Activity Prediction of Schiff Base Compounds using Improved QSAR Models of Cinnamaldehyde Analogues and Derivatives

Hui Wang, Haijian Yuan, Shujun Li,* Zhuo Li, Mingyue Jiang, and Jiafa Tang

In past work, QSAR (quantitative structure-activity relationship) models of cinnamaldehyde analogues and derivatives (CADs) have been used to predict the activities of new chemicals based on their mass concentrations, but these approaches are not without shortcomings. Therefore, molar concentrations were used instead of mass concentrations to determine antifungal activity. New QSAR models of CADs against *Aspergillus niger* and *Penicillium citrinum* were established, and the molecular design of new CADs was performed. The antifungal properties of the designed CADs were tested, and the experimental Log *AR* values were in agreement with the predicted Log *AR* values. The results indicate that the improved QSAR models are more reliable and can be effectively used for CADs molecular design and prediction of the activity of CADs. These findings provide new insight into the development and utilization of cinnamaldehyde compounds.

Keywords: Cinnamaldehyde analogues and derivatives; Molar concentration; Activity prediction; Quantitative structure-activity relationship (QSAR); Cinnamaldehyde Schiff base

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INTRODUCTION

Cinnamaldehyde is a major constituent of cinnamon essential oil, an extract from the bark of cinnamon trees of the *Cinnamomum* genus (Yen and Chang 2008). It has been shown to have strong antifungal activities and has been widely studied as a potential natural wood preservative (Wang *et al*. 2005; Pánek *et al*. 2014). However, its volatility and pungent odor limit its use as a wood preservative, which has led many scholars to attempt to modify cinnamaldehyde (Teng and Bi 2003; Singh and Singh 2012).

The Quantitative Structure-Activity Relationship (QSAR) system is a computational approach that is used to mathematically analyze information of active compounds, using mathematical formulae to describe the relationship between the biological molecular organization and the physicochemical and structural parameters of organic compounds (Sun *et al*. 2009). QSAR plays a central role in computational molecular modeling methodology and is currently applied in many disciplines, many of which pertain to drug design and environmental risk assessment (Sun *et al*. 2009; Vaidya *et al*. 2014).

In the last decade, research concerning Schiff bases has garnered tremendous interest, primarily due to their bioactivity and strong metal coordination ability. Schiff base compounds have been extensively applied in the field of biochemistry (Halli and Sumathi 2012). N,N'-bis(*α*-methyl trans cinnamaldehyde) ethylenediamine ($C_{22}H_{24}N_2$), synthesized by Shreaz *et al.* exhibits putative bioactivity, and compared to methyl

cinnamaldehyde, it is 4.48 times more effective against *Candida* in liquid medium (Shreaz *et al*. 2011).

The QSAR model of CADs' inhibition of *Aspergillus niger* by Zhang *et al*. (2013) was established based on mass concentration. A cinnamaldehyde Schiff base compound (N,N'-bis(*p*-methoxy cinnamaldehyde) ethylenediamine) was designed and synthesized, and its bioactivity was predicted (Zhang 2013). Unfortunately, experimental results revealed that the calculated activity of the designed compound deviated significantly from the experimental value. The calculated value of cinnamaldehyde Schiff base compound (N,N'-bis(*p*-methoxy cinnamaldehyde) ethylenediamine) was 3.5868 for *Aspergillus niger*; whereas the experimental result was 1.9098 (Zhang 2013). The absolute error and relative error were 1.6770 and 87.81%, respectively. These errors were so significant that Zhang's models were unable to predict the bioactivity of new compounds (Zhang 2013).

In terms of QSAR, some vital features related to bioactivity can be identified, which are related to the molar concentration. The molar concentration varied greatly with the different molecular weight of compounds, although their mass concentrations were the same. The greater the molecular weight of the compounds, the lower will be their molar concentrations, and the lesser the active they will show. There were a few active functional groups that were found to contribute to antifungal activity in a manner proportional to mass concentration. However, in Zhang's model, the same mass concentration was used to measure the antifungal activity, which resulted in a big difference between calculated and experiment values (Zhang *et al*. 2013).

In the present study, mass concentration was replaced by molar concentration in the modified QSAR models of CADs against *Aspergillus niger* and *Penicillium citrinum*. The activity of four cinnamaldehyde Schiff base compounds was predicted, and the reliability of the new models was further validated.

EXPERIMENTAL

Materials

Cinnamon oil (cinnamaldehyde content, 95%) was produced by the Zhenxing spices oil refinery of Ji'an City, Jiangxi Province, China. The cinnamyl alcohol was chemically pure, and the cinnamic acid, acetophenone, benzaldehyde (Akimoto *et al*. 1988), ethyl alcohol, diethyl ether ethylenediamine, and sucrose were all analytical grade reagents. Cinnamamide, *α*-methylcinnamaldehyde, *p*-methoxy cinnamaldehyde, *p*-chlorocinnam-aldehyde, *p*-nitrocinnamaldehyde, *iso*-propyl cinnamate, and cinnamaldehyde diethyl acetal were all produced by Wuhan Yuancheng Technology Development Co., Ltd. Phenylpropyl aldehyde, *m*-nitrobenzaldehyde, and *o*-methoxylbenzaldehyde were purchased from Wuhan Ding Lixin Chemical Co., Ltd. *α*-bromocinnamaldehyde, *o*-nitrocinnamaldehyde, and ethyl cinanmate were synthesized according to the methods of Zhuang (1991). *D*-(+)-Glucosamine hydrochloride was biochemical regent.

