Synthesis and QSAR study of novel α-methylene-γ-butyrolactone derivatives as antifungal agents

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Title: Synthesis and QSAR study of novel α-methylene-γ-butyrolactone derivatives as antifungal agents

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Abstract: Thirty-six new α-benzylidene-γ-lactone compounds based α-methylene-γ-butyrolactone substructure were prepared and characterized by spectroscopic analysis. All compounds were evaluated for antifungal activities *in vitro* against six plant pathogenic fungi and the half maximal inhibitory concentration (IC_{50}) against *Botrytis cinerea* and *Colletotrichum lagenarium* were investigated. Compounds **5c-3** and **5c-5** with the halogen atom exhibited excellent fungicidal activity against *B. cinerea* (IC_{50} = 22.91, 18.89 μM). The structure-activity relationships (SARs) analysis indicated that the derivatives with electron-withdrawing substituents at the meta- or para-positions improves the activity. Via the heuristic method, the generated quantitative structure-activity relationship (QSAR) model (R^2 = 0.961) revealed a strong correlation of antifungal activity against *B. cinerea* with molecular structures of these compounds. Meanwhile, the cytotoxicity of 20 representative derivatives was tested in the human tumor cells line (HepG2) and the hepatic L02 cells line, the result indicated that the synthesized compounds showed significant inhibitory activity and limited selectivity. Compound 5c-5 has the highest fungicidal activity with IC_{50} = 18.89 μM (against *B. cinerea*.) but low cytotoxicity with IC_{50} = 35.4 μM (against HepG2 cell line) and IC_{50} = 68.8 μM (against Hepatic L02 cell line). These encouraging results can be providing an alternative, promising use of α-benzylidene-γ-lactone through the design and exploration of eco-friendly fungicides with low toxicity and high efficiency.

Keywords: α-benzylidene-γ-lactone derivatives; antifungal activity; QSAR; heuristic method; cytotoxicity

Plant fungal pathogens have significant impacts on the crops, and often lead to significant yield reduction and dramatic economic losses in agriculture.[1-2] Furthermore, many of the fungi can produce mycotoxins harmful to animal and human health.[3] Meanwhile, many commercial chemical fungicides have several detrimental effects, such as residual toxicity, severe pesticide resistance, and environmental pollution.[4] Thus, there is a growing need to develop new antifungal agents to effectively control agricultural diseases.

Using natural products as lead compounds to develop new pesticides with novel structures and mechanism is one of the most effective methods for pesticide design. Sesquiterpene lactones (STLs) have been considered interesting leads to develop a new class of potential agents in the past, such as costunolide, andrographolide and carabrone (isolated from fruits of *Carpesium macrocephalum*) (Figure **1**).[5-7] The functional unit of α-methylene-γ-butyrolactone substructure is one of the commonly chemical scaffolds among numerous natural products because it's electrophilic α, β-unsaturated carbonyl structure could react with biological nucleophiles.[8-9] STLs natural products containing the α-methylene-γ-butyrolactone substructure always were found to possess a broad spectrum of biological activities, including anticancer, antibacterial, anti-inflammatory, and antimicrobial properties.[10-13] While, great biological activities of this class of compounds have been extensively reported in the pharmaceutical field but not in the agrochemical field.[14] In our previous research, we demonstrated carabrone and a series of carabrol derivatives exhibited prominent antifungal activity, and we also found aromatic substituents directly fused to the γ-position of the α-methylene-γ-lactone ring boost their antifungal potency more effectively.[15-16] Therefore, compounds containing α-methylene-γ-lactone can

be used to develop novel and improved crop-protection agents.

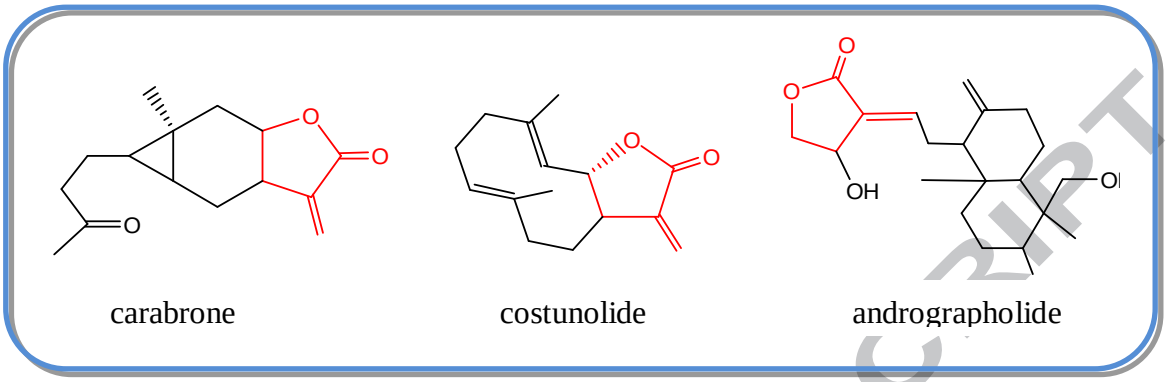
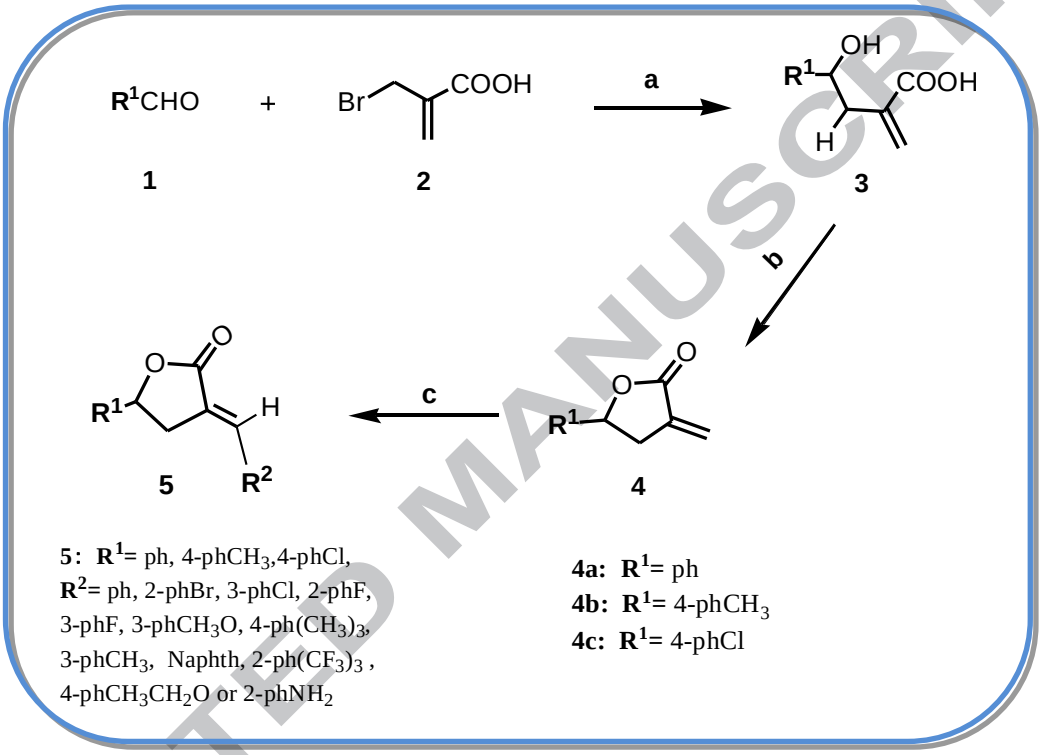


