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ORIGINAL PAPER

3D-QSAR studies on caspase-mediated apoptosis activity of phenolic analogues

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Abstract Phenols and its analogues are known to induce caspase-mediated apoptosis activity and cytotoxicity on various cancer cell lines. In the current work, two types of molecular field analysis techniques were used to perform the three dimension quantitative structure activity relationship (3D-QSAR) modeling between structural characters and anticancer activity of two sets of phenolic compounds, which are comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA). Then two 3D-QSAR models for two sets of phenolic analogues were obtained with good results. The first QSAR model, which was derived from CoMFA for phenols with caspase-mediated apoptosis activity against L1210 cells, had good predictability (q^2=0.874, r^2=0.930), and the other one was derived from CoMSIA for electron-attracting phenols with cytotoxicity in L1210 cell (q^2= 0.836, r^2=0.950). In addition, the CoMFA and CoMSIA contour maps provide valuable guidance for designing highly active phenolic compounds.

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Introduction

The phenolic compounds have an aromatic ring bearing one or more hydroxyl groups which are obtained from plant sources. In recent years, many research groups have focused on the phenolic hydroxyl group due to its wide radius of activity. For example, the phenolic compounds exhibit many pharmacologic actions like antitumor, antiviral, antibacterial, cardio-protective, pro-oxidant, and antimutagenicity, meanwhile, it demonstrates significant toxicity. These activities are believed to be associated with its hydrogen abstraction and subsequent formation of aryloxyl free radicals [1, 2].

Recently, many quantitative structure activity relationship (QSAR) models have been constructed with different descriptors and modeling techniques by some research groups. Selassie and colleagues [3] observed that some phenols cause inhibition of fast-growing murine leukemia L1210 cells, and constructed QSAR model with Hammett constant (σ^+) and hydrophobic parameter (Log P). Thakur and coworkers [4] modeling the phenolic activity (log 1/C) of large series of phenols against L1210 leukaemia using physicochemical parameters, such as parachor (Pc), surface tension (ST), density (D), polarizability (Pol) parameters, molar refraction (MR), molar volume (MV), index of refraction (η) and hydrophobic parameter (log P). Their research representing hydrophobic parameter has significant influence for activity. The cytotoxicity of one set of substituted phenols is modeled by Loader and colleagues

[5] using quantum topological molecular similarity (QTMS), and the cytotoxicity of these phenols is dependent primarily on electronic and radical effects. Roy and Popelier [6] construct serials predictive QSAR models for hepatocyte toxicity data of phenols using quantum topological molecular similarity (QTMS) descriptors along with hydrophobicity (logP) as predictor variables. The QTMS descriptors are calculated at different levels of theory including AM1, HF/3-21G(d), HF/6-31G(d), B3LYP/6-31+G(d,p), B3LYP/6-311+G(2d,p) and MP2/ 6-311+G(2d,p). Reis *et al*. [7] calculate a set of molecular properties for 41 phenol compounds with antioxidant activity using quantum chemical calculations at the DFT/B3LYP, HF, and AM1 and PM3 semiempirical levels, including vertical ionization potentials (IPvs), electron affinity (EA), electronegativity (χ), hardness (η), softness (S), electrophilic index (ω), partition coefficient (Log P), charges and other properties, and the serials QSAR model between descriptors and antioxidant activity indicate that vertical ionization potentials (IPvs) and the charge on oxygen atom 7 have significant effect.

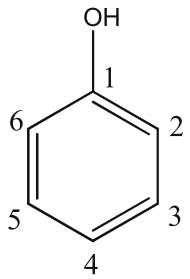
Phenol and its analogues were found to induce caspase-mediated apoptosis activity and cytotoxicity on murine leukemia cell line (L1210), human submandibular gland carcinoma cells (HSG), human promylolytic cell line (HL-60), human breast cancer cell line (MCF-7), parenteral human acute lymphoblastic cells (CCRFCEM), and multidrug-resistant subline of CCRF-resistant to vinblastine (CEM/VLB) cells. Apoptosis is characterized by programmed cell death because of activation of caspases that are a specific class of proteases. Various signaling pathways can trigger apoptosis, ultimately converging toward permeabilization of the mitochondrial membrane [1, 8–10]. Apoptosis, radical-scavenging, antioxidant and pro-oxidant characteristics are primarily responsible for the antitumor activities of phenolic compounds. In this paper, comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) were used to study phenol and its analogues, and two 3D-QSAR model with good predictability obtained for phenols with caspase-mediated apoptosis activity against L1210 cells (q^2=0.874, r^2=0.930) and electron-attracting phenols with cytotoxicity in L1210 cell respectively (q^2=0.836, r^2= 0.950). The CoMFA and CoMSIA contour map can provide valuable guidance for design phenolic compounds with highly anticancer activity.