Antifungal Experiment

Two wood fungi were used in this study: *Aspergillus niger* and *Penicillium citrinum*. A paper disc method was applied to determine the antifungal activities of 18

CADs and 4 designed compounds. Both strains were grown in 1% potato dextrose agar (PDA) medium.

The pre-made strain suspensions were poured into molten PDA and mixed thoroughly. The cultures were then decanted into petri dishes and allowed to cool. Autoclaved 8-mm-diameter filter discs were soaked in a 0.25-mmol/mL solution of CADs for 10 min and air-dried prior to use. The dried filter discs were placed on the center of solid agar with forceps and cultured in an incubator at 30 °C for 2 to 4 days. The diameter of the inhibition zone was recorded and used to determine the antifungal activity (Liang *et al*. 2012).

In the present study, sample 1227 (dodecyl dimethyl benzyl ammonium chloride) was used as a control. Experiments were carried out in triplicate for each tested compound and the average diameters of the zone of inhibition were calculated. The antifungal ratio (AR) of each sample was obtained using the following Eq. 1,

$$AR = [d/d_0] \times 100\% \quad (1)$$

where d and d_0 are the antifungal diameters of the tested compound and control 1227, respectively. The antifungal diameters of compound 1227 for *Aspergillus niger* and *Penicillium citrinum* were 11 and 13 mm, respectively. The *AR* and log *AR* of each cinnamaldehyde analogue or derivate against *Aspergillus niger* (Table 1) and *Penicillium citrinum* (Table 2) are listed.

QSAR Model Calculation

To further investigate the relationship between the antifungal activity and the molecular structure, AMPAC Agui 9.2.1 (Ren and Kong 2009) was applied to perform geometric optimization, and the data obtained were imported into Codessa 2.7.16 software to calculate structural descriptors (Feng *et al*. 2007). The best multi-linear regression (BMLR) method was used to generate the QSARs of CADs and to construct a series of models. The "breaking point" approach was adopted to determine the number of descriptors. The "internal validation" and "leave-one-out validation" methods were used to validate the derived models (Golbraikh *et al*. 2000).

Based on their molecular features, the descriptors were divided into six groups: structural, topological, geometrical, thermodynamic, electrostatic, and quantum-chemical. The descriptors obtained in the study included a large amount of molecular structure information of the CADs, which provided a strong calculation basis for seeking suitable QSAR models (Wang *et al*. 2008).

Synthesis of Cinnamaldehyde Schiff Base Compounds

Using the model descriptors, four cinnamaldehyde Schiff base compounds were designed, with each demonstrating desirable antifungal activity. The title compounds were identified by FT-IR spectroscopy, ^{1}H NMR, and ESI-MS. The new compounds, by design, do not possess the disadvantages of cinnamaldehyde such as high volatility, strong irritating odor, or high susceptibility for oxidization; in other words, the designed compounds can greatly expand the application of cinnamaldehyde.

The synthesis routes of the designed compounds are depicted in Figs. 1 and 2, respectively. Antifungal activity was detected as described in the Antifungal Experiment section.

Syntheses of compound A

Eighteen mmol D-(+)-Glucosamine hydrochloride was dissolved in a 30 mL mixture of methanol/ distilled water (v: v= 1:2) in a 100 mL three-neck flask by magnetic stirring. Next, 22.5 mmol of cinnamon oil (95% cinnamaldehyde) was dissolved in methanol (30 mL) and added to the three-neck flask dropwise within 1 h at room temperature.

The pH of the mixture was simultaneously adjusted to 8 by addition of 1 mol/L NaOH solution. After reacting for 2 h at room temperature, the glucosamine cinnamaldehyde Schiff base formed a light yellow precipitate. The precipitate was washed with diethyl ether three times; then the solid was vacuum-dried 24 h under 0.01 MPa at 35 °C to obtain the final title compounds.

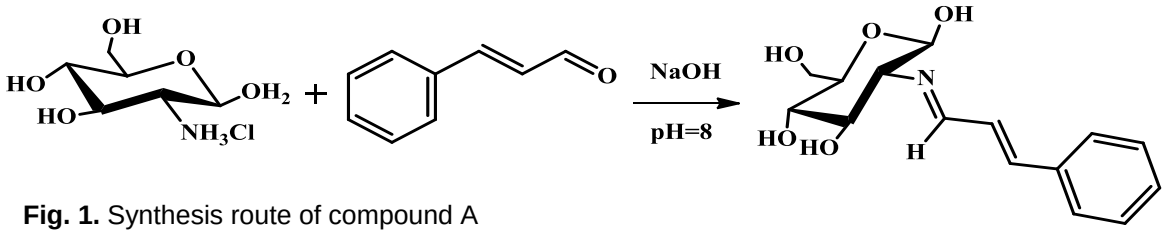


Fig. 1. Synthesis route of compound A

Syntheses of compounds B, C, and D

The syntheses route of compounds B, C, and D are shown in Fig. 2. The synthesis process used here was the same as that discussed in a previous study (Yuan *et al*. 2015).

