Comparative Molecular Field Analyses (CoMFA) on the Melanogenesis Inhibitory Activities of Alkyl-3,4-dihydroxybenzoyl Derivatives.

Sang-Jin Kim[1], Nack-Do Sung [2] and Tack-Hyuck Lee [3]

[1]*Dept. of Cosmetic Science, Daejeon Health Sciences College, Daejeon, 300-711, Korea*
[2]*Division of Applied Biology & Chemistry, College of Agriculture & Life Sciences, Chungnam National University, Daejeon, 305-764, Korea*
[3]*Dept. of Chemistry, College of Natural Sciences, Paichai University, Daejeon, 302-735, Korea*

Synopsis

To search and development a new material with superior melanogenesis inhibitory activity, the bioactivities (obs. pI_{50}) of alkyl-3,4-dihydroxybenzoyl esters and N-alkyl-3,4-dihydroxybenzoyl amides as substrate molecules were measured in mouse melanoma cells. And also, we have studied that 3-D QSARs (3 dimensional Quantitative Structure-Activity Relationships) between molecular interaction field of substrates and the bioactivities were analyzed using CoMFA (Comparative Molecular Field Analyses) method. When cross-validation value (q^2) is 0.68 at 3 components, the Pearson correlation coefficient (r^2) is 0.900. From the basis on the findings, the model was appeared by the contour map such as steric field and electrostatic field relationships between quantitative structure and the bioactivity of the various substrate derivatives. Measured bioactivities (obs. pI_{50}) of unknown compounds are very similar to predicted activity (pred. pI_{50}) according to the CoMFA model. As the results of prediction, we could conclude that the bioactivities were increased by creation of R_1 substitution of 5,5-dimethylhexoxy, 6,6-dimethylheptyl, 1-amino-6,6-dimethylheptyl group etc and R_2 substitution of hydroxy, methyl, methoxy group etc.

Keywords :CoMFA, 3D-QSARs, Alkyl-3,4-dihydroxybenzoyl esters, N-alkyl-3,4-dihydroxybenzoyl amides, Melanogenesis inhibitor

Introduction

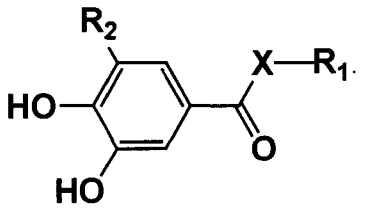
Skin color of a person is determined by melanin of the dark brown order, carotene of the yellow order and hemoglobin of the red order. Melanin is made by reaction of tyrosine and tyrosinase[1)]. Even though melanin can protect skin from the ultraviolet (UV) rays, some people don't like it because it makes people's skin look black and dark. Human-beings have wanted the whiter and lighter skin. So, we need to develop whitening cosmetics and skin whitening agents that inhibit melanin production. The basic researching methods[2)] to develop new melanogenesis inhibitors are viewed in Table 1.

Recently a lot of researches about sun screening agents, peeling of keratin, bleaching of melanin, cytokine network regulation and tyrosinase activity inhibitors for prevention of melanin production have been done and also researches about inhibition of signal transduction[3)] of cells and inactivation of the gene[4)] of tyrosinase synthesis. To inhibit the activity of tyrosinase, we usually use two methods which cover the active site of tyrosinase with same sized and shaped substance and activate the formation of chelate between copper(II) ion in the active site and inhibitor. Also we can change the active form tyrosinase into inactive form or prohibit inactive form from changing into active form of tyrosinase. The substances that have the similar structure and action to tyrosinase have been searched to invent tyrosinase activity inhibitors because OH group of tyrosine makes chelate with copper(II) ion in tyrosinase and forms DOPA, DOPA quinone and melanin.

Table I. Melanogenesis and screening methods for the development of whitening agents.

