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ORIGINAL ARTICLE

Microwave-assisted Synthesis and antifungal activity of coumarin[8,7-*e*][1,3]oxazine derivatives

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Abstract The synthesis of novel coumarin[8,7-*e*][1,3]oxazine derivatives through a microwave-assisted three-component one-pot Mannich reaction is described in this study. All the target compounds were evaluated *in vitro* for their antifungal activity against *Botrytis cinerea*, *Colletotrichum capsici*, *Alternaria solani*, *Gibberella zeae*, *Rhizoctonia solani*, and *Alternaria mali*. The preliminary bioassays showed that **5e**, **5m**, and **5s** exhibited good antifungal activity and the most active compound was **5m** with an EC_{50} value as low as 2.1 nM against *Botrytis cinerea*.

Keywords Coumarin · Oxazine · Microwave-assisted synthesis · Mannich reaction · Antifungal activity · SAR

Introduction

Coumarins, an important class of heterocyclic compounds, are widely distributed throughout nature as secondary metabolites from plants. They are frequently associated with a wide range of biological activities, such as anticoagulant, anticancer, and antifungal activities [1–3]. As the structural core, coumarin is used regularly as a scaffold in medicinal and agricultural chemistry. Examples, such as Warfarin and Acenocoumarol (Fig. 1), are anticoagulant agents that function as vitamin K antagonists used in the prevention of thrombosis and thromboembolism [4–6]. Osthole (Fig. 1), a natural *O*-methylated coumarin found in many plants, shows antifungal activity against *Rhizoctonia solani* and a broad spectrum of other phytopathogenic fungi [7–9], and has a long history of use in China as a fungicide. The recently introduced strobilurin fungicide Coumoxystrobin (Fig. 1) is also a coumarin derivative. It contains an (*E*)-methyl 3-methoxy-2-phenylacrylate substructure and displays a broad spectrum of antifungal activity [10–12].

In our previous work, Osthole was used as the lead structure to carry out structural optimization [13–15], as the 2*H*-pyran-2-one substructure in Osthole can be considered as configuration ring-closed analog of (*E*)-methyl 3-methoxy-2-phenylacrylate (shown in Fig. 2), which is the pharmacophore of the strobilurin fungicides. In our lab we observed that designed compounds containing the coumarin scaffold exhibited high potency against phytopathogenic fungi (unpublished results). In this paper, we focus not only on the side-chain cyclization, but also on the introduction of an oxazine ring to the coumarin structure through a microwave-assisted three-component one-pot Mannich reaction (as shown in Fig. 2), as compounds containing an oxazine subunit usually demonstrate favorable drug-like properties, including good solubility and balanced lipophilicity [16–19]. Some coumarin[8,7-*e*][1,3]oxazine derivatives have also been reported to exhibit anti-inflammatory and antitumour properties [20–24]. Aside from this, further structural optimization and antifungal activity of novel coumarin[8,7-*e*][1,3]oxazine derivatives have not been reported before.

Aiming to discover promising compounds for their use as antifungal agents with a broad spectrum of activity and a new mode of action, several 7-hydroxycoumarins were used as the parent structure to generate a series of

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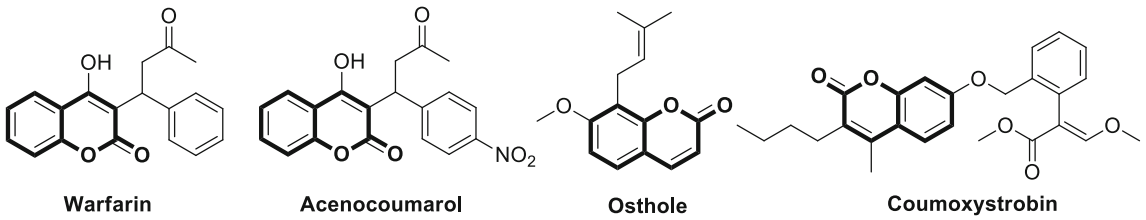
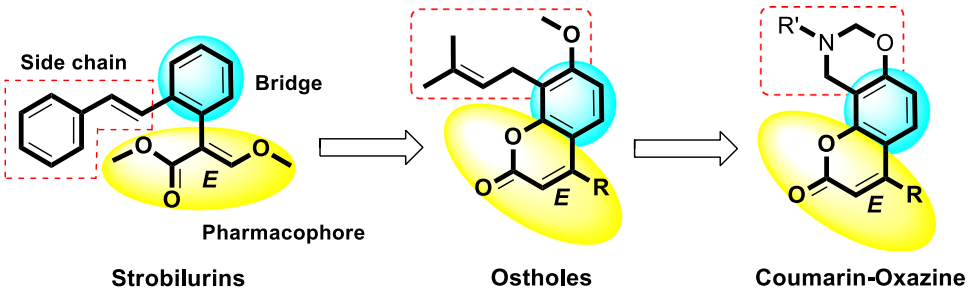
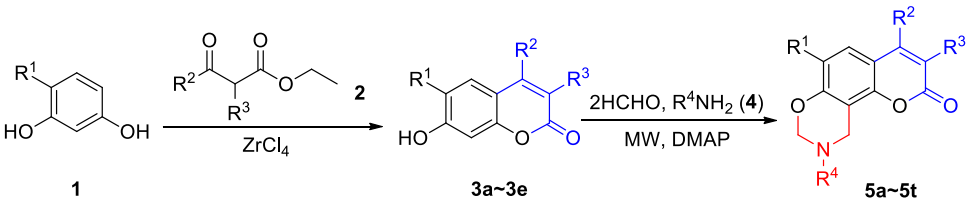


