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PII: S0223-5234(16)30655-9

DOI: 10.1016/j.ejmech.2016.08.012

Reference: EJMECH 8808

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 10 June 2016

Revised Date: 29 July 2016

Accepted Date: 8 August 2016

Please cite this article as: M.-Z. Zhang, R.-R. Zhang, J.-Q. Wang, X. Yu, Y.-L. Zhang, Q.-Q. Wang, W.-H. Zhang, Microwave-assisted synthesis and antifungal activity of novel fused osthole derivatives, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.08.012.

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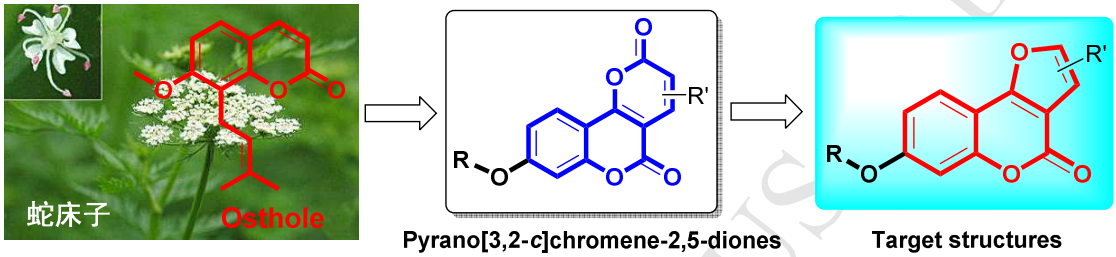


A series of novel furo[3,2-*c*]coumarins as fused Osthole derivatives were designed and synthesized through an optimized microwave-assisted protocol. Some target compounds exhibited potential activity in antifungal screening, and **6c** was identified as the most promising candidate with better antifungal activity than Azoxystrobin.

# Microwave-assisted synthesis and antifungal activity of novel fused Osthole derivatives

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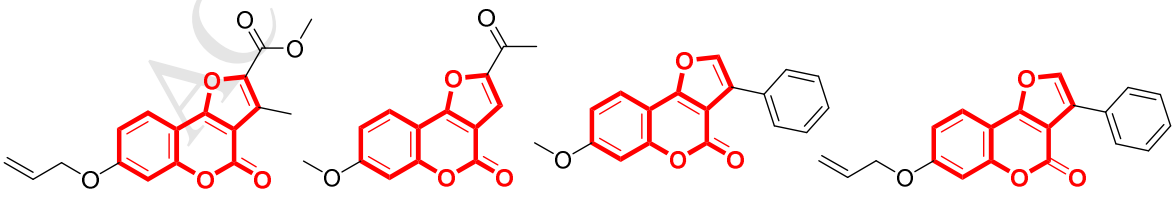
**Abstract:** Based on the microwave-assisted synthetic protocol developed in our previous work, we have synthesized a series of novel furo[3,2-*c*]coumarins as fused Osthole derivatives, via the reaction of 4-hydroxycoumarins and *β*-ketoesters catalyzed by DMAP. All the target compounds were evaluated *in vitro* for their antifungal activity against six phytopathogenic fungi, some compounds exhibited potential activity in the primary assays. Especially compounds **6c**, **7b**, **8b** and **8c** (shown in **Figure 1**) were the most active ones, $EC_{50}$ values of these four compounds against *Colletotrichum capsica*, *Botrytis cinerea* and *Rhizoctonia solani* were further investigated. **6c** was identified as the most promising candidate with the $EC_{50}$ value at 0.110 μM against *Botrytis cinerea* and 0.040 μM against *Colletotrichum capsica*, respectively, representing better antifungal activity than that of the commonly used fungicide Azoxystrobin.



**6c** **7b** **8b** **8c**

**Figure 1.** Structures of the most active synthetic furo[3,2-*c*]coumarin derivatives

**Key words :** Osthole; Microwave-assisted; Furo[3,4-*c*]coumarin; Synthesis; Antifungal activity.

## 1. Introduction

Osthole, a natural occurring coumarin derivative, was reported as the biologically active ingredient of Cnidium Monnieri, a traditional Chinese herbal medicine. As shown in **Figure 2**, Osthole is chemically known as an *O*-methylated coumarin, which is also found in many plants and possesses a broad scope of pharmacological and biochemical activities[1-4], including antiarrhythmia, antidiabetic, anticancer, antiosteoporosis, antiinflammatory, hepatoprotection and neuroprotection. It has been widely used as a fungicide in China for a long history[5], displaying a range of antifungal activities against *Rhizoctonia solani* and other phytopathogenic fungi[6].

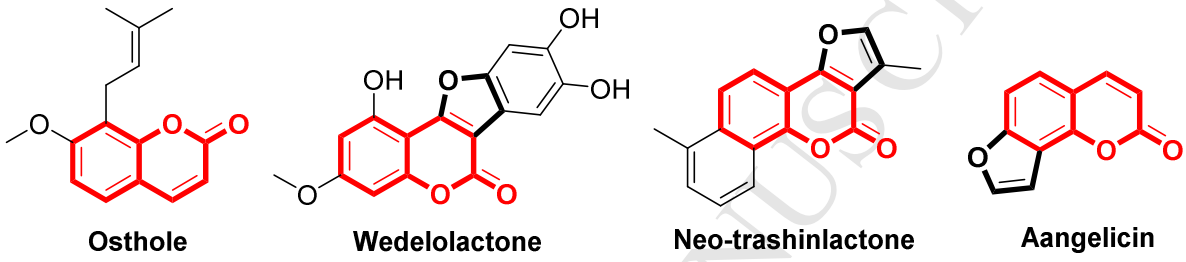



**Figure 2.** Structures of osthole and furocoumarins

Recently, some novel structures and their antifungal activity have been reported[7-9], osthole and its analogues have attracted great research interest for agricultural chemists, as it could be a promising lead structure for exploiting new pesticides for the treatment of agricultural diseases[10, 11]. Meanwhile, osthole derivatives (**Figure 2**), such as furo[3,2-*c*]coumarin, exhibited interesting biological acivities because of their capability to form covalent bonds with DNA and other biological macromolecules[12], such as **Wedelolactone**, is a antihepatotoxic coumestan from Eclipta prostrata[13], **Neo-tanshinlactone** showed significant inhibition against two estrogen receptor positive human breast cancer cell lines[14, 15], while **Angelicin** is a furocoumarin naturally occurring tricyclic aromatic compound, it is reported to exhibit anticancer, antiviral, antiinflammatory activities[16-18].

In our previous work, we have accomplished various structural modifications to osthole, including coumarino[8,7-*e*][1,3]oxazine derivatives[19], and novel fused coumarin analogues pyrano[3,2-*c*]chromene-2,5-diones[20]. Bioscreening results showed that some of the synthesized compounds exhibited effective control to certain phytopathogenic diseases. However, their potency is too low to be used as agricultural fungicide. In a continuation of our studies aimed at the

discovery of diversified analogues with improved antifungal activity, we now describe our research which has focused on the synthesis and antifungal activity of novel furo[3,2-*c*]coumarins (**Figure 3**).