Figure 1. Chemicial structures of the representative sesquiterpene lactones

While, during the design and modification of potential antifungal agents derived from α-methylene-γ-butyrolactone chemical scaffolds, the exocyclic carbon-carbon double bond in α, β-unsaturated carbonyl system need to be considered. In order to analysis the roles of electron density and steric hindrance influence on the exocyclic carbon-carbon double bond, a series of α-benzylidene-γ-lactone were prepared and their activity were evaluated in this study. In addition, developing and screening candidates with antifungal activity from thousands of compounds is virtually and economically impossible, and employing quantitative structure-activity relationship (QSAR) study is a method to reduce the problems on cost and time requirements for screening.[17] Meanwhile, after the essential structural features for the activity were defined, the mechanism of action can be elaborated advantageously. [18-19]

In continuation of our investigation on the design and synthesis of bioactive compounds, six important crops threatening pathogenic fungus in agriculture were choosed to initial screen all the compounds antifungal activities, and with the higher preliminary activity the half maximal inhibitory concentration (IC_{50}) against *B. cinerea* and *C.*

lagenarium were investigated. Meanwhile, the cytotoxicity was tested to ensure selectivity of the antifungal effects. In addition, a QSAR study was also performed on all of the derivatives using some quantification software packages, which can correlate their structural features with their antifungal activities.



scheme 1. Synthesis route of the target compounds **5**.

Reagents and conditions: (a) indium powder, THF, rt. (b) 6 M HCl, rt, 6 h. (c) iodobenzene, $Pd(OAc)_2$, Et_3N, DMF, 80°C, rt.

As shown in **scheme 1**, three kinds of intermediate compounds **4a-4c** were obtained under milder aqueous reaction conditions through indium-mediated Barbier allyl addition to aldehydes.[20] This versatile and simple method was used to prepare γ-substituted α-methylene-γ-butyrolactones in good yields (72–93%) under markedly milder aqueous reaction conditions than other reported methods.[15] The palladium-catalyzed arylation of different α-methylene-γ-lactone lactones was shown to produce *E*-olefin coupling products selectively in moderate to excellent yields.[21] Herein, through the previously reported Heck

reaction conditions (0.04 mmol of $Pd(OAc)_2$ with Et_3N in DMF at 80 °C), substituted α-methylene-γ-butyrolactones (**4a-4c**) were reacted with a series of readily available aryl iodides to obtained corresponding α-benzylidene-γ-lactones compounds **5** (table 1). We also strategically selected aryl iodides that contained different substitution pattern and the presence of electron-donating or electron-withdrawing substituents on the aromatic ring to understand the structure-activity relationships of substituents on the aromatic ring of the analogues. Meanwhile, the substituents on the aromatic ring did not affect the reaction yields. Moreover, the structures of all the derivatives were characterized by ^{1}H NMR, ^{13}C NMR and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS).

Table 1 Initial Antifungal Activity of Compounds **5** at100 μg/mL [a]

