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CoMFA and CoMSIA 3D QSAR Studies on Pimarane Cyclooxygenase-2 (COX-2) Inhibitors

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Comparative molecular field analysis and comparative molecular similarity indices analysis were performed on twenty five analogues of pimarane COX-2 inhibitor to optimize their cyclooxygenase-2 (COX-2) selective anti-inflammatory activities.

Key words: 3D QSAR, Pimarane diterpenoids, COX-2 inhibitors

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) (Mantri *et al.*, 1994; Vane *et al.*, 1996) are of immense benefit in the treatment of inflammatory diseases. The principal pharmacological effects of NSAIDs are due to their inhibitory activity of cyclooxygenase which catalyze the oxidative conversion of arachidonic acid into prostaglandin H_2. (Smith *et al.*, 1996) It is well known that COX has two isoforms, i.e., COX-1 and COX-2. (Marnett, 2000) COX-1 is the constitutive isoform and is mainly responsible for the synthesis of cytoprotective prostaglandins (PG) in the gastrointestinal tract (GI) and of the proaggregatory thromboxane in blood platelets. (Allison *et al.*, 1992) COX-2 is inducible and short-lived; Its expression is stimulated in response to endotoxin, cytokines, and mitogen. (Kujubu *et al.*, 1991; Lee *et al.*, 1992) COX-2 plays a pivotal role in PG biosynthesis in inflammatory cells and in the central nervous cells. (Smith *et al.*, 1998) Thus, the identification of a novel COX-2 selective inhibitor should offer an excellent anti-inflammatory activity with minimal side effects including GI toxicity. (Laneuville *et al.*, 1994) Previously, we have reported acanthoic acid anologues as novel COX-2 inhibitors and their Structure-Activity Relationships (SAR) suggesting that the substitutions at C4 and C16 lead to the variations in COX-2 inhibition activity. (Suh, Y. G. *et al*, 2004)

In this work, we have conducted 3D-QASR studies on a series of acanthonic acid derivatives that act as COX-2 inhibitors, using two different methods: comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA). The resulting QSAR models were in good agreement with the experimental data of the COX-2 inhibitory activities of these compounds, and rationalized the SAR of acanthoic acid analogues as novel COX-2 inhibitors.

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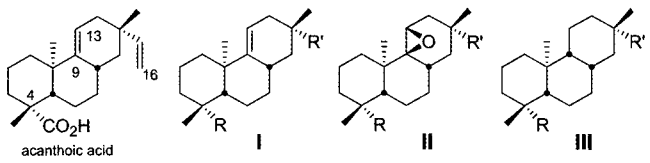
3D-QSAR Modeling

The chemical structures and COX-2 inhibitory activities of the twenty five analogues are given in Table I. The activities were expressed as log(IC_{50}) values in Table III, and 3D-QSAR by CoMFA and CoMSIA were carried out using SYBYL 6.8. The partial least squares (PLS) method was used to derive predictive relationship models, and the analyses were conducted by correlating variations in the compounds activities with variations in their CoMFA or CoMSIA fields

Molecular 3D structure building

All molecules were minimized using Tripos forcefield parameters and the conjugate gradient algorithm with a gradient convergence value of 0.005 kcal/mol Å. Partial atomic charges were calculated using the Gasteiger-Hückel method. Low energy conformation was searched by a systematic conformational search.

Table I. The chemical structures and biological activities of acanthoic acid analogues



Compds	Type	R[a]	R'	$IC_{50}(\mu M)$[b]
1	I	CO_2H	$CHCH_2$	790.4
2	I	$CH=CHCO_2H$	$CHCH_2$	69.7
3	I	$CH_2CH_2CO_2H$	$CHCH_2$	105
4	III	CO_2H	CH_2CH_3	425
5	II	CO_2H	$CHCH_2$	3144
6	II	CO_2H	oxirane	2492
7	I	CH_2CO_2H	$CHCH_2$	82.3
8	I	CONHOH	$CHCH_2$	818.9
9	I	$(CH_2)_4CO_2H$	$CHCH_2$	38.7
10	I	$CH_2CH=CHCO_2H$	$CHCH_2$	32
11	I	$(CH_2)_3CO_2H$	$CHCH_2$	49.4
12	I	CO_2H	CH_2CH_2OAc	1420
13	I	$(CH_2)_2CONHSO_2CH_3$	$CHCH_2$	179.8
14	I	$CH_2CH=CHCH=CHCO_2H$	$CHCH_2$	20.7
15	I	$(CH_2)_3CH=CHCO_2H$	$CHCH_2$	37.8
16	I	$(CH_2)_5CO_2H$	$CHCH_2$	27.5
17	I	$CH=CH-CH=CH-CH=CHCO_2H$	$CHCH_2$	25.4
18	I	$(CH_2)_6CO_2H$	$CHCH_2$	60.1
19	I	CO_2H	CH_2CH_2F	1679.5
20	I	COF	CH_2CH_2F	193.7
21	I	CO_2H	CH_2CHF_2	796
22	I	$CH=CH-CH=C(CH_3)CO_2H$	$CHCH_2$	197
23	I	$CH_2CH=C(CH_3)CH=C(CH_3)CO_2H$	$CHCH_2$	83.2
24	I	$CH_2CH=C(CH_3)CH_2CH(CH_3)CO_2H$	$CHCH_2$	81.7
25	I	$CH_2CH=CH-CH=CHCONHSO_2CH_3$	$CHCH_2$	102.9