Materials and methods

All molecular modeling techniques and 3D-QSAR studies described here were performed on Silicon Graphics fuel workstations using SYBYL6.9 molecular modeling software [11, 12].

Materials

The phenolic compounds with caspase-mediated apoptotic activities against L1210 and cytotoxicities ytotoxicity versus L1210 have been reported in the literature [1, 2], which is a series of *mono*-, *di*-, and *tri*-substituted phenolic derivatives were obtained with the introduction of different aliphatic and aromatic substituents in *ortho*-, *meta*- and *para*-positions of the phenolic nucleus. Chemically, the phenolic nucleus is 1-hydroxy benzene.



Phenolic nucleus (1-hydroxy benzene)

The phenols are classified into two main groups according to electron-releasing or electron-attracting attributes, namely electron-releasing phenols that cause inhibition of cell growth by electron-releasing substituents and electron-attracting phenols that inhibit cell growth, by electron-attracting substituents. The biological activity data may vary depending on the different cell lines to which phenols are enacted. The molar concentration of X-phenol is represented by ID_{50} indicating 50% growth inhibition of cancer cells and thus $\log 1/ID_{50}$ has been taken as a measure of biological activity in the proposed study. The activities of both electron-attracting and electron-releasing phenols have been utilized in the formation of structure-property correlations. There are 52 phenols with caspase- mediated apoptosis against L1210 cells have been shown in Table 1, and 27 electron- attracting phenols with cytotoxicity versus L1210 cells have been shown in Table 2 [1, 2].

Molecular modeling

The use of a reasonably low energy conformation in the alignment is a useful starting point for statistical comparisons of flexible structures within the CoMFA and CoMSIA models. In this study, each structure of two sets of compounds was fully geometry-optimized using MMFF94 molecular mechanics force field and energy gradient convergence criterion, which meant molecules were minimized till root-mean square (rms) deviation (0.05 kcal mol^{-1}) has been achieved. Besides, the partial atomic charges required for calculation of the electrostatic potential were assigned using the MMFF94 formation [13].

Table 1 Caspase-mediated apoptosis activity of phenol against L1210 cells (log $1/ID_{50}$)