Fig. 1 Structures of coumarin-containing drugs and agrochemicals

Fig. 2 Design strategy for the target molecules



Scheme 1 Synthetic route to coumarin[8,7-*e*][1,3]oxazine derivatives



R^1 = H, R^2 = Me, R^3 = H: R^4 = Ph (**5a**), 4-Me-C_6H_4 (**5b**), 4-Cl-C_6H_4 (**5c**), $CH_2C_6H_4$ (**5d**)
R^1 = Cl, R^2 = Me, R^3 = H: R^4 = Ph (**5e**), 4-Me-C_6H_4 (**5f**), 4-Cl-C_6H_4 (**5g**), $CH_2C_6H_4$ (**5h**)
R^1 = H, R^2 = Ph, R^3 = H: R^4 = Ph (**5i**), 4-Me-C_6H_4 (**5j**), 4-Cl-C_6H_4 (**5k**), $CH_2C_6H_4$ (**5l**)
R^1 = Cl, R^2 = CF_3, R^3 = H: R^4 = Ph (**5m**), 4-Me-C_6H_4 (**5n**), 4-Cl-C_6H_4 (**5o**), $CH_2C_6H_4$ (**5p**)
R^1 = Cl, R^2 = Me, R^3 = Me: R^4 = Ph (**5q**), 4-Me-C_6H_4 (**5r**), 4-Cl-C_6H_4 (**5s**), $CH_2C_6H_4$ (**5t**)

coumarin[8,7-*e*][1,3]oxazine derivatives. Herein, we report the novel and efficient microwave-assisted synthesis of coumarin[8,7-*e*][1,3]oxazine derivatives from readily available starting materials and their in vitro antifungal activity against six phytopathogenic fungi.

Results and discussion

Synthetic chemistry [25]

The coumarin ring was prepared via a Pechmann condensation using $ZrCl_4$ as catalyst, and the 1,3-oxazine ring was introduced through a microwave-assisted Mannich reaction. Aniline, 4-methylaniline, 4-chloroaniline, and benzylamine were used as the amine input in the condensation step for the construction of the oxazine unit (Scheme 1).

In our initial study, we utilized microwave irradiation to synthesize the designed coumarin[8,7-*e*][1,3]oxazines, as opposed to using the conventional heating (Entries $l - o$). Reaction times can be reduced from about 6 hours to 30 min, and isolated yields increased up to 65 % (Table 1). The reaction of 7-hydroxycoumarins (**3a**–**3e**) with different amines using catalytic amounts of 4-*N*,*N*-dimethylaminopyridine (DMAP) to make **5a** was chosen as the model reaction to optimize the reaction conditions (shown in Scheme 2). 1,4-Dioxane was found to be the best solvent for this reaction (Entries $a - d$) under a microwave power of 400 W, with the yields increasing consistently with time, from 17 % (10 min) to a maximum of 65 % (30 min) (Entries f, h, and i). Increasing the microwave power and reaction time did not lead to a significant improvement in yield. The results are summarized in Table 1.

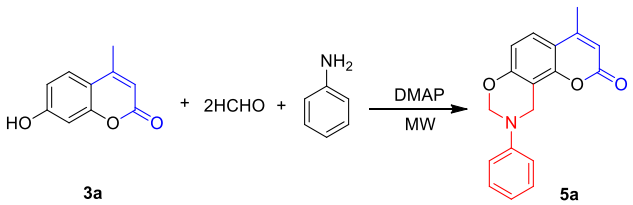
Biological assays

The materials and methods used in the biological assays are the same as those reported in the literature [26]. The antifungal activity of the synthesized compounds was assessed using solutions at a concentration of 50 ppm and Osthole as positive control. The biological results for the compounds tested against six phytopathogenic fungi are reported in Table 2. In

Table 1 Optimization parameters for formation of the oxazine ring (**5a**)