[a]All olefin bonds have an *E* geometry. [b]*in vitro* COX-2 inhibitory activity. (The purified COX-2 enzyme assay was performed according to Bohlin protocol with a slight modification)

Alignment

The most active compound 17 was used as the template, and the lowest energy conformations of the remaining molecules were aligned with respect to the core structure, fragment 1 (Fig. 1). The alignment of all twenty five compounds is shown in Fig. 2.

CoMFA method

The CoMFA analysis was carried out using the standard options of SYBYL 6.8. A total of twenty five analogues (Table III) were divided into two groups; a training set consisting of twenty compounds and a test set consisting of five compounds. Both sets contain low, moderate, and high activity compounds in approximately equal proportions.

The results of CoMFA are shown in Table II and Fig. 3, and the actual, predicted activities, and residuals are shown in Table III.

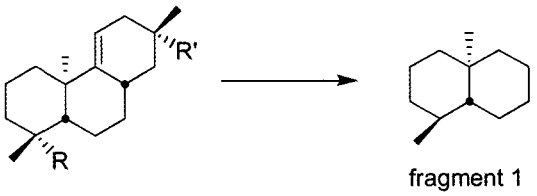


Fig. 1. The structure of fragment 1

Fig. 2. The alignment of pimarane analogues

Table II. Summary of CoMFA and CoMSIA results of the training set

Fields	CoMFA	CoMSIA (SEHDA)
Opt. No. of components	3	3
Probe atom	$C(SP^3,+1)$	$C(SP^3,+1)$
Cross-validated r^2	0.733	0.847
Standard error of estimate	0.205	0.118
Conventional r^2	0.933	0.978
F values	73.901[a]	232.302[b]

[a]F-test value and Prob. of $R^2=0$ (n1=3, n2=16) [b]F-test value and Prob. of $R^2=0$ (n1=3, n2=16)

CoMSIA method

The same grid constructed for the CoMFA calculation was used for the CoMSIA field calculation. The CoMSIA combined with five fields (steric, electrostatic, hydrophobic, hydrogen-bond acceptor and donor properties; SEHDA) was performed employing the standard options of SYBYL 6.8. The results of CoMSIA are shown in Table II and Fig. 3, and the predicted activities, and residuals are shown in Table III.

Table III. Actual and predicted activities and residuals from CoMFA and CoMSIA analysis

Compds	Actual Log(IC_{50})	CoMFA		CoMSIA (SEHDA)[b]	
		Predictied	Residual	Predictied	Residual
1	2.90	2.74	0.16	2.85	0.05
3	2.02	2.01	0.01	2.06	-0.04
5	3.50	3.24	0.26	3.29	0.21
6	3.40	3.39	0.01	3.54	-0.14
7	1.92	2.42	-0.50	2.05	-0.13
8	2.91	2.74	0.17	3.00	-0.09
9	1.59	1.75	-0.16	1.65	-0.06
10	1.51	1.79	-0.28	1.54	-0.03
11	1.69	1.56	0.13	1.66	0.03
12	3.15	3.32	-0.17	3.20	-0.05
13	2.25	2.10	0.15	2.09	0.16
14	1.61	1.84	-0.23	1.78	-0.17
15	1.58	1.51	0.07	1.57	0.01
16	1.44	1.29	0.15	1.47	-0.03
17	1.40	1.37	0.03	1.37	0.03
18	1.78	1.77	0.01	1.72	0.06
19	3.23	3.20	0.03	3.09	0.14
21	2.90	2.96	-0.06	2.99	-0.09
23	1.92	1.79	0.13	1.73	0.19
25	2.01	1.91	0.10	2.05	-0.04
2[a]	1.84	2.69	-0.85	2.56	-0.72
4[a]	2.63	3.03	-0.40	2.94	-0.31
20[a]	2.29	3.08	-0.79	2.22	0.07
22[a]	2.29	1.56	0.73	1.89	0.40
24[a]	1.91	1.35	0.56	1.71	0.20

[a]Test set [b]CoMSIA with different field combinations such as steric (S), electrostatic (E), hydrophobic (H), donor (D), and acceptor (A) fields.

