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3D-QSAR study on ether and ester analogs of artemisinin 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 correlations between the physicochemical properties and the *in vitro* activities of a series of ether and ester analogs of artemisinin. Four alignment models were used in the CoMFA investigation. The correlations derived from CoMFA analysis with the four alignments proved all to have good predictive values. The steric field predictive model of alignment B is accordant with the experimental results of Avery M A, *et al*: J Med Chem 1993; **36**: 4264 — 75. The electrostatic field predictive results of alignments A, B, and C are consistent with our previous result of quantum chemical calculation. The highest r^2_{cross} of alignment D, indicated that the side chain of $-C_8-O_2-O_1-C_{10}-O_3-C_7-O_4-C_{12}-O_5-$ and atom C_{14} are important groups of artemisinin analogs for antimalarial activity.

KEY WORDS artemisinin; antimalarials; molecular models; 3D-structure-activity relationship

Artemisinin (qinghaosu, 1), a unique sesquiterpene, isolated from Chinese herb *Artemisia Annua* L, is an antimalarial with novel structure, high potency, and low toxicity. Its antimalarial activity, especially against *Plasmodium falciparum*, provided a major lead in an area where resistance to existing drug treatment is increasing alarmingly[1-3].

Our Institute has modified the structure of 1 and synthesized a series of ether and ester derivatives[4]. With pharmacological screen, the quantitative biological activity, designated by SD_{90} (Fig 1), was determined[5]. Dihydroartemisinin (2) and its ether and ester analogs (3) were more active than 1.

In our previous studies on the ether and ester analogs of artemisinin with quantum chemical methods, we have suggested a pharmacophore and an interaction model of the drugs with receptor[6]. In order to demonstrate the relationship between the three-dimensional structure properties and the activities *in vitro*, we carried out a three-dimensional (3D) quantitative structure-activity relationship (3D-QSAR) study on the ether and ester analogs of artemisinin with comparative molecular field analysis (CoMFA). Based on the calculation results, the 3D-QSAR model of artemisinin analogs was derived, which could be used to explain the differences of biological activity among artemisinin analogs by the steric and electrostatic fields present to the receptor, presented the suggestion of structure modification to the artemisinin analogs. The 3D-QSAR derived from CoMFA proved to have good predictive ability and is in consistent with the experimental result of Avery and coworkers[7] and our previous quantum chemical calculation result[6].

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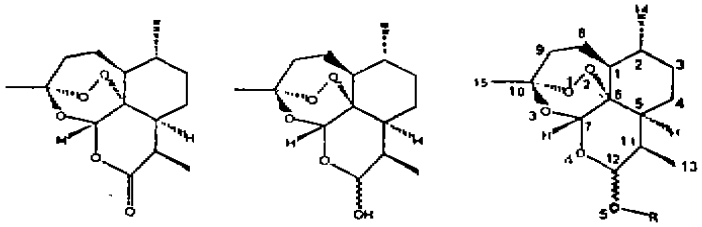
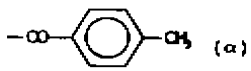
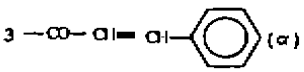
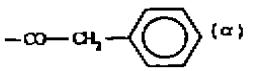


1 2 3

C_{16} connects with O_5

Comp	R	SD_{90}/mg.kg.$^{-1}$.d^{-1}	-lgC*
DH	-H(β)	3.65	4.89
SM 229	$-CH_3$(α)	1.16	5.41
SM 224	$-CH_3$(β)	1.02	5.47
SM 227	$-C_2H_5$(β)	1.95	5.21
SM 220	$-n-C_3H_7$(β)	1.70	5.28
SM 245	$-CH(CH_3)_2$(β)	2.24	5.16
SM 247	$-CH_2CH_2CH(CH_3)_2$(β)	5.65	4.80
SM 105	$-COCH_3$(α)	1.20	5.44
SM 108	$-COC_2H_5$(α)	0.66	5.71
SM 241	$-COC_3H_7$(α)	0.65	5.74
SM 223	$-COOC_2H_5$(α)	0.63	5.75
SM 242	$-COOC_3H_7$(α)	0.50	5.87
SM 242a	$-COOC_3H_7$(β)	1.32	5.45
SM 273		1.73	5.37
SM 233		0.74	5.75
SM 374		0.95	5.62
SM 280	$-CH_2CH_2OCH_3$(α)	2.28	5.18
SM 232		3.42	5.04
SM 249	$-CH_2CH_2OCH_3$(β)	4.10	4.92
SM 272		6.40	4.76
SM 270		4.70	4.89
SM 277	$CH_2CH_2OCH_2CH_2OCH_3$(β)	3.71	5.02

