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A 3D-QSAR STUDY ON GINKGOLIDES AND THEIR ANALOGUES WITH COMPARATIVE MOLECULAR FIELD ANALYSIS

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Abstract Comparative molecular field analysis (CoMFA), a three-dimensional quantitative structure-activity relationship (3D-QSAR) paradigm was used to study the correlation between the physicochemical properties and the *in vitro* bioactivities of ginkgolide analogues. The correlation derived from CoMFA analysis has a good predictive capability. Based on the result of CoMFA analysis, we designed some compounds. Pharmacological assay indicated that three of these new designed compounds are 2 and 4 times more potent than that of ginkgolides.

Introduction

Platelet activating factor (PAF) is a potent bioregulator which appears to play a key role in *acute inflammation, asthma, ischemic injury* and *tissue rejection* through its action at high affinity receptors (EC_{50}~10^{-10}M)[1]. Consequently, the development of PAF antagonists which are suitable for therapeutic use has assumed considerable importance. Among the known types of PAF antagonists ginkgolide B is especially interesting because of its long history of human user, its notable task of toxicity, and its total resistance to metabolism. Review of the therapeutic potential of ginkgolide B, the limited amounts of ginkgolide available from the *ginkgo tree*, and the poor absorption of orally administered ginkgolide B., Corey et al[2] investigated a range of synthetic analogues to provide insights regarding the structural features of ginkgolide B which enhance anti-PAF activity. In order to obtain further insights into the structural requirements of the PAF receptor, we carried out 3D-QSAR studies using the comparative molecular field analysis (CoMFA)[3] method. The CoMFA model can describe the steric and electrostatic requirements for recognition forces characterizing receptor site.

Here we present studies which applied CoMFA methodology to rationalize the structure-activity relationship of ginkgolides and their analogues. The "alignment rule", the positioning of the ligand within the fixed lattice, is by far the most important input variable in CoMFA since the relative interaction energies between the ligand and the PAF receptor depend strongly on their relative molecule position. Furthermore, in the case of conformational flexible molecules, CoMFA requires that a single conformation be selected for each molecule. In this work, 25 molecules were studied: most of them are composed of rigid condensed ring and some flexible side chain, and ginkgolide B was used as the template family for the alignment rules. The CoMFA model was used finally to identify a putative bioactive conformation of the different ligands from

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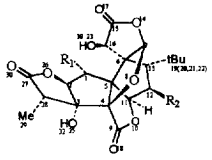
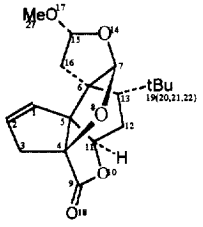
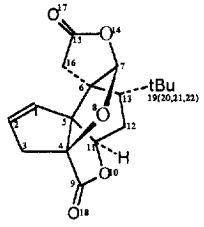
Table 1. The structure and bioactivitiesof ginkgolides and analogues



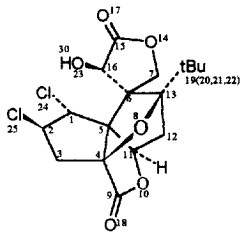
1. R_1=O(24)H(31), R_2=H (0.6μM)
2. R_1=H, R_2=H (17.8μM)
3. R_1=O(24)H(31), R_2=O(26)H(17.0μM)
4. R_1=H, R_2=O(26)H (129.6μM)



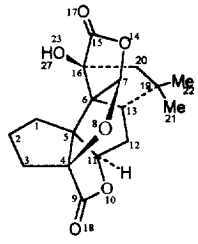
5 (120.0μM)



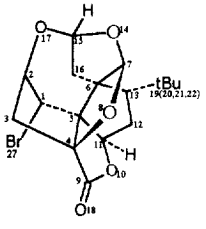
6 (80.0μM)



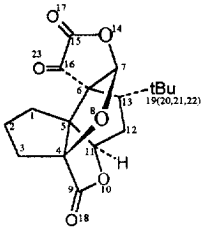
7 (38.0μM)