Entry	Solvent	MW power	Temperature	Time	Yield (%)[a]
a	THF	320W	r.t.	30 min	32
b	Ethanol	320 W	r.t.	30 min	41
c	Toluene	320 W	r.t.	30 min	16
d	1,4-dioxane	320 W	r.t.	30 min	58
e	1,4-dioxane	240 W	r.t.	30 min	24
f	1,4-dioxane	400 W	r.t.	30 min	65
g	1,4-dioxane	480 W	r.t.	30 min	59
h	1,4-dioxane	400 W	r.t.	10 min	17
i	1,4-dioxane	400 W	r.t.	20 min	35
j	1,4-dioxane	400 W	r.t.	35 min	63
k	1,4-dioxane	400 W	r.t.	40 min	62
l	Toluene	–[b]	80 °C	6 h	26
m	Ethanol	–[b]	Reflux	6 h	41
n	1,4-dioxane	–[b]	80 °C	6 h	43
o	1,4-dioxane	–[b]	100 °C	6 h	60

[a]Yields after purification by recrystallization
[b]Conventional heating, no microwave irradiation



Scheme 2 Synthesis of **5a**

addition, the EC_{50} values of the most active compounds were obtained and are shown in Table 3.

Antifungal activity and the structure-activity relationships (SAR)

As shown in Table 2, these target compounds exhibited different rates of growth inhibition against the six fungi, and some showed strong activity against *Botrytis cinerea* and *Rhizoctonia solani*. Compounds **5e**, **5m**, **5s**, and Osthole showed 88.2, 98.7, 78.6, and 86.8 % inhibition, respectively, against *Botrytis cinerea*. Compounds **5g** and **5m** displayed 66.7 and 79.6 % inhibition, respectively, against *Colletotrichum capsici*, and were more active than the positive control Osthole. Compounds **5e**, **5m**, **5o** exhibited 73.3, 88.2, and 81.5 % inhibition, respectively, against *Rhizoctonia solani*.

Although the antifungal activity of most of the coumarin derivatives proved to be quite poor, making it difficult to elucidate a detailed structure-activity relationship analysis, some broad observations can still be drawn. First, it was noticeable that where compounds were active it was most commonly against *Botrytis cinerea*, *Colletotrichum capsici*, *Gibberella zeae* and *Rhizoctonia solani*, but lacking potency against *Alternaria solani* and *Alternaria mali*, as illustrated by the absence of activity against theses two kinds of fungi, shown in Table 2. Nonetheless, compound **5p** showed equivalent activity with the positive control Osthole against *Alternaria mali*. Second, the spectrum of antifungal activity is generally improved by introducing a Cl to the 6-position of the coumarin ring. Indeed, compounds **5e**, **5i**, and **5m** displayed a broader spectrum of activity compared with compound **5a**. Thirdly, compounds exhibited better activity against *Botrytis cinerea* and *Rhizoctonia solani* when R^4 was a phenyl group rather than substituted benzene or benzyl groups, for example, compounds **5a**, **5e**, **5i**, **5m**, and **5q** were much more active against *Botrytis cinerea* and *Rhizoctonia solani* than the other R^4 compounds. Analogs **5e** and **5m** even showed improved activity compared with the positive control. However, for the other substituents of the coumarin ring, the SAR was rather flat, i.e., marginally affected by structural alterations.

In addition, as shown in Table 3, the EC_{50} values of these active compounds were measured. The EC_{50} values of **5m** against *Botrytis cinerea*, *Colletotrichum capsici* and *Rhizoctonia solani* were 2.1, 19, and 5.8 nM, respectively, more potent than Osthole. Compound **5g** displayed similar activity to Osthole against *Colletotrichum capsici*. Compounds **5e** and **5o** showed weaker inhibition than Osthole against *Rhizoctonia solani*. It is worth mentioning that the most active compound **5m** was identified as the most promising candidate for further study with a broad-spectrum of antifungal activity.

Table 2 Antifungal activity of compounds **5a**–**5t** (Inhibition rates %, at 50 ppm)