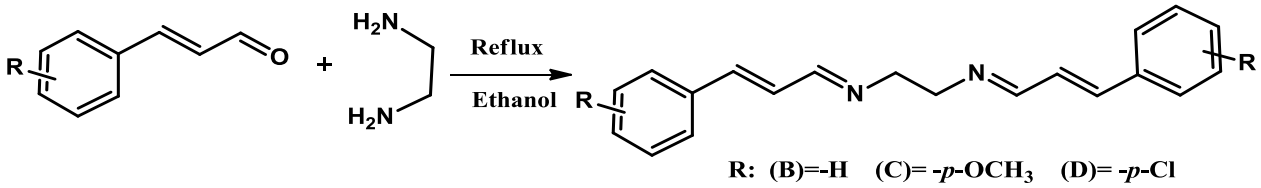


Fig. 2. Synthesis route of compounds B, C, and D

RESULTS AND DISCUSSION

Antifungal Activity

The *AR* and Log *AR* data of the 18 CADs against *Aspergillus niger* and *Penicillium citrinum* are listed in Tables 1 and 2, respectively. The *p*-chloro cinnamaldehyde exhibited the best antifungal ability: its inhibition diameters against *Aspergillus niger* and *Penicillium citrinum* were 43 and 40 mm, respectively. Cinnamaldehyde exhibited the second best inhibitory effects, with antifungal diameters of 39 mm against both *Aspergillus niger* and *Penicillium citrinum.*

Table 1. Antifungal Ratios and Structure Descriptors of Cinnamaldehyde Analogues and Derivatives (CADs) against *Aspergillus niger*

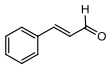
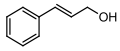
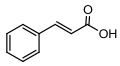
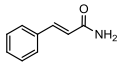
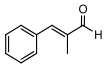
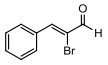
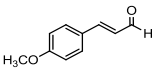
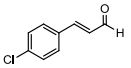
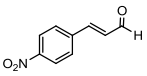
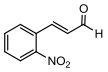
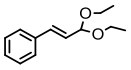
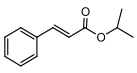
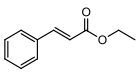
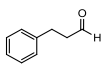
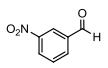
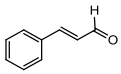
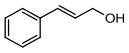
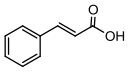
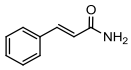
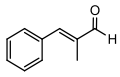
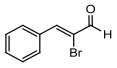
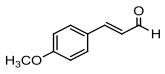
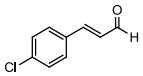
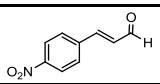
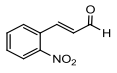
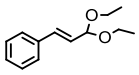
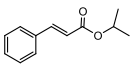
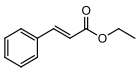
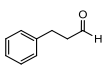
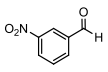
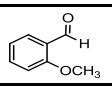
ID	Structure	*AR*	Log *AR*	ESP-Min net atomic charge for an H atom, d1	Tot molecular 1-center E-E repulsion/ # of atoms, d2	Max 1-electron react Index for a C atom, d3	FPSA-3 Fractional PPSA (PPSA-3/TMSA), d4
1		334.78	2.5248	0.0585	50.3745	0.0236	0.0739
2		121.74	2.0854	0.0966	46.0827	0.0229	0.0839
3		69.57	1.8424	0.1701	59.3897	0.0194	0.0892
4		73.91	1.8687	0.1632	52.8337	0.0243	0.0907
5		73.91	1.8687	0.1363	53.6558	0.0189	0.0756
6		86.96	1.9393	0.1084	47.0522	0.0215	0.0801
7		115.65	2.0631	0.1553	62.9429	0.0196	0.0733
8		200.00	2.3010	0.0850	54.4129	0.0208	0.0705
9		373.91	2.5728	0.0925	63.2311	0.0215	0.0556
10		217.39	2.3372	0.1133	72.3933	0.0216	0.1122
11		95.65	1.9807	0.1022	72.4024	0.0126	0.1120
12		295.65	2.4708	0.0192	44.1650	0.0237	0.0788
13		208.70	2.3195	0.0576	48.9465	0.0211	0.0809
14		108.70	2.0362	0.1368	54.7486	0.0206	0.0802
15		69.57	1.8424	0.1620	49.1786	0.0179	0.0764
16		217.39	2.3372	0.0888	45.8571	0.0307	0.0763
17		226.09	2.3543	0.1415	80.7103	0.0254	0.1092
18		86.96	1.9393	0.1391	57.9169	0.0158	0.0757

Table 2. Antifungal Ratios and Structure Descriptors of CADs against *Penicillium citrinum*

