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Structural modification, fungicidal and insecticidal activity of 5-arylbenzofuran neolignan from *Magnolia officinalis*

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ABSTRACT

As shown in a number of previous researches, the extracts of *Magnolia officinalis* exhibited broad-spectrum fungicidal and insecticidal activity. In our preliminary study, 5-arylbenzofuran neolignans, the secondary metabolite of *Magnolia officinalis*, was found to inherit the activity of *Magnolia officinalis*. For developing novel agrochemicals, natural 5-arylbenzofuran neolignan was selected as lead compound. After structural optimization, the fungicidal and insecticidal activity and selectivity were significantly improved. Compounds **5a**, **5b**, **6c** and **6d** displayed similar or equivalent activity compared with the positive controls against corresponding fungi or pests. Especially, compounds **6c** and **6d** exhibited the most promising insecticidal ability against *Aphis fabae* with the LC_{50} values of 3.56 mg/L and 5.96 mg/L, respectively. The preliminary structure-activity relationship was obtained, and the promising derivatives showed low toxicity in the model of human cells. The results of research indicated great potential of 5-arylbenzofuran neolignan derivatives in the field of fungicide and insecticide.

1. Introduction

Agricultural chemicals have been used all over the world to guarantee the food supplies. However, with the emergence of resistance of harmful organisms and the increasing environmental and toxicological requirement, a great deal of traditional agrochemicals were faced with elimination (Paulraj et al., 2017; Shi et al., 2016). It's important to develop new agrochemicals with novel skeleton, high activity and low toxicity.

In the long evolutionary process, plants produced many secondary metabolites to protect themselves from pathogens, pests and weeds. Those plant-sourced bioactive substances often have moderate to strong activity and high safety for crops and environments (Benelli et al., 2017). Some secondary metabolites of plants have been directly used in the agricultural production, such as Toosendanin (Xu et al., 2018) and nicotine (Yang et al., 2018). Some other secondary metabolites became the lead compounds of modern agrochemicals (Fig. 1), such as Physostigmine which was the lead of carbamate insecticides, Pyrethrin I which was the origin of pyrethroids insecticides, and indole-3-acetic acid, the lead of auxin herbicides (Sparks et al., 2017).

Magnolia officinalis is a species of Magnoliaceae. According to the literatures, the extracts of *Magnolia officinalis* showed broad-spectrum inhibitory activity against plant pathogenic fungi and insect pests (Boulogne et al., 2012; Choi et al., 2009; Hu et al., 2013; Ye et al., 2015; Vásquez-Morales et al., 2015; Yang et al., 2015). 5-Arylbenzofuran neolignans are the secondary metabolites produced by *Magnolia officinalis* and a newfound class of natural compounds (Kuo et al., 2013). In our preliminary research, 5-arylbenzofuran neolignan (**1**, Fig. 1) was also found having moderate to potent fungicidal and insecticidal activity. In consideration of the activity and low molecular weight, it's appropriate to be used as the lead compound for the development of agrochemical with novel skeleton, high effect and low toxicity. Therefore, in this work, a variety of 5-arylbenzofuran neolignan derivatives were synthesized and investigated for their fungicidal and insecticidal activity.

2. Results and discussion

2.1. Chemistry

As outlined in Scheme 1, natural 5-arylbenzofuran neolignan **1**, which can only be extracted from *Magnolia officinalis* in a very low yield

