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Comparative molecular field analysis for inhibitory activities of lamellrins against tyrosine kinase

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Abstract. The common structural requirements for inhibitory activity of lamellarins against tyrosine kinase (TK) were determined using comparative molecular field analysis (CoMFA) technique. Twenty five lamellarins were selected to serve as the training set, whereas another group of 7 compounds were used as the test set. The best CoMFA yielded satisfactory predictive ability with r^2_{cv} values in the range of 0.660. Additionally, the contour maps obtained from the CoMFA suggested structural requirements of various substituents such as methoxy groups at C7 and C14, and oxygen atom at C8, C9, and C21 around the lamellarin skeleton for their inhibitory activity against tyrosine kinase.

1. Introduction

In the battle against cancer, the epidermal growth factor receptor EGFR has investigated as cancer therapy protein targeted such as TK domain. In past decade, there are numerous reports to find the novel active compounds against EGFR-TK. In anticancer drug development, anticancer agents are derived from natural sources, including plants, marine organisms and micro-organisms. Especially, lamellarins are one of promising anti-cancer molecules which have been isolated from marine organisms. Their inhibitory activities against several kinds of cancer relevant protein kinases have been highlighted [1-3]. Lamellarins are polyaromatic pyrrole alkaloids that have been isolated from different sources, such as ascidians, molluscs, and sponges. A family of lamellarins consists of three structural groups, including saturated D-ring fused, unsaturated D-ring fused, and unfused central pyrrole ring group [4-5]. In previous study, the fifty percent inhibitory concentrations (IC_{50}) of lamellarins against TK were measured by using fluorescence. The set of some lamellarin were screened and were selected as potent compounds for investigate more molecular basis by using 3D-QSAR. These methods were employed for understanding the mechanism of the interactions between ligands and an unknown receptor. In order to use these methods, the physicochemical properties of lamellarin molecules were represented in the form of molecular fields, which could then be effectively correlated with their inhibitory activities using partial least squares (PLS) regression analysis. Especially, CoMFA suited to describe ligand-receptor interactions, because it considers the properties of the ligands in their bioactive conformations. CoMFA calculates steric and electrostatic properties according to the Lennard-Jones and Coulomb potentials. This method was explored the molecular basis for selective cytotoxicity of lamellarins against human hormone dependent T47D and hormone-independent MDA-MB 231 breast cancer cells [6].

From Choowongkomon group, the fifty percent inhibitory concentrations (IC_{50}) of 32 lamellarins which have difference substituent groups as in Table 1 were measured TK inhibition assay by using fluorescence. The values thus obtained were then transformed by calculating their negative logarithm ($-\log IC_{50}$), which is a standard notation to make very small numbers fit into a more comprehensible range, and larger numbers indicate more potent inhibitory activities.

In this work, CoMFA technique was applied to examine molecular basis for the differences between each lamellarins against TK.

2. Methods

2.1 Data set

The 32 lamellarin molecules were separated into two groups. The first group consisting of 25 to 28 compunds, served as the training set. On the other hand, 4 to 7 compounds comprising the test set were chosen randomly such as Lam 24, Lamellarin J, Lam 15, Lam 19, Lam 32, Lamellarin η, and Lam 56. The starting geometries of all 32 lamellarin structures were fully optimized at the AM1 level using the HyperChem program (Hypercube, Inc., Gainesville, FL, USA). The partial atomic charges required for calculations of electrostatic interactions were subsequently computed using the Gasteiger-Hückel method in the SYBYL-X 2.0 program (Tripos, L.P., St. Louis, MO, USA). Molecular alignments were then carried out using the fit atom method which available in the SYBYL-X 2.0 software. The common structure used for fit atom alignments involves the atoms constituting the A-, B-, and C-rings, as denoted with asterisks in Fig. 1. Additionally, the most active molecule, Lamellarin W, was used as the template for alignments.

2.2 CoMFA

To calculate the CoMFA descriptor fields, a cubic lattice was first generated around each lamellarin molecule based on its molecular volume, and a grid spacing of 2 Å was used to ensure that the grid extended by 4.0 Å beyond the molecular dimensions in all directions. The steric and electrostatic fields of each aligned lamellarin were generated based on their Lennard-Jones and Coulomb potentials, respectively. The interactions between these three-dimensional fields with each probe atom were then calculated using the CoMFA standard scaling technique. All the calculated data were then put into a CoMFA table.

The three-dimensional properties of lamellarins determined using CoMFA were then correlated with their inhibitory activities against TK using PLS regression analysis, and various 3D-QSAR models were subsequently derived.

The predictive ability of the derived CoMFA models was evaluated by leave-one-out (LOO) cross-validation, and is expressed in terms of r^2_{cv} (also called q^2), which is defined as shown below.

$$r^2_{cv} = (\text{SSY-PRESS})/\text{SSY}$$

where SSY represents the variance of the cytotoxic activities of molecules around the mean value, and PRESS is the prediction error sum of squares derived from the leave-one-out method.