ID	Structure	AR	LogAR	FNSA-2 Fractional PNSA, d5	HDSA H-donors surface area, d6	Max 1-electron react. for a C atom, d3	ESP-Min net atomic charge for an H atom, d1
1		300.00	2.4771	-0.1117	0.0000	0.0236	0.0585
2		92.31	1.9652	-0.1153	30.5363	0.0229	0.0966
3		92.31	1.9653	-0.1572	29.1049	0.0194	0.1701
4		61.54	1.7892	-0.1400	53.4385	0.0243	0.1632
5		69.23	1.8403	-0.0861	0.0000	0.0189	0.1363
6		84.62	1.9275	-0.1022	0.0000	0.0215	0.1084
7		184.62	2.2663	-0.1520	0.0000	0.0196	0.1553
8		238.46	2.3774	-0.1417	0.0000	0.0208	0.0850
9		307.69	2.4881	-0.1587	0.0000	0.0215	0.0925
10		194.62	2.2892	-0.1554	0.0000	0.0216	0.1133
11		123.08	2.0902	-0.1610	0.0000	0.0126	0.1022
12		263.85	2.4214	-0.1114	0.0000	0.0237	0.0192
13		200.00	2.3010	-0.1121	0.0000	0.0211	0.0576
14		115.38	2.0621	-0.1172	0.0000	0.0206	0.1368
15		61.54	1.7892	-0.0854	0.0000	0.0179	0.1620
16		192.31	2.2840	-0.0937	0.0000	0.0307	0.0888
17		238.46	2.3774	-0.1427	0.0000	0.0254	0.1415
18		92.31	1.9652	-0.1244	0.0000	0.0158	0.1391

Quantitative Structure-Activity Relationship

Determination of QSAR models

The "breaking point" rule was used to determine the number of descriptors. The correlation coefficients, R^2, of the models obtained with different numbers of descriptors were generated using the Codessa software and the results were plotted. The appropriate number of descriptors was decided by observing the "breaking point" of the two trend lines. As shown in Fig. 3, the breaking points of *Aspergillus niger* and *Penicillium citrinum* with respect to their R^2 values were both at the 4-descriptor site. There was further increase in R^2 as the number of descriptors increased, but the rate of increase was attenuated when the number of descriptors exceeded four. The optimal models with four descriptors corresponded to multivariate linear regression, $n \geq 3 (k + 1)$ (where n is the number of samples and k is the factor number in the final model) (Wang *et al*. 2008).

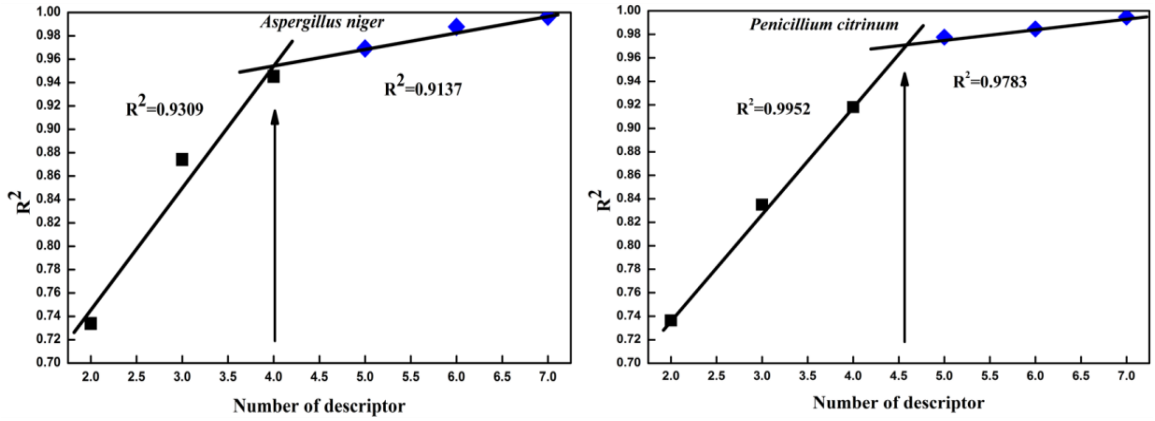


Fig. 3. The "breaking point" rule for determining the number of descriptors (left: *Aspergillus niger;* right: *Penicillium citrinum*)

Table 3. Multilinear QSAR Models Obtained for CADs against *Aspergillus niger* and *Penicillium citrinum*

Fungus	X	ΔX	t test	Name of descriptor
Aspergillus niger	1.6498e+00	1.5898e-01	10.3767	Intercept
	-4.8491e+00	4.2843e-01	-11.3205	ESP-Min net atomic charge for an H atom, d1
	1.7874e-02	2.1822e-03	8.3962	Tot molecular 1-center E-E repulsion/ # of atoms, d2
	2.8286e+01	4.4791e+00	6.3151	Max 1-electron reaction Index for a C atom, d3
	-6.7965e+00	1.3804e+00	-4.9237	FPSA-3 Fractional PPSA (PPSA-3/TMSA) [Quantum-Chemical PC], d4
Penicillium citrinum	1.1165e+00	1.9487e-01	5.7296	Intercept
	-5.8733e+00	7.9911e-01	-7.3499	FNSA-2 Fractional PNSA (PNSA-2/TMSA), [Zefirov's PC] ,d5
	-8.3442e-03	1.4530e-03	-5.7426	HDSA H-donors surface area [Quantum-chemical PC], d6
	2.9915e+01	5.5919e+00	5.3496	Max 1-electron react. for a C atom, d3
	-2.5800e+00	5.3113e-01	-4.8577	ESP-Min net atomic charge for a H atom , d1