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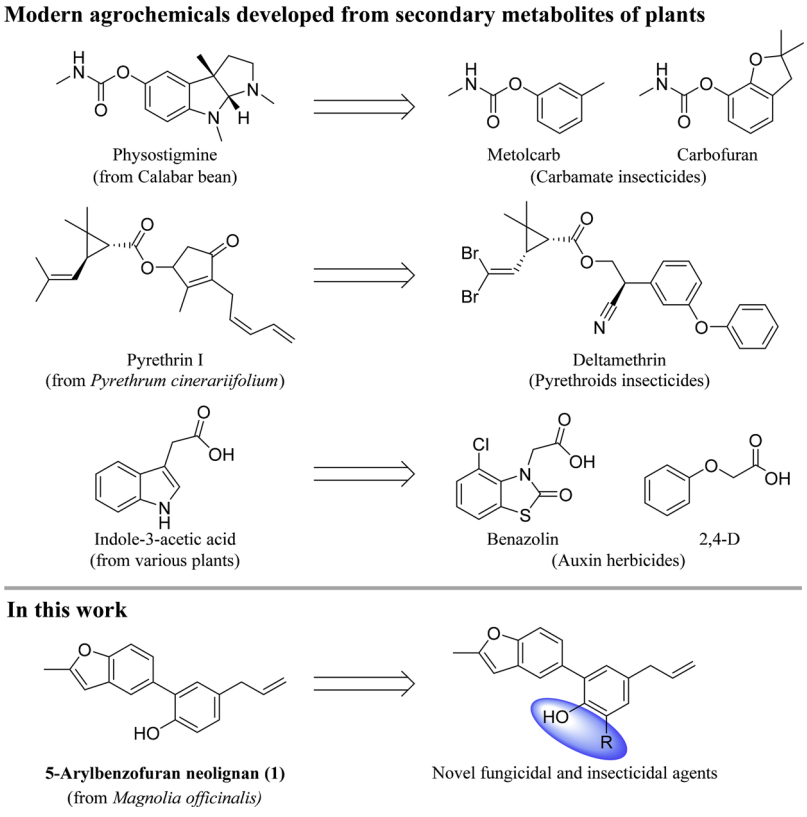
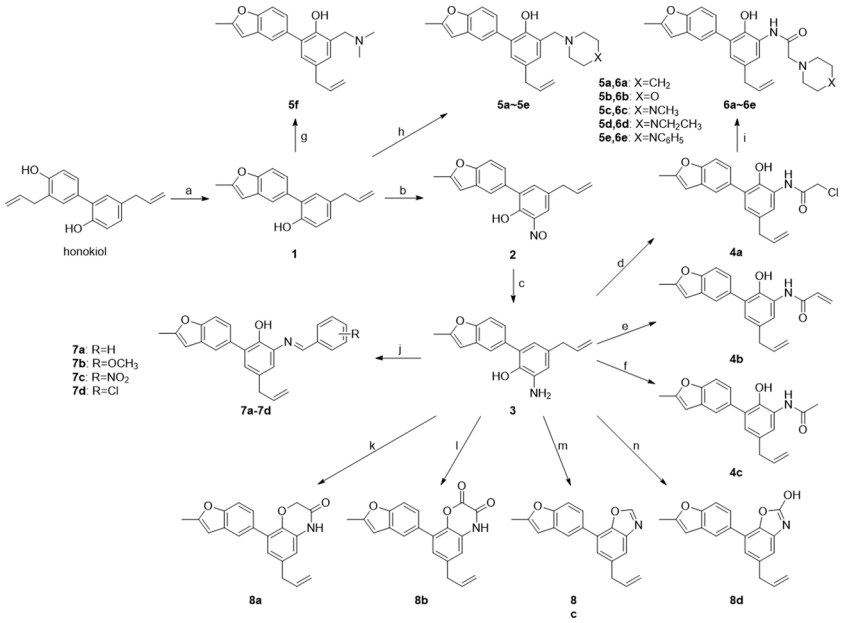


Fig. 1. Design strategy of the novel fungicidal and insecticidal agents developed from 5-arylbenzofuran neolignan.



Scheme 1. Reagents and conditions: (a) $PdCl_2$, NaOAc, O_2, DMA/H_2O, 60 °C, 86%; (b) $NaNO_2$, concentrated hydrochloric acid(36–38%), $MeCN/H_2O$, r.t., 77%; (c) Zn, NH_4Cl, HOAc, $EtOH/H_2O$, r.t., 73%; (d) chloroacetyl chloride, K_2CO_3, CH_2Cl_2, r.t., 81%; (e) acryloyl chloride, K_2CO_3, CH_2Cl_2, r.t., 46%; (f) acetic anhydride, K_2CO_3, ethyl acetate, r.t., 88%; (g) dimethylamine, HCHO(37%), MeOH, 60 °C, 53%; (h) appropriate cyclic secondary amine, HCHO(37%), MeOH, 60 °C, 55–83%; (i) appropriate cyclic secondary amine, NEt_3, THF, r.t., 48–85%; (j) substituted benzaldehyde, acetic acid, EtOH, r.t., 29–73%; (k) chloroacetyl chloride, DMAP, NEt_3, THF/CH_2Cl_2, r.t. to reflux, 44%; (l) oxalyl chloride, NEt_3, CH_2Cl_2, r.t., 42%; (m) triethyl orthoformate, NEt_3, 60 °C, 76%; (n) triphosgene, NEt_3, CH_2Cl_2, r.t., 59%.

