Comparative Molecular Similarity Indices Analysis (CoMSIA) on the Melanogenesis Inhibitory Activities of Alkyl-3,4-dihydroxybenzoate and *N*-alkyl-3,4-dihydroxybenzamide Derivatives.

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Synopsis

To find a new substance with superior melanogenesis inhibitory activity, the bioactivities of alkyl-3,4-dihydroxy-5-substituted benzoate (A) and *N*-alkyl-3,4-dihydroxy-5-substituted benzamide (B) derivatives as substrate of tyrosinase were measured in mouse melanoma cells. And the bioactivities analyzed using comparative molecular similarity indices analysis (CoMSIA). From the CoMSIA model, when cross-validation value (q^2) is 0.713 at four components, the pearson correlation coefficient (r^2) is 0.900. Unknown compounds were predicted, using QSAR analyzed results from the CoMSIA methods. Excellent agreement was obtained between the measured and the predicted bioactivities of unknown compounds. As the results of prediction from CoMSIA, we could conclude that the bioactivities were increased from pI_{50}=3.18~4.80 to above 5.17 by creation of 6-methylheptyl, n-pentylphenyl and 2-hydroxypentylphenyl group etc,.

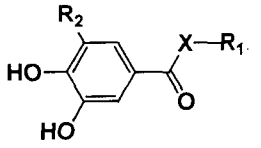
Keywords : CoMSIA, 3D-QSARs, Melanogenesis inhibitor, Alkyl-3,4-dihydroxy-5-substituted benzoate, *N*-alkyl-3,4-dihydroxy-5-substituted benzamide.

Introduction

Generally, to inhibit the formation of melanin, we usually use two methods which masking the active site of tyrosinase with similar sized and shaped substance and activate the formation of chelate between copper ion in the active site and inhibitors. The substances that have the similar structure and action to tyrosin have been searched to invent tyrosinase activity inhibitors because hydroxy moiety of tyrosine makes chelate with copper ion in tyrosinase and forms melanin[1]. Therefore, search of tyrosinase inhibitor should be begun with interaction between tyrosine and copper ion in active site of tyrosinase, by using substrate with hydroxy or carboxylic acid moiety capable of having a bond with cupper ion[2]. Moreover, it is anticipated that dihydroxy derivatives have better inhibitory activity than monohydroxy derivatives by forming stronger chelate with copper ion[3].

Till now, a lot of studies about the structural and physicochemical properties, biological mode of action of synthetic or natural compounds in vivo have been done, but recently the studies about quantitative structure-activity relationships (QSARs)[4], which can explain the relationship between structure and biological activity, have been increasing. QSARs allow us to predict the biological activity, toxicity and nature of the pharmacophore fast and exactly, so we can minimize time, money and effort to search a new highly active compound. CoMSIA method is the way with which we can design the new active compounds or new medicine from the contour map of steric field, electrostatic field and hydrophobicity that was obtained when ligands was arrayed in three-dimensional (3-D) space. The QSARs was based on the three-dimensional structures of the ligands and CoMSIA method is known as one of the new 3-D QSAR descriptors. In this study, we compared the melanogenesis inhibitory activities that measured in mouse melanoma cells with feature that predicted from CoMSIA. And then, we could get the new compounds that have superior bioactivity.

In this study, alkyl-3,4-dihydroxybenzoate and *N*-alkyl-3,4-dihydroxybenzamide derivatives are anticipated to enter tyrosinase's active site and form firm chelate with copper ion in active site of tyrosinase with two hydroxy moiety in Figure 1. Therefore, to find a new substance with superior melanogenesis inhibitory activity we use the alkyl-3,4-dihydroxybenzoate and *N*-alkyl-3,4-dihydroxybenzamide derivatives as melanogenesis inhibitor and CoMSIA method.



X=O or NH

Figure 1. General structure of alkyl-3,4-dihydroxy-5-substituted benzoate (A) and *N*-alkyl-3,4-dihydroxy-5-substituted benzamide (B) derivatives as substrate molecule of tyrosinase inhibitor.