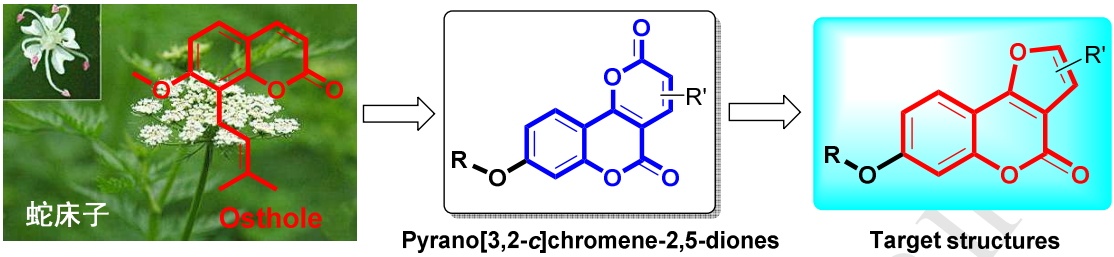



**Figure 3**. Design strategy for target molecules

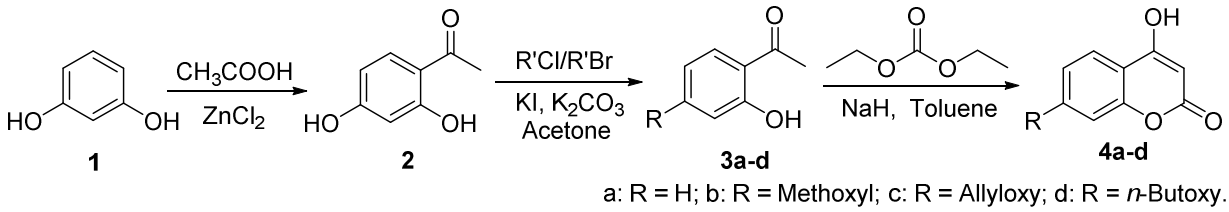
## 2. Materials and methods

### *2.1 Chemicals and Instruments*

All chemicals including 2-Hydroxyacetophenone were purchased from commercial sources (e.g., Crystal Chemicals) and used without further purification unless otherwise stated. The microwave treatment was carried out in a microwave oven (WBFY-201, Gongyi Yuhua Instrument Co., Ltd.) with an emission frequency of 2450 MHz and a maximum output power of 800 W. The course of reactions and the purity of products were monitored by TLC using silica gel GF/UV 254. The melting points of these coumarin derivatives were determined on an X-4 apparatus (uncorrected), which was bought from Shanghai Tech. Infrared (IR) spectra were recorded on a Bruker Tensor 27 spectrometer, and samples were prepared as KBr plates. $^{1}$H NMR and $^{13}$C NMR spectra were obtained with a Bruker Avance 400 MHz spectrometer in $CDCl_3$ solution with TMS as an internal standard. HR-MS (ESI) spectra were carried out with a Thermo Exactive spectrometer, and X-Rays were measured at 296 K on a Bruker SMART APEX2 CCD area detector diffractometer.

#### *2.1.1 General procedure for the preparation of compounds 4a-d (**Scheme 1**)*

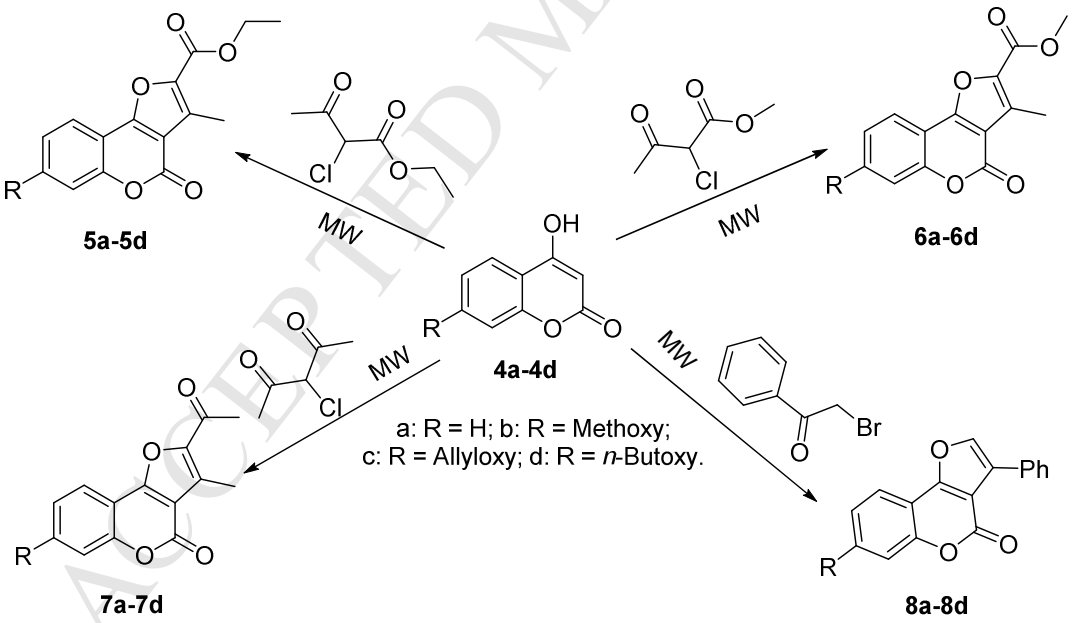
4-Hydroxycoumarins **4a-4d** were synthesized through the reported synthetic route[20], yields for **4b**, **4c**, and **4d** are 85%, 86%, and 82%, respectively.




**Scheme 1.** Synthetic route for 4-Hydroxycoumarins **4a**-**4d**

*2.1.2 Microwave-assisted synthetic procedure for the preparation of compounds 5a-8d (**Scheme 2**)*

Based on the method reported in our previous work[20], compounds **5-8** could be prepared in moderate yields with toluene as the solvent, using 640W microwave irradiation, and DMAP as the catalyst. The yields vary from 61% to 77% considerably depending upon the irradiative time, which determined by the TLC monitor. After the reaction was complete, toluene was removed by rotavapor, and the resulting residue was recrystallized from ethanol and water (10:1 in volume) or purified by column chromatography. The experimental results were summarized in **Table 1** reaction, reaction yields were not optimized.




**Scheme 2.** Synthetic routes for the target compounds

**Table 1**. The structures and yields of target compounds **5a-8d**

| **Product** | **Reaction Time** | **R** | **Yield** (%) |
|---|---|---|---|
| 5a | 10min | H | 65 |
| 5b | 15min | Methoxy | 77 |
| 5c | 10min | Allyloxy | 67 |

| | | | |
|---|---|---|---|
| 5d | 10min | *n*-Butoxy | 63 |
| 6a | 10min | H | 67 |
| 6b | 15min | Methoxy | 73 |
| 6c | 15min | Allyloxy | 65 |
| 6d | 15min | *n*-Butoxy | 61 |
| 7a | 10min | H | 69 |
| 7b | 15min | Methoxy | 73 |
| 7c | 15min | Allyloxy | 65 |
| 7d | 15min | *n*-Butoxy | 62 |
| 8a | 15min | H | 67 |
| 8b | 15min | Methoxy | 73 |
| 8c | 15min | Allyloxy | 65 |
| 8d | 15min | *n*-Butoxy | 61 |

Yields after recrystallization or isolation by column chromatography.