* C = SD_{90}/(molecular weight) × 1000 (mol)

Fig 1. Compounds and their antimalarial activities.

Comparative molecular field analysis is a recent development in the area of 3D-QSAR[8]. The underlying assumption in CoMFA is that since the interactions between a ligand and its receptor are primarily non-bonded interactions, differences in biological activities among a series of drug molecules can be explained by differences in the steric and electrostatic fields present to the active site of their receptor. In a CoMFA study, molecules are represented by their steric and electrostatic sampled at the intersection of a three-dimensional lattice, calculated parameters are the energies of steric (van der Waals 6—12) and electrostatic (Coulombic, with an r^{-1} dielectric) interaction between the compound of interest and a "probe atom" placed at the various intersections of a regular three-dimensional lattice, large enough to surround all of the compounds in the series. The data is analyzed using Partial Least Squares (PLS)[9] and with cross - validation . The derived 3 D - QSAR model is likely to have predictive validity.

METHODS

A series of 22 artemisinin ether and ester analogs were chosen. Their structural formulae, antimalarial activities SD_{90}, and $-lgC$ are shown in Fig 1.

Molecular 3D structure building The 3D structures of compounds were constructed on Silicon Graphic IRIS Indigo XZ4000 computer system with molecular modeling software SYBYL 6. 0[10]. The rigid conforms of tetracyclic nucleus were represented by the conform of artemisinin's crystal structure. The Search routine of SYBYL was performed for the systematic conformational search of the side chain of the studied compound. The conformation corresponding to global energy was selected by quantum chemical calculation with semiempirical quantum chemistry methods AM1[11] and PM3[12]. The optimized configurations of the artemisinin's analogs were obtained, on which a CoMFA analysis was performed.

CoMFA: alignment rule It is very important for CoMFA analysis to select a proper alignment rule[13], and 4 alignments were selected. From the result of our previous study[6], the probable pharmacophore of the ether and ester analogs of artemisinin was obtained, We selected the atoms which formed the pharmacophore, i e, the oxygen atoms (O_1 and O_2) of the peroxide linkage and atom C_{16} which linked O_5 (Fig 2), as the pairs between the molecules for alignment in the least square-fit protocol[8] (alignment A). We selected the tetracyclic nucleus as the pairs between the molecules for alignment in the CoMFA analysis because the rigid tetracyclic nucleus of the artemisinin analogs are almost unchanged (alignment B). Combined alignments A and B, we selected O_1, O_2, O_3 and C_{16} as the pairs between the molecules in the CoMFA analysis (alignment C). Because the chain of $-C_6-O_2-O_1-C_{16}-O_5-C_7-O_4-C_{12}-O_5-$ is a very important substructure for antimalarial activity[2], we selected this chain and atom C_{16} as the alignment pairs between molecules for CoMFA analysis (alignment D).

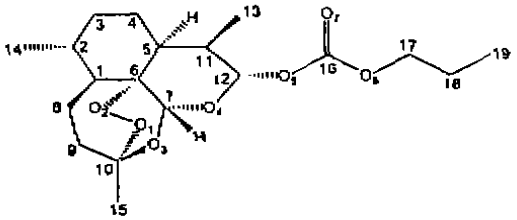


Fig 2. Atom ID of SM242.