No.	Substituents	Obsd. Act.	Cald. Act. (CoMSIA)	Cald. Act. (CoMFA)
1	4-OCH_3	0.02	0.37	0.06
2	4-CN	0.10	0.45	0.17
3	4-NO_2	1.00	0.62	0.92
4	4-OC_4H_9	0.14	0.10	0.02
5	4-OC_6H_5	0.19	0.28	0.17
6	4-OC_3H_7	0.09	0.14	0.40
7	4-$C(CH_3)_3$[a]	0.11	−0.18	0.35
8	4-$COCH_3$	0.03	0.21	0.40
9	H	−0.20	0.54	0.64
10	3-NO_2	0.79	1.16	0.71
11	3-$NHCOCH_3$	1.10	1.19	1.40
12	3-Cl[a]	0.74	0.24	0.84
13	3-Br[a]	1.00	0.13	0.78
14	3-F	0.82	0.40	0.97
15	3-NH_2	1.25	0.95	0.57
16	3-CN	1.11	0.67	1.00
17	3-OCH_3	1.31	0.99	1.20
18	3-CH3	0.45	0.53	0.67
19	3-OH	0.79	0.84	0.83
20	2,6-$(C(CH_3)_3)_2$, 4-$OCOCH_3$	2.80	2.50	2.86
21	2,6-$(C(CH_3)_3)_2$, 4-C_2H_5	2.43	2.26	2.31
22	2,6-$(C(CH_3)_3)_2$, 4-CHO	2.49	2.61	2.76
23	2,6-$(C(CH_3)_3)_2$	1.90	2.62	2.31
24	2,6-$(C(CH_3)_3)_2$, 4-CH_2OCH_3[a]	2.90	2.38	2.20
25	2,6-$(C(CH_3)_3)_2$, 4-OH[a]	2.50	2.57	2.24
26	2,4,6-$(C(CH_3)_3)_3$	2.10	1.96	2.20
27	2,6-$(C(CH_3)_3)_2$, 4-CN	2.88	2.53	2.27
28	2,6-$(C(CH_3)_3)_2$, 4-CH_2OH	2.39	2.53	2.46
29	2,6-$(C(CH_3)_3)_2$, 4-$COCH_3$	2.41	2.31	2.48
30	2,6-$(C(CH_3)_3)_2$, 4-Br	2.58	2.44	2.42
31	2,6-$(C(CH_3)_3)_2$, 4-CH_3	2.09	2.40	2.32
32	2,6-$(C(CH_3)_3)_2$, 4-NO_2	2.49	2.72	2.85
33	2,6-$(C(CH_3)_3)_2$, 4-OCH_3	2.87	2.52	2.30
34	2,6-$(OCH_3)_2$	2.70	2.72	2.75
35	2,4,6-$(OCH_3)_3$	2.20	2.63	2.32
36	2,6-$(OCH_3)_2$, 4-CH=CHCHO	2.60	2.58	2.50
37	2,6-$(OCH_3)_2$, 4-NH_2[b]	3.10	2.83	2.65
38	2,6-$(OCH_3)_2$, 4-$COCH_3$	2.80	2.52	2.74
39	2,6-$(OCH_3)_2$, 4-$NHCOCH_3$	2.50	2.72	2.79
40	2,6-$(OCH_3)_2$, 4-CH_3	2.40	2.55	2.40
41	2,6-$(OCH_3)_2$, 4-CHO	2.90	2.72	2.98
42	2-NH_2	2.29	2.29	2.10
43	2-$C(CH_3)_3$[a]	2.42	2.63	2.26
44	2-$CH(CH_3)_2$	2.38	2.40	2.29
45	2-CH_3[a]	1.87	0.87	0.92
46	2-I[ao]	2.38	−2.08	1.02
47	2-C_2H_5[a]	2.11	1.61	1.54
48	2-C_3H_7	2.16	2.35	2.24
49	2-NH_2, 4-NO_2	2.46	2.38	2.45
50	2-NH_2, 4-CH3	2.09	2.04	2.02
51	2-OCH_3	2.41	2.26	2.27
52	2-$C(CH_3)_3$, 4-OCH_3[a]	2.71	2.47	1.76

[a] Compounds selected in test set

[b] Template structure for molecular alignment

[o] Outlier

Alignment rules

Structural alignment is perhaps the most subjective, yet critical step in the CoMFA and CoMSIA study. To obtain a more compact alignment, the phenol was used as the reference atoms for alignment in this study. The selected template molecule is the typical one as follows [14]: (1) the most active compound; (2) the lead and/or commercial compound; (3) the compound containing the greatest number of functional groups. In this study, the template molecule was the highest activity compound. The molecular alignment used for the studies was obtained by means of the SYBYL6.9 common structural alignment protocol and the common structure was phenol (1-hydroxy benzene). According to the alignment rules, the alignment formation of phenols with caspase-mediated apoptosis against L1210 cells and electron-attracting phenols with cytotoxicity versus L1210 cells was shown in Figs. 1 and 2 respectively.

CoMFA and CoMSIA analysis

In the typical CoMFA calculation, the ligands were mutually aligned and were placed in a common 3D lattice. The steric and electrostatic fields of each ligand were sampled at the various grid points of the lattice.

Table 2 Cytotoxicity of electron-attracting phenols versus L1210 cells

No.	Substituents	Obsd. Act.	Cald. Act. (CoMSIA)	Cald. Act. (CoMFA)
1	H	3.27	3.40	3.59
2	4-$CONH_2$[a]	2.48	3.66	3.14
3	4-NO_2	3.45	3.50	3.52
4	4-I[a]	3.86	4.02	3.95
5	4-SO_2NH_2	2.50	2.45	2.44
6	4-CHO	3.08	3.04	3.14
7	4-Cl	4.29	4.32	4.35
8	4-Br	4.20	4.12	3.99
9	4-CN	3.44	3.44	3.44
10	3-NO_2	3.48	3.50	3.54
11	3-$NHCOCH_3$	2.65	2.66	2.65
12	3-Cl	3.87	3.86	3.88
13	3-OCH_3	3.71	3.73	3.67
14	3-Br	3.82	3.75	3.80
15	3-CN	3.11	3.15	3.00
16	3-F[a]	3.46	3.88	3.84
17	3-OH	3.46	3.55	3.50
18	2-Cl	3.22	3.52	3.39
19	2-CN	3.30	3.29	3.36
20	2-NO_2[a]	3.34	3.46	3.17
21	2-Br	3.44	3.49	3.42
22	2-I	3.95	3.48	3.65
23	2-CF_3	3.22	3.20	3.17
24	2,6-$(C(CH_3)_3)_2$, 4-NO_2[b]	4.90	4.90	4.96
25	2-CH_3, 4-NO	3.49	3.52	3.48
26	2-CH_3, 4-$COCH_3$[a]	3.14	3.40	2.96
27	2,6-$(C(CH_3)_3)_2$, 4-CN	4.68	4.67	4.58