*2.1.3 Compounds data*

**3-Methyl-4-oxo-4H-furo[3,2-*c*]chromene-2-carboxylic acid ethyl ester（5a）：** a white solid, mp: 140.2-140.6℃， $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, J = 7.9, 1.4 Hz, 1H), 7.69 – 7.58 (m, 1H), 7.56 – 7.37 (m, 2H), 4.50 (q, J = 7.1 Hz, 2H), 2.75 (s, 3H), 1.50 (t, J = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl3 ) δ 159.01, 158.07, 157.79, 153.50, 141.85, 132.09, 130.31, 124.74, 121.90, 117.40, 112.18, 111.52, 61.45, 14.36, 10.15; IR (KBr) ν (cm-1) 1708, 1631, 1446, 1321, 1139, 1087; HR-MS (ESI): m/z calcd for $C_{15}H_{12}O_5$([M+H]$^+$) 273.07630, found 273.07575.

**7-Methoxy-3-methyl-4-oxo-4H-furo[3,2-*c*]chromene-2-carboxylic acid ethyl ester（5b）：** a white solid, mp: 186.4-187.1℃， $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.69 – 7.58 (m, 1H), 7.56 – 7.37 (m, 2H), 4.50 (q, *J* = 7.1 Hz, 2H), 2.75 (s, 3H), 1.50 (t, *J* = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 163.07, 159.14, 158.71, 158.10, 155.37, 141.03, 130.45, 122.95, 113.03, 109.18, 105.41, 101.37, 61.32, 55.85, 14.37, 10.17; IR (KBr) ν ($cm^{-1}$) 1739, 1627, 1442, 1323, 1241, 963; HR-MS (ESI): m/z calcd for $C_{16}H_{14}O_6$ ([M+H]$^+$ ) 303.08686, found 303.08631.

**7-Allyloxy-3-methyl-4-oxo-4H-furo[3,2-*c*]chromene-2-carboxylic acid ethyl ester（5c）：** a white solid, mp: 155.5-156.2℃, $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.7 Hz, 1H), 7.00 (dd, *J* = 12.6, 3.8 Hz, 2H), 6.10 (ddd, *J* = 16.6, 10.4, 5.2 Hz, 1H), 5.45 (dd, *J* = 39.7, 13.9 Hz, 2H), 4.67 (d, *J* = 5.2 Hz, 2H), 4.49 (q, *J* = 7.1 Hz, 2H), 2.73 (s, 3H), 1.49 (t, *J* = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 162.01, 159.14, 158.70, 158.11, 155.30, 141.08, 132.02, 130.45, 122.97, 118.66, 113.57, 109.25, 105.55, 102.25, 69.35, 61.31, 14.37, 10.17; IR (KBr) ν ($cm^{-1}$) 1720, 1607, 1450, 1317, 1244, 1083, 994; HR-MS (ESI): m/z calcd for $C_{18}H_{16}O_6$ ( [M + H]$^+$ ) 329.10252, found 329.10196.

**7-Butoxy-3-methyl-4-oxo-4H-furo[3,2-*c*]chromene-2-carboxylic acid ethyl ester（5d）：** a white solid, mp :189.3-192.3℃, $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.69 – 7.58 (m, 1H), 7.56 – 7.37 (m, 2H), 4.50 (q, *J* = 7.1 Hz, 2H), 2.75 (s, 3H), 1.50 (t, *J* = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 173.09, 165.25, 162.73, 155.77, 155.45, 126.39, 122.94, 113.73, 113.48, 108.54, 101.87, 100.77, 68.54, 61.30, 30.95, 19.16, 14.38, 13.78, 10.20; IR (KBr) ν ($cm^{-1}$) 1732, 1696, 1623, 1442, 1309, 1135, 962; HR-MS (ESI): m/z calcd for $C_{19}H_{20}O_6$ ($[M + H]^+$) 345.13382, found 345.13331.

**3-Methyl-4-oxo-4H-furo[3,2-*c*]chromene-2-carboxylic acid methyl ester（6a）:** a white solid, mp: 152.2-152.4℃, $^{1}$H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.8 Hz, 1H), 7.59 (t, *J* = 26.3 Hz, 1H), 7.43 (dt, *J* = 34.3, 17.6 Hz, 2H), 4.04 (t, *J* = 16.4 Hz, 3H), 2.76 (t, *J* = 16.6 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 159.34, 158.12, 157.72, 153.50, 141.59, 132.16, 130.64, 124.78, 121.85, 117.41, 112.11, 111.49, 52.21, 10.12; IR (KBr) ν ($cm^{-1}$) 1700, 1623, 1446,1325, 1232, 1131, 1083; HR-MS (ESI): m/z calcd for $C_{14}H_{10}O_5$ ($[M+H]^+$) 259.06065, found 259.06010.

**7-Methoxy-3-methyl-4-oxo-4H-furo[3,2-*c*]chromene-2-carboxylic acid methyl ester（6b）：** a white solid, mp: 216.0℃, $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.6 Hz, 1H), 7.31 (t, *J* = 14.7 Hz, 1H), 6.96 (d, *J* = 11.6 Hz, 1H), 4.01 (dd, *J* = 17.9, 11.4 Hz, 3H), 3.95 – 3.88 (m, 3H), 2.72 (dd, *J* = 17.9, 11.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 163.15, 159.51, 158.83, 158.09, 155.45, 140.83, 130.83, 122.95, 113.10, 109.21, 105.41, 101.43, 55.87, 52.12, 10.16; IR (KBr) ν ($cm^{-1}$) 1736, 1699, 1617, 1445, 1325, 1235, 1082, 960; HR-MS (ESI): m/z calcd for $C_{15}H_{10}O_5$ ($[M + H]^+$) 289.07122, found 289.07066.

**7-Allyloxy-3-methyl-4-oxo-4H-furo[3,2-*c*]chromene-2-carboxylic acid methyl ester（6c）：** a white solid, mp: 151.0-154.9℃, $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.98 – 7.85 (m, 1H), 7.04 – 6.89 (m, 2H), 6.12 – 5.99 (m, 1H), 5.43 (dd, *J* = 49.7, 13.8 Hz, 2H), 4.65 (s, 2H), 4.07 – 3.94 (m, 3H), 2.77 – 2.65 (m, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 162.05, 159.46, 158.73, 158.02, 155.31, 140.82, 132.00, 130.79, 122.91, 118.74, 113.55, 109.22, 105.47, 102.25, 69.46, 52.09, 10.13; IR (KBr) ν ($cm^{-1}$) 1732, 1623, 1446, 1333, 1260, 1091, 994; HR-MS (ESI): m/z calcd for $C_{17}H_{14}O_6$ ($[M + H]^+$) 315.08686, found 315.08631.

**7-Butoxy-3-methyl-4-oxo-4H-furo[3,2-*c*]chromene-2-carboxylic acid methyl ester（6d）:** a white solid, mp: 145.9-146.8℃, $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.90 (dd, *J* = 8.6, 3.5 Hz, 1H), 6.94 (dd, *J* = 11.6, 5.9 Hz, 2H), 4.10 – 4.03 (m, 2H), 4.00 (d, *J* = 3.5 Hz, 3H), 2.70 (t, *J* = 9.4 Hz, 3H), 1.84 (s, 2H), 1.56 (dd, *J* = 16.8, 5.8 Hz, 3H), 1.10 – 0.96 (m, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 159.34,

158.12, 157.72, 153.50, 141.59, 132.16, 130.64, 124.78, 121.85, 117.41, 112.11, 111.49, 68.46, 52.21, 30.98, 19.18, 13.80, 10.12; IR (KBr) ν ($cm^{-1}$) 1758, 1711, 1614, 1453, 1329, 1132, 999; HR-MS (ESI): m/z calcd for $C_{18}H_{18}O_6$ ($[M + H]^+$) 331.11816, found 331.11761.