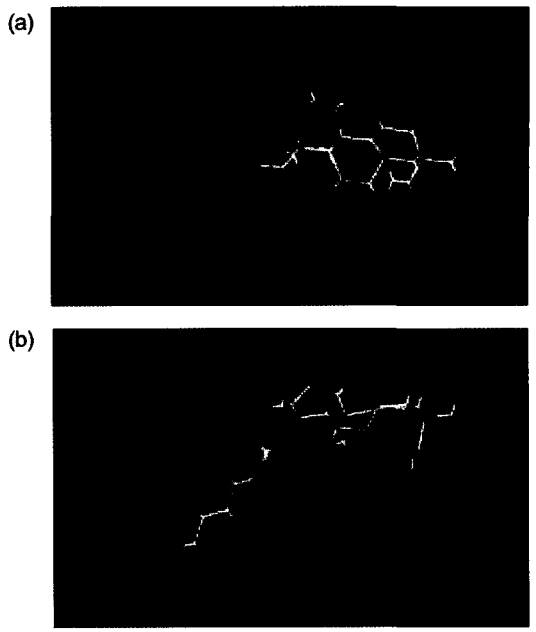


Fig. 3. (a) Contour maps from the final CoMFA analysis. Steric con-tour map. Green contours refer to sterically favored regions. Electro-static contour map. Blue contours refer to regions where negatively charged substituents are disfavored; red contours indicate regions where negatively charged substituents ard favored. (b) Contour maps from the final CoMSIA(SEHDA) analysis. Yellow contours refer to regions where hydrophobic substituents are favored. Red contours refer to regions where hydrogen bonding acceptor are disfavored. Purple contours refer to regions where hydrogen bonding donor are disfavored. Green contours refer to sterically favored regions.

RESULTS

CoMFA analysis

The CoMFA study on the test set gave a good cross-validated value of 0.733 (q^2) with an optimized components of 3, the conventional correlation coefficient is r^2=0.933, F=73.901, and the estimated standard error is 0.205. The steric and electrostatic field contributions are 60:40 indicating a nearly equal influence of these two fields on ligand-receptor interactions. The CoMFA steric and electrostatic fields for the analysis are presented as contour map in Fig. 3(a). For reference, analogue 17 is displayed in the map. The bulky substituents in the regions (shaded green) at C4 of pimarane compound are well expected to enhance the COX-2 inhibitory activity.

The electronegative substituents in regions (shaded red) at C ring and C4 of pimarane analogue are anticipated to increase the COX-2 inhibitory activity by the enforced electrostatic interaction of the ligand and the active site of COX-2 enzyme, which was shown in the previous docking model of COX-2 and acanthoic acid. (Suh *et al.*, 2001)

CoMSIA analysis

The CoMSIA model was generated with five field combinations (SEHDA). A correlation coefficient of r^2 = 0.978 and a cross-validated coefficient of q^2 = 0.847 were obtained (Table II). CoMSIA analyis provided a better 3D-QSAR model, and is revealed by the small deviation of the calculated from the experimental values of IC_{50} (Table III). The contour map of CoMSIA(SEHDA) analysis are presented in Fig. 3(b). The hydrophobic substituents in the regions (shaded yellow) at the C4 linker and C16 of the pimarane skeleton are expected to increase COX-2 inhibtory activity. The hydrogen bonding donor group (cyan region) is likely to increase the inhibitory activity, while the hydrogen bonding donor group (purple region) is likely to decrease the inhibitory activity. The red and the purple contours show the regions of negative hydrogen bonding acceptor and donor interactions, respectively.

CONCLUSION

CoMFA and CoMSIA analysis of the twenty-five pimarane analogues produced the good models with the high predictive abilities. The CoMSIA model showed the improved prediction abilities in comparison with the CoMFA model. It is well revealed that the COX-2 inhibitory activity is influenced by the character of steric, electrostatic, hydrophobic, hydrogen bonding donor, and hydrogen bonding acceptors, particularly, at the C4 linker and C16 of the pimarane skeleton. These results are highly consistent with our previous SAR studies (Suh *et al.*, 2001) and provide the crucial information for the design and development of new pimarane COX-2 inhibitors as the excellent anti-inflammatory agents. Currently, the development of the new and potent COX-2 inhibitors based on the 3D QSAR of acanthoic acid is in good progress. The updated results will be reported in due courses.

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