CoMFA: interaction energies and regression technic All CoMFA studies were carried out on Silicon Graphic IRIS Indigo XZ 4 0 0 0 computer system running SYBYL 6. 0/CoMFA rou tine. The steric and electrostatic field energies (AM1 charge[11]) 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. 2 nm) grid. Steric and electrostatic contributions were truncated at 30 kcal · mol^{-1}. All regression analyses were done using Partial Least Squares (PLS) algorithms in SYBYL 6. 0. Initial analyses were performed using cross-validation of compounds leave-one out method and 8 principal

components. 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^{-1}.

RESULTS AND DISCUSSION

CoMFA analysis For alignment A, The first CoMFA of 22 compounds gave a poor cross-validated r^2 (r^2_{cross}) value of 0.186 (Tab 1). Residual values of activity showed that 3 compounds, SM272, DH, and SM270, were at least partially responsible for the poor r^2_{cross}. Omission of the analog SM272 from the 22 compounds led to $r^2_{cross} = 0.494$, and residual values for DH was at a highest value of -0.657. Recalculation without SM272 and DH gave an $r^2_{cross} = 0.663$, and a residual value for SM272 was at the highest value of -0.395. The next CoMFA analysis without the above 3 compounds resulted in an $r^2_{cross} = 0.713$ with 5 optimal components. The conventional correlation coefficient was $r_2 = 0.992$, $F = 327.7$, and the standard error estimate was 0.032. The last predictive result and residual values are presented in Tab 2 and Fig 3.

Tab 1. CoMFA for artemisnin ether and ester analogs of alignment A, B, C, and D.

Alignment	Compound number	Cross-validated r^2	Cross-validated optimal component	Conventional r^2	Conventional s	Conventional F
A	22	0.186	1	0.473	0.256	18.0
	21[a]	0.494	5	0.989	0.040	264.1
	20[b]	0.663	5	0.988	0.041	235.6
	19[c]	0.713	5	0.992	0.032	327.7
B	22	0.281	2	0.754	0.180	29.1
	21[a]	0.498	1	0.650	0.201	35.2
	20[b]	0.622	2	0.902	0.107	78.0
	19[d]	0.717	3	0.984	0.047	157.5
C	22	0.343	5	0.964	0.075	85.0
	21[a]	0.519	5	0.978	0.057	131.8
	20[b]	0.621	5	0.976	0.059	112.4
	19[e]	0.662	5	0.990	0.036	255.5
	18[f]	0.713	5	0.989	0.038	206.8
D	22	0.316	5	0.977	0.060	136.3
	21[a]	0.522	5	0.985	0.047	195.1
	20[b]	0.652	5	0.986	0.045	191.8
	19[d]	0.744	5	0.986	0.046	182.9
	18[g]	0.774	5	0.987	0.043	185.8

[a] Omit SM272 from the 22 compounds. [b] Omit SM272 and DH from the 22 compounds.
[c] Omit SM272, DH and SM247 from the 22 compounds. [d] Omit SM272, DH and SM270 from the 22 compounds.
[e] Omit SM272, DH and SM247 from the 22 compounds. [f] Omit SM272, DH, SM232 from the 22 compounds.
[g] Omit SM272, DH, SM242a, and SM270 from the 22 compounds.

Tab 2. Experimental activities and predictive activities with the model of CoMFA.