**2-Acetyl-furo[3,2-*c*]chromen-4-one（7a）:** a white solid, mp: 178.7-179.5℃, $^1$H NMR (400 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.66 – 7.56 (m, 1H), 7.52 – 7.35 (m, 2H), 2.75 (s, 3H), 2.65 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 188.73, 157.79, 157.33, 153.66, 149.14, 132.28, 129.54, 124.84, 121.57, 117.54, 112.12, 112.04, 27.66, 10.24; IR (KBr) ν ($cm^{-1}$) 1739, 1683, 1623, 1548, 1442, 1360, 1229, 1185, 994; HR-MS (ESI): m/z calcd for $C_{14}H_{10}O_4$ ($[M+H]^+$) 243.06573, found 243.06519.

**2-Acetyl-7-methoxy-furo[3,2-*c*]chromen-4-one(7b):** a white solid, mp: 200.9-203.2℃, $^1$H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.6 Hz, 1H), 7.03 – 6.90 (m, 2H), 3.96 – 3.87 (m, 3H), 2.74 (s, 3H), 2.62 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 188.56, 163.23, 158.06, 158.03, 155.61, 148.61, 129.77, 122.62, 113.22, 109.69, 105.35, 101.46, 55.95, 27.60, 10.28; IR (KBr) ν ($cm^{-1}$) 1744, 1627, 1546, 1438, 1276, 1087; HR-MS (ESI): m/z calcd for $C_{15}H_{12}O_5$ ($[M+H]^+$) 273.07630, found 273.007575.

**2-Acetyl-7-allyloxy-furo[3,2-*c*]chromen-4-one(7c):** a white solid, mp: 146.2-146.3℃, $^1$H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.7 Hz, 1H), 6.99 – 6.87 (m, 2H), 4.05 (t, *J* = 6.5 Hz, 2H), 2.77 – 2.66 (m, 3H), 2.58 (d, *J* = 17.9 Hz, 3H), 1.87 – 1.76 (m, 2H), 1.52 (dt, *J* = 14.8, 7.5 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 188.58, 162.14, 158.10, 158.04, 155.50, 148.61, 131.95, 129.76, 122.63, 118.76, 113.73, 109.72, 105.44, 102.30, 69.39, 27.62, 10.29; IR (KBr) ν ($cm^{-1}$) 1736, 1607, 1438, 1333, 1252, 1099, 998; HR-MS (ESI): m/z calcd for $C_{17}H_{14}O_5$ ($[M+H]^+$) 289.09195, found 289.09140.

**2-Acetyl-7-butoxy-furo[3,2-*c*]chromen-4-one(7d):** a white solid, mp: 149.7℃, $^1$H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.7 Hz, 1H), 6.99 – 6.87 (m, 2H), 4.05 (t, *J* = 6.5 Hz, 2H), 2.77 – 2.66 (m, 3H), 2.58 (d, *J* = 17.9 Hz, 3H), 1.87 – 1.76 (m, 2H), 1.52 (dt, *J* = 14.8, 7.5 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 188.52, 162.84, 158.12, 158.09, 155.62, 148.56, 129.78, 122.54, 113.59, 109.58, 105.11, 101.89, 68.50, 30.98, 27.58, 19.18, 13.80, 10.28; IR (KBr) ν ($cm^{-1}$) 1744, 1667, 1627, 1595, 1458, 1256, 1011; HR-MS (ESI): m/z calcd for $C_{18}H_{18}O_5$ ($[M+H]^+$) 315.12325, found 315.12270.

**3-Phenyl-furo[3,2-*c*]chromen-4-one(8a):** a white solid, mp: 209.1-210.2℃, $^1$H NMR (500 MHz, $CDCl_3$) δ 7.95 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.80 (dd, *J* = 6.3, 2.0 Hz, 2H), 7.57 (ddd, *J* = 8.8, 7.3, 1.5

Hz, 1H), 7.51 – 7.47 (m, 3H), 7.44 – 7.38 (m, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 158.80, 157.86, 152.61, 141.28, 130.97, 129.09, 128.68, 128.59, 128.39, 126.74, 124.51, 120.99, 117.13, 112.77, 108.48; IR (KBr) ν ($cm^{-1}$) 1732, 1627, 1490, 1438, 1317, 1079, 962; HR-MS (ESI): m/z calcd for $C_{17}H_{10}O_3$ ($[M+H]^+$) 263.07082, found 263.07027.

**7-Methoxy-3-phenyl-furo[3,2-*c*]chromen-4-one(8b):** a white solid, mp: 162.1-162.4□, $^1$H NMR (500 MHz, $CDCl_3$) δ 7.95 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.80 (dd, *J* = 6.3, 2.0 Hz, 2H), 7.57 (ddd, *J* = 8.8, 7.3, 1.5 Hz, 1H), 7.51 – 7.47 (m, 3H), 7.44 – 7.38 (m, 3H), $^{13}$C NMR (101 MHz, $CDCl_3$) δ 162.18, 159.46, 158.17, 154.39, 140.38, 129.29, 128.62, 128.54, 128.27, 126.47, 121.95, 112.88, 106.14, 106.05, 101.04, 55.78; IR (KBr) ν ($cm^{-1}$) 1732, 1627, 1454, 1337, 1236, 1131; HR-MS (ESI): m/z calcd for $C_{18}H_{12}O_4$ ($[M + H]^+$) 293.08139, found 293.08084.

**7-Allyloxy-3-phenyl-furo[3,2-*c*]chromen-4-one(8c):** a white solid, mp: 167.4-168.5℃, $^1$H NMR (500 MHz, $CDCl_3$) δ 7.80 (ddd, *J* = 14.9, 11.1, 6.3 Hz, 3H), 7.72 (d, *J* = 1.7 Hz, 1H), 7.53 – 7.38 (m, 3H), 6.99 (td, *J* = 4.6, 2.3 Hz, 2H), 6.09 (ddt, *J* = 17.3, 10.6, 5.3 Hz, 1H), 5.55 – 5.34 (m, 2H), 4.69 – 4.62 (m, 2H), $^{13}$C NMR (101 MHz, $CDCl_3$) δ 161.11, 159.44, 158.14, 154.31, 140.40, 132.22, 129.29, 128.62, 128.54, 128.27, 126.49, 121.95, 118.55, 113.41, 106.29, 106.13, 101.96, 69.29; IR (KBr) ν ($cm^{-1}$) 1739, 1611, 1451, 1232, 1138, 1068, 941; HR-MS (ESI): m/z calcd for $C_{20}H_{14}O_4$ ($[M+H]^+$) 319.09704, found 319.09649.

**7-Butoxy-3-phenyl-furo[3,2-*c*]chromen-4-one(8d):** a white solid, mp: 182.0-182.1℃, $^1$H NMR (500 MHz, $CDCl_3$) δ 7.84 – 7.77 (m, 3H), 7.71 (d, *J* = 1.6 Hz, 1H), 7.51 – 7.44 (m, 2H), 7.44 – 7.38 (m, 1H), 6.98 – 6.94 (m, 2H), 4.06 (t, *J* = 6.5 Hz, 2H), 1.91 – 1.79 (m, 2H), 1.55 (td, *J* = 14.8, 7.3 Hz, 3H), 1.06 – 0.97 (m, 3H), $^{13}$C NMR (101 MHz, $CDCl_3$) δ 161.80, 159.56, 158.20, 154.44, 140.30, 132.17, 129.35, 128.62, 128.52, 128.24, 126.49, 121.87, 113.29, 105.97, 101.54, 68.36, 31.04, 19.21, 13.83; IR (KBr) ν ($cm^{-1}$) 1728, 1623, 1510, 1446, 1272, 1244; HR-MS (ESI): m/z calcd for $C_{21}H_{18}O_4$ ($[M+H]^+$) 335.12834, found 335.12779.