For all models, a maximum number of components was first used and subsequently decreased until an optimal number was obtained when the resulting cross-validated r^2_{cv} differed from the previous value by less than 0.05. The optimal number of component was then used to perform non cross-validated analyses. Briefly, the conventional correlation coefficient, r^2, was calculated based on compounds in the training set. Then, CoMFA contour maps were created by using the best r^2 model and most active molecule Lamellarin W as a template. These maps provide detailed of the binding pockets which show the best biological acitivity against TK.

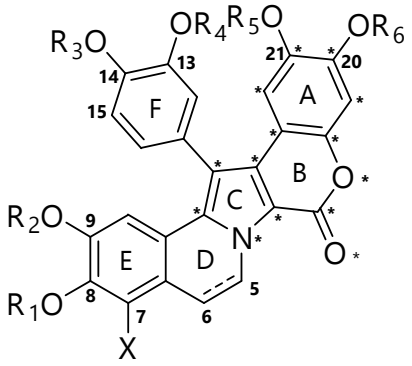


Fig. 1. Core structure of lamellarins, in which the common atoms used for CoMFA fit atom alignments are denoted by asterisks (*).

Table 1 Substituent groups of saturated D-ring (group 1) and unsaturated D-ring lamellarins (group 2)

Lamellarin										
Group 1 (C5–C6)	Group 2 (C5=C6)	C5	C7	C8	C9	C13	C14	C15	C20	C21
Lamellarin χ	-	H	H	OH	OMe	OMe	OH	H	OH	OMe
-	Lamellarin X	H	OH	OMe	OMe	OH	OMe	H	OH	OMe
Lamellarin J	-	H	H	OH	OMe	OMe	OMe	H	OH	OMe
Lamellarin L	-	H	H	OH	OMe	OH	OMe	H	OH	OMe
-	Lamellarin W	H	OMe	OMe	OMe	OH	OMe	H	OH	OMe
-	Lamellarin α	H	H	OMe	OMe	OH	OMe	H	OH	OMe
-	Dehydrolam Y	H	H	OMe	OH	OH	OMe	H	OH	OMe
-	Dehydrolam γ	H	OH	OMe	OMe	OMe	H	OMe	OH	OMe
-	Lamellarin ϕ	H	OMe	OMe	OH	OMe	OH	H	OH	OMe
-	Lamellarin η	H	H	OMe	OMe	OMe	OMe	H	OH	OMe
Lam 1	-	H	H	OH	OMe	H	H	H	OH	OMe
Lam 9	-	H	H	OH	OMe	H	OMe	H	OH	OMe
Lam 11	Lam 12	H	H	OH	OMe	H	F	H	OH	OMe
-	Lam 14	H	H	OH	OMe	F	F	H	OH	OMe
Lam 15	Lam 16	H	H	OMe	OH	H	H	H	OH	OMe
Lam 19	Lam 20	H	H	OMe	OMe	H	H	H	OH	OMe
Lam 21	-	H	OH	OMe	OMe	H	H	H	OH	OMe
Lam 23	Lam 24	H	OH	OMe	OMe	OH	H	H	OH	OMe
Lam 25	-	H	OH	OMe	OMe	OMe	H	H	OH	OMe
Lam 27	-	H	OH	OMe	OMe	H	OH	H	OH	OMe
Lam 29	Lam 30	H	OH	OMe	OMe	H	OMe	H	OH	OMe
Lam 31	Lam 32	H	OMe	OMe	OMe	H	H	H	OH	OMe
Lam 54	Lam 56	H	OH	OMe	OMe	OH	H	H	OH	OMe
Lam 55	-	H	OH	OMe	OMe	-	-	H	OH	OMe
-	Lam 57	H	OMe	OMe	H	H	OH	H	OH	OMe

3. Results and discussion

3.1 CoMFA models

The CoMFA models were generated using the difference compounds in the training and test sets as shown in Table 2. All the models indicated that the changes in the electrostatic interactions accounted for around half of the changes in the inhibitory activities of lamellarins towards TK and the remaining 45-48% was contributed by the steric interactions. Among the four CoMFA models, model 4 calculated using seven lamellarins in test set yielded the highest predictive ability, as indicated by the r^2_{cv} value = 0.660, the r^2 value = 0.989, s = 0.064, and F value = 296.310.