Based on the results, the optimal models for CADs designed to inhibit *Aspergillus niger* and *Penicillium citrinum* (Table 3) were decided. In both models, there were four descriptors. The corresponding numbers of descriptors for each optimum model are listed in Tables 1 and 2, respectively. The important descriptors of Zhang's model for *Aspergillus niger* were different from the descriptors listed in Table 3, which were Max nucleoph. React. index for a C atom, ESP-Min net atomic charge, PNSA-1 Partial negative surface area and Min partial charge for a C atom (Zhang *et al*. 2013). The new models have the following statistical characteristics: R^2 = 0.9452, F = 56.10 and s^2 = 0.0045 (*Aspergillus niger*) and R^2 = 0.9181, F = 36.41, and s^2 = 0.0062 (*Penicillium citrinum*). Compared to Zhang's model against *Aspergillus niger*, which has statistical characteristics: R^2= 0.9099, F = 32.82 and s^2 = 0.0405, the new model exhibited high correlation coefficient and Fisher value, and low standard deviation.

The predicted Log *AR* values for the compounds obtained using the models described in Table 3 are listed in Table 4. Graphical representations of these predictions are shown in Fig. 4.

Table 4. Experimental log *AR* and Predicted log *AR* for *Aspergillus niger* and *Penicillium citrinum*

Aspergillus niger				*Penicillium citrinum*			
ID	Exp. log *AR*	Calc. log *AR*	Difference	ID	Exp. log *AR*	Calc. log *AR*	Difference
1	2.5248	2.4318	-0.0930	1	2.4771	2.3277	-0.1494
2	2.0854	2.0821	-0.0033	2	1.9652	1.9746	0.0094
3	1.8424	1.8280	-0.0144	3	1.9653	1.9373	-0.0280
4	1.8687	1.8740	0.0053	4	1.7892	1.7991	0.0099
5	1.8687	1.9692	0.1005	5	1.8403	1.8363	-0.0040
6	1.9393	2.0303	0.0910	6	1.9275	2.0820	0.1545
7	2.0631	2.0774	0.0143	7	2.2663	2.1943	-0.0720
8	2.3010	2.3201	0.0191	8	2.3774	2.3524	-0.0250
9	2.5728	2.5625	-0.0103	9	2.4881	2.4538	-0.0343
10	2.3372	2.2426	-0.0946	10	2.2892	2.3831	0.0939
11	1.9807	2.0444	0.0637	11	2.0902	2.1766	0.0864
12	2.4708	2.4818	0.0110	12	2.4214	2.4310	0.0096
13	2.3195	2.2914	-0.0281	13	2.3010	2.2570	-0.0440
14	2.0362	2.0034	-0.0328	14	2.0621	2.0691	0.0070
15	1.8424	1.7306	-0.1118	15	1.7892	1.7359	-0.0533
16	2.3372	2.3880	0.0508	16	2.2840	2.3555	0.0715
17	2.3543	2.3830	0.0287	17	2.3774	2.3497	-0.0277
18	1.9393	1.9430	0.0037	18	1.9652	1.9608	-0.0044

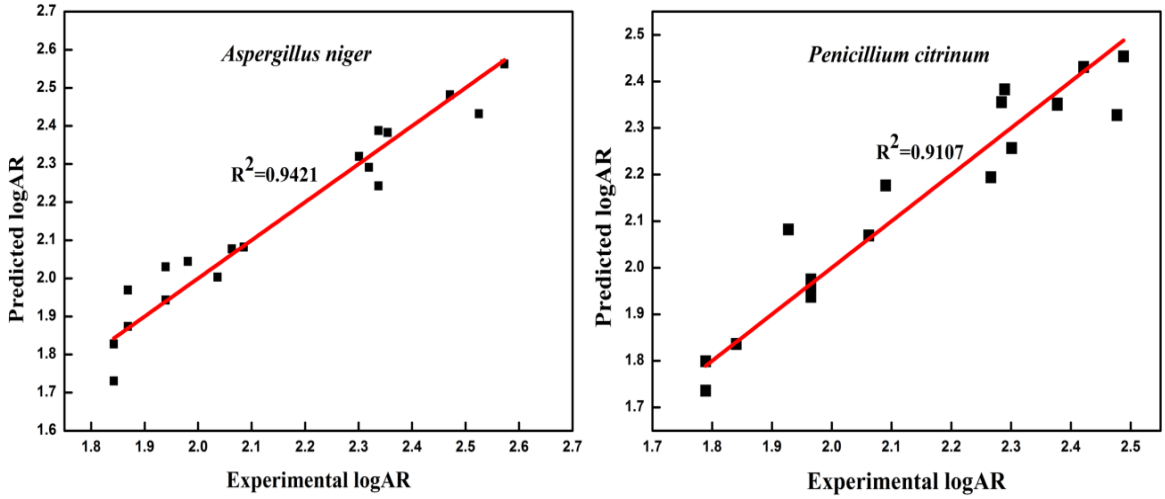


Fig. 4. Experimental *versus* predicted values according to the optimum models for *Aspergillus niger* and *Penicillium citrinum*