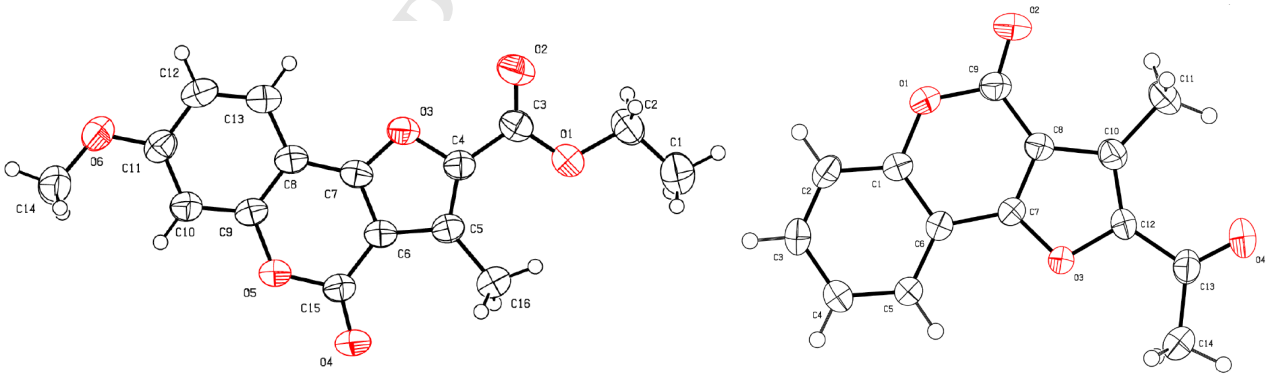
*2.2. X-ray diffraction analysis*

White crystals of compound **5b** (0.280 × 0.260 × 0.220 $mm^3$) were mounted on a quartz fiber with protection oil. Cell dimensions and intensities were measured at 296 K on a Bruker SMART APEX2 CCD area detector diffractometer with graphite monochromated MoKα radiation (λ= 0.71073 Å); $\theta_{max}$ = 25.010; 2016 independent reflections ($R_{int}$ = 0.0408). The structure was solved by direct methods using SHELXS-97; all other calculations were performed with Bruker SAINT System and Bruker SMART programs. Full-matrix least-squares refinement based on $F^2$ using the

weight of ω= 1/[σ2(Fo2) + (0.0659P)2 + 0.0500P] gave final values of R = 0.0408, ωR = 0.1331, in the title compound, the moiety formula is $C_{16}H_{14}O_6$.

White crystals of compound **7a** (0.500 × 0.480 × 0.360 $mm^3$) were mounted on a quartz fiber with protection oil. Cell dimensions and intensities were measured at 296 K on a Bruker SMART APEX2 CCD area detector diffractometer with graphite monochromated MoKα radiation (λ= 0.71073 Å); $\theta_{max}$ = 27.517;1993 independent reflections ($R_{int}$ = 0.0453).Data were corrected for Lorentz and polarization effects and for absorption ($T_{min}$ = 0.942; $T_{max}$ = 0.961). The structure was solved by direct methods using SHELXS-97; all other calculations were performed with Bruker SAINT System and Bruker SMART programs. Full-matrix least-squares refinement based on $F^2$ using the weight of ω= 1/[σ2(Fo2) + (0.0633P)2 + 0.4557P] gave final values of R = 0.0453, ωR = 0.1377, max/min residual electron density = 0.205 and -0.171 e.$Å^{-3}$. Hydrogen atoms were observed and refined with a fixed value of their isotropic displacement parameter. In the title compound, the moiety formula is $C_{14}H_{10}O_4$.

The crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC) as supplementary publication number: CCDC 1055787 (**5b**), CCDC 1417754 (**7a**), Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB12 1EZ, UK (Fax + 441223336033 or E-mail: deposit@ccdc.cam.ac.uk). The structures are shown in **Figure 4**.




**Compound 5b** **Compound 7a**

**Figure 4**. X-ray structures of compounds **5b** and **7a**

*2.3. Biological assays*

The antifungal activities of all the synthesized target compounds were carried out at the concentration of 50 μg/mL using mycelia growth inhibitory rate methods, with Azoxystrobin used as the positive control. The tested fungi were provided by the Laboratory of Plant Disease Control, Nanjing Agricultural University, and they are representative fungal strains in the outbreaks region in China and US, the detailed procedure of experimental methods for the antifungal activity refer to the paper from Department of Plant Pathology, Nanjing Agriculture University[21]. After retrieval from the storage tube, the strains were then incubated in PDA at 25 □ for a week to get new mycelia for the antifungal assay. All the test compounds were first dissolved in 5 mL DMF to generate a 100 μg/mL stock solution. The needed test solutions were prepared by diluting the above solution. The antifungal results of all the compounds against *Botrytis cinerea, Collecterichu mcapsica, Alternaria solani, Gibberella zeae, Cucumber anthrax* and *Rhizoctorzia solani* are listed in **Table 2**.

**Table 2**. Antifungal activity of coumarin analogues (inhibitory rate, %)

| Species [a] | | BOT | COL | ALT | GIB | CUC | RHI |
|---|---|---|---|---|---|---|---|
| Compd | Rate (μg/mL) | 50 | 50 | 50 | 50 | 50 | 50 |
| 5a | | 10.7 [b] | 31.7 | 10.0 | 13.2 | 20.1 | 33.4 |
| 5b | | 11.8 | 25.7 | 10.0 | 11.8 | 11.4 | 23.8 |
| 5c | | 10.1 | 31.4 | 10.0 | 14.7 | 15.7 | 30.2 |
| 5d | | 12.8 | 20.1 | 10.0 | 13.2 | 18.6 | 22.8 |
| 6a | | 20.7 | 32.9 | 10.0 | 16.7 | 18.6 | 34.7 |
| 6b | | 22.6 | 24.3 | 10.0 | 11.8 | 15.7 | 37.1 |
| 6c | | **54.2** | **65.7** | 14.2 | 41.2 | 27.1 | **54.7** |
| 6d | | 14.1 | 21.4 | 10.0 | 11.8 | 15.7 | 24.7 |
| 7a | | 25.7 | 32.9 | 10.1 | 16.2 | 15.7 | 32.8 |
| 7b | | **40.8** | 35.7 | 10.1 | 23.5 | 21.4 | **64.3** |
| 7c | | 22.1 | 24.5 | 10.0 | 11.8 | 25.7 | 33.2 |
| 7d | | 20.7 | 21.7 | 10.0 | 11.8 | 15.7 | 27.4 |
| 8a | | 16.1 | 30.1 | 10.0 | 11.8 | 22.9 | 33.8 |
| 8b | | 27.1 | 34.7 | 10.0 | 11.8 | 20.1 | 47.8 |
| 8c | | 15.7 | 35.7 | 10.0 | 14.7 | 22.9 | 42.9 |
| 8d | | 20.7 | 20.1 | 10.0 | 27.5 | 20.1 | 33.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Azoxystrobin | 33.8 | 43.6 | 15.7 | 67.6 | 82.9 | 62.9 |

[a] BOT, Botrytis cinerea; COL, Collecterichum capsica; ALT, Alternaria solani; GIB, Gibberella zeae; CUC, Cucumber anthrax; RHI, Rhizoctorzia solani.