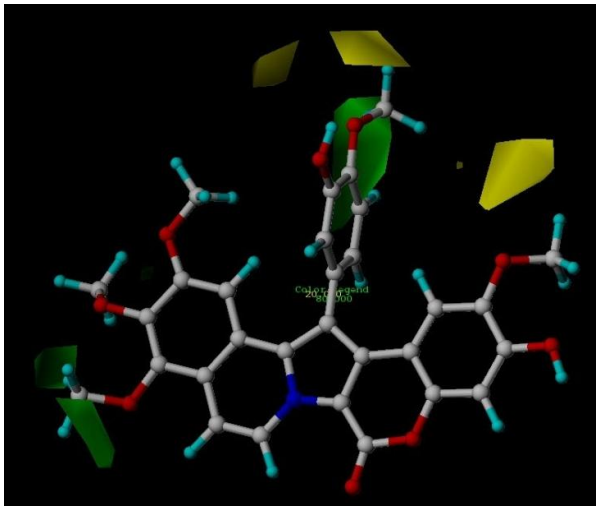
3.2 CoMFA contour maps

The CoMFA contour maps were created using the best CoMFA model 4 as shown in Fig. 2. The resulting contours on these maps not only highlighted the key structural features correlated with the inhibitory activity of the molecule being considered, but also provided detailed understanding of the binding pockets. The green and yellow regions represented the areas where steric bulk enhanced and diminished inhibitory activity, respectively. And, blue and red regions represented the areas where electropositive and electronegative enhanced inhibitory activity, respectively. The green regions were found around methoxy groups at C7 and C14. Yellow regions located near methoxy groups at C14 and methoxy groups at C21. Red contours occupy the space between the oxygen atom of the methoxy group at C7, C8, C9, C14, and C21. Additionally, blue contours located around methoxy groups at C8, C14, and C21. These indicate that a steric group with electronegative oxygen atom such as methoxy groups at C7, electropositive group with electronegative oxygen atom such as methoxy groups at C8, and electronegative oxygen atom at C9 would increase the activity. Moreover, the yellow, green, red, and blue contours near C14 position led to the idea that bulky and electropositive group with electronegative oxygen atom at this position would increase the activity, but the size of this group should not be too large. The yellow and blue contour near methoxy and red contour around oxygen atom at C21 indicate that occupancy of nonsteric and electropositive group with electronegative oxygen atom would increase the activity. Hence, it might suggest that the methoxy groups at C7 and C14, and oxygen atom at C8, C9, and C21 would play an important role in increasing inhibitory activity of lamellarin against TK.

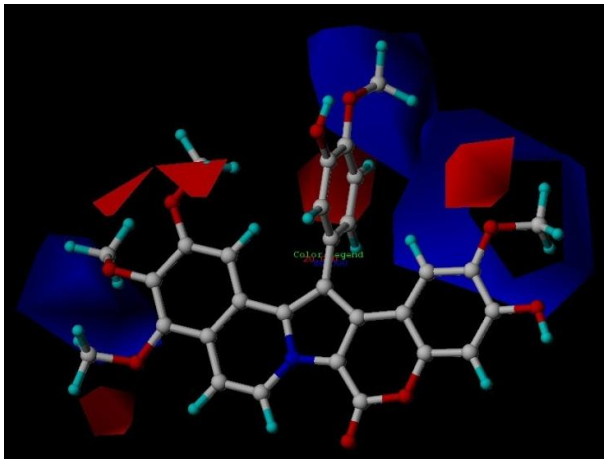
Table 2 Summary of various parameters associated with the CoMFA models

Model	Test set compound	noc	r^2_{cv}	r^2	s	F	Contributions
1	Lam 24, Lamellarin J, Lam 15, and Lam 19	6	0.461	0.984	0.078	209.382	St = 47.9 El = 52.1
2	Lam 24, Lamellarin J, Lam 15, Lam 19, and Lam 32	6	0.522	0.988	0.068	268.936	St = 45.6 El = 54.4
3	Lam 24, Lamellarin J, Lam 15, Lam 19, Lam 32, and Lamellarin η	6	0.583	0.989	0.066	285.217	St = 45.8 El = 54.2
4	Lam 24, Lamellarin J, Lam 15, Lam 19, Lam 32, Lamellarin η, and Lam 56	6	0.660	0.989	0.064	296.310	St = 45.2 El = 54.8

noc = number of components; r^2_{cv} = cross-validated correlation coefficient; r^2 = conventional correlation coefficient; s = standard error of estimation; F = F-value, St = steric field, El = electrostatic field



(a)



(b)

Fig. 2. Stereoviews of steric (a) and electrostatic (b) contour maps, obtained from CoMFA model 4. Lamellarin W is presented inside the contour. Sterically favored and unfavored areas are shown by green and yellow regions, respectively. Electropositive and electronegative areas are shown by blue and red regions, respectively.

4. Conclusion

CoMFA method revealed specific structural requirement of lamellarins for their inhibitory activity against tyrosine kinase. Especially, the importance of methoxy groups at C7 and C14, and oxygen atom at C8, C9, and C21 were also found.

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