Compd.	*Botrytis cinerea*	*Colletotrichum capsici*	*Alternaria solani*	*Gibberella zeae*	*Rhizoctonia solani*	*Alternaria mali*
5a	63.9	14.1	31.4	7.1	61.5	55.4
5b	33.4	18.2	17.5	17.9	48.8	7.8
5c	76.2	33.3	19.4	16.9	57.5	29.8
5d	30.4	14.1	5.6	**69.8**	14.9	16.1
5e	**88.2**	34.4	21.3	26.8	**73.3**	45.3
5f	28.6	16.7	3.4	17.1	60.3	7.1
5g	27.1	**66.7**	14.5	34.1	51.4	42.3
5h	62.0	41.7	3.5	31.8	28.6	61.9
5i	76.9	22.4	3.9	19.5	40.4	9.5
5j	37.8	31.3	11.3	**74.5**	29.3	16.7
5k	23.9	37.5	12.1	29.2	45.7	22.0
5l	16.5	37.5	4.5	41.5	28.0	42.3
5m	**98.7**	**79.6**	37.5	**69.8**	**88.2**	44.6
5n	53.9	47.2	26.7	19.5	62.4	10.1
5o	69.7	45.3	18.1	34.1	**81.5**	20.3
5p	57.8	25.5	10.6	**40.6**	31.0	**78.6**
5q	62.6	37.2	13.8	30.8	62.7	49.1
5r	54.5	16.6	9.4	15.3	38.2	8.6
5s	78.6	28.9	21.5	**47.4**	64.3	25.2
5t	28.1	14.3	6.2	27.5	22.9	34.7
Osthole	**86.8**	**66.4**	**62.5**	**41.7**	**66.7**	**72.3**

Bold means better activity than the control Osthole

Table 3 EC_{50} of the most active compounds

Pathogen	Compound	Toxic regression	R	EC_{50}(nM)	95 % Confidence interval
Botrytis cinrea	**5e**	Y = 4.1882 + 1.1207X	0.9864	**16**	13–20
Botrytis cinrea	**5m**	Y = 5.0878 + 1.2236X	0.9939	**2.1**	1.6–3.2
Botrytis cinrea	**5s**	Y = 3.7502 + 1.1866X	0.9987	**3.0**	2.8–3.2
Botrytis cinrea	**Osthole**	Y = 4.3172 + 0.8435X	0.9629	**26**	18–38
Collecterichum capsica	**5g**	Y = 4.4428 + 0.5918X	0.9815	**24**	19–30
Collecterichum capsica	**5m**	Y = 4.1642 + 0.9702X	0.9952	**19**	17–22
Collecterichum capsica	**Osthole**	Y = 3.9221 + 0.8790X	0.9886	69	58–82
Rhizoctonia solani	**5e**	Y = 2.9245 + 1.5630X	0.9797	65	50–84
Rhizoctonia solani	**5m**	Y = 4.2009 + 0.8875X	0.9983	**5.8**	5.0–6.5
Rhizoctonia solani	**5o**	Y = 3.3057 + 1.4442X	0.9835	36	29–44
Rhizoctonia solani	**Osthole**	Y = 4.5097 + 0.5854X	0.9899	28	23–34

Bold means better activity than the control Osthole

Conclusion

In summary, we have developed a microwave-assisted synthetic method for the preparation of coumarin[8,7-*e*][1,3]oxazine derivatives which proceeds in acceptable yields and short reaction times. The *in vitro* antifungal activity of these compounds was evaluated. The preliminary bioassay showed that compounds **5e**, **5m** and **5s** exhibit good antifungal activity against *Botrytis cinerea* and *Rhizoctonia solani*, better than or similar to the positive control Osthole. The EC_{50} values of **5m** against *Botrytis cinerea*, *Colletotrichum capsici* and *Rhizoctonia solani* were 2.1, 19, and 5.8 nM, respectively, much lower than that of Osthole, and compound **5m** was identified as the most promising candidate for further study.

Experimental

All materials were obtained from commercial sources and were used as received. Melting points were obtained on a WRS-1B melting-point apparatus and are uncorrected. NMR spectra were recorded on a Bruker DRX-400 instrument in $CDCl_3$ or DMSO-d_6 using TMS as the internal reference (400 and 100 MHz for ^{1}NMR and ^{13}C NMR, respectively). Infrared (IR) spectra were recorded on a Bruker Tensor 27 spectrometer, and samples were prepared as KBr plates. High-resolution mass spectra (HRMS) were acquired in positive mode on a JMS-AX505HA (JEOL, Japan). Microwave irradiation was carried out using 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. Reaction yields were not optimized.

Synthesis of 7-hydroxycoumarin analogs 3a–3e [27,28]

A mixture of resorcinol **1** (or 4-chlororesorcinol) (0.1 mol) and β-keto-ester **2** (0.12 mol) was cooled to 0°C, and concentrated sulfuric acid (40 mL) was added dropwise. The mixture was held at room temperature for 12 hours with mechanical stirring. Then the mixture was poured into 250 mL ice water to give a precipitate, which was filtered off and crystallized from ethanol to give compounds **3a**–**3e**.

*7-Hydroxy-4-methyl-2H-chromen-2-one (**3a**)*

Yield: 68 %, m.p. 187.6–187.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.55 (s, 1H), 7.60 (d, J = 8.7 Hz, 1H), 6.81 (d, J = 11.0 Hz, 1H), 6.71 (s, 1H), 6.14 (s, 1H), 2.37 (s, 3H).