[b] All the data was the average value of three replications, 10.0 indicate the data below 10% inhibitory.

**Table 3**. $EC_{50}$ determination of compounds **6c**, **7c**, **8b** and **8c**

| Pathogen | Compound | Toxic regression | R | $EC_{50}$ (μM) | 95% confidence interval |
|---|---|---|---|---|---|
| *Botrytis cinerea* | 6c | Y=4.0226+0.6349x | 0.9996 | **0.110** | 0.1069-0.1136 |
| | 7b | Y=3.0842+0.9859x | 0.9999 | 0.322 | 0.3139-0.3312 |
| | 8b | Y=3.3405+0.7564x | 0.9995 | 0.533 | 0.482-0.590 |
| | 8c | Y=3.2146+0.6974x | 0.9994 | 1.140 | 0.978-1.325 |
| | Azoxystrobin | Y=3.0886+1.0598x | 0.9997 | 0.158 | 0.1453-0.1708 |
| *Collecterichum capsica* | 6c | Y=4.2672+0.6668x | 0.9998 | **0.040** | 0.0385-0.0414 |
| | 7b | Y=3.7545+0.6248x | 0.9998 | 0.362 | 0.3492-0.3947 |
| | 8b | Y=3.3566+0.7364x | 0.9999 | 0.584 | 0.5697-0.5981 |
| | 8c | Y=3.4286+0.7088x | 1.0000 | 0.518 | 0.5112-0.5253 |
| | Azoxystrobin | Y=3.9631+0.5312x | 0.9985 | 0.222 | 0.1936-0.2543 |
| *Rhizoctorzia solani* | 6c | Y=3.6804+0.8438x | 0.9996 | 0.110 | 0.1069-0.1136 |
| | 7b | Y=4.2512+0.6556x | 0.9995 | **0.051** | 0.0485-0.0537 |
| | 8b | Y=3.5907+0.7947x | 0.9999 | 0.203 | 0.1999-0.2065 |
| | 8c | Y=3.8061+0.5964x | 0.9998 | 0.316 | 0.3049-0.3263 |
| | Azoxystrobin | Y=3.8654+0.8546x | 0.9995 | 0.053 | 0.0506-0.0548 |

The $EC_{50}$ value was the average value of three replications.

## 3. Results and discussion

### *3.1 Synthetic chemistry*

In our previous study, the molecule annulation at the 3, 4 position of coumarin structure resulting in Pyrano[3,2-*c*]coumarin derivatives was reported, the application of microwave irradiation has been employed as an efficient and green synthetic approach which dramatically reduces reaction time. Based on our aim to discover novel Osthole analogues with improved antifungal activity, our previous research was focused on a series of fused coumarin analogues:

pyrano[3,2-*c*]chromene-2,5-diones. In this study, we design and synthesize furo[3,2-*c*]coumarin as the scaffold for the further structural modification. With the optimized microwave-assisted synthetic method, some *β*-ketoesters including 3-chloropentane-2,4-dione, ethyl 2-chloroacetoacetate, methyl 2-chloro-3-oxobutanoate and 2-bromoacetophenone were employed to react with 4-Hydroxycoumarins to extend the variety of furocoumarin derivatives, then a focused library of 16 compounds diversified on furan ring and 7-position of coumarin was set up for the antifungal screening.

All of the target molecules listed in **Table 1** were characterized by IR, $^{1}$H NMR, $^{13}$C NMR and HR-MS. In addition, the structures of two typical compounds **5b** and **7a** were further confirmed by X-ray crystallographic diffraction analysis (**Figure 4**).

*3.2 Antifungal activity and the structure-activity relationships (SAR)*

The results of the antifungal activity screening against six phytopathogenic fungi are shown in **Table 2**. For the purposes of analysis of structure-activity relationships, the antifungal activity of all target compounds were compared with the positive control Azoxystrobin, a broad spectrum fungicide used for protecting plants and food crops from fungal diseases.

In general, data presented in **Table 2** indicate that the synthesized furocoumarin derivatives exhibit certain activities against *Botrytis cinerea*, *Collecterichumcapsica* and *Rhizoctorzia solani* at 50 μg/mL, while, most of the target compounds showed rather poor activity against *Alternaria solani, Gibberella zeae* and *Cucumber anthrax*.

As four compounds (**6c**, **7b**, **8b** and **8c**) showed relatively effective control against *Botrytis cinerea, Colletotrichum capsica* and *Rhizoctorzia solani*, therefore, we further tested the $EC_{50}$ values of these compounds together with Azoxystrobin. As shown in **Table 3**, it was noticed that the $EC_{50}$ values of compounds **6c** was as low as 0.110 and 0.040 μM against *Botrytis cinerea* and *Colletotrichum capsica*, respectively, which proves it's more effective than Azoxystrobin (0.158 μM). Compound **7b** (0.051 μM) exhibited slightly better activity than the control Azoxystrobin (0.053 μM) against *Rhizoctorzia solani*.

Although the antifungal activity for most synthesized derivatives has been proven to be slightly poor, making it hard to extract a distinct structure-activity relationship analysis, we still can draw some broad conclusions from the presented data. Firstly, these fused coumarin compounds

were noticeably more active against *Botrytis cinerea*, *Colletotrichum capsici* and *Rhizoctorzia solani*, but they usually lacked potency against *Alternaria solani*, *Gibberella zeae* and *Cucumber anthrax*, as illustrated by the absence of activity against these three kinds of fungi. Secondly, the alkoxy group on the 7-position of coumarin is essential for the antifungal activity, as compounds (**5a**, **6a**, **7a** and **8a**) showed very weak activity or even totally inactive against the tested phytopathogenic fungi, however, their equivalents with methoxy, allyloxy or *n*-butoxy on 7-position of the coumarin ring showed greatly improved antifungal activity, highlighted by compounds **6c**, **7b**, **8b** and **8c**. Thirdly, though the influence of substituents on the furan ring is not clear due to the limited diversity, we still noticed that the antifungal activity of ethyl ester (**5a**-**5d**) is usually weaker than their equivalents with methyl ester (**6a**-**6d**) or acetyl group (**7a**-**7d**).

## 4. Conclusions

In summary, aiming to discover novel Osthole analogues with improved antifungal activity, we have efficiently synthesized a series of novel furo[3,2-*c*]coumarins through the microwave-assisted protocol developed in our previous research. Biological testing data showed that the fused Osthole analogues displayed an altered pattern of biological activity, compounds **6c**, **7b**, **8b** and **8c** were identified as the most active ones, the $EC_{50}$ values of these compounds together with Azoxystrobin were further tested. Compared to Azoxystrobin, compound **6c** displayed improved activity against *Botrytis cinerea*, *Collecterichum capsica*, and equivalent activity against *Rhizoctorzia solani*, compound **7b** also showed slightly better activity against *Rhizoctorzia solani*. Compound **6c** was identified as the most promising candidate for further study. Further structural optimization of fused Osthole analogues is well underway, alongside more detailed biological testing of the most active compounds, with the aim to improve their levels of antifungal activity.