Descriptor analysis in the optimal QSAR models

The optimal QSAR model in the tables indicates that four descriptors are critical for the design of CADs inhibiting *Aspergillus niger*. According to the t-test, the most important descriptor in this model was ESP-Min net atomic charge for an H atom, d1. The minimum net atomic charge for an H atom reflects the hydrogen bonding and electrostatic interactions between the cation and anion (Yu *et al*. 2013). In Table 3, d1 has a coefficient with a negative sign, which implies hydrogen bonding and electrostatic interactions of molecules may decrease the inhibitory activities of the compounds against *Aspergillus niger*.

The second significant descriptor, a quantum-chemical descriptor, is the total molecular 1-center E-E repulsion/# of atoms (TMEER1), d2. The electron-electron repulsion energy describes the electron repulsion-driven processes taking place in a molecule and may be related to conformational (rotational, inversional) changes or the atomic reactivity of the molecule (Xue *et al*. 2004). In the model, d2 has a positive-sign coefficient, meaning that an increase in the magnitude of d2 enhances the inhibitory activity of the CADs.

The third descriptor for the model is the maximum 1-electron reaction index for a C atom, d3. It is a quantum-chemical descriptor determined by the frontier orbital energies and gives an estimate of the susceptibility of a molecule to radical reactions (Katritzky *et al*. 2006; Colombo *et al*. 2008). The positive regression coefficient in the model implies that increasing the value of this descriptor yields greater Log *AR*.

The fourth descriptor is FPSA-3, or Fractional PPSA (PPSA-3/TMSA), d4. It belongs to the partial charge surface descriptor group and is the ratio of PPSA-3 (partial positive surface area) to TMSA (total molecular surface area), reflecting the surface area and positive charge distribution of a molecule. With respect to molecular shape and electronic information, it describes the polarization between molecules and the molecule itself (Ji *et al.* 2009). It can be mathematically expressed as follows (Eq. 2),

$$\text{FPSA-3} = \text{PPSA-3/TMSA} \quad (2)$$

The negative correlation coefficients in the model suggest that the increasing the number of descriptors reduces the Log *AR* of the studied compounds.

The best QSAR model, as shown in Table 3, utilized four descriptors (d1, d3, d5, and d6), reflecting the biological activity of CADs against *Penicillium citrinum*. The most statistically significant descriptor, according to the t-test shown in the Table 3 is FNSA-2, Fractional PNSA-2(PNSA-2/TMSA d5). d5 is the total charge-weighted partial negatively charged molecular surface area. This value reflects the negative charge redistribution on cations and is most likely correlated to the presence of hetero atoms and their capability to participate in donor-acceptor or dipole-dipole interactions with the anion (Katritzky *et al*. 2007; Yu *et al*. 2013). The negative coefficient of this descriptor implies that enhancing the magnitude of d2 decreased inhibitory activity against *Penicillium citrinum*.

The second important descriptor is HDSA (d6) and is defined as HDSA/TMSA, where HDSA is the H-donor surface area. With increasing HDSA/TMSA, the proportion of the H-donor surface area of the total molecular surface area increases, meaning the formation of H-bonds becomes likelier and the bulk of a molecule increases. This leads to a decrease in viscosity (Liu *et al*. 2006; Ravindranath *et al*. 2007). d6 has a negative effect on the Log *AR* value against *Penicillium citrinum.* The third and the fourth descriptors were mentioned above.

Validation of models

The internal and leave-one-out validation methods were employed to verify the models that were obtained. Internal validation was carried out by dividing the 18 CADs into three subgroups (A, B, and C), which were then prepared as combinations of two subsets, and the 18 compounds were numbered from 1 to 18. Compounds 1, 4, 7, 10, 13, and 16 formed subgroup A; subgroup B was composed of compounds 2, 5, 8, 11, 14, and 17; the remaining compounds were designated subgroup C. The A+B, A+C, and B+C combinations were imported into the Codessa 2.7.16 software to obtain two four-descriptor models for *Aspergillus niger* and *Penicillium citrinum*, respectively. Based on the models generated, the Log *AR* values of the remaining, corresponding compounds were calculated. The predicted value was input into the Codessa 2.7.16 software for verification, yielding relative R^2, F, and s^2 values. Comparisons and evaluations were carried out and the results of the internal validation are listed in Table 5.