*6-Chloro-7-hydroxy-4-methyl-2H-chromen-2-one (**3b**)*

Yield: 76 %, m.p. 285.3–285.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6)δ: 11.34 (s, 1H), 7.74 (s, 1H), 6.88 (s, 1H), 6.19 (s, 1H), 2.37 (s, 3H).

*7-Hydroxy-4-phenyl-2H-chromen-2-one (**3c**)*

Yield: 80 %, m.p. 250.2–250.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.71 (s, 1H), 7.66–7.46 (m, 5H), 7.29 (d, J = 8.6 Hz, 1H), 6.88-6.69 (m, 2H), 6.17 (s, 1H).

*7-Hydroxy-4-(trifluoromethyl)-2H-chromen-2-one (**3d**)*

Yield: 33 %, m.p. 185.5–185.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.99 (s, 1H), 7.56 (d, J = 10.5 Hz, 1H), 6.91 (d, J = 8.9 Hz, 1H), 6.84 (s, 1H), 6.77 (s, 1H).

*6-Chloro-7-hydroxy-3,4-dimethyl-2H-chromen-2-one (**3e**)*

Yield: 68 %, m.p. 263.7–264.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6)δ: 11.17 (s, 1H), 7.71 (s, 1H), 6.85 (s, 1H), 2.31 (s, 3H), 2.04 (s, 3H).

Synthesis of coumarin[8,7-e][1,3]oxazine 5a–5t

A solution of primary amine **4** (5 mmol) in dioxane (15 mL) was treated with formalin solution (35 %, 10 mmol). The resulting mixture was held at room temperature and stirred vigorously for 1 hour, then treated with 7-hydroxycoumarin analogs **3a**–**3e** (5 mmol) and a catalytic amount of DMAP (20 mg), and irradiated at 400 W for 30 min in the microwave synthesizer. The solvent was evaporated, and the residue was crystallized from ethanol to give the title compounds **5a**–**5t**.

4 -Methyl -9 -phenyl - 9,10-dihydrochromeno[8,7 -e][1,3] oxazin -2(8H)- one (**5a**)

Yield: 65 %, m.p. 149.7–150.0 °C. IR (KBr) cm^{-1}: 3071, 2915, 1727, 1595, 1495, 1449. ^{1}H NMR (400 MHz, $CDCl_3$): δ: 7.37 (d, J = 8.8 Hz, 1H), 7.32–7.23 (m, 2H), 7.18–7.11 (m,2H), 6.96 (t, J = 7.3 Hz, 1H), 6.78 (d, J= 8.8 Hz, 1H), 6.13 (s, 1H), 5.44 (s, 2H), 4.83 (s, 2H), 2.37 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 161.02, 157.61, 153.12, 151.16, 147.92, 129.40, 123.59, 122.03, 118.52, 113.64, 113.25, 111.71, 108.89, 79.83, 46.36, 18.77. HRMS calcd for $C_{18}H_{16}O_3N[M+H]^+$ 294.1125, found 294.1122.

4-Methyl-9-p-tolyl-9,10-dihydrochromeno[8,7-e][1,3] oxazin-2(8H)-one (**5b**)

Yield: 63 %, m.p. 160.1–161.2 °C. IR (KBr) cm^{-1}: 3023, 2883, 1713, 1596, 1512, 1493.^{1}H NMR (400 MHz, $CDCl_3$): δ: 7.36 (d, J = 8.8 Hz, 1H), 7.12–7.01(m, 4H), 6.77 (d, J = 8.8 Hz, 1H), 6.12 (s, 1H), 5.41 (s, 2H), 4.79 (s, 2H), 2.37 (s, 3H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 161.05, 157.54, 153.13, 151.18, 145.58, 131.66, 129.91, 123.54, 118.78, 113.60, 113.21, 111.63, 108.85, 80.26, 46.46, 20.59, 18.76. HRMS calcd for $C_{19}H_{18}O_3N[M+H]^+$308.1281, found 308.1279.

9-(4-Chlorophenyl)-4-methyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5c**)

Yield: 57 %, m.p. 137.4–137.8 °C. IR (KBr) cm^{-1}: 3071, 2955, 1724, 1593, 1492, 1437. ^{1}H NMR (400 MHz, DMSO-d_6): δ: 7.52 (d, J = 8.8 Hz, 1H), 7.34–7.14 (m, 4H), 6.80 (d, J = 10.3 Hz, 1H), 6.21 (s, 1H), 5.54 (s, 2H), 4.74 (s, 2H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 160.92, 157.28, 153.12, 151.10, 146.55, 129.32, 127.10, 123.77, 119.86,

113.62, 113.39, 111.81, 108.54, 79.67, 46.50, 18.79. HRMS calcd for $C_{18}H_{15}O_3NCl[M+H]^+$ 328.0735, found 328.0729.