## Acknowledgement

The project was supported by the National Natural Science Foundation of China (21272116) and the Fundamental Research Funds for the Central Universities (KYTZ201604). We thank Dr. Fenger Zhou from Chemistry Department at Texas A&M University (US) for his kind help on the manuscript revision.

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**Figure 1.** Structures of the most active synthetic furo[3,2-*c*]coumarin derivatives

**Figure 2.** Structures of osthole and furocoumarins

**Figure 3**. Design strategy for target molecules

**Figure 4**. X-ray structures of compounds **5b** and **7a**

**Scheme 1.** Synthetic route for 4-Hydroxycoumarins **4a-4d**

**Scheme 2.** Synthetic routes for the target compounds

**Table 1**. The structures and yields of target compounds **5a-8d**

**Table 2**. Antifungal activity of coumarin analogues (inhibitory rate, %)

**Table 3**. $EC_{50}$ determination of compounds **6c**, **7c**, **8b** and **8c**

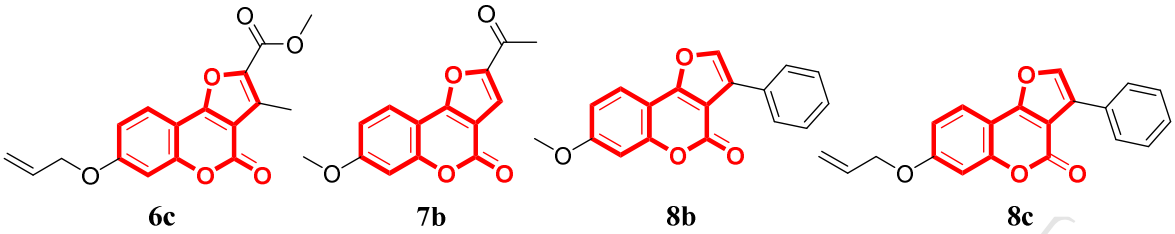



**Figure 1.** Structures of the most active synthetic furo[3,2-*c*]coumarin derivatives

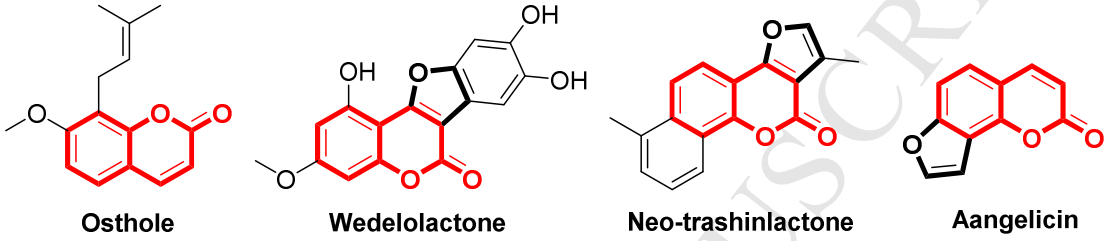



**Figure 2.** Structures of osthole and furocoumarins

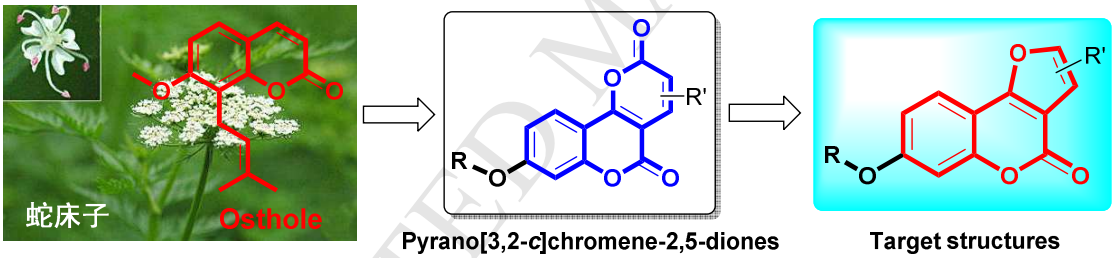



**Figure 3**. Design strategy for target molecules

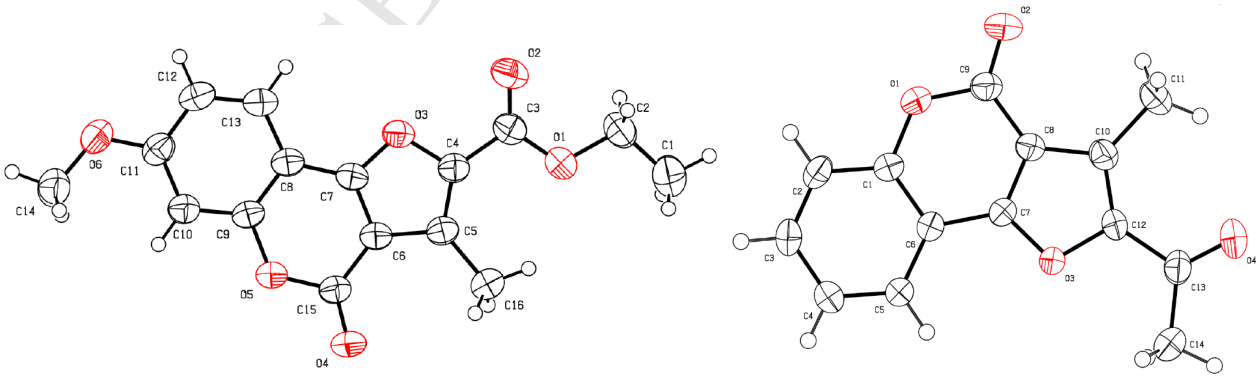
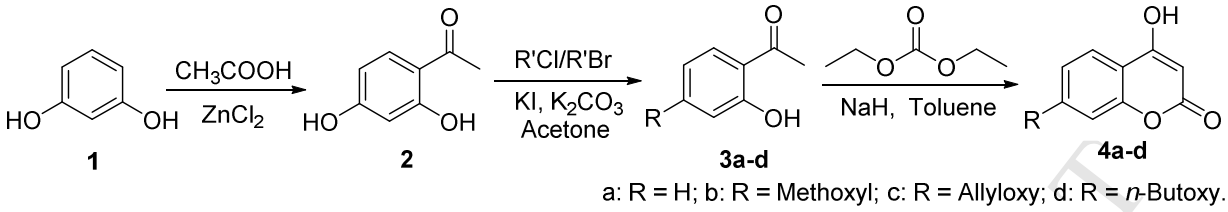
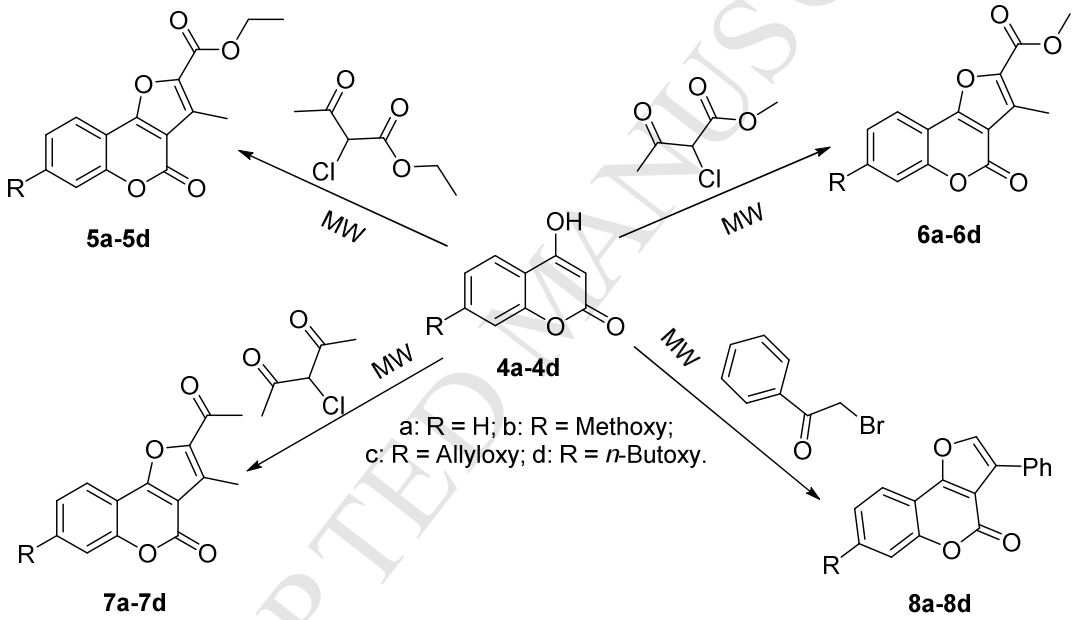