before (Kuo et al., 2013), was now semi-synthesized from honokiol, an easily available natural product, in a high yield by Wacker-type intramolecular cyclization (Mitsudome et al., 2006). Compound **3** was synthesized by the reduction of compound **2**, which was prepared via the nitrosation of **1**. Compounds **4** were obtained from **3** by acylation reaction with chloroacetyl chloride, acryloyl chloride or acetic anhydride, respectively. Compounds **5** were generated through Mannich reaction of **1** with formalin and appropriate secondary amine. Compounds **6** were prepared by the substitution reaction between compound **4a** and appropriate cyclic secondary amine. Schiff base derivatives **7** were synthesized through the condensation reaction of compound **3** with corresponding substituted benzaldehyde. Additionally, oxazine and oxazole derivatives **8** were obtained by the cyclization of compound **3** with chloroacetyl chloride, oxalyl chloride, triethyl orthoformate or triphosgene, respectively. The synthetic procedures and characterizations are provided in the Supplementary material.

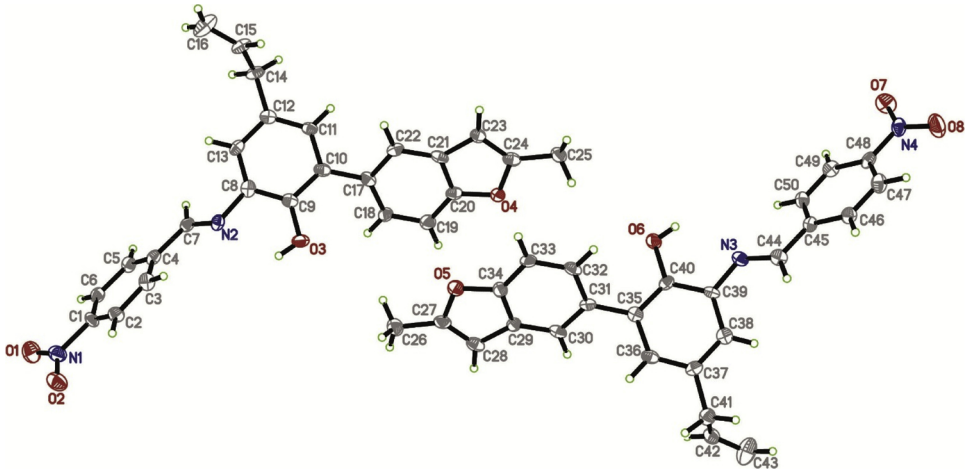


Fig. 2. Single crystal of compound **7c**.

Table 1
The fungicidal activities of target compounds tested at the concentration of 25 mg/L for 72 h.

Compd.	Inhibition rate / %				
	Pc	*Aa*	*Gz*	*Bc*	*Ss*
1	26.1	20.0	28.8	42.2	40.2
3	14.5	2.5	8.5	31.3	33.8
4a	23.0	17.1	27.4	24.1	26.2
4b	19.7	17.1	12.9	37.0	37.6
4c	19.7	19.5	16.1	48.2	45.6
5a	57.8	41.3	9.5	40.0	46.3
5b	50.7	47.5	25.4	34.4	35.8
5c	36.2	27.5	25.4	46.9	43.3
5d	39.4	39.1	25.7	51.1	59.4
5e	18.3	15.2	18.9	13.3	23.5
5f	14.5	10.0	0	31.3	30.2
6a	25.4	23.9	0	40.0	35.7
6b	4.9	2.4	16.1	3.7	5.3
6c	0	4.9	14.5	87.0	85.8
6d	32.4	26.1	17.6	48.9	50.3
6e	19.7	17.4	4.1	6.7	4.3
7a	7.3	2.5	1.7	23.4	20.0
7b	11.5	0	4.8	22.2	20.1
7c	3.3	7.3	12.9	20.4	22.4
7d	7.3	5.0	25.4	23.4	22.5
8a	16.4	9.8	27.4	27.8	28.7
8b	18.0	22.0	16.1	37.0	37.6
8c	18.2	30.2	26.9	47.4	38.8
8d	0	14.6	21.0	3.7	4.3
Thifluzamide	63.0	–	14.0	–	36.9
Boscalid	5.3	–	37.0	–	100
Azoxystrobin	56.1	–	–	47.6	93.5
Dimethachlon	–	100	–	–	–