[a] Compounds selected in test set

[b] Template structure for molecular alignment

The resulting field matrix is analyzed by the PLS method, from which a QSAR model can be constructed. An energy cutoff with 30 kcal mol^{-1} was applied, which meant the steric and electrostatic energies greater than 30 kcal mol^{-1} were truncated to that value, thus, can avoid infinity of energy values inside the molecule.

Likewise, in CoMSIA calculation, the alignment served to compute similarity index fields for CoMSIA analysis. The analysis depended on the evaluation of five structural properties: steric, electrostatic, hydrophobic, hydrogen bond donator, and hydrogen bond acceptor. These fields are selected to cover the major contributions to ligand binding.

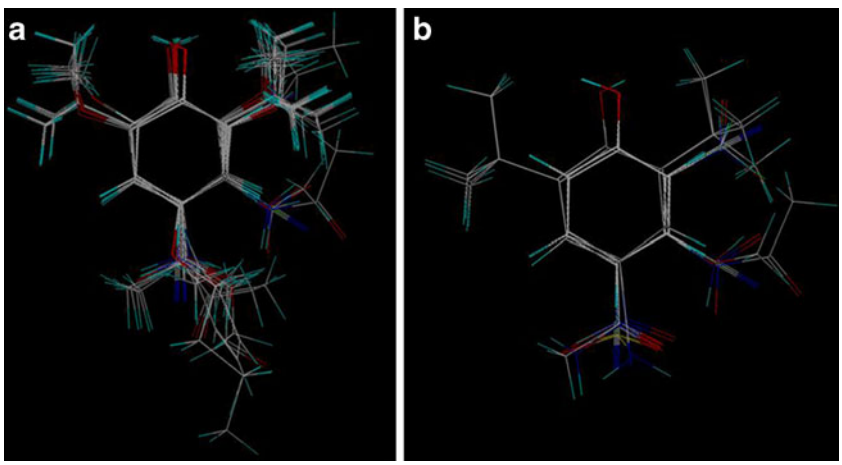


Fig. 1 Alignment of all **a** phenols and **b** electron-attracting phenols compounds