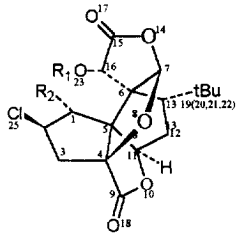
8 (9.2μM)



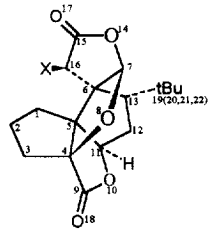
9 (2.9μM)



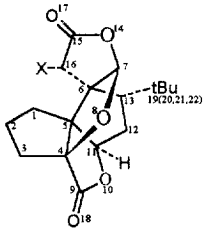
10 (18.0μM)



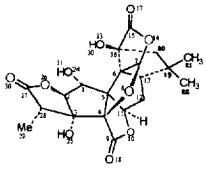
11. R_1=H, R_2=H (1.1μM)
12. R_1=OCH_2OCH_3, R_2=OCH_2OCH_3 (0.3μM)
13. R_1=H, R_2=Cl (1.1μM)
14. R_1=OCH_2OCH_3, R_2=Cl (0.3μM)



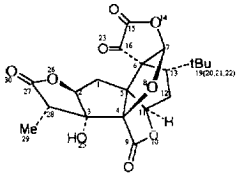
15. X=OH (76.0μM)
16. X=$OCOCH_3$ (13.0μM),
17. X=OCH_2OCH_3 (9.4μM)



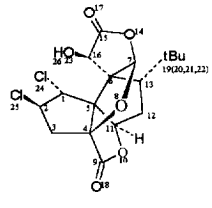
18. X=OH (13.0μM)
19. X=$OCOCH_3$ (11.0μM)
20. X=OCH_2OCH_3 (21.0μM)



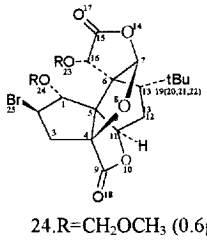
21 (0.7μM)



22 (3.9μM)



23 (1.3μM)



24. R=CH_2OCH_3 (0.6μM)
25. R=H (14.0μM)

the possible alternatives. In order to assess better the robustness of the 3D-QSAR, some new analogues of ginkgolide have been synthesized according to the CoMFA model. The observed values of binding affinity of these new compounds against PAF are higher than the lead, ginkgolide B.

Method

A serious of 25 ginkgolides and their analogues are chosen. Their structure and bioactivity are show in Table 1.

Molecular 3D Structure Building The entire set of ginkgolide analogues was built using the SKETCH option in SYBYL 6.2[4] starting from X-ray coordinates data of ginkgolide B and fully geometry optimized using the standard TRIPOS molecular mechanics force field, with a 0.001 kcal/mol energy gradient convergence criterion and a distance-dependent dielectric constant on silicon graphic IRIS indigo XZ4000 computer system. The search routine of SYBYL was used for the systematic conformational search of the side chain of the studied compound. The conformation corresponding to local energy was selected by quantum chemical calculation with semiempirical quantum chemistry methods AM1 and PM3. While it is recognized that the low-energy conformations may not necessarily be adopted in the drug-receptor complex, the use of a reasonable low-energy conformation in the alignment is a useful starting point for statistical comparisons of flexible structures within the SYBYL CoMFA model.

CoMFA: Alignment Rule It is very important for CoMFA analysis to select a proper alignment rule. The training compounds are mainly composed of condensed rings and some side chains. We selected the condensed ring as the pairs between the molecules for alignment in the CoMFA analysis because the rigid condensed ring of the ginkgolides' compounds are almost unchanged. In addition, we selected some important atoms which formed the side chains, i.e., the oxygen atoms(O_{17}, O_{18}, O_{23} and O_{24}), atom C_{19}, to be as the alignment pairs between two molecules.