Compound				average inhibition rate (%) (100 μg/mL; 72h)					
	no.	R^1	R^2	*B. c.*	C. *l.*	*P. c.*	*F. o.*	*S. s.*	*F. g.*
1	5a-1	H	ph	80.1 ± 0.7	76.2 ± 0.3	48.3 ± 0.8	53.3 ± 0.3	70.3 ± 0.1	67.7 ± 0.7
2	5a-2	H	2-phBr	81.0 ± 0.1	77.3 ± 0.5	46.9 ± 0.4	51.7 ± 0.3	72.2 ± 0.3	68.3 ± 0.5
3	5a-3	H	3-phCl	88.9 ± 0.2	85.5 ± 0.3	49.5 ± 0.5	54.8 ± 0.6	73.8 ± 0.4	74.5 ± 0.6
4	5a-4	H	2-phF	86.8 ± 0.3	81.6 ± 0.8	51.7 ± 0.7	57.3 ± 0.0	71.9 ± 0.4	70.6 ± 0.5
5	5a-5	H	3-phF	91.7 ± 0.1	86.2 ± 0.5	65.8 ± 0.9	64.6 ± 0.9	77.3 ± 0.6	79.2 ± 0.3
6	5a-6	H	3-phCH_3O	75.2 ± 0.6	67.9 ± 0.3	46.3 ± 0.0	47.3 ± 0.8	65.5 ± 0.4	64.3 ± 0.6
7	5a-7	H	4-ph$C(CH_3)_3$	71.3 ± 0.2	65.1 ± 0.7	44.6 ± 0.2	46.9 ± 0.6	64.6 ± 0.3	60.9 ± 0.4
8	5a-8	H	3-phCH_3	76.8 ± 0.2	71.7 ± 0.1	49.5 ± 0.1	54.8 ± 0.2	66.1 ± 0.1	67.1 ± 0.2
9	5a-9	H	2-naphthyl	83.2 ± 0.8	68.7 ± 0.2	53.8 ± 0.4	53.5 ± 0.4	65.0 ± 0.2	71.8 ± 0.1
10	5a-10	H	2-phCF_3	83.9 ± 0.9	77.2 ± 0.4	53.2 ± 0.3	56.6 ± 0.6	73.8 ± 0.8	69.7 ± 0.0
11	5a-11	H	4-phCH_3CH_2O	67.7 ± 0.2	65.9 ± 0.3	46.6 ± 0.6	47.8 ± 0.9	58.7 ± 0.3	62.4 ± 0.8
12	5a-12	H	2-phNH_2	69.1 ± 0.3	66.6 ± 0.8	43.5 ± 0.8	45.2 ± 0.7	62.2 ± 0.5	61.6 ± 0.7
13	5b-1	CH_3	ph	75.0 ± 0.5	75.2 ± 0.7	48.7 ± 0.6	50.5 ± 0.9	67.1 ± 0.6	65.1 ± 0.4
14	5b-2	CH_3	2-phBr	83.8 ± 0.5	81.8 ± 0.3	54.2 ± 0.4	53.6 ± 0.4	70.3 ± 0.9	66.5 ± 0.5
15	5b-3	CH_3	3-phCl	85.7 ± 0.1	84.2 ± 0.5	56.3 ± 0.1	55.8 ± 0.6	73.2 ± 0.2	73.7 ± 0.2
16	5b-4	CH_3	2-phF	85.1 ± 0.3	83.3 ± 0.9	55.1 ± 0.3	55.6 ± 0.1	72.7 ± 0.1	70.2 ± 0.4
17	5b-5	CH_3	3-phF	89.3 ± 0.2	85.6 ± 0.5	60.6 ± 0.6	58.1 ± 0.2	75.3 ± 0.1	76.2 ± 0.8
18	5b-6	CH_3	3-phCH_3O	70.5 ± 0.8	74.4 ± 0.3	46.2 ± 0.8	47.3 ± 0.2	64.6 ± 0.4	57.7 ± 0.4
19	5b-7	CH_3	4-ph$C(CH_3)_3$	67.0 ± 0.5	71.5 ± 0.4	44.2 ± 0.5	43.3 ± 0.3	61.1 ± 0.5	55.6 ± 0.6
20	5b-8	CH_3	3-phCH_3	74.2 ± 0.2	75.7 ± 0.7	49.6 ± 0.6	49.6 ± 0.5	65.3 ± 0.8	63.7 ± 0.7

21	5b-9	CH_3	2-naphthyl	77.5 ± 0.9	68.1 ± 0.6	45.2 ± 0.3	47.6 ± 0.6	66.4 ± 0.5	64.2 ± 0.2
22	5b-10	CH_3	2-phCF_3	81.3 ± 0.4	84.0 ± 0.3	49.6 ± 0.7	51.6 ± 0.8	68.8 ± 0.7	67.1 ± 0.3
23	5b-11	CH_3	4-phCH_3CH_2O	65.1 ± 0.6	65.6 ± 0.4	43.2 ± 0.4	41.2 ± 0.2	47.2 ± 0.3	47.7 ± 0.3
24	5b-12	CH_3	2-phNH_2	66.2 ± 0.7	65.4 ± 0.0	46.3 ± 0.2	47.9 ± 0.6	59.6 ± 0.2	54.2 ± 0.5
25	5c-1	Cl	ph	80.7 ± 0.8	77.3 ± 0.2	50.6 ± 0.1	51.2 ± 0.4	63.7 ± 0.1	67.3 ± 0.8
26	5c-2	Cl	2-phBr	87.3 ± 0.4	84.6 ± 0.4	57.7 ± 0.6	57.0 ± 0.4	73.8 ± 0.6	71.4 ± 0.4
27	5c-3	Cl	3-phCl	90.9 ± 0.1	87.5 ± 0.6	63.8 ± 0.6	60.1 ± 0.8	76.1 ± 0.5	77.8 ± 0.6
28	5c-4	Cl	2-phF	89.2 ± 0.3	87.1 ± 0.8	60.6 ± 0.3	59.4 ± 0.7	74.0 ± 0.3	75.1 ± 0.3
29	5c-5	Cl	3-phF	97.6 ± 0.4	93.0 ± 0.1	70.7 ± 0.7	68.9 ± 0.4	77.3 ± 0.2	83.8 ± 0.8
30	5c-6	Cl	3-phCH_3O	79.5 ± 0.7	75.3 ± 0.2	50.5 ± 0.4	45.8 ± 0.7	67.6 ± 0.6	65.1 ± 0.5
31	5c-7	Cl	4-ph$C(CH_3)_3$	77.3 ± 0.9	76.2 ± 0.4	49.3 ± 0.5	44.7 ± 0.0	52.5 ± 0.1	54.0 ± 0.2
32	5c-8	Cl	3-phCH_3	81.2 ± 0.4	77.4 ± 0.5	52.2 ± 0.2	47.3 ± 0.3	68.2 ± 0.6	70.2 ± 0.3
33	5c-9	Cl	2-naphthyl	82.8 ± 0.5	77.8 ± 0.7	50.6 ± 0.1	55.8 ± 0.4	72.7 ± 0.8	70.7 ± 0.6
34	5c-10	Cl	2-phCF_3	85.7 ± 0.3	80.6 ± 0.6	55.7 ± 0.2	54.6 ± 0.8	67.6 ± 0.0	72.4 ± 0.7
35	5c-11	Cl	4-phCH_3CH_2O	76.6 ± 0.8	75.1 ± 0.3	48.2 ± 0.6	43.6 ± 0.7	52.5 ± 0.2	53.3 ± 0.3
36	5c-12	Cl	2-phNH_2	77.3 ± 0.2	76.5 ± 0.5	49.3 ± 0.3	45.6 ± 0.2	53.4 ± 0.4	55.8 ± 0.1
37	Tulipalin A[b]			19.4 ± 0.9	21.5 ± 0.8	25.1 ± 0.8	17.8 ± 0.9	13.3 ± 0.6	22.8 ± 0.3

Note: [a] *B. c., Botrytis cinerea; C.l., Colletotrichum lagenarium; P.c. ,Phytophthora capsici; F.o., Fusarium oxysporum.sp.cucumebrium; S.s., Sclerotinia sclerotiorum; F.g., Fusarium graminearum.* [b] Natural lead compound tulipalin A was used as the positive control.