Compd	EA[a]	Alignment A		Alignment B		Alignment C		Alignment D	
		PA[b]	δ[c]	PA[b]	δ[c]	PA[b]	δ[c]	PA[b]	δ[c]
DH	4.89								
SM 229	5.51	5.388		5.409	0.001	5.396	0.014	5.401	0.009
SM 224	5.47	5.425	0.022	5.487	−0.017	5.467	0.003	5.430	0.040
SM 227	5.21	5.283	0.045	5.255	−0.045	5.270	−0.060	5.288	−0.078
SM 220	5.28	5.276	−0.073	5.229	0.051	5.287	−0.007	5.230	0.050
SM 245	5.16	5.176	0.004	5.174	−0.014	5.134	0.026	5.194	−0.034
SM 247	4.80		−0.016	4.790	0.010			4.783	0.017
SM 105	5.44	5.474	0.035	5.522	−0.082	5.479	−0.039	5.476	−0.036
SM 108	5.71	5.675	0.004	5.603	0.107	5.645	0.065	5.625	0.085
SM 241	5.74	5.736	−0.005	5.723	0.017	5.737	0.003	5.723	0.017
SM 223	5.75	5.755	0.022	5.758	−0.008	5.788	−0.038	5.770	−0.020
SM 242	5.87	5.848	0.005	5.875	−0.005	5.847	0.023	5.882	−0.012
SM 242a	5.45	5.445	0.008	5.446	0.004	5.435	0.015		
SM 273	5.37	5.362	−0.034	5.358	0.012	5.396	−0.026	5.377	−0.007
SM 233	5.75	5.784	−0.012	5.786	−0.036	5.725	0.025	5.748	0.002
SM 374	5.62	5.632	−0.005	5.585	0.035	5.649	−0.029	5.648	−0.028
SM 280	5.18	5.185	0.020	5.218	−0.038	5.179	0.001	5.188	−0.008
SM 232	5.04	5.020	−0.005	5.053	−0.013			5.039	0.001
SM 249	4.92	4.925		4.916	0.004	4.954	−0.034	4.909	0.011
SM 272	4.76		0.008						
SM 270	4.89	4.882	0.012			4.863	0.027		
SM 277	5.02	5.008		5.002	0.018	4.988	0.032	5.030	−0.010

[a] Experimental activities, [b] Prediction, [c] Residual values.

For alignments B—D, the CoMFA analyses was carried out with similar methods to omit the compounds with the highest residual values (Tab 1). The last predicative activities and residual values are presented Tab 2 and in Fig 3.

These values indicated a good conventional statistical correlation, and we also found that the resultant CoMFA model had a fair predictive ability. But from the r^2_{cross} values, alignment D was the best model. The chain of $-C_6-O_2-O_1-C_{10}-O_3-C_7-O_4-C_{12}-O_5-$ and atom C_{16} were definitely important sub-structure for antimalarial activity of artemisinin analogs.

CoMFA coefficient contour maps The QSAR produced by CoMFA, with its hundreds or thousands of terms, was usually represented as a 3D "coefficient contour" map. The CoMFA steric and electrostatic fields for the analysis based on alignments A − D are presented as contour plots in Fig 4. To aid in visualization, the potent ester analog of artemisinin SM 242 is displayed in each of the maps. Fig 2 shows the SM242's atom ID.

In general, the color polyhedra in the maps surrounded all lattice points where the QSAR strongly associated changes in the analogs' field values with changes in antimalarial potency. Green polyhedra surrounded regions where more bulk is "good" for increasing potency while yellow polyhedra surrounded regions where less bulk was "good." Red and blue contours showed regions of desirable negative and positive electrostatic interaction, respectively. All the suggested structural modifying information by above 4 alignment models was around the side chain of atom C_{12}. The steric fields were similar, and