CoMFA: Interaction Energies and Regression Technic The CoMFA study was carried out on Silicon Graphic IRIS XZ4000 computer system running SYBYL 6.2/CoMFA routine. The steric and electrostatic field energies (AM1 charge) were calculated using an sp^3 carbon probe atom with a charge of +1 and a distance-dependent dielectric constant at all intersection of a regularly-spaced (0.2nm) grid. Steric and electrostatic contributions were truncated at 30 kcal/mol. Regression analysis was performed using cross-validation of compounds leave-one out method. The optimal number of component to be used in the non-cross-validated(conventional) analyses was defined as that which yielded the highest cross-validated r^2 value. For component models with identical values, the component number producing the smallest standard error of prediction(SEP) was selected. All cross-validated analyses were performed with a minimum σ (column filter) value of 2.00 kcal/mol.

Results and Discussion

CoMFA analysis The first CoMFA of 25 compounds gave a poor cross-validated r^2_{cross} value of 0.542. Residual values of activity showed that compound 25 was at least partially responsible for the poor r^2_{cross} (Table 2). Omission of the analogue 25 from the 25 compounds led to r^2_{cross}=0.732. The conventional correlation coefficient was r^2=0.928, F=68.851 and standard error estimate was 0.218 (Table 2). The last predictive result

and residual values are presented in Table 3 and Figure 1. These values indicating a good conventional statistical correlation have been obtained, and we also found that the resultant CoMFA model had a fair predictive ability.

Table 2. CoMFA analysis results about the analogues of GINKGOLIDE

		cross-validated		Conventional		
	compounds number	r^2_{cross}	Optimal component	r^2	s	F
model-A	25	0.574	4			
model-B	24	0.723	4	0.928	0.218	68.851

Table 3. Experimental activities and predictive activities with the model of CoMFA

compounds	EAa	model -A		model-B	
		PAb	δc	PAb	δc
1	6.22	5.78	0.44	5.63	0.59
2	4.75	4.80	-0.05	4.71	0.04
3	4.77	5.11	-0.34	5.08	-0.31
4	3.89	4.09	-0.02	4.04	-0.15
5	3.92	3.87	0.05	3.85	0.07
6	4.07	4.05	0.02	3.92	0.15
7	4.42	4.49	-0.07	4.48	-0.06
8	5.04	4.83	0.21	5.16	-0.12
9	5.54	5.78	-0.24	5.41	0.13
10	4.74	4.77	-0.03	4.64	0.01
11	5.96	5.70	0.26	5.98	-0.02
12	6.52	6.38	0.14	6.34	0.18
13	6.40	6.01	0.39	6.32	0.08
14	6.70	6.94	-0.24	6.69	0.01
15	4.12	4.28	-0.16	4.27	-0.15
16	4.89	4.93	-0.04	5.02	-0.13
17	5.03	5.00	0.03	5.03	-0.00
18	4.89	4.55	0.34	4.59	0.30
19	4.96	4.90	0.06	5.21	-0.25
20	4.68	4.65	0.03	4.70	-0.02
21	6.15	6.01	0.14	6.34	-0.19
22	5.41	5.32	0.09	5.41	0.00
23	5.89	6.30	-0.41	6.08	-0.19
24	6.22	6.23	-0.01	6.29	-0.07
25	3.89	4.09	-0.20	-	-

CoMFA coefficient contour maps The QSAR produced by CoMFA, with its hundreds of thousands of terms, was usually represented as a 3D "coefficient contour" map. The CoMFA steric and electrostatic fields for the analysis are presented as contour plots in Figure 2. To aid in visualization, the potent ester analogue of ginkgolde B is displayed in each of the maps.

In general, the color polyhedra in the maps surrounded all lattice points where the QSAR strongly associated changes in the analogues' field values with changes in anti-PAF potency. Green polyhedra surrounded regions

where more bulk is "good" for increasing potency while yellow polyhedra surrounded regions where less bulk is "good". Red and blue contours showed regions of desirable negative and positive electrostatic interaction, respectively.