Table 5. Internal validation of the QSAR models

Training set	N	R^2(fit)	F(fit)	s^2(fit)	Test set	N	R^2(pred)	F(pred)	s^2(pred)
Validation for the model in Table 3									
A+B	12	0.9532	35.61	0.0058	C	6	0.9031	46.68	0.0180
A+C	12	0.9424	28.61	0.0047	B	6	0.9401	78.41	0.0271
B+C	12	0.9449	30.00	0.0050	A	6	0.8972	43.63	0.0317
Average		0.9468	31.41	0.0052			0.9135	56.24	0.0256
Validation for the model in Table 3									
A+B	12	0.9383	26.60	0.0070	C	6	0.8756	35.21	0.0236
A+C	12	0.9167	19.25	0.0070	B	6	0.9020	46.04	0.0342
B+C	12	0.9124	18.22	0.0069	A	6	0.9280	64.49	0.0261
Average		0.9225	21.36	0.007			0.9019	48.58	0.0279

The leave-one-out approach was similar in execution to the internal validation method. Every four compounds formed a group. Compounds 4, 8, 12, and 16 were selected for an external test. The remaining 14 compounds were used for calculation to generate two four-descriptor models. For *Aspergillus niger*, R^2=0.9369, and for *Penicillium citrinum*, R^2=0.9021. The external four CADs were then used for verification. The correlation coefficients of the external tests were R^2=0.9752 and R^2=0.9185, respectively. According to the internal and leave-one-out validation methods, the models obtained were adequate.

The best linear regression equations describing the CADs activities against *Aspergillus niger* and *Penicillium citrinum* are shown as Eqs. 3 and 4:

$$\text{Log } AR = (1.6498 \pm 1.5898 \times 10^{-1}) - (4.8491 \pm 4.2843 \times 10^{-1}) \times d1 + (1.7874 \times 10^{-2} \pm 2.1288 \times 10^{-3}) \times d2 + (2.8286 \times 10^{1} \pm 4.4791) \times d3 - (6.7965 \pm 1.3804) \times d4 \quad (3)$$

$$\text{Log } AR = (1.1165 \pm 1.9487 \times 10^{-1}) - (5.8733 \pm 7.9911 \times 10^{-1}) \times d5 - (8.3442 \pm 1.4530) \times 10^{-3} \times d6 + (2.9915 \times 10^{1} \pm 5.5919) \times d3 - (2.5800 \pm 5.3113 \times 10^{-1}) \times d1 \quad (4)$$

Activity of Designed Compounds and the Best Calculation Values of QSAR models

Structure analysis of designed compounds

The glucosamine cinnamaldehyde Schiff base (A)

Light yellow powder, yield: 52.05%, FT-IR (cm^{-1}): 1633 (C=N), 1610 (C=C), 1033 (C-N), 754 (Ar-H), 692 (Ar-H), ^{1}H NMR (500 MHz, DMSO): δ = 7.93 (d, *J*=8.8 Hz, 1H, -CH=N), 7.60 (d, *J*=7.4 Hz, 2H, Ar-H), 7.37 (dt, *J*=28.6 Hz, 7.2 Hz, 3H, Ar-H), 7.12 (d, *J*=16.1 Hz, 1H, -C=CH), 6.91 (dd, *J*=16.1 Hz, 8.9 Hz, 1H, -C=CH), 6.55 (d, *J*=6.8 Hz, 1H, O-CH-O), 4.95 (d, *J*=5.3 Hz, 1H, -OH), 4.86 (dd, *J*=23.9 Hz, 5.3 Hz, 1H, -OH), 4.64 (t, *J*=7.3 Hz, 1H, -OH), 4.55 (t, *J*=5.8 Hz, 1H, -OH), 3.71 (dd, *J*=9.9 Hz, 5.5 Hz, 1H, CH-O), 3.47 (dt, *J*=11.8 Hz, 6.0 Hz, 1H, CH-O), 3.41 – 3.35 (m, 1H, CH-O), 3.24 – 3.17 (m, 1H, CH-O), 3.12 (td, *J*=9.1 Hz, 5.4 Hz, 1H, CH-O), 2.70 (t, *J*=8.5 Hz, 1H, -CH-N), ESI-MS *m/z* calcd. for $C_{15}H_{19}NO_5$, M= 293.3, found 294.5 $[M+H]^+$.

N,N'-bis(*trans*-cinnamaldehyde) ethylenediamine (B)

Yellow powder, yield: 94.15%, FT-IR (cm^{-1}): 1627 (C=N, C=C), 987 (C-N), 752 (Ar-H), 691 (Ar-H), ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05 (d, *J*=7.5 Hz, 2H, 2(CH=N)), 7.46 (d, *J*=7.1 Hz, 4H, Ar-H), 7.38 – 7.27 (m, 6H, Ar-H), 6.99 – 6.86 (m, 4H, 2(CH=CH)), 3.85 (s, 4H, CH_2-CH_2), ESI-MS *m/z* calcd. for $C_{20}H_{20}N_2$, M=288.2, found 289.4 $[M+H]^+$.