According to the mycelium linear growth rate method reported previously, all α-benzylidene-γ-lactones compounds **5** were screened for antifungal activity against six plant pathogenic fungi *in vitro* at 100 μg/mL. The results are listed in Table **1**. Almost all of the test compounds exhibited some inhibition activity against each of the fungi at 100 μg/mL, and all derivatives exhibited higher activity than the natural lead compound tulipalin A. Among these compounds, compounds **5a-5**, **5b-3-5**, **5c-2-5** showed broad spectra antifungal activities against all tested phytopathogens, especially, most compounds were more active against *B. cinerea* and *C. lagenarium*. On the basis of these results, all compounds with the higher preliminary activity against *B. cinerea* and *C.lagenarium* were further assayed for the half maximal inhibitory concentration (IC_{50}).

The results of the antifungal activity against *B. cinerea* are summarized in Table **2**,

from which it can be seen that the substitution pattern and the halogen atom containing derivatives exhibited significant antifungal activity against *B. cinerea*. The following four main structure-activity relationships (SARs) obtained. First, it is easy to see that all the compounds (5**a-1-12**, **5b-1-12**, and **5c-1-12**) were found to have higher activity than the corresponding intermediate compounds (**4a**, **4b**, and **4c**). Among all the derivatives, compounds **5c** with a chlorine atom intermediate were more active than those of the other compounds (**5a** and **5b**, respectively). Second, the introduction of the electron-withdrawing groups F, Cl, and Br to the benzene ring dramatically increased the potency. Compounds **5a-2-5**, **5b-2-5**, and **5c-2-5** (IC_{50} = 18.89-44.67 μM) exhibited antifungal activity approximately four to ten-fold higher than intermediate compounds (**4a**, **4b** and **4c**, respectively). It was notable that the IC_{50} values of **5c-3** and **5c-5** were approximately threefold less than carbendazim. While, the electron-donating groups CH_3, $C(CH_3)_3$, NH_2, CH_3O and CH_3CH_2O introduced to the benzene ring to give **5a-6-8**, **5a-11-12, 5b-6-8**, **5b-11-12, 5c-6-8**, and **5c-11-12,** (IC_{50} = 42.69-125.89 μM) greatly weakened the potency. Third, the result suggested that the steric hindrance on the benzene ring have an important influence on the antifungal activity. It is worth noting that the compounds **5c-10** (-CF_3) (IC_{50} = 33.88 μM) containing an ortho substituent on the aryl ring was found less active than compounds **5c-5** (-F) (IC_{50} = 18.89 μM) with a meta substituent. Also, the presence of a *t*-Bu group (**5a-7**, **5b-7** and **5c-7**) on the benzene ring decreases the activity in all cases. Fourth, the effects of aromatic properties of the compounds have an important influence on the antifungal activity. Compound (**5a-9**, **5b-9** and **5c-9**) with the alphy-naphthyl group increases the activity in all or most cases. Overall, the derivatives with

electron-withdrawing substituents at the meta- or para-positions improves the activity, in contrast, the presence of an electron-donating group at the ortho-positions on the aryl ring decreases the activity.

Table 2 In vitro fungicidal activity of compounds against *B.cinerea* and *C. lagenarium*.

Compd.	*B. cinerea*		*C. lagenarium*	Compd.	*B. cinerea*		*C. lagenarium*
	IC_{50} [a], μM	pIC_{50}	IC_{50} [a], μM		IC_{50} [a], μM	pIC_{50}	IC_{50} [a], μM
5a-1	61.66	-1.79	74.37	**5b-9**	61.66	-1.79	73.99
5a-2	38.02	-1.58	46.95	**5b-10**	54.95	-1.74	61.00
5a-3	27.54	-1.44	35.56	**5b-11**	125.89	-2.10	137.61
5a-4	34.67	-1.54	46.59	**5b-12**	102.33	-2.01	112.18
5a-5	26.30	-1.42	36.08	**5c-1**	35.48	-1.55	43.54
5a-6	63.10	-1.85	77.90	**5c-2**	29.51	-1.47	38.32
5a-7	83.18	-1.92	93.42	**5c-3**	22.91	-1.36	27.99
5a-8	66.07	-1.82	75.16	**5c-4**	27.54	-1.44	38.09
5a-9	51.29	-1.71	59.98	**5c-5**	18.89	-1.22	29.18
5a-10	38.90	-1.59	47.25	**5c-6**	44.67	-1.65	64.00
5a-11	97.72	-1.99	106.08	**5c-7**	52.98	-1.69	53.21
5a-12	85.11	-1.93	96.94	**5c-8**	42.69	-1.62	51.08
5b-1	72.44	-1.86	81.84	**5c-9**	37.15	-1.57	47.80
5b-2	44.67	-1.65	52.30	**5c-10**	33.88	-1.53	45.96
5b-3	30.90	-1.49	38.67	**5c-11**	58.88	-1.77	67.36
5b-4	41.69	-1.62	55.10	**5c-12**	56.23	-1.75	61.88
5b-5	28.84	-1.46	42.50	**4a**	164.30	-2.22	189.00
5b-6	95.50	-1.98	103.19	**4b**	196.19	-2.29	215.33
5b-7	97.72	-1.99	110.88	**4c**	104.31	-2.02	128.83
5b-8	77.62	-1.89	87.01	**carbendazim** [b]	8.38	-0.92	10.99

Note: [a] All half maximal inhibitory concentration (IC_{50}) values are presented as the means ± SD (n = 3), μM; [b] Commercial fungicide, carbendazim was used as the positive control.