Experimental

Materials and Equipments

All molecular modeling techniques used in this research were performed with SYBYL software package Ver. 6.7 (Tripos Ins., St. Louis, MO, USA)[5] and O_2 workstation (Silicon Graphics, Inc,.). Experimental reagents[6] including substrate molecules were the one's Sigma, Fluka, Gibco life technologies company etc. Mouse melanoma cell was offered by Korea Research Institute of Bioscience & Biotechnology and absorbance measurement was done by model UV-1601PC ultraviolet spectrophotometer of Shimadzu company.

Methods

Measurement of melanogenesis inhibitory activity

The melanogenesis inhibitory activities of alkyl-3,4-dihydroxybenzoate and *N*-alkyl-3,4-dihydroxybenzamide derivatives were measured by comparing the amount of melanin produced when the inhibitor was added with one without inhibitor. The methods are as follows[7]: Mouse melanoma cells were cultivated in dulbecco's modified eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 100 nM of 12-O-tetradecanoylphorbol 13-acetate (TPA) and 1 nM of cholera toxin, in the condition at 37°C and 5% of CO_2 and until 96 cells/well. Next, we add 10 mg/L of inhibitor solutions 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 absorbance in 400nm. Finally, by comparing absorbance we obtained pI_{50} by calculating each absorption coefficient from measured absorbance about the trial set compounds and gaining 50% inhibitory concentration from absorbance value in 50% inhibition. We converted the 50% inhibitory concentration (IC_{50}) to molarity and took reciprocal of logarithm. The equation is as follows:

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

Molecular 3-D structure building and CoMSIA analysis.

The CoMSIA approach implies moving from field descriptors based on well established and generally accepted potentials to some arbitrary descriptors considering similarity or dissimilarity of molecules. 3D-QSAR is a method to map and pin down similarities or dissimilarities of molecules. The descriptors used in 3D-QSAR need not necessarily display partitions of interaction energy terms. They have only to correlate in a uniform manner with contributions determining binding affinity. Keeping the design of novel molecules in mind, this spatial interpretation of 3D-QSAR results is out of utmost importance; it allows us to understand what really matters in terms of structural features. With CoMSIA, substantially improved contour maps are obtained. They can easily be interpreted and used as a visualization tool in designing novel compounds[11].

The acceptor field contains information about where hydrogen bond donating groups should be on the receptor and the donor field describes where hydrogen bond acceptor groups should be located on the receptor. And so, if hydrogen bond fields have been created, the acceptor field is in the steric field, and the donor field is in the electrostatic field. Any hydrophobic field will be in the steric field[9)10)].

The 3-D structures of entire trial sets of alkyl-3,4-dihydroxybenzoate and *N*-alkyl-3,4-dihydroxybenzamide analogues were constructed using standard geometric parameters of molecular modeling software package SYBYL (Ver. 6.7) program. We used simulated annealing methods to search minimized energy for conformer. Simulated annealing conditions were summarized as follows; used 10 cycles, Gasteiger-Huckel partial atomic charges were used, temperatures range were from 200°C to 1000°C. With full minimization by the tripos force field, the optimized structures of these analogues were obtained. Comparative molecular similarity indices analysis is developed by Gerhard Klebe and Thomas Mietzner that is a new descriptor to bioactivity, toxicity and docking etc,. CoMSIA expressed by contour map, the contour maps derived from CoMSIA models permitted an understanding of the steric, electrostatic and hydrophobic fields requirements for ligand binding. In CoMSIA analysis, ligands are placed in a three-dimensional lattice and each field was calculated at each lattice grid point. The three-dimensional lattice set up was 18×20×22 Å with a 2 Å grid spacing for the each fields, the default cut-off used was 30 kcal/mol. The similarity indices is calculated as follows[11)];