9-Benzyl-4-methyl-9,10-dihydrochromeno[8,7-e][1,3] oxazin-2(8H)-one (**5d**)

Yield: 62 %, m.p. 133.5–133.5 °C. IR (KBr) cm^{-1}: 3071, 2947, 1717, 1596, 1489, 1437. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.56 (d, J = 10.4 Hz, 1H), 7.43–7.22 (m, 5H), 6.85 (d, J = 10.2 Hz, 1H), 6.18 (s, 1H), 4.99 (s, 2H), 4.02 (s, 2H), 3.88 (s, 2H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 161.12, 157.51, 153.09, 152.06, 137.49, 128.88, 128.62, 127.67, 123.34, 113.31, 113.26,111.61, 108.04, 82.63, 55.95, 45.12, 18.77. HRMS calcd for $C_{19}H_{18}O_3N[M+H]^+$ 308.1281, found 308.1281.

6-Chloro-4-methyl-9-phenyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5e**)

Yield: 75 %, m.p. 173.7–174.1 °C. IR (KBr) cm^{-1}: 3007, 2871, 1715, 1598, 1569, 1493. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.72 (s, 1H), 7.34–7.32 (m,2H), 7.21–7.11 (m, 2H), 6.93 (t, J = 7.2 Hz, 1H), 6.29 (s, 1H), 5.69 (s, 2H), 4.79 (s, 2H), 2.37 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 160.35, 152.88, 152.08, 149.43, 147.52, 129.49, 123.51, 122.42, 118.67, 118.10, 113.34, 112.82, 110.35, 80.74, 46.43, 18.70. HRMS calcd for $C_{18}H_{15}O_3NCl[M+H]^+$ 328.0735, found 328.0732.

6-Chloro-4-methyl-9-p-tolyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5f**)

Yield: 68 %, m.p. 199.6–199.9 °C. IR (KBr) cm^{-1}: 3055, 2971, 1725, 1596, 1565, 1510, 1485. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.72 (s, 1H), 7.33–6.80 (m, 4H), 6.29 (s, 1H), 5.65 (s, 2H), 4.74 (s, 2H), 2.38 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 160.40, 152.87, 152.13, 149.46, 145.18, 132.09, 129.98, 123.45, 118.91, 118.06, 113.27, 112.71, 110.33, 81.17, 46.62, 20.59, 18.69. HRMS calcd for $C_{19}H_{17}O_3NCl[M+H]^+$ 342.0891, found 342.0888.

6-Chloro-9-(4-chlorophenyl)-4-methyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5g**)

Yield: 78 %, m.p. 198.5–198.8 °C. IR (KBr) cm^{-1}: 3063, 2923, 1717, 1591, 1493, 1421. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.74 (s, 1H), 7.35–7.16 (m, 4H), 6.30 (s, 1H), 5.68 (s, 2H), 4.78 (s, 2H), 2.38 (s, 3H). ^{13}CNMR (100 MHz, $CDCl_3$): 152.65, 152.10, 146.16, 129.43, 127.59, 123.68, 120.04, 118.12, 113.48, 112.90, 110.03, 80.60, 46.62, 18.71. HRMS calcd for $C_{18}H_{14}O_3NCl_2[M+H]^+$ 362.0345, found 362.0341.

9-Benzyl-6-chloro-4-methyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5h**)

Yield: 71 %, m.p. 153.5–154.4 °C. IR (KBr) cm^{-1}: 3051, 2955, 1707, 1598, 1485, 1441. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.76 (s, 1H), 7.46–7.16 (m, 5H), 6.26 (s, 1H), 5.16 (s, 2H), 4.04 (s, 2H), 3.90 (s, 2H), 2.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 159.86, 153.60, 152.67, 150.37, 138.25, 128.97, 127.87, 124.06, 116.82, 113.36, 112.26, 109.55, 84.41, 55.34, 43.87, 18.60. HRMS calcd for $C_{19}H_{17}O_3NCl[M+H]^+$ 342.0891, found 342.0891.

4,9-Diphenyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5i**)

Yield: 75 %, m.p. 183.1–183.1 °C. IR (KBr) cm^{-1}: 3067, 2959, 1716, 1589, 1488, 1445. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.64–7.44 (m, 5H), 7.35–7.14 (m, 5H), 6.92 (t, J= 7.2 Hz, 1H), 6.79 (d, J = 8.9 Hz, 1H), 6.25 (s, 1H), 5.60 (s, 2H), 4.83 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 129.69, 129.44, 128.86, 128.42, 126.13, 122.20, 118.64, 113.69, 111.62, 109.06, 79.90, 46.50. HRMS calcd for $C_{23}H_{18}O_3N[M+H]^+$ 356.1281, found 356.1277.