The results of the antifungal activity against *C. lagenarium* are summarized in Table **2**, from which we can see that compounds **5a-2-5**, **5b-3**, **5b-5**, **5c-1-5**, and **5c-9-10** exhibited moderate antifungal activity against *C. lagenarium*. Most of the test compounds showed less effective than against *B. cinerea*.

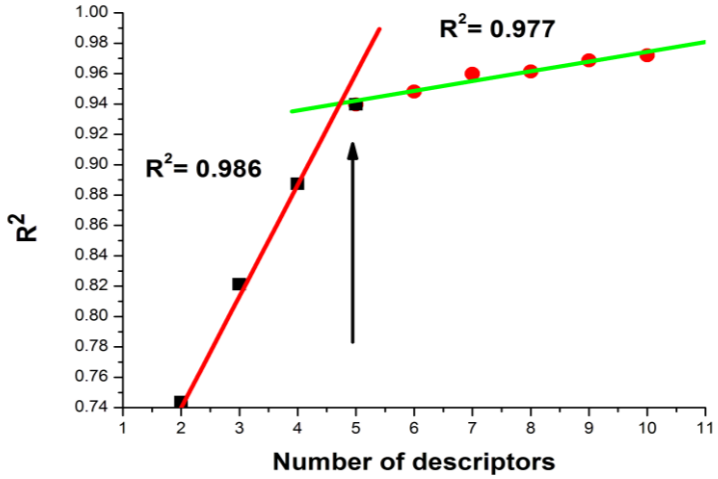


Figure 2 The "breaking point" rule results

After conformer optimizing, minimum energy calculating and format converting, the best regression relation between antifungal activity against *B. cinerea* and descriptors can be established. Therefore, it is necessary for selecting exact regression analysis method in CODESSA 2.7.15 software, which has a large number of regression analysis methods like heuristic regression analysis, multi-linear regression analysis, etc. In this paper, 36 α-methylene-γ-butyrolactones analogues were used as samples and 5 groups of descriptors were calculated. In view of the above results, the heuristic regression analysis method was considered for constructing the QSAR model. The number of the descriptors was confirmed using the "breaking point" graph rule (Figure. **2**). The heuristic regression analysis indicated a statistically significant improvement in the correlation coefficient (R^2 = 0.986) when the descriptors varied from 2 to 5. Nevertheless, no obvious improvement (R^2 = 0.977) was observed when the descriptors varied from 5 to 10. After the number of the descriptors reached a certain value, this statistical improvement in regression equation became less unimportant (ΔR^2 < 0.02–0.04).[22] Meanwhile, the numbers of samples and descriptors also meet the equation (3D $\leqslant$ S-3, S means the number of samples; D means the number of

descriptors). Thus, the final 5-descriptor model was generated according to the above results.

Table 3 The difference between the experimental pIC_{50} and predicted pIC_{50}

No.	Compd.	Calc.pIC_{50}	Exp.pIC_{50}	Difference
1	**5a-1**	-1.7563	-1.7900	0.0337
2	**5a-2**	-1.5405	-1.5800	0.0395
3	**5a-3**	-1.4717	-1.4400	-0.0317
4	**5a-4**	-1.5907	-1.5400	-0.0507
5	**5a-5**	-1.4460	-1.4200	-0.0260
6	**5a-6**	-1.8556	-1.8500	-0.0056
7	**5a-7**	-1.9483	-1.9200	-0.0283
8	**5a-8**	-1.8206	-1.8200	-0.0006
9	**5a-9**	-1.7431	-1.7100	-0.0331
10	**5a-10**	-1.5514	-1.5900	0.0386
11	**5a-11**	-1.9558	-1.9900	0.0342
12	**5a-12**	-1.9006	-1.9300	0.0294
13	**5b-1**	-1.8145	-1.8600	0.0455
14	**5b-2**	-1.6291	-1.6500	0.0209
15	**5b-3**	-1.4476	-1.4900	0.0424
16	**5b-4**	-1.6779	-1.6200	-0.0579
17	**5b-5**	-1.4744	-1.4600	-0.0144
18	**5b-6**	-1.9897	-1.9800	-0.0097
19	**5b-7**	-1.9657	-1.9900	0.0243
20	**5b-8**	-1.8666	-1.8900	0.0234
21	**5b-9**	-1.7947	-1.7900	-0.0047
22	**5b-10**	-1.7663	-1.7400	-0.0263
23	**5b-11**	-2.0828	-2.1000	0.0172
24	**5b-12**	-2.0473	-2.0100	-0.0373
25	**5c-1**	-1.4967	-1.5500	0.0533
26	**5c-2**	-1.4395	-1.4700	0.0305
27	**5c-3**	-1.3633	-1.3600	-0.0033
28	**5c-4**	-1.5062	-1.4400	-0.0662
29	**5c-5**	-1.3513	-1.3200	-0.0313
30	**5c-6**	-1.6753	-1.6500	-0.0253
31	**5c-7**	-1.6972	-1.6900	-0.0072
32	**5c-8**	-1.5774	-1.6200	0.0426
33	**5c-9**	-1.5059	-1.5700	0.0641
34	**5c-10**	-1.4896	-1.5300	0.0404
35	**5c-11**	-1.7927	-1.7700	-0.0227
36	**5c-12**	-1.7476	-1.7500	0.0024

This optimized model showed that the predicted values of pIC_{50} (negative log IC50) can be calculated, which was shown in Table **3**. Moreover, the comparison chart of predictive and practical activity of 36 derivatives is shown in Figure. **3**. This model included five descriptors in descending order according to their statistical significance (*t* values), which is shown in Table **4**, and the regression coefficients *X* and their standard errors Δ*X* are also listed. The final QSAR model with 5 descriptors was shown in Eq. (**1**) as

$$pIC_{50} = -8.6656 + 2.7387 \times MAOEP - 0.1016 \times \mu + 0.6023 \times q^{C}_{min} + 0.8535 \times n_o - 2.3901 \times q^{O}_{max} \quad (1)$$