$$A^{q}_{F,K,(J)} = -\Sigma W_{probe,k} W_{ik} e^{-\alpha r2iq}$$

In this equation, A is the similarity index at grid point q, summed over all atoms (i) of the molecule (J). $W_{probe,k}$ is the probe atom of radius 1 Å charge +1, hydrophobicity index +1, hydrogen bond donating and hydrogen bond accepting +1. W_{ik} is the value of the physicochemical properties (k) of atom (i). r_{iq} is distance between the probe atom at grid point (q) and atom (i) of the test molecule. α is the attenuation factor. CoMSIA incorporated in descriptor values as well as ClogP (calculated hydrophobicity) values. From the contour map, the structural variations in the training set that give rise to variation in the molecular fields at particular regions of the space are correlated to the biological properties[12)]. To fine the optimum number of principal compounds corresponding to the smallest error of prediction, a leave-one-out (LOO) cross-validation procedure was performed. And to obtain better Pearson correlation coefficient (r^2) value, the column filtering value was assigned. Analyses result, does to sort compounds to core molecular, are derived by partial least square (PLS) methods between creation activities of sorted compounds and correlation equations of explanation factors.

Result and Discussion

CoMSIA models for melanogenesis inhibition activity.

To find out highly effective melanogenesis inhibitors, a trial set of 40 compounds was used. Observed melanogenesis inhibitory activity values (obs. pI_{50}) of alkyl-3,4-dihydroxybenzoate and *N*-alkyl-3,4-dihydroxybenzamide derivatives were pI_{50}=3.18~4.80 range. To substrate, A19 (obs.pI_{50}=4.80) and B8 (obs.pI_{50}=4.72) caused the highest melanogenesis inhibitory activities, B1 (obs.pI_{50}=3.18) showed the lowest melanogenesis inhibitory activity. And we aligned by setting a trial set of 40 compounds which has by common substrate. With core molecule, the alignment results confirmed substrates exited with a variety of forms plane or space around core molecule. CoMSIA and ClogP values were used as descriptors and then the predicted activities (pred. pI_{50}) were compared to the observed melanogenesis inhibitory activities as dependent column against mouse melanoma cell lines.

Table I shows melanogenesis inhibitory activities (obs.pI_{50}). They measured in response to derivatives esters (A) and amides (B) that are expected to have melanogenesis inhibitory activity.

Table. I Observed (obs.pI_{50}) and predicted (pre.pI_{50}) melanogenesis inhibitory activities of alkyl-3,4-dihydroxy-5-substituted benzoate (A) and *N*-alkyl-3,4-dihydroxy-5-substituted benzamide (B) derivatives.

No	R_1	R_2	PI_{50}		No	R_1	R_2	PI_{50}	
			Obs[a]	Pre[b]				Obs[a]	Pre[b]
A1	H	OCH_3	3.98	4.01	B1	CH_3	OCH_3	3.18	3.48
A2	CH_3	OCH_3	4.05	4.00	B2	C_2H_5	OCH_3	3.80	3.74
A3	C_3H_7	OCH_3	4.35	4.18	B3	C_3H_7	OCH_3	3.53	3.95
A4	$CH_2CHC(CH_3)_2$	OCH_3	4.40	4.40	B4	C_4H_9	OCH_3	4.19	4.20
A5	CH_3	OC_2H_5	3.99	4.11	B5	C_5H_{11}	OCH_3	4.56	4.40
A6	CH_3	OC_3H_7	4.07	4.24	B6	C_6H_{13}	OCH_3	4.66	4.53
A7	CH_3	OC_4H_9	4.51	4.38	B7	C_7H_{15}	OCH_3	4.70	4.73
A8	CH_3	$OCH_2CHC(CH_3)_2$	4.49	4.44	B8	C_8H_{17}	OCH_3	4.72	4.76
A9	CH_3	OCH_2-C_6H_5	4.55	4.50	B9	$CH(CH_3)_2$	OCH_3	3.77	3.80
A10	CH_3	OC_2H_4-C_6H_5	4.70	4.61	B10	$CH(CH_3)C_2H_5$	OCH_3	4.01	3.96
A11	CH_3	OH	4.18	4.05	B11	OH	OCH_3	3.97	3.81
A12	$CH(CH_3)_2$	OH	3.95	4.04	B12	C_2H_4OH	OCH_3	3.38	3.38
A13	C_2H_5	H	4.43	4.29	B13	C_3H_6OH	OCH_3	3.50	3.48
A14	$CH(CH_3)_2$	H	4.29	4.40	B14	C_6H_5	OCH_3	4.65	4.60
A15	C_3H_7	H	4.45	4.37	B15	CH_2-C_6H_5	OCH_3	4.37	4.41
A16	C_4H_9	H	4.51	4.54	B16	C_2H_4-C_6H_5	OCH_3	4.49	4.40
A17	C_6H_{13}	H	4.61	4.75	B17	C_3H_6-C_6H_5	OCH_3	4.67	4.45
A18	C_8H_{17}	H	4.71	4.63	B18	C_6H_4-*p*-OH	OCH_3	3.43	3.41
A19	3,7-$(CH_3)_2$-C_8H_{17}	H	4.80	4.85	B19	C_6H_4-*m*-OH	OCH_3	3.43	3.29
A20	C_6H_5	H	4.56	4.50	B20	$C_2H_4NH_2$	OCH_3	3.44	3.39