4-Phenyl-9-p-tolyl-9,10-dihydrochromeno[8,7-e][1,3] oxazin-2(8H)-one (**5j**)

Yield: 68 %, m.p. 152.9–153.0 °C. IR (KBr) cm^{-1}: 3071, 2915, 1718, 1609, 1585, 1515, 1441. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.68–7.43 (m, 5H), 7.18 (d, J = 8.9 Hz, 1H), 7.14–6.93 (m, 4H), 6.76 (d, J= 8.9 Hz, 1H), 6.24 (s, 1H), 5.54 (s, 2H), 4.77 (s, 2H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 161.04, 157.70, 156.39, 151.87, 145.57, 135.56, 131.77, 129.95, 129.64, 128.87, 128.43, 126.08, 118.86, 113.67, 112.20, 111.52, 109.06,80.32,46.64, 20.62. HRMS calcd for $C_{24}H_{20}O_3N[M+H]^+$ 370.1438, found 370.1435.

9-(4-Chlorophenyl)-4-phenyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5k**)

Yield: 74 %, m.p. 186.5–187.3 °C. IR (KBr) cm^{-1}: 3075, 2887, 1717, 1614, 1585, 1492. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.61–7.52 (m, 3H), 7.52–7.44 (m, 2H), 7.35–7.26 (m, 2H), 7.26–7.15 (m, 3H), 6.79 (d, J = 8.9 Hz, 1H), 6.25 (s, 1H), 5.58 (s, 2H), 4.82 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 160.90, 157.45, 156.35, 151.79, 146.57, 135.46, 129.70, 129.36, 128.89, 128.42, 127.21, 126.28, 119.95, 113.67, 112.38, 111.70, 108.77, 79.74, 46.68. HRMS calcd for $C_{23}H_{17}O_3NCl[M+H]^+$ 390.0892, found 390.0888.

9-Benzyl-4-phenyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5l**)

Yield: 62 %, m.p. 66.3–66.6 °C. IR (KBr) cm^{-1}: 3059, 2959, 1720, 1592, 1553, 1493. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.69–7.47 (m, 5H), 7.43–7.28 (m, 5H), 7.23 (d, J = 8.9 Hz, 1H), 6.84 (d, J= 8.8 Hz, 1H), 6.22 (s, 1H), 5.03 (s, 2H), 4.08 (s, 2H), 3.91 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 160.15, 157.74, 156.02, 152.61, 138.40, 135.47, 130.11, 129.31, 129.01, 128.90, 127.83, 125.93, 113.71, 111.84, 111.35, 108.35, 83.15, 55.36, 44.13, 31.17. HRMS calcd for $C_{24}H_{20}O_3N[M+H]^+$ 370.1438, found 370.1434.

6-Chloro-9-phenyl-4-(trifluoromethyl)-9,10-dihydrochromeno[8,7-e[1,3]oxazin-2(8H)-one (**5m**)

Yield: 72 %, m.p. 152.9–153.3 °C. IR (KBr) cm^{-1}: 3075, 2975, 1732, 1595, 1557, 1485. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.52 (s, 1H), 7.36–7.13 (m, 4H), 7.02–6.88 (m, 2H), 5.75 (s, 2H), 4.81 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 158.35, 153.95, 150.18, 147.15, 141.20, 140.87, 129.43, 123.97, 122.65, 119.27, 118.71, 112.93 (q, J=5.7Hz), 110.68, 106.57, 80.98, 46.37. HRMS calcd for $C_{18}H_{12}O_3NClF_3[M+H]^+$ 382.0452, found 382.0450.

6-Chloro-9-p-tolyl-4-(trifluoromethyl)-9,10-dihydrochromeno[8,7-e[1,3]oxazin-2(8H)-one (**5n**)

Yield: 80 %, m.p. 178.7–179.7 °C. IR (KBr) cm^{-1}: 3083, 2963, 1745, 1589, 1509, 1481. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.51 (s, 1H), 7.14–7.02 (m, 4H), 6.96 (s, 1H), 5.70 (s, 2H), 4.76 (s, 2H), 2.20 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 158.55, 154.10, 150.37, 144.95, 141.35, 141.02, 132.52, 130.07, 124.09, 119.37, 119.07, 113.00 (q, J = 5.7 Hz), 110.80, 106.65, 81.55, 46.70, 20.61. HRMS calcd for $C_{19}H_{14}O_3NClF_3[M+H]^+$ 396.0609, found 396.0607.