N= 36, R^2 = 0.961, F = 84.05, S^2 = 0.0021

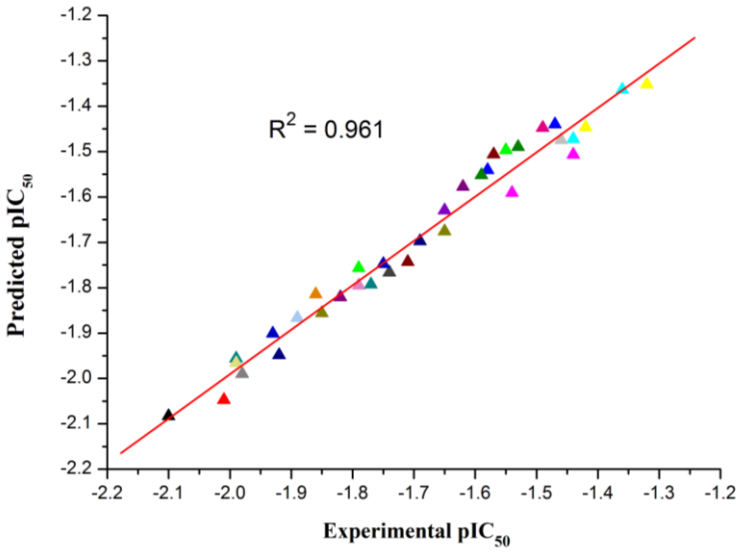


Figure 3. Experimental pIC_{50} *vs.* predicted pIC_{50}.

Table 4 The best five-descriptor model

Descriptor No.	*X*	±Δ*X*	*t*-Text	Descriptor
0	-8.6656	6.2626×10^{-1}	13.8371	Intercept
1	2.7387	3.5882×10^{-1}	-7.6452	*MAOEP* [a]
2	-1.0164×10^{-1}	1.1442×10^{-2}	8.8830	μ [b]
3	6.0228×10^{-1}	5.6884×10^{-1}	-1.0588	q^{C}_{min} [c]
4	8.5350×10^{-1}	1.8621×10^{-1}	-4.5835	n_o [d]
5	-2.3901	8.9065×10^{-1}	-2.6836	q^{O}_{max} [e]

Note: [a] Max. atomic orbital electronic population. [b] The total dipole moment of the molecule. [c] Min. net atomic charge for a C atom. [d] Number of occupied electronic levels of atoms. [e] Max. net atomic charge for a O atom.

The internal validation and the "leave-more-out" cross-validation methods were used to validate the developed QSAR model.[23] The internal validation was carried out by dividing the compound data into three subsets A–C. The compounds 1, 4, 7, 10, etc., went into the first subset (A); 2, 5, 8, 11, etc., went into the second subset (B); and 3, 6, 9, 12, etc., went into the third subset (C). Two of the three subsets, (A and B), (A and C), and (B and C), consist the training set while the remaining subset was treated as a test set. The correlation equations were derived from each of the training sets using the same descriptors and then used to predict values for the corresponding test set.[24] Internal validation results are presented in Table **5.** The $R_{Training}^2$ and R_{Test}^2 are within 5% for all three sets, and the average values of $R_{Training}^2$ = 0.964 and R_{Test}^2 = 0.963 were close to the overall R^2 value. Thus, the obtained QSAR model obtained demonstrated the predictive power of 3-fold cross-validation. Meanwhile, the "leave-more-out" method was completed in a similar manner to the internal validation. Every fourth compound (1, 5, 9, 13, etc.) was put into an external test set, and the remaining compounds were left in the training set. The QSAR model containing the same five descriptors was obtained with R^2 = 0.968 from the training set. When the same QSAR model was applied on the test set, R^2 = 0.959 was observed. Hence, the "leave-more-out" cross-validation results were also satisfactory.

Table 5 Internal validation of the QSAR model [a]

Training set	***N***	R^2	***F***	S^2	**Test set**	***N***	R^2	***F***	S^2
A+B	24	0.958	91.56	0.0028	C	12	0.967	84.65	0.0026
B+C	24	0.969	87.87	0.0022	A	12	0.958	83.82	0.0024
A+C	24	0.965	82.83	0.0024	B	12	0.963	87.31	0.0025
Average		0.964	87.42	0.0025	Average		0.963	85.26	0.0025

Note: [a] Compds. A: 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34. Compds. B: 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35. Compds. C: 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36.

Some insights into the structure of α-methylene-γ-butyrolactones derivatives structure that influences antifungal activity may be gained by explaining the descriptors referred to the constructed QSAR model. The 1st and 4th most important descriptors obtained in the model were the maximum atomic orbital electronic population and the number of occupied electronic levels of atoms, which have a significant effect on the antifungal activity. Maximum atomic orbital electronic population for a given atomic species in the molecule is an important index to describe the nucleophilicity of the molecule, which is directly related to molecular nucleophilic capacity and characterizes the susceptibility of the molecule to electrophilic attack.[25] The number of occupied electronic levels of atoms depends directly on the quantum-chemically calculated charge distribution in the molecules, and therefore describes the polar interactions between molecules.[26-27] In fact, α-methylene-γ-butyrolactones derivatives with the α, β-unsaturated carbonyl system (Michael acceptor), which had higher electron deficiency induced by electron-withdrawing groups, can be easily attacked by bionucleophiles.[12-13] In eqn (1), these two descriptors appearance with a positive sign in this model, and the electron withdrawing substitution groups in a molecule with a higher descriptor value would has a higher pIC_{50}, which indicated the obtained QSAR study result partially met the above SAR study conclusion.