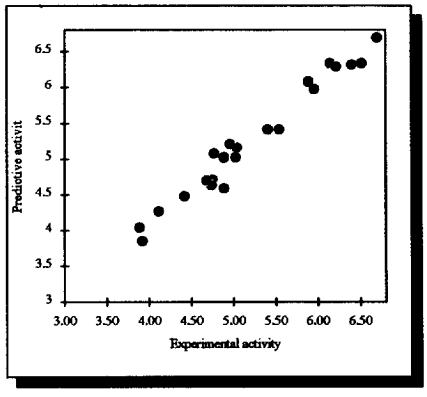


Figure 1. Experimental activities vs predictive values of ginkgolide analogs

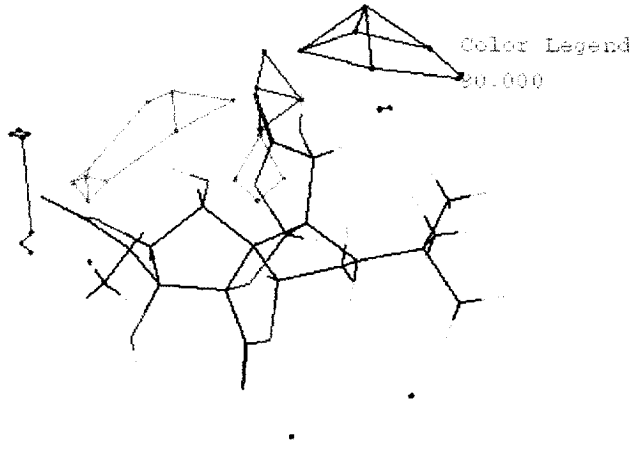
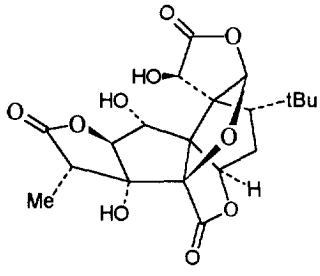


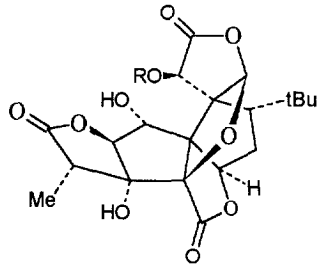
Figure 2. CoMFA contour maps from analysis. Sterically favored areas (contribution level of 80%) in green color. Sterically unfavored areas (contribution level of 20%) in yellow color. Positive potential favored areas (contribution level of 80%) in blue color. Positive potential unfavored areas (contribution level of 20%) in red color.

Of particular interesting we noted that green color polyhedra surrounded around atoms 1,2 of ginkgolide B (Figure 2). This means more bulk and hydrophobic substituents are beneficial to the activity. According to this structural modification clue, we have designed and synthesized some new analogues of ginkgolide B. Pharmacological assay released that a serious of new analogues with higher activities were involved in our designed compounds. Here we reported three new analogues that are more active than that of lead ginkgolide B as PAF antagonists, respectively (Table 4).

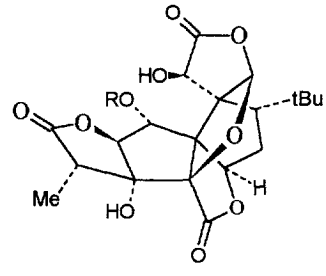
Table 4. Anti-PAF bioactivity of some new compounds and ginkgolide B



Ginkgolide B



New1



New2

Compounds	R	IC_{50} (μM)
ginkgolide B		0.128
New 1	$PhCH_2OCH_2$-	0.0404
New 2a	$PhCH_2OCH_2$-	0.0588
New 2b	p-$ClC_6H_5CH_2$-	0.0289

References:

1. Hanahan, D.J., *Ann Rev. Biochem.*, 1986, **55**:483
2. Corey, E.J., V. Gavai Ashvinikumar, *Tetrahedron Lett.*, 1989, **30**(50):6959
3. Cramer III, R. D., Patterson, D.E., Bunce J.D., *J. Am. Chem. Soc.*, 1988, **110**: 5959
4. Sybyl [computer program]. version 6.2. St Louis (MO): Tripos Associates, 1995