4.16	4.191	−0.031	**27**	44	4.36	4.402	−0.042
8	160	3.8	3.881	−0.081	**28**	16	4.8	4.898	−0.098
9	62	4.21	4.147	0.063	**29**	33	4.48	4.351	0.129
10	75	4.12	4.129	−0.009	**30**	45	4.35	4.353	−0.003
11	111	3.95	3.987	−0.037	**31**	25	4.6	4.546	0.054
12	46	4.34	4.225	0.115	**32**	37	4.43	4.43	0
13	40	4.4	4.325	0.075	**33**	11	4.96	5.087	−0.127
14	47	4.33	4.34	−0.01	**34**	41	4.39	4.468	−0.078
15	64	4.19	4.167	0.023	**35**	23	4.64	4.547	0.093
16	41	4.39	4.284	0.106	**36**	19	4.72	4.726	−0.006
17	51	4.29	4.302	−0.012	**37**	25	4.6	4.721	−0.121
18	24	4.62	4.709	−0.089	**38**	30	4.52	4.466	0.054
19	72	4.14	4.15	−0.01	**39**	2	5.7	5.543	0.157
20	65	4.19	4.111	0.079	**40**	5.4	5.27	5.211	0.059

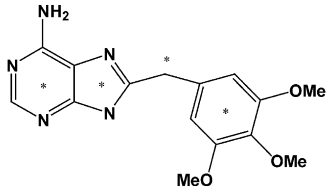

Figure 2. General structures of Hsp90 inhibitors. Stars indicate the group and atom selected as the fitting centers.

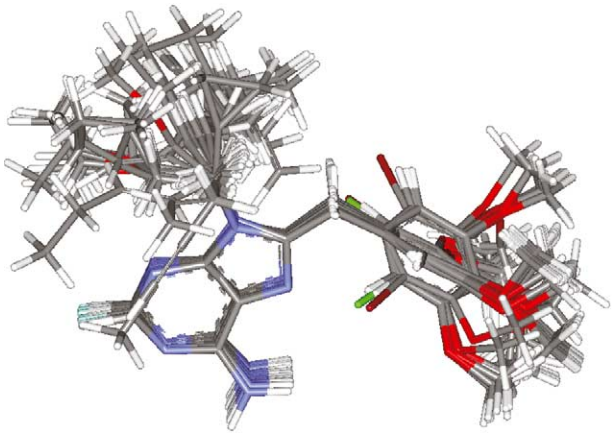

Figure 3. Stereoview of the superimposed complexes based on alignment (aggregate).

would yield a higher activity. It was found that molecules **36**, **37**, **38**, **39**, and **40** which have negative charge group like chlorine or bromine atom show higher inhibition activities than the others. Indeed the red region near the C-2 position of the adenine suggests that substituting a group with more negative charge would have higher activity. This fact is consistent with molecules **39** and **40** with fluorine atom have higher inhibition activities than the others.[17] The red regions around the R group in the electrostatic contour map suggests that the negative charge group will increase activity, which is consistent with the fact that molecules **28** and **40** with

Table 2. Statistical Indexes of CoMFA

	Cross-validate		Conventional		
	q^2	No. of components	r^2	s	F
CoMFA	0.513	6	0.947	0.087	98.546

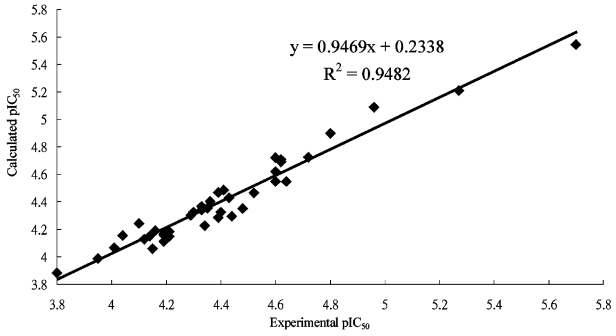

Figure 4. Correlation between the calculated pIC_{50} and the experimental pIC_{50}.

2-isopropoxyethyl group give rise to higher inhibition activities than the others. The predicted IC_{50} was 5 μM when the C-2 position chlorine of compound **39** was replaced with fluorine in light of the contour map. Similarly, predictions showed 3, 2 and 2 μM while fluorine, chlorine and bromine were in place of the C-6 position hydrogen of compound **39**, respectively.

3. Conclusions

In this work, we successfully aligned structures of Hsp90 inhibitors, minimized by molecular mechanics using standard Tripos force field, for the CoMFA study. A satisfactory model was obtained with LOO cross-validation q^2 and conventional r^2 values of 0.513 and 0.947, respectively. The effects of the electrostatic and

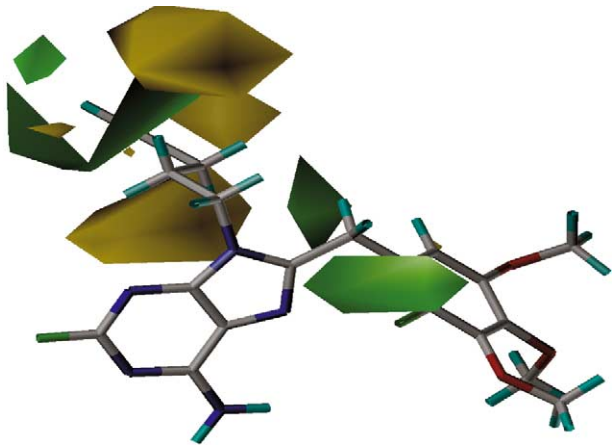

Figure 5. The CoMFA contour map for Hsp90 inhibitors: steric map in which green and yellow polyhedra indicate regions where more steric bulk or less steric bulk, respectively, will enhance the affinity.

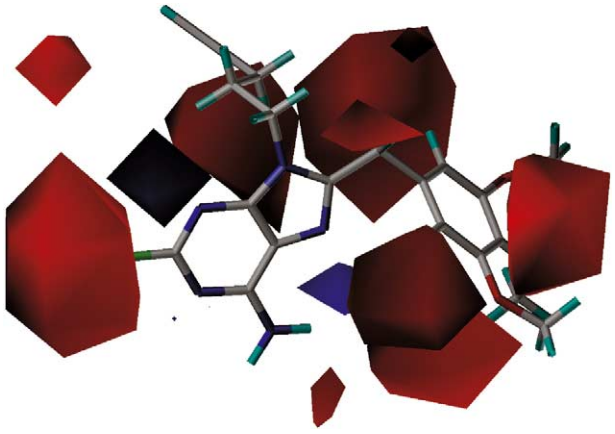

Figure 6. The CoMFA contour map for Hsp90 inhibitors: Electrostatic map indicating red and blue polyhedra where more high electron density (negative charge) or low electron density (partial positive charge), respectively, will enhance the affinity.

steric fields around the aligned molecules on their activities are clarified by analyzing the CoMFA contour maps. The information obtained in this study provides the tools for predicting the affinity values of structurally similar analogues, and for guiding further structure modifications and synthesizing potent Hsp90 inhibitors.

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