[a]observed values, [b]predicted values.

From the PLS analyses, using steric fields, electrostatic fields and hydrophobicity as descriptors, the q^2 value of CoMSIA was 0.713 at four 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 would have been comparatively accurate itself if q^2 had over 0.5. The q^2=0.71 showed the level to which the predicted activity approximated to the biological activity, so r^2 value was correct. Therefore, CoMSIA result seems to be very reliable predictors of the melanogenesis inhibitory activity. The steric field descriptors explain 18.8% of variance, electrostatic 1.8%, hydrophobic 53.8%, hydrogen bond donor 17.1% and hydrogen bond acceptor explain 8.6%, respectively.

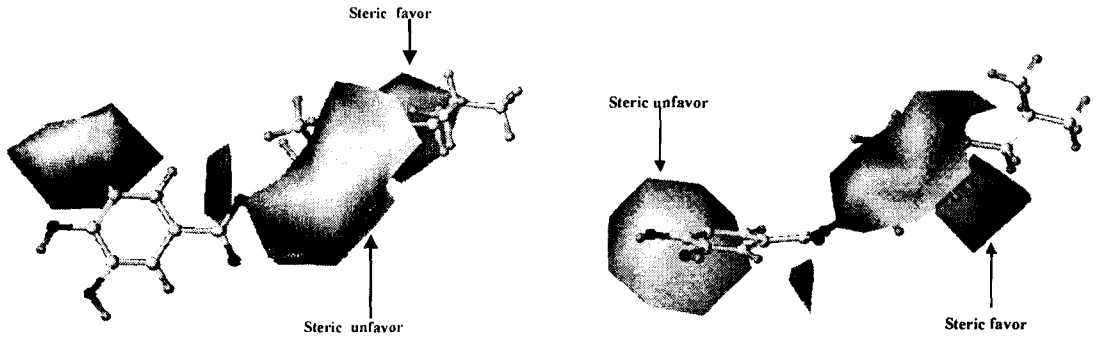
Table II. The results of PLS analyses and the relative contribution (%) for melanogenesis inhibitory activities.

n^a	o^b	q^{2c}	r^{2d}	s^e	cn^f	RC(%)[g]				
						steric[h]	elect[i]	ClogP[j]	HD[k]	HA[l]
40	2	0.713	0.900	0.153	4	18.8	1.8	53.8	17.1	8.6