N,N'-bis(*p*-methoxy cinnamaldehyde) ethylenediamine (C)

Yellow powder, yield: 71.81%, FT-IR (cm^{-1}): 1635 (C=N), 1604 (C=C), 1025 (C-N), 822 (Ar-H), ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.01 (d, *J*=8.6 Hz, 2H, 2(CH=N)), 7.40 (d, *J*=8.7 Hz, 4H, Ar-H), 6.88 (dd, *J*=12.3 Hz, 3.5 Hz, 6H, Ar-H, CH=CH), 6.76 (dd, *J*=16.0 Hz, 8.6 Hz, 2H, CH=CH), 3.87 – 3.80 (m, 10H, -OCH_3, -CH_2-CH_2), ESI-MS *m/z* calcd. for $C_{22}H_{24}N_2O_2$ M=348.2, found 349.4 $[M+H]^+$.

N,N'-bis(*p*-chloro cinnamaldehyde) ethylenediamine (D)

Yellow powder, yield: 78.72%, FT-IR (cm^{-1}): 1636 (C=N, C=C), 1090 (C-N), 816 (Ar-H), ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03 (d, *J*=7.2 Hz, 2H, 2(CH=N)), 7.38 (d, *J*=8.6 Hz, 4H, Ar-H), 7.32 (d, *J*=8.5 Hz, 4H, Ar-H), 6.92 – 6.84 (m, 4H, 2(CH=CH)),

3.84 (s, 4H, -CH_2-CH_2), ESI-MS *m/z* calcd. for $C_{20}H_{18}Cl_2N_2$ M=337.3, found 357.1 $[M+H]^+$.

Antifungal ability of designed compounds

The inhibition diameters of the designed compounds against the test fungi are given in Table 6. The results indicate that all designed compounds exhibited good antimycotic activity against both species. Compound A exhibited the best inhibition activity against *Aspergillus niger*, and its average inhibition diameter was 23.00 mm. Compound C exhibited the weakest activity with an inhibition diameter of 11.75 mm. Compound B functioned best against *Penicillium citrinum* with an inhibition diameter of 39.33 mm. Compound D had the weakest antifungal ability with a diameter of 18.5 mm. As shown in Table 6, the inhibition diameters suggest that *Penicillium citrinum* is generally more sensitive to the inhibitory compounds.

Analysis and verification of the QSAR models

The antifungal diameters of the compounds with a concentration of 0.25 mmol/mL were used in Eq. 1 to calculate the experimental Log *AR* value.

The approaches mentioned above were used to calculate the six descriptors of the four design compounds. The results were input into Eqs. 3 and 4 to obtain the predicted Log *AR* values. The comparisons of the predicted and experimental Log *AR* values are shown in Table 6.

As shown in Table 6, the experimental Log *AR* value of the designed compounds was in agreement with the predicted value. The minimum differences between the experimental and predicted Log *AR* values were 0.0796 and 0.2664 for *Aspergillus niger* and *Penicillium citrinum*, respectively. For the anti-*Aspergillus niger* activity, the largest difference between the experimental and calculated Log *AR* values was 0.5055 for compound B. For the anti-*Penicillium citrinum* activity, compound D displayed the biggest difference (0.9270) between the experimental and predicted values. The average errors against *Aspergillus niger* and *Penicillium citrinum* were 0.2661 and 0.6025, respectively. Over all, the QSAR model of the CADs against *Aspergillus niger* was more reliable than that against *Penicillium citrinum*.

As shown in Table 6, the difference between experimental Log *AR* and predicted Log *AR* of compound C was 0.0796 against *Aspergillus niger*. This difference was much lower than the difference of 1.6770 calculated using Zhang's model (Zhang *et al.* 2013). These results indicated that the modified QSAR model of CADs is more reliable and demonstrates more sensitive predictive ability than Zhang's model.

Table 6. Experimental and Predicted Log *AR* values for the designed compounds

No	*Aspergillus niger*			Absolute error	*Penicillium citrinum*			Absolute error
	diameter	Exp.LogAR	Calc.LogAR		diameter	Exp.LogAR	Calc.LogAR	
A	23.00	2.3010	2.1698	0.1312	35.00	2.4301	3.2197	0.7896
B	16.67	2.1620	1.6565	0.5055	39.33	2.4808	2.0537	0.4271
C	11.75	2.0093	1.9297	0.0796	21.67	2.2219	2.4883	0.2664
D	14.00	2.0854	2.4337	0.3483	18.50	2.1532	3.0802	0.9270

CONCLUSIONS

1. The QSAR models of CADs were modified and new QSAR models of CADs, based on mass concentration, were established with regard to *Aspergillus niger* and *Penicillium citrinum*.
2. According to the new models, four cinnamaldehyde Schiff base compounds were designed and synthesized and their antifungal activities against *Aspergillus niger* and *Penicillium citrinum* were determined.
3. The four cinnamaldehyde Schiff base compounds exhibited satisfactory results. The experimental Log *AR* of the designed compounds was consistent with the predicted value. The average errors of the models against *Aspergillus niger* and *Penicillium citrinum* were 0.2661 and 0.625, respectively. The findings of this study suggest that the modified QSAR models of CADs are highly reliable and have good predictive ability. This study provides a theoretical basis for the future study and application of cinnamaldehyde.

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