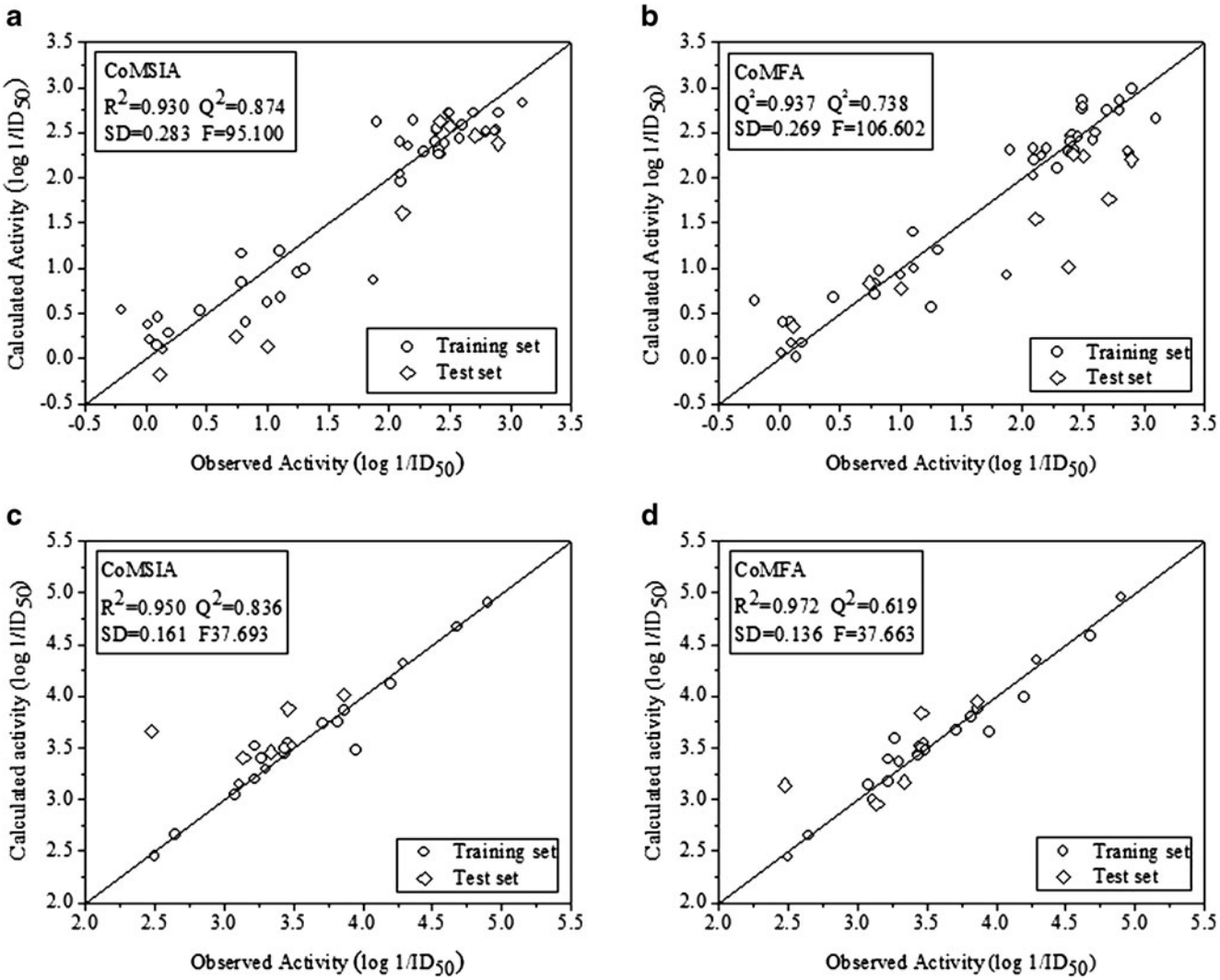


Fig. 2 Correlation diagram between observed and calculated activity. **a** CoMSIA for phenols, **b** CoMFA for phenols, **c** CoMSIA for electron-attracting phenols, **d** CoMFA for electron-attracting phenols

In this study, the phenols and its analogues were divided into a training and test set respectively, the training set was used for model construction and the test set was used to validate the model. The compounds were sorted by the ascending of activity, and then one per five was selected as the test set. Using all five CoMSIA descriptors for the explanatory variables, a leave one out (LOO) running and a no validation PLS analysis were performed.

Results and discussion

Results of CoMFA and CoMSIA analysis based on phenols with caspase-mediated apoptosis against L1210 cells

The results of CoMFA and CoMSIA analysis were listed in Table 3, and the calculated activity by using the QSAR models were shown in Table 1. The CoMSIA model was constructed with steric and hydrophobic fields and CoMFA model was established using steric and electrostatic fields. The results showed that the CoMSIA model had better self-consistency (r^2=0.930) and predictability (q^2=0.874), and the correlation between observed and calculated activity with CoMSIA and CoMFA models were illustrated by Fig. 2a and b.

As we know, the final PLS QSAR models are used not only for predicting the missing data but also for generating 3D contour maps. The contour maps of steric and hydrophobic fields of CoMSIA were shown in Fig. 3, and the contour maps of steric and electrostatic fields of CoMFA were illustrated with Fig. 4. In Figs. 3a, 4a, the steric contour map was shown with green (favored level, 80%) and yellow (disfavored level 20%), and in Fig. 3b, the hydrophobic contour map was shown with orange (favored) and white (disfavored), and in Fig. 4b, the electrostatic

Table 3 Results of CoMFA, CoMSIA and literature for phenols and electron-attracting phenols

Compound	methods	LOO		Conventional		
		q^2	nc	r^2	SD	F
Phenols	COMFA	0.738	5	0.937	0.269	106.602
	COMSIA	0.874	5	0.930	0.283	95.100
	MLR[1]	0.909	/	0.947	0.263	56.778
	Liner[2]	0.866	/	0.886	0.349	121.72
Electron-attracting phenols	COMFA	0.619	7	0.972	0.136	37.663
	COMSIA	0.836	7	0.950	0.161	37.693
	MLR[1]	0.872	/	0.957	0.138	61.397
	Liner[2]	0.812	/	0.848	0.233	66.910

contour map was shown with blue (favored) and red (disfavored). The higher activity is correlated with more bulk near green, larger hydrophobicity in the orange, more electronegativity in the blue, and vice versa [15].