6-Chloro-9-(4-chlorophenyl)-4-(trifluoromethyl)-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5o**)

Yield: 61 %, m.p. 203.3–204.4 °C. IR (KBr) cm^{-1}: 3091, 2955, 1739, 1601, 1497, 1485. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.52 (s, 1H), 7.35–7.27 (m, 2H), 7.26–7.17 (m, 2H), 6.98 (s, 1H), 5.73 (s, 2H), 4.80 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 158.41, 153.56, 150.76, 146.38, 142.99, 129.42, 126.06, 123.15, 120.35, 117.76, 115.22 (q, J= 4.9 Hz), 111.59, 106.94, 99.97, 81.23, 45.18. HRMS calcd for $C_{18}H_{11}O_3NCl_2F_3[M+H]^+$ 416.0063, found 416.0060.

9-Benzyl-6-chloro-4-(trifluoromethyl)-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5p**)

Yield: 69 %, m.p. 150.6–151.0 °C. IR (KBr) cm^{-1}: 3003, 2859, 1737, 1593, 1485, 1445, 1405. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.55 (s, 1H), 7.43–7.20 (m, 5H), 6.93 (s, 1H), 5.26 (s, 2H), 4.07 (s, 2H), 3.91 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 158.60, 154.09, 151.24, 141.35, 141.02, 136.83, 128.80, 128.77, 127.95, 123.84, 119.07, 112.98 (q, J = 5.6 Hz), 110.00, 106.68, 84.28, 56.10, 44.73. HRMS calcd for $C_{19}H_{14}O_3NClF_3[M+H]^+$ 396.0609, found 396.0605.

6-Chloro-3,4-dimethyl-9-phenyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5q**)

Yield: 66 %, m.p. 187.5–188.0 °C. IR (KBr) cm^{-1}: 3051, 2908, 1701, 1596, 1496, 1453. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.73 (s, 1H), 7.40–7.10 (m, 4H), 6.93 (t, J= 7.2 Hz, 1H), 5.69 (s, 2H), 4.79 (s, 2H), 2.34 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 161.57, 151.69, 147.75, 147.60, 145.70, 129.45, 123.28, 122.30, 120.03, 118.63, 117.80, 113.98, 110.00, 80.60, 46.40, 15.15, 13.38. HRMS calcd for $C_{19}H_{17}O_3NCl[M+H]^+$ 342.0892, found 342.0886.

6-Chloro-3,4-dimethyl-9-p-tolyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5r**)

Yield: 58 %, m.p. 212.7–214.1 °C. IR (KBr) cm^{-1}: 3030, 2915, 1704, 1593, 1510, 1483.^1H NMR (400 MHz, DMSO-d_6): δ: 7.73 (s, 1H), 7.25–6.91(m, 4H), 5.64 (s, 2H), 4.74 (s, 2H), 2.35 (s, 3H), 2.19 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$):161.62, 151.69, 147.78, 145.72, 145.26, 131.96, 129.95, 123.23, 119.96, 118.87, 117.76, 113.92, 109.98, 81.02, 46.58, 20.60, 15.14, 13.37. HRMS calcd for $C_{20}H_{19}O_3NCl[M+H]^+$ 356.1048, found 356.1042.

6-Chloro-9-(4-chlorophenyl)-3,4-dimethyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5s**)

Yield: 63 %, m.p. 213.7–214.1 °C. IR (KBr) cm^{-1}: 2924, 1707, 1586, 1487, 1428, 1328. ^1H NMR (400 MHz, DMSO-d_6): δ: 7.75 (s, 1H), 7.39–7.10 (m, 4H), 5.68 (s, 2H), 4.78 (s, 2H), 2.35 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$):147.73, 146.25, 145.68, 129.39, 127.45, 123.47, 120.21, 120.00, 117.83, 114.14, 109.68, 80.47, 46.59, 15.18, 13.40. HRMS calcd for $C_{19}H_{16}O_3NCl_2[M+H]^+$ 376.0502, found 376.0496.

9-Benzyl-6-chloro-3,4-dimethyl-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one (**5t**)

Yield: 76 %, m.p. 178.0–178.5 °C. IR (KBr) cm^{-1}: 3030, 2902, 1700, 1620, 1591, 14788. ^1H NMR (400 MHz,

DMSO-d_6): δ: 7.76 (s, 1H), 7.50–7.22 (m, 5H), 5.15 (s, 2H), 4.03 (s, 2H), 3.89 (s, 2H), 2.36 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 161.67, 151.61, 148.66, 145.67, 137.25, 128.81, 128.65, 127.76, 123.00, 119.95, 117.44, 113.96, 109.17, 83.86, 56.03, 44.85, 15.16, 13.37. HRMS calcd for $C_{20}H_{19}O_3NCl[M+H]^+$ 356.1048, found 356.1044.

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