–: not determined; *Pc: Phytophythora capsici*; *Aa*: *Alternaria alternata*; *Gz*: *Gibberella zeae*; *Bc*: *Botrytis cinerea*; *Ss*: *Sclerotonia sclerotiorum*.

Table 2
The insecticidal activities of target compounds tested at the concentration of 500 mg/L for 72 h.

Compd.	Mortality rate/%		
	Ms	*Af*	*Tu*
1	75	0	0
3	39	0	0
4a	25	0	0
4b	45	0	0
4c	5	0	0
5a	0	7	2
5b	60	0	0
5c	90	18	0
5d	0	50	21
5e	0	13	0
5f	85	40	0
6a	0	6	0
6b	65	5	0
6c	25	100	0
6d	0	100	21
6e	0	22	9
7a	55	7	0
7b	70	3	0
7c	55	20	0
7d	70	13	0
8a	80	8	15
8b	30	21	0
8c	5	28	0
8d	90	13	0
Chlorantraniliprole	100	–	–
Imidacloprid	–	100	–
Spirotetramat[α]	–	–	83

–: not determined; *Ms*: *Mythimna separate*; *Af*: *Aphis fabae*; *Tu*: *Tetranychus urticae*; [α]Spirotetramat was tested at the concentration of 20 mg/L.

2.2. Crystal structure

For well characterization of target compounds, compound **7c** was determined by single-crystal X-ray diffraction. The data obtained from X-ray investigation are in good agreement with the structure of compound **7c** (Fig. 2). It's showed that the double bond of compound **7c** is "*E*" configuration. The crystal of compound **7c** is made up of triclinic unit cells, each containing two molecules which are stabilized by π-π conjugation of benzofuran rings. The bond distances and experimental parameters are supplied in the Supplementary material. Additional data are available from the Cambridge Crystallographic Data Centre (www.ccdc.cam.ac.uk) as **CCDC 1886118**.

2.3. Biological activities

2.3.1. Fungicidal activity

The in vitro fungicidal activities of target compounds against *Phytophythora capsici*, *Alternaria alternate*, *Gibberella zeae*, *Botrytis cinerea* and *Sclerotonia sclerotiorum* were tested according to our previously reported method (Yan et al., 2018) at the concentration of 25 mg/L for 72 h. The results of fungicidal activities were listed in Table 1. Thifluzamide, Boscalid, Azoxystrobin and Dimethachlon were selected as the positive controls. Natural product **1** exhibited moderate antifungal activity against all tested fungi. With modification, the activities and selectivity were changed in some extent. The introduction of Mannich base always enhanced the inhibitory activity against *Pc* (*Phytophythora capsici*) and *Aa* (*Alternaria alternate*), especially

Table 3
The LC_{50} values of compounds **6c** and **6d** against *Aphis fabae*.

Compd.	Regression Equation	Correlation	95% Confidence Intervals (mg/L)	MW	LC_{50}	
6c	y = 4.7504 + 0.4522x	0.9855	2.61–4.86	419.52	3.56 mg/L	8.48 μM
6d	y = 4.4630 + 0.6918x	0.8912	2.95–12.11	433.55	5.96 mg/L	13.75 μM
Spirotetramat	y = 4.0389 + 2.2634x	0.9693	1.96–3.60	373.45	2.66 mg/L	7.12 μM

Table 4
Cytotoxicity of natural compound **1** and promising compounds **5a**, **5b** and **6c**.