Figure 3a showed that moderate bulk substituent at position 2 and 6 was favored for activity, such as methoxy and tert-butyl, and Fig. 4a showed that less bulk substituent at position 3 and 4 was a benefit to increase activity, such as methoxy and aldehyde. Figure 3b showed that larger hydrophobicity at position 2 and less hydrophobicity at position 4 was favored for activity, but less bulk and large hydrophobicity at position 2 was disfavored for activity, such as methoxy, tert-butyl at position 2 and methoxy at position 4. Figure 4b indicated more electronegativity at position 4 and less electronegativity at position 3 was a benefit to increase activity; however, more bulk and electronegativity, and less bulk and electronegativity at position 2 was favored for activity, such as amino, formyl , methoxy at position 4. All of the above, compounds 24, 37 and 61 have higher activity, which are suitable groups at position 2, 4 and 6. In additional, there was one outlier phenolic compound with the QSAR models, which was 2-I phenol. The outlier had been reported in the literature [1, 2], which may be an error of detection.

Results of CoMFA and CoMSIA analysis based on electron-attracting phenols with cytotoxicity versus L1210 cells

The results of CoMFA and CoMSIA analysis were summarized in Table 3, the calculated activity using the QSAR models were shown in Table 2. The CoMSIA and CoMFA models were constructed with electrostatic field. The CoMSIA model has better self-consistency (r^2=0.950) and predictability (q^2=0.836). The correlation between observed and calculated activity with CoMSIA and CoMFA models were illustrated by Fig. 2c and d.

The contour maps of electrostatic field of CoMSIA and CoMFA were shown in Fig. 5, and the influence of substituents was shown with blue (favored level, 80%) and

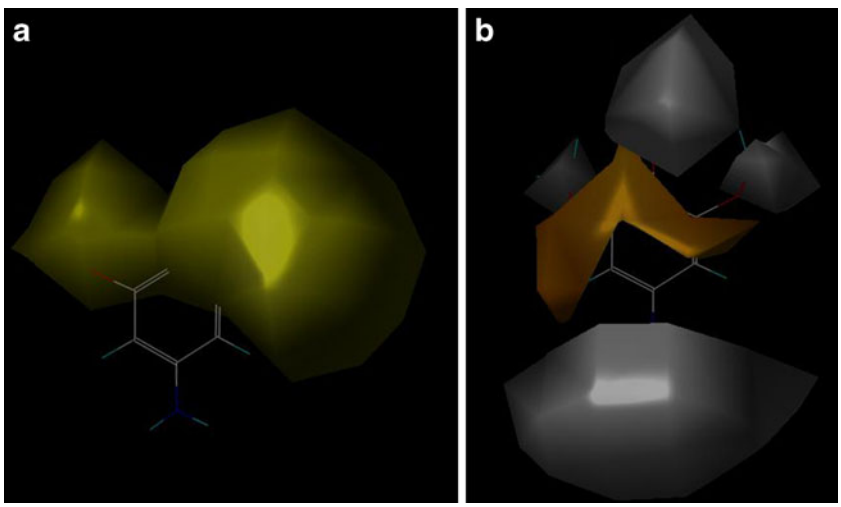


Fig. 3 Contour maps of the CoMSIA models for phenols (**a** Steric, **b** Hydrophobic, template is compound 37)