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

CoMSIA contour map

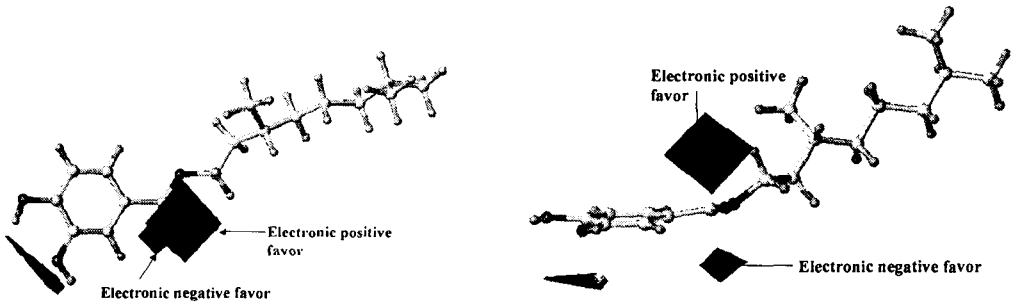
In the CoMSIA contour map of steric field (Figure 2), sterically favored areas are represented by green polyhedral, the green colored contour surrounding the R_1 group is increase of melanogenesis inhibitory activity by introducing steric bulky substituents in this region. In contrast, sterically disfavored areas are represented by yellow polyhedral, the yellow colored contour surrounding the R_1 and R_2 groups indicate that steric occupancy with bulky groups in this region will decrease activity. The steric field descriptors explain 18.8 % of variance[13].



(1) Front view (2) Side view

Figure 2. CoMSIA contour map of steric field for melanogenesis inhibitory activity. Green contours refer to sterically favored regions and yellow contours indicated disfavored region.

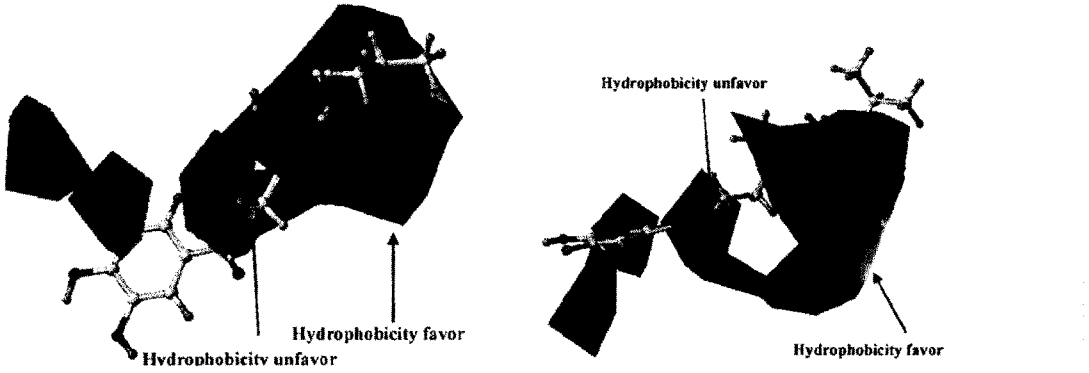
In the CoMSIA contour map of electrostatic field (Figure 3), electropositive favored area are represented by blue polyhedral, the blue colored contour surrounding the CO group (Figure 1) is increase of melanogenesis inhibitory activity by introducing electropositive group in this region and, electronegative favored areas are represented by red polyhedral, the red colored contour indicate electronegative groups under the CO group will increase activity. From the CoMSIA field analyses, the electrostatic field descriptors explain 1.8% of variance. It can be concluded that electrostatic field may be low influence than another fields.



(1) Front view (2) Side view

Figure 3. CoMSIA contour map of electrostatic field for melanogenesis inhibitory activity. Blue contours refer to electropositive favored regions and red contours indicated electronegative favored region.