Compd.	IC_{50}/μM		
	HepG2	A549	K562
1	125.60 ± 2.26	115.55 ± 2.05	126.20 ± 1.56
5a	> 128	> 128	> 128
5b	> 128	> 128	> 128
6c	98.59 ± 7.80	75.41 ± 2.27	61.27 ± 5.63

compounds **5a** and **5b**, the activities of which against *Pc* were similar to the positive controls Thifluzamide and Azoxystrobin. Compound **6c** displayed the best fungicidal activity and selectivity against *Bc* (*Botrytis cinerea*) and *Ss* (*Sclerotonia sclerotiorum*) with the inhibition rates of 87.0% and 85.8% respectively, which were similar or stronger compared to the positive controls Boscalid and Azoxystrobin. Conversely, the amide (**4a–4c**), Schiff base (**7a–7d**), oxazine (**8a**,**8b**) and oxazole (**8c**,**8d**) derivatives showed low fungicidal activity against all tested fungi.

2.3.2. Insecticidal activity

The in vivo insecticidal activities of target compounds against *Mythimna separate*, *Aphis fabae* and *Tetranychus urticae* were evaluated according to our previously reported method (Huang et al., 2017) at the concentration of 500 mg/L for 72 h. Chlorantraniliprole, Imidacloprid and Spirotetramat was selected as the positive control. As shown in Table 2, natural product **1** displayed potent insecticidal activity against *Ms* (*Mythimna separate*) with the mortality rate of 75%, but have no effect on *Af* (*Aphis fabae)* and *Tu* (*Tetranychus urticae*). After modification, compounds **5c**, **5f**, **8a** and **8d** showed better insecticidal activity against *Ms* than natural compound **1**. What is more surprising is that compounds **6c** and **6d** exhibited 100% kill rate against *Af*, which is worthy to be further investigated. Therefore, **6c** and **6d** were singled out to evaluate the LC_{50} against *Af*. As displayed in Table 3, the LC_{50} values of **6c** and **6d** were 3.56 mg/L (8.48 μM) and 5.96 mg/L (13.75 μM) respectively, which were equivalent or similar to that of the commercial insecticide Spirotetramat (7.12 μM) and showed great application potential.

2.3.3. Cytotoxicity

Magnolia officinalis has been used as a herbal medicine for centuries (Shih et al., 2016), which means it's relatively safe for human. For further evaluating the safety of promising compounds, we investigated their cytotoxicity, as the cytotoxicity always be used as a parameter for assessing the security of agrochemicals for human or mammal (Yun et al., 2017). The cytotoxicities of several promising compounds were evaluated by typical MTT assay or MTS assay according to our previous method (Wu et al., 2016; Fang et al., 2016) and the results were displayed in Table 4. Lead compound **1** and all of the promising compounds showed very low toxicity against HepG2, A549 and K562 cell lines with the IC_{50} values larger than 60 μM, which were greatly lower than that of Toosendanin, a widely used plant-sourced insecticide which showed high cytotoxicity with the IC_{50} values of 5.4–150 nM against various cell lines (Zhang et al., 2005).

3. Conclusions

In conclusion, natural 5-arylbenzofuran neolignan **1** was semi-synthesized and 23 kinds of derivatives were designed and prepared. All the target compounds were evaluated for their fungicidal and insecticidal activity. Several compounds exhibited more potent inhibitory activity than natural compound **1** against tested fungi and pests. Thereinto, compounds **5a**, **5b** and **6c** displayed promising antifungal activity against certain fungi at the concentration of 500 mg/L. Compounds **6c** and **6d** showed similar insecticidal ability with the commercial insecticide against *Aphis fabae* with the LC_{50} values of 8.48 μM and 13.75 μM, respectively. Additionally, the lead compound **1** and promising compounds showed low toxicity in the model of human cells. And the further research is underway. All the research results above suggested great potential of 5-arylbenzofuran neolignan derivatives as novel fungicides or insecticides with high effect and low toxicity. The research will also contribute to explore other biological activities of 5-arylbenzofuran neolignan and its derivatives.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2019.01.016.

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