Melanogenesis	Screening methods
Ultraviolet ray	Sun screening
Signal transduction	Cytokine network regulation
Melanosome formation	Melanosome degradation
Tyrosinase synthesis	Inhibition of tyrosinase synthesis
	Inhibition of tyrosinase activity
Oxidation	Reduction or antioxidation
Melanin synthesis	Peeling of keratin
	Bleaching of melanin

Therefore, search of tyrosinase inhibitor should begin with interaction between tyrosine and copper(II) ion in active site of tyrosinase, using inhibitor such as kojic acid, ascorbic acid, hydroxy benzoic acid etc and hydroxy phenol kind, catechol kind etc which have substrate such as OH and COOH group capable of having a bond with copper(II) ion[5]. Moreover it is anticipated that dihydroxy derivatives have better inhibitory activity than monohydroxy derivatives[6] to form strong chelate with copper(II) ion. Toluene, however, has a little melanogenesis inhibitory activity even though it doesn't have an OH group[7]. That's because the shape and size of toluene are similar to the active site of tyrosinase. On the contrary, the inhibitory activity of EDTA, which is a strong chelator, is low. That's because the shape and size of EDTA are not similar to the active site of tyrosinase. In summary, alkyl-3,4-dihydroxybenzoyl derivatives (Figure 1) are anticipated to enter tyrosinase's active site and form firm chelate with copper(II) ion in active site of tyrosinase with two OH groups[8].



X = O & NH

Figure 1. General structure of alkyl(R_1)-3,4-dihydroxy-5-sub(R_2)-benzoyl ester and N-alkyl(R_1)-3,4-dihydroxy-5-sub(R_2)-benzoyl amide derivative as substrate molecule of tyrosinase.

QSAR methodologies[9] have attempted to analyses of interactions between ligand molecules and receptor which method will minimize the number of compounds for test, the time needed to search a new material and money. The CoMFA was one method for QSAR based on the three dimensional structures of the ligands which is allows us to predict unknown compound's biological activity, toxicity and nature of pharmacophor etc and powerful method for design of new drugs, to search a new material, for molecular modeling etc,.

In this studies, the melanogenesis inhibitory activities (obs. pI_{50}) of alkyl-3,4-dihydroxybenzoyl esters and N-alkyl-3,4-dihydroxybenzoyl amides as substrate molecules were measured in mouse melanoma cells and then 3-D QSARs (3 dimensional Quantitative Structure-Activity Relationships) between molecular interaction field of substrate molecules and the bioactivities were analyzed in order to search and development a new material with superior melanogenesis inhibitory activity using CoMFA (Comparative Molecular Field Analyses) method[10].

Experimental

Meterials and Equipments.

For conformer search, Tripos[11] Company's SYBYL program (Ver. 6.7) and silicon graphic Company's O_2 workstation were used. Experimental agents[2] including substrate molecules were the ones Sigma, Fluka, Gibco life Technologies company etc. Mouse melanoma cells was offered by Korea Research Institute of Bioscience & Biotechnology and absorbency measurement was done by model UV-1601PC ultraviolet spectrophotometer of Shimadzu Company.

Methods

Measurement of melanogenesis inhibitory activity.

In measurement of melanogenesis inhibitory activity, the amount of produced melanin without melanogenesis inhibitor in mouse melanoma cells was compared with the one of melanin produced with melanogenesis inhibitor in the cells. Specific experimental method is as follows. Mouse melanoma cells were cultivated in Dulbecco's Modified Eagle's Media (DMEM) with 10% fetal bovine serum (FBS), 100 nM 12-O-tetradecanoylphorbol-13-acetate (TPA) and 1 nM cholera toxin (CT), in the condition of 37°C and 5% of CO_2 and until 96 cells/well. After, we add 10 mg/L inhibitor solution in cell culture media and cultivate for 3 days and then removed culture media. In the same breath, we washed it with Dulbecco's Phosphate Buffered Saline (PBS), melt melanin with 1N NaOH, and measured absorbency in 400nm[12]. By comparing absorbency in mouse melanoma cells without inhibitor add with the one with inhibitor add, we calculate melanogenesis inhibitory activity. Melaninogenesis inhibitory activity value (pI_{50}) was gotten by calculating from absorbance coefficient that are measured about substrate molecules, and found 50% inhibitory concentration (IC_{50}, mg/L = 0.2501/extinction coefficient x 1) by substituting absorbency value at 50% inhibition. Continuously, converted this 50% inhibitory concentration into mol concentration and adopted reciprocal in log of this values and finally found from the next equation.

$$pI_{50} = -\log\{IC_{50}/M.Wt \times 1000\}$$

Molecular modeling and CoMFA analyses.