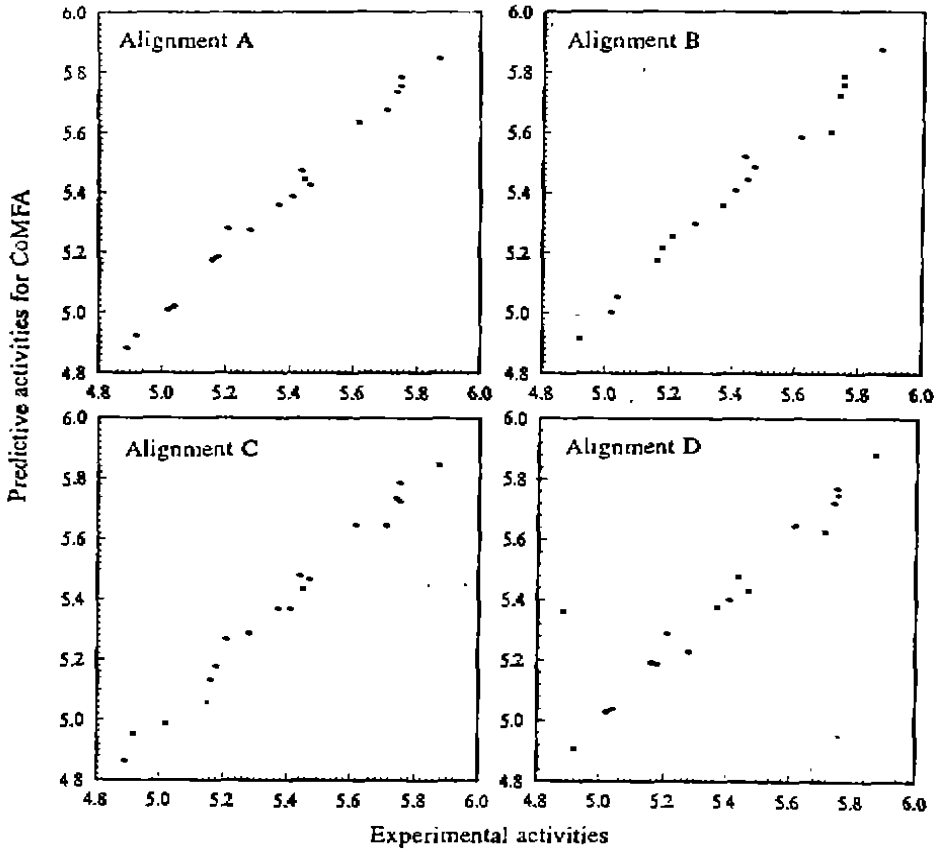


Fig 3. Experimental activities *vs* predictive values of artemisinin analogs.

bulky substituents could be added at the terminal of the side chain which might increase the activity. The steric contour map of alignment B (Fig 4, Plate 1) suggested that buck group was favorable for increasing potency near the substituted methyl of atom C_{11}. This predictive result was in agreement with the result of Avery *et al*.[7]

Alignment D almost gave no information about the electrostatic fields. The blue broken lines in the contour maps of alignments A, B, and C indicated that positive fields existed near the C_{16} and O_5 of artemisinin analogs, and the presence of positive potential increases the antimalarial activity of artemisinin analogs. It means that the positive region of artemisinin analogs interacts with the negative domain of their receptor by electrostatic interaction, and the potency of artemisinin analogs increase with the increase of positive potential. This conclusion is consistent with our previous result of quantum chemical calculation[6].

CONCLUSION

According to 4 alignments, CoMFA models with good predictive ability have been derived for the ether and ester analogs of artemisinin. The predictive result from alignment B is consistent with the result of Avery *et al*[7]. The electrostatic field predictive results from alignments A—C are in accordance with our previous quantum chemical calculations[6].

Alignment D has the highest r^2_{cross}, which indicates that the chain of $-C_6-O_2-O_1-C_{10}-O_3-C_7-O_4-C_{12}-O_5-$ and atom C_{16} are the important groups for antimalarial activity.

From this CoMFA study, a few modified compounds of artemisinin which might have higher activities were suggested. The synthesis and pharmacological test work is now in progress.

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用比较分子力场分析法研究青蒿素醚类和酯类衍生物的三维定量结构—活性关系

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摘要 运用三维定量结构—活性关系分析方法—比较分子力场分析法(CoMFA)，研究了青蒿素醚类和酯类衍生物的理化性质与抗疟活性的关系。选用的四种分子重叠模型，其计算结果均有较强的预测能力，其中模型 B 得到的分子立体场分布与 Avery 等的实验结果一致；模型 A、B 和 C 的静电场分布计算结果与我们的量子化学计算结果一致；模型 D 的预测结果表明，$-C_6-O_2-O_1-C_{10}-O_3-C_7-O_4-C_{12}-O_5-$和 C_{16} 是抗疟活性的重要基团。

关键词 青蒿素；抗疟药；分子模型；三维定量结构—活性关系
CoMFA