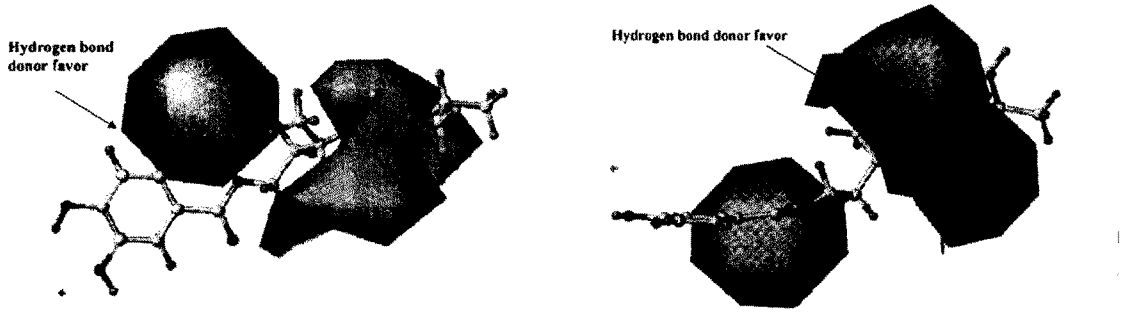
One of the advantages of CoMSIA is that hydrophobic contributions, which cannot be completely treated using Lennard-Jones and coulombic fields, are evaluated using a hydrophobic similarity index field. In the CoMSIA contour map of hydrophobic field (Figure 4), hydrophobic favored areas are represented by blue polyhedral, the blue colored contour surrounding the R_1 and R_2 groups is increase of melanogenesis inhibitory activity by introducing hydrophobic substituents in this region. In contrast, hydrophobic disfavored areas are represented by brown polyhedral, the brown colored contour surrounding the X (Figure 1) indicates that hydrophobic substratents in this region will decrease activity. From the CoMSIA field analyses, the hydrophobic field descriptors explain 53.8% of variance. This is very influential hydrophobicity for melanogenesis inhibitory activity, and then materials of strong hydrophobicity would pointed to enhance melanogenesis inhibitory activity.



(1) Front view (2) Side view

Figure 4. CoMSIA contour map of hydrophobic field for melanogenesis inhibitory activity. Blue contours refer to hydrophobicity favored regions and brown contours indicated to hydrophobicity disfavored regions.

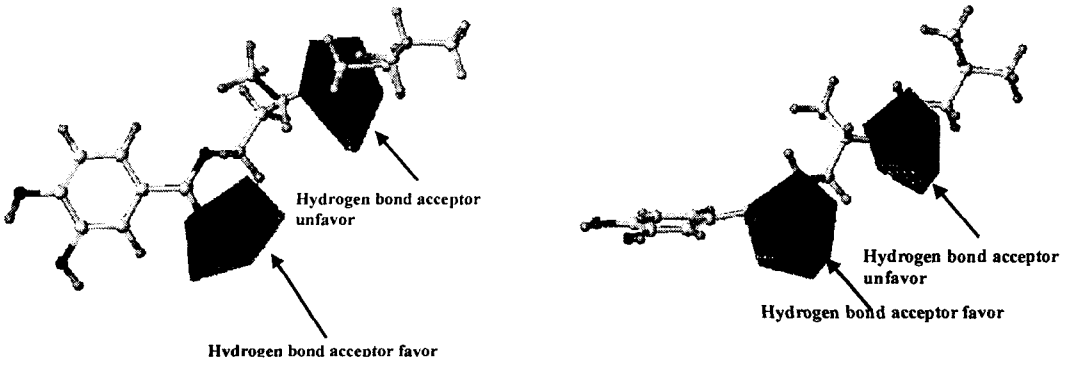
In the CoMSIA contour map of hydrogen bond donor (Figure 5), hydrogen bond donor favored areas are represented by dark sky blue polyhedral, the dark sky blue colored contour surrounding the R_1 group is increase of melanogenesis inhibitory activity by introducing the hydrogen bond donor in this region. From the CoMSIA field analyses, the hydrogen bond donor descriptors explain 17.1% of variance.



(1) Front view (2) Side view

Figure 5. CoMSIA contour maps of hydrogen bond donor for melanogenesis inhibitory activity. Dark sky blue contours refer to hydrogen bond donor favored regions.