All computations were performed using molecular modeling software package Tripos Company's SYBYL (Ver. 6.7) program. We used simulated Annealing methods to search minimized energy conformer. Simulated Annealing conditions were summarized as follows; used 10 cycles, gasteriger-Huckel partial atomic charges were used, temperatures range were 200°C to 1000°C. CoMFA incorporated in steric and electrostatic values as well as ClogP (calculated Hydrophobicity) values. In CoMFA analysis, ligands are placed in a three-dimensional lattice and their steric and electrostatic field is calculated at each lattice grid point. The resulting field matrix is analyzed by Partial Least Squares (PLS) method[13] for the compounds.

The three-dimensional lattice set up was 18×20×22 Å with a 2 Å grid spacing for both the steric and electrostatic field, the default cut-off used was 30 kcal/mol. CoMFA expresses by contour map of three-dimensional spaces between steric field and electrostatic field when it arranged each atom to appear compounds for three-dimensional space. Analyses result, does to sort compounds to core molecular, are derived by partial least square methods between creation activities of sorted compounds and correlation equations of explanation factors.

Result and Discussion

CoMFA models for melanogenesis inhibition activity.

To find out highly effective[14] melanogenesis inhibitors, we made alignment by setting-3,4-dihydroxybenzoyl which has by common substrate. As core molecule, the alignment results confirmed substrates exited with a variety of forms plane or space around core molecule (Figure2).

(I) Front view (II) Side view

Figure 2. Stereo diagram (I & II) of the structural superposition of potential energy minimized substrate molecular structures according to a least-squares atom fit.

Table II shows melanogenesis inhibitory activities (obs. pI_{50}) measured in response to derivatives A-series (esters) and B-series (amides) that are expected to have melanogenesis inhibitory activity. Observed melanogenesis inhibitory activity value (obs. pI_{50}) of A-series was 3.95~4.80 (Ave. pI_{50}= 4.38), B-series showed value of 3.18~4.72 (Ave. pI_{50}=4.03) range. To substrate, A19 (obs. pI_{50}=4.80) and B8 (obs. pI_{50}=4.72) caused the highest melanogenesis inhibitory activity, A12 (obs. pI_{50}=3.95) and B1 (obs.pI_{50}=3.18) showed the lowest melanogenesis inhibitory activity.

Table II. Melanogenesis inhibitory activities (obs. pI_{50}) of alkyl-3,4-dihydroxybenzoyl esters and N-alkyl-3,4-dihydroxybenzoyl amides as substrate molecules.

Esters				Amides			
No.	R_1	R_2	pI_{50}	No.	R_1	R_2	pI_{50}
A1	H	Methoxy	3.98	B1	Methyl	Methoxy	3.18
A2	Methyl	Methoxy	4.05	B2	Ethyl	Methoxy	3.80
A3	n-propyl	Methoxy	4.35	B3	n-Propyl	Methoxy	3.53
A4	3-Methyl-2-butenyl	Methoxy	4.40	B4	n-Butyl	Methoxy	4.19
A5	Methyl	Ethoxy	3.99	B5	n-Pentyl	Methoxy	4.56
A6	Methyl	n-Propoxy	4.07	B6	n-Hexyl	Methoxy	4.66
A7	Methyl	n-Butoxy	4.51	B7	n-Heptyl	Methoxy	4.70
A8	Methyl	3-Methyl-2-butenoxy	4.49	B8	n-Octyl	Methoxy	4.72
A9	Methyl	Phenylmethoxy	4.55	B9	Iso-propyl	Methoxy	3.77
A10*	Methyl	Phenylethoxy	4.70	B10	1-Methylpropyl	Methoxy	4.01
A11	Methyl	Hydroxy	4.18	B11	Hydroxy	Methoxy	3.97
A12	Iso-propyl	Hydroxy	3.95	B12	2-Hydroxyethyl	Methoxy	3.38
A13	Ethyl	H	4.43	B13	3-Hydroxypropyl	Methoxy	3.50
A14	Iso-propyl	H	4.29	B14*	Phenyl	Methoxy	4.65
A15	n-Propyl	H	4.45	B15	Benzyl	Methoxy	4.37
A16	n-Butyl	H	4.51	B16	Ethylphenyl	Methoxy	4.49
A17	n-Hexyl	H	4.61	B17	Propylphenyl	Methoxy	4.67
A18	n-Heptyl	H	4.71	B18	*p*-Hydroxyphenyl	Methoxy	3.43
A19	3,7-Dimethyloctyl	H	4.80	B19	*m*-Hydroxyphenyl	Methoxy	3.43
A20	Phenyl	H	4.56	B20	2-Aminoethyl	Methoxy	3.44