The second important descriptor was the total dipole moment of the molecule. This descriptor was important in modulating antifungal activity because of the presence of C=O in the molecule, which exhibited permanent polarization due to an electronegativity difference between the atoms.[28-29] The C(C=O) atoms may be involved in binding interactions with cells present at the target site. The total dipole moment of the molecule

thus played a critical role in modulating the antifungal of the test compounds. In Eq. (**1**), appearance with a positive sign in the model indicated that molecule with higher descriptor value had a higher pIC_{50}. In contrary, a negative sign in the model indicated that molecule with lower descriptor value had a higher pIC_{50}.

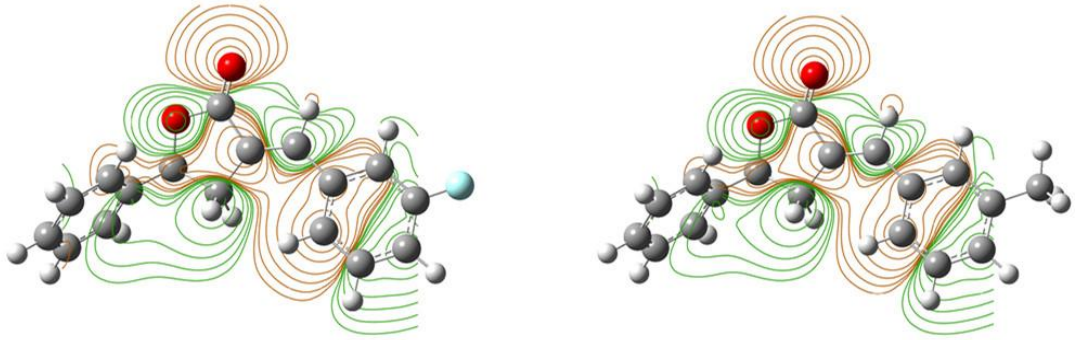
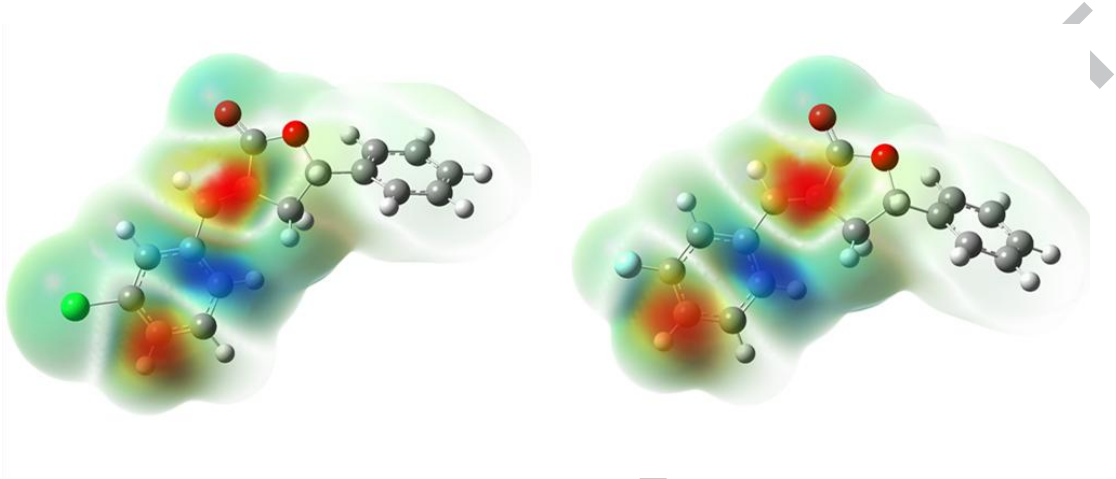


Figure 4 Optimal conformer, charge distribution and contour map of compounds 5a-5and 5a-8

The 3rd and 5th descriptors obtained in the model were min. net atomic charge for a C atom and max. net atomic charge for a O atom. These two descriptors belonged to electrostatic descriptors and they reflect the charge distribution of the molecules as shown in the contour maps (Figure. **4**), the green lines represent electron density decrease part, and the red lines represent electron density increase part which exhibits the strongest electron attraction.[30-31] Meanwhile, as the presence of the of electron activity difference between the atoms, the permanent polarization was shown in the molecular electrostatic potential map (Figure. **5**), the exocyclic carbon-carbon double bond exhibit the greater negative electrostatic potential which was easily occurred necleophilic reaction.[32-33] This finding was consistent with those of electrophilic α, β-unsaturated carbonyl structure in the α-methylene-γ-butyrolactone could reaction with biological nucleophiles.[12-13] These observations suggested that the electrostatic properties of C atom and O atom were

important elements affect the antifungal activity of the test compounds.

Figure 5 molecular electrostatic potential map of compounds **5a-3** and **5a-5**



5a-3 **5a-5**

In general, sesquiterpene lactone with the α-methylene- γ-butyrolactone structure often showed a high toxicity potential against mammalian cells.[34-35] In order to ensure the selectivity of the antifungal effects, the cytotoxicity of 20 representative derivatives was tested in the human tumor cells line (HepG2) and Hepatic L02 cells. The result is listed in Table **6**, which indicated that the QSAR underlying the antifungal and cytotoxic effects of these representative compounds are different. For instance, compound **5c-5** has the highest fungicidal activity with IC_{50} = 18.89 μM (against *B. cinerea*.) but low activity with IC_{50} = 35.4 μM (against HepG2 cell line) and IC_{50} = 68.8 μM (against Hepatic L02 cell line). On the contrary, low antifungal activity compound **5a-6** (IC_{50} = 83.18 μM) has moderate cytotoxic activity with IC_{50} = 18.7μM (against HepG2 cell line) and IC_{50} = 42.1 μM (against Hepatic L02 cell line). The results also showed that compound **5c-5** displayed high selectivity for the activity against Hepatic L02 cell line to the *B. cinerea*. (selectivity index>3). Through QSAR studies on antifungal and cytotoxicity of

α-benzylidene-γ-butyrolactone derivatives, these are important points that need further investigation to seek high activity derivatives with non-cytotoxicity.