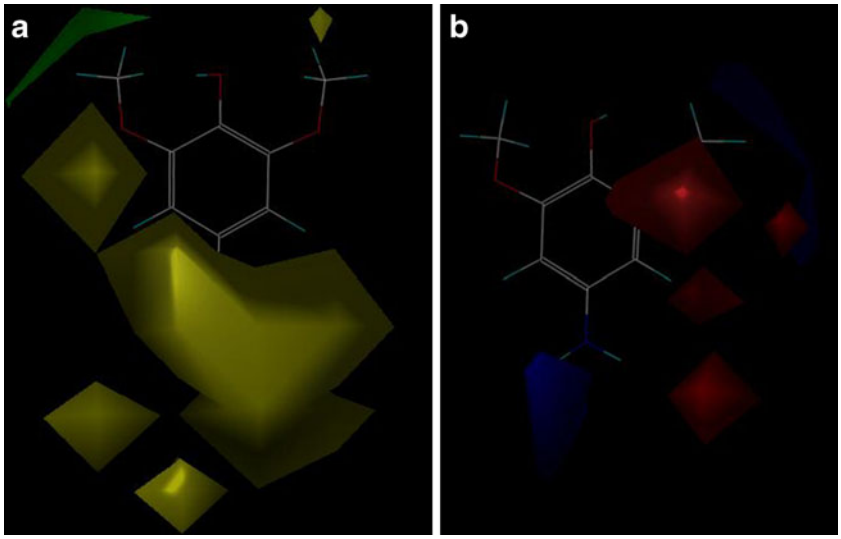


Fig. 4 Contour maps of the CoMFA models for phenols (**a** Steric, **b** Electrostatic, Template is compound 37)

red (disfavored level 20%). Figure 5a showed that more electronegativity at position 1 and 5, and less bulk and more electronegativity at position 3 and 4 were favored for activity. Figure 5b indicated that more electronegativity at position 3, 4 and 5, and less bulk and electronegativity were a benefit to increase activity. Consequently, the compounds are higher activity if there have chlorine, bromine, nitryl or cyan at position 4, for example, compounds 7, 8, 24 and 27 have higher activity.

Conclusions

In the present 3D-QSAR studies, CoMFA and CoMSIA methodologies were employed to study two sets of phenolic compounds with good results. The CoMSIA model constructed with steric and hydrophobic fields showed good predictability for the phenol with caspase-mediated apoptosis activity (q^2=0.874), similarly, the CoMSIA model established with electrostatic field had a good predictive ability for the electron-attracting phenols cytotoxicity (q^2= 0.836). Obviously, the caspase-mediated apoptosis activity was significantly correlated with steric, hydrophobic and electrostatic properties of substituent, especially volume of substituent, and the cytotoxicity depended on the electrostaric properties of substituent. In this study, the quality of 3D-QSAR model was no more better than that of literature, but it can provide more 3D information of substituents that contributed to activity of phenol and phenolic analogues. Furthermore, the 3D-QSAR model can guide the structural modification of phenol and its analogues directly. Therefore, it has more opportunity to design phenolic analogues with higher biological activity, if combining the advantages of the 3D- and 2D-QSAR model.

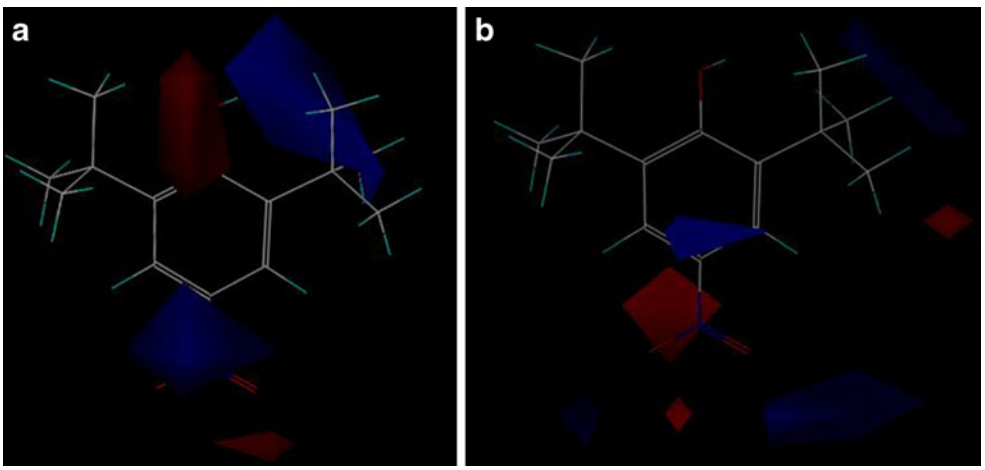


Fig. 5 Contour maps of the **a** CoMSIA and **b** CoMFA for electron-attracting phenols (Template is compound 24)

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