*Outliers

A trial set of 40 compounds (ester: 20 & amide: 20) was used. CoMFA and ClogP values were used as descriptors and the predicted activities (pred. pI_{50}) were compared to the melanogenesis inhibitory activity as dependent column. In the studies, the q^2 value was 0.758, r^2 value was 0.934 of esters and the q^2 value was 0.677, r^2 value was 0.896 of amides, respectively. In case of total compounds, when outliers used 2 compounds, the q^2 value of CoMFA was 0.681 at three components and Pearson correlation coefficient, r^2 value was 0.900 which means that the analyzed results have a 90.0% fitness compared to the biological test results. The r^2 (above 0.9) would have been comparatively accurated itself if q^2 had over 0.5. The q^2=0.68, showed the level to which the predicted activity approximated to the biological activity. Therefore, CoMFA result seem to be very reliable predictors of the melanogenesis inhibitory activity by the order of ester>(ester & amide)>amide. Table III shows the results of PLS analyses and relative contribution by CoMFA analyses. CoMFA results for melanogenesis inhibitory activity was shown as follow. pI_{50}=0.726(logP)+[steric]+[electrostatic]+3.845 (n=40, s=0.15, F=103.585 & r^2=0.900)

Table III. Results of PLS analyses and relative contribution (%) for melanogenesis inhibitory activities.

	n[a]	o[b]	q^{2c}	r^{2d}	s[e]	Com[f]	RC(%)[g]		
							Steric[h]	Elect[i]	ClogP[j]
Esters	20	0	0.758	0.934	0.068	3	42.4	26.0	44.4
Amides	20	1	0.677	0.896	0.183	2	26.0	36.7	37.3
Total	40	2	0.681	0.900	0.150	3	44.4	29.0	26.6

[a]total number of used compounds, [b]outlier, [c]predicted cross validate value, [d]Pearson correlation coefficient, [e]standard deviation, [f]number of component, [g]percent of relative contribution, [h]steric effect, [i]electrostatic field, [j]calculated logP.

In the CoMFA contour map (Figure 3), the green colored contour surrounding the R_1 group is increase of melanogenesis inhibitory activity by introducing steric bulky substituents in this region. In contrast, the yellow contour at the under position of R_1 group indicate that steric occupancy with bulk groups in this region will decrease activity. And blue colored contour surrounding the R_1 group is increase of activity by introducing electropositive group. Conversely, the red colored small region that was located in X (Figure 1) is increase of activity by introducing electronegative substituents in this region.

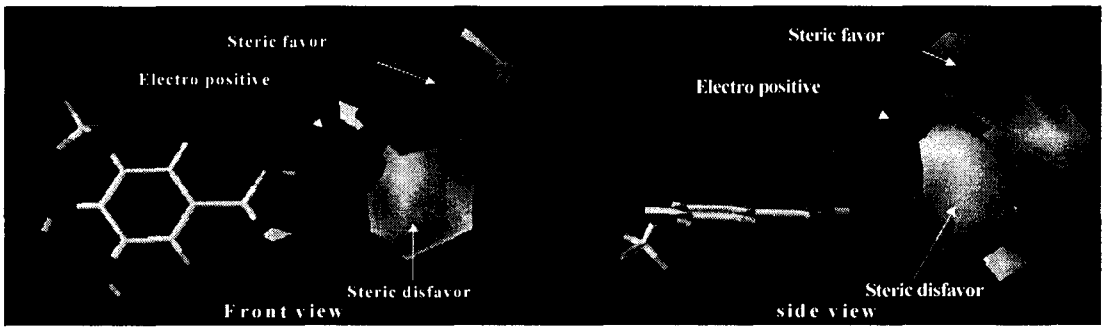


Figure 3. CoMFA contour maps for melanogenesis inhibitory activity. Green color is a steric favor region, yellow is a steric disfavor region , blue is a electropositive favor region and red is a electronegative favor region.