In the CoMSIA contour map of hydrogen bond acceptor (Figure 6), hydrogen bond acceptor favored areas are represented by pink polyhedral, the pink colored contour surrounding the CO group (Figure 1) is increase of melanogenesis inhibitory activity by introducing the hydrogen bond acceptor in this region. In contrast, hydrogen bond acceptor disfavored areas are represented by dark red polyhedral, the dark red colored contour surrounding the R_1 group indicate that hydrogen bond acceptor in this region will decrease activity. From the CoMSIA field analyses, the hydrogen bond acceptor descriptors explain 8.6% of variance. Therefore, R_1 position were located to strong hydrophobicity, hydrogen bond donor groups and R_2 position were located to not only strong hydrophobicity but also less bulky. Surrounding the CO position should increased melanogenesis inhibitory activity because of substituents of the hydrogen bond acceptor.



(1) Front view (2) Side view

Figure 6. CoMSIA contour map of hydrogen bond acceptor for melanogenesis inhibitory activity. Pink contours refer to hydrogen bond acceptor favored regions and dark red contours indicated disfavored region.

Predictions for test set compounds.

From the CoMSIA model, compound that has higher melanogenesis inhibitory activity was predicted and database were constructed. To know agreements between predicted compounds and CoMSIA results, consecutively, calculation progresses were enforced repeatedly. As the results of prediction, the predicted values (pre.pI_{50}) were well agreed with observed values (obs.pI_{50}). Finally, correlations between predicted values and observed values were investigated (Table III).

Table III. Predicted values of test set compounds .

No.	R_1	R_2	Obs. pI_{50}[d]	Pred. pI_{50}[e]	Dev.[f]
1[a]	*N*-2-cyclohexylethyl	H	3.65	3.60	-0.05
2[a,c]	*N*-Phenyl	H	4.65	4.67	+0.02
3[b]	Propylphenyl	H	4.67	4.89	+0.22
4[b]	Benzyl	H	4.51	4.49	-0.02

[a]Amide, [b]Ester, [c]outlier, [d]observed values, [e]predicted values, [f]deviation between observed and predicted pI_{50}.

From a variety of analyses, a lot of new compounds were predicted and then Table IV showed the results of predicted and observed melanogenesis inhibitory activities of predicted compounds. Predicted values were calculated according to the CoMSIA model and melanogenesis inhibitory activities of predicted compounds from CoMSIA contour map were observed according to the same method. Observed values are very similar to predicted values. Therefore, we got to know that our prediction was right and then confirm the predicted compounds.

Table IV. Predicted and observed melanogenesis inhibitory activities for new alkyl-3,4-dihydroxy-5-substituted benzoate derivatives.*

R_1	R_2	Pred. pI_{50}[a]	Obs. pI_{50}[b]	Dev.[c]
6-methylheptyl	H	5.31	5.28	-0.03
n-pentylphenyl	H	5.34	5.30	-0.04
2-hydroxypentylphenyl	H	5.12	5.17	+0.05

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

Conclusions

Alkyl-3,4-dihydroxy-5-substituted benzoate derivatives and *N*-alkyl-3,4-dihydroxy-5-substituted benzamide 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 (CoMSIA). When cross-validation value (q^2) is 0.713 at four components, the pearson correlation coefficient (r^2) is 0.900. The CoMSIA results (n=40, s=0.153, F=79.612, r^2= 0.900) was obtained with the explanations in 53.8% of hydrophobicity field, 18.8% of steric field, 17.1% of hydrogen bond donor field, 8.6% of hydrogen bond acceptor field and 1.8% of electrostatic field, respectively. Melanogenesis inhibitory activity field for relative contribution (%) in molecular interaction field was explained by the order of hydrophobicity> steric field> hydrogen bond donor field > hydrogen bond acceptor field > electrostatic field.

As the results of prediction from the CoMSIA analyses, we could conclude that the melanogenesis inhibitory activity was increased by creation of R_1 substitution of 6-methylheptyl, n-pentylphenyl and 2-hydroxypentylphenyl group etc,. And finally predicted compound has good, above 5.17, biological activities. It was suggest that the QSAR technique is capable of providing a model for designing new melanogenesis inhibitors.

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