**Figure 4**. X-ray structures of compounds **5b** and **7a**




**Scheme 1.** Synthetic route for 4-Hydroxycoumarins **4a**-**4d**




**Scheme 2.** Synthetic routes for the target compounds

**Table 1**. The structures and yields of target compounds **5a-8d**

| **Product** | **Reaction Time** | **R** | **Yield** (%) |
|---|---|---|---|
| 5a | 10min | H | 65 |
| 5b | 15min | Methoxy | 77 |
| 5c | 10min | Allyloxy | 67 |
| 5d | 10min | *n*-Butoxy | 63 |
| 6a | 10min | H | 67 |
| 6b | 15min | Methoxy | 73 |
| 6c | 15min | Allyloxy | 65 |
| 6d | 15min | *n*-Butoxy | 61 |
| 7a | 10min | H | 69 |
| 7b | 15min | Methoxy | 73 |
| 7c | 15min | Allyloxy | 65 |
| 7d | 15min | *n*-Butoxy | 62 |
| 8a | 15min | H | 67 |
| 8b | 15min | Methoxy | 73 |
| 8c | 15min | Allyloxy | 65 |
| 8d | 15min | *n*-Butoxy | 61 |

Yields after recrystallization or isolation by column chromatography.

**Table 2**. Antifungal activity of coumarin analogues (inhibitory rate, %)

| Species [a] | | BOT | COL | ALT | GIB | CUC | RHI |
|---|---|---|---|---|---|---|---|
| Compd | Rate (μg/mL) | 50 | 50 | 50 | 50 | 50 | 50 |
| 5a | | 10.7 [b] | 31.7 | 10.0 | 13.2 | 20.1 | 33.4 |
| 5b | | 11.8 | 25.7 | 10.0 | 11.8 | 11.4 | 23.8 |
| 5c | | 10.1 | 31.4 | 10.0 | 14.7 | 15.7 | 30.2 |
| 5d | | 12.8 | 20.1 | 10.0 | 13.2 | 18.6 | 22.8 |
| 6a | | 20.7 | 32.9 | 10.0 | 16.7 | 18.6 | 34.7 |
| 6b | | 22.6 | 24.3 | 10.0 | 11.8 | 15.7 | 37.1 |
| 6c | | **54.2** | **65.7** | 14.2 | 41.2 | 27.1 | **54.7** |
| 6d | | 14.1 | 21.4 | 10.0 | 11.8 | 15.7 | 24.7 |
| 7a | | 25.7 | 32.9 | 10.1 | 16.2 | 15.7 | 32.8 |
| 7b | | **40.8** | 35.7 | 10.1 | 23.5 | 21.4 | **64.3** |
| 7c | | 22.1 | 24.5 | 10.0 | 11.8 | 25.7 | 33.2 |
| 7d | | 20.7 | 21.7 | 10.0 | 11.8 | 15.7 | 27.4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8a | 16.1 | 30.1 | 10.0 | 11.8 | 22.9 | 33.8 |
| 8b | 27.1 | 34.7 | 10.0 | 11.8 | 20.1 | 47.8 |
| 8c | 15.7 | 35.7 | 10.0 | 14.7 | 22.9 | 42.9 |
| 8d | 20.7 | 20.1 | 10.0 | 27.5 | 20.1 | 33.8 |
| Azoxystrobin | 33.8 | 43.6 | 15.7 | 67.6 | 82.9 | 62.9 |

[a] BOT, Botrytis cinerea; COL, Collecterichum capsica; ALT, Alternaria solani; GIB, Gibberella zeae; CUC, Cucumber anthrax; RHI, Rhizoctorzia solani.

[b] All the data was the average value of three replications, 10.0 indicate the data below 10% inhibitory.

**Table 3**. $EC_{50}$ determination of compounds **6c**, **7c**, **8b** and **8c**

| Pathogen | Compound | Toxic regression | R | $EC_{50}$ (μM) | 95% confidence interval |
|---|---|---|---|---|---|
| *Botrytis cinerea* | 6c | Y=4.0226+0.6349x | 0.9996 | **0.110** | 0.1069-0.1136 |
| | 7b | Y=3.0842+0.9859x | 0.9999 | 0.322 | 0.3139-0.3312 |
| | 8b | Y=3.3405+0.7564x | 0.9995 | 0.533 | 0.482-0.590 |
| | 8c | Y=3.2146+0.6974x | 0.9994 | 1.140 | 0.978-1.325 |
| | Azoxystrobin | Y=3.0886+1.0598x | 0.9997 | 0.158 | 0.1453-0.1708 |
| *Collecterichum capsica* | 6c | Y=4.2672+0.6668x | 0.9998 | **0.040** | 0.0385-0.0414 |
| | 7b | Y=3.7545+0.6248x | 0.9998 | 0.362 | 0.3492-0.3947 |
| | 8b | Y=3.3566+0.7364x | 0.9999 | 0.584 | 0.5697-0.5981 |
| | 8c | Y=3.4286+0.7088x | 1.0000 | 0.518 | 0.5112-0.5253 |
| | Azoxystrobin | Y=3.9631+0.5312x | 0.9985 | 0.222 | 0.1936-0.2543 |
| *Rhizoctorzia solani* | 6c | Y=3.6804+0.8438x | 0.9996 | 0.110 | 0.1069-0.1136 |
| | 7b | Y=4.2512+0.6556x | 0.9995 | **0.051** | 0.0485-0.0537 |
| | 8b | Y=3.5907+0.7947x | 0.9999 | 0.203 | 0.1999-0.2065 |
| | 8c | Y=3.8061+0.5964x | 0.9998 | 0.316 | 0.3049-0.3263 |
| | Azoxystrobin | Y=3.8654+0.8546x | 0.9995 | 0.053 | 0.0506-0.0548 |

The $EC_{50}$ value was the average value of three replications.

## Highlights

The microwave-assisted synthesis of novel furo[3,2-*c*]coumarins was described.

Antifungal activity screening of the designed compounds against six fungi was screened.

Some compounds exhibited potent antifungal activity with **6c** as the most promising candidate for further study.

The SAR of synthesized furo[3,2-*c*]coumarins were summarized.