From the CoMFA field analyses, steric properties (44.4%) were exclusive contributors to the activities and then electrostatic (29%) and ClogP (26.6%) values. Figure 4 shows observed and calculated melanogenesis inhibitory activity (pI_{50}) values against mouse melanoma cell.

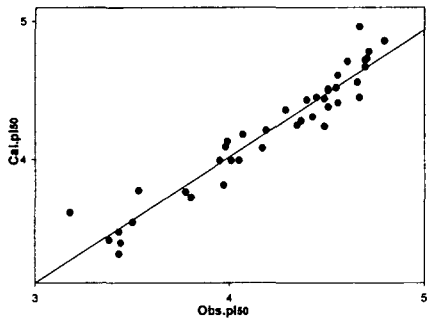


Figure 4. *Relationships between observed (obs. pI_{50}) and predicted (Cal.pI_{50}) melanogenesis inhibitory activity (pI_{50}) values against mouse melanoma cell according to equation.*

Predictions for test set compounds.

From the equation and CoMFA contour map, compounds that has higher melanogenesis inhibitory activity were predicted and database were constructed. To know agreements between predicted compounds and CoMFA results, consecutively, calculation progresses were enforced repeatedly. Finally, correlations between predicted values and observed values were investigated (Table IV).

Table IV. Predicted values of test set compounds in alkyl-(R_1)-3,4-dihydroxy-5-sub-(R_2)-benzoyl derivatives*

No.	R_1	R_2	Obs. pI_{50}	Pred. pI_{50}[b]	Dev.[c]
1[a]	Methyl	Phenylethoxy	4.70	4.67	-0.03
2[a]	Phenyl	Methoxy	4.65	4.67	+0.02
3	Propylphenyl	H	4.67	4.70	+0.03
4	Benzyl	H	4.51	4.51	0.00

*Ester: No. 1 & 3, Amide: No. 2 & 4., [a]outlier, [b]predicted values were according to the equation, [c]deviation between observed and predicted pI_{50}.

From a variety of analyses, a lot of new compounds were predicted and then Table V showed the results of predicted and observed melanogenesis inhibitory activities of predicted compounds. Melanogenesis inhibitory activities of predicted compounds from CoMFA contour map and CoMFA equation were observed according to the same method. Observed pI_{50} values are very similar to predicted pI_{50}. Therefore, we got to know that our prediction was right and then confirm the predicted compounds.

Table V. Predicted and observed melanogenesis inhibitory activities of new alkyl-(R_1)-3,4-dihydroxy-5-sub-(R_2)-benzoyl ester derivatives.*

R_1	R_2	Pred. pI_{50}	Obs. pI_{50}	Dev.[a]
5,5-Dimethylhexoxy	Hydroxy	5.13	5.10	-0.03
1-Amino-6,6-dimethylheptyl	Methoxy	4.92	4.94	+0.02
6,6-Dimethylheptyl	Methyl	4.90	5.00	+0.10

*The compounds were not included in test set data., [a]deviation between observed and predicted pI_{50}.

Conclusions

Alkyl-3,4-dihydroxy-5-sub-benzoyl derivatives as the substrate were used to evaluate the melanogenesis inhibitory activity. The Quantitative Structure-Activity Relationships (QSARs) between above inhibitory activity and substrates were investigated by using 3D-QSAR (CoMFA). When cross-validation value (q^2) is 0.68 at 3 components, the Pearson correlation coefficient (r^2) is 0.900. The CoMFA equation, Obs.pI_{50}=0.73(logP)+[steric]+[electro]+3.845 (n=40, s=0.15, F=103.59, r^2= 0.900) was obtained with the explanations in 44.4% of steric field, 29% of electrostatic field and 26.6.0% of hydrophobicity, respectively. Melanogenesis inhibitory activity field for relative contribution (%) in molecular interaction field was explained by the order of steric field> electrostatic field> hydrophobic.

As the results of prediction from the CoMFA analyses, we could conclude that the melanogenesis inhibitory activity was increased by creation of R_1 substitution of 5,5-dimethylhexoxy, 6,6-dimethylheptyl, 1-amino-6,6-dimethylheptyl group etc and R_2 substitution of hydroxy, methyl, methoxy group etc. And finally predicted compounds has good, above 4.94, biological activities. It was suggest that the QSAR technique is capable of providing a model for designing new melanogenesis inhibitors.

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