Table 6 In vitro fungicidal activity of compounds against *B.cinerea* and cytotoxic activity against human tumor cells lines (HepG2) and Hepatic L02 cells

No.	Compd.	IC_{50} (μM) (against *B.cinerea*)	IC_{50} (μM) (against HepG2 cell line)	IC_{50} (μM) (against Hepatic L02 Cells)	Selectivity index (activity against Hepatic L02 Cells / *B.cinerea*)
1	5a-1	61.66	32.6	44.5	<1
2	5a-3	27.54	44.8	25.3	<1
3	5a-4	34.67	35.3	56.6	1
4	5a-6	83.18	18.7	42.1	<1
5	5a-7	66.07	38.3	24.0	<1
6	5a-10	38.90	45.3	26.9	<1
7	5b-2	44.67	24.5	63.6	1
8	5b-4	41.69	33.1	45.6	1
9	5b-5	28.84	21.7	32.8	1
10	5b-8	77.62	42.2	77.6	<1
11	5b-9	61.66	85.2	>112.6	1
12	5b-10	54.95	98.6	78.5	1
13	5c-3	22.91	43.6	55.9	2
14	5c-5	18.89	35.4	68.8	3
15	5c-7	52.98	78.4	89.9	1
16	5c-9	37.15	55.2	27.1	<1
17	5c-11	58.88	20.9	65.6	1
18	5c-12	56.23	>106.2	96.2	1
19	4a	164.30	>128.6	>166.1	1
20	4c	104.31	87.9	84.8	<1

In this study, 36 α-benzylidene-γ-butyrolactone derivatives, namely, **5a-1-12**, **5b-1-12** and **5c-1-12** were synthesized. The antifungal activity results showed compounds **5c** with a chlorine atom intermediate were more active than those of the other compounds. Meanwhile, the antifungal activity against *B. cinerea* and *C.lagenarium* of compounds **5c-3**

and **5c-5** was excellent. Moreover, the result of the SARs and QSAR studies exhibited that the higher electron density around the α-methylene-γ-butyrolactone backbone structure and smaller steric hindrance on the benzene ring played great beneficial effect on the antifungal activity. Although all the compounds were not so effective against *B. cinereal* and *C.lagenarium* compared with the positive control, the promising results obtained from SARs, QSAR and cytotoxicity studies based α-benzylidene-γ-butyrolactone derivatives will inspire us to carry on further work for seek the high-activity and low-toxic fungicides candidate.

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Supporting Information

Supplementary data associated with this article can be found, in the online version, at …

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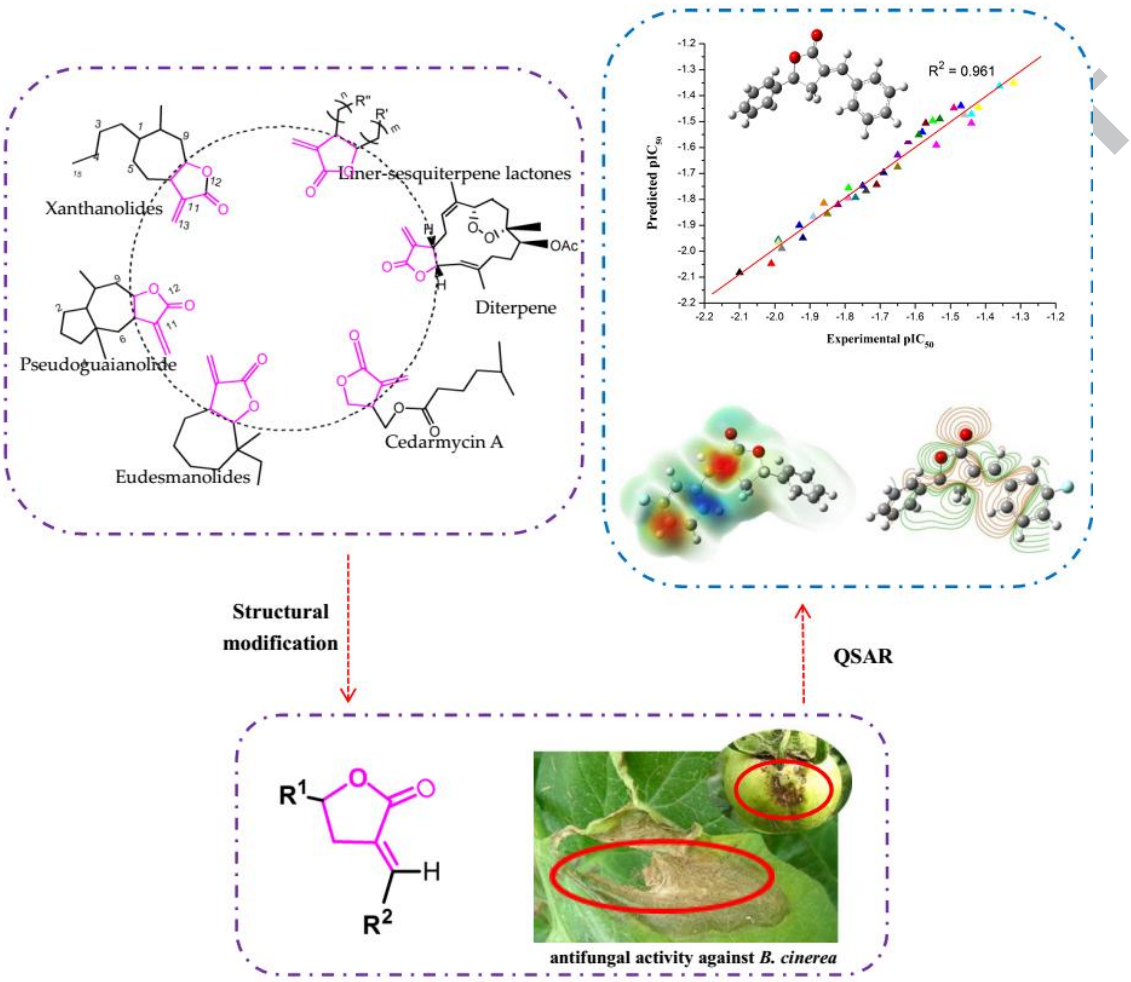

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