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Novel trifluoromethyl sydnone derivatives: Design, synthesis and fungicidal activity

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ABSTRACT

Crop pathogens reduce the yield and quality of agricultural production. The development of new fungicides will help to sustain this protection and overcome fungicide resistance. Sydnone is a kind of mesoionic, which has a wide range of biological activities. The application of sydnones in agriculture is less, and the study of these compounds will lead to the discovery of new active compounds. In this study, we designed and synthesized a series of noval sydnone mesoionic derivatives by active substructure splicing. All compounds were characterized using ^{1}H and ^{13}C NMR spectroscopy. Among them, trifluoromethyl compound **D17** showed good bioactivity against *Pseudoperonospora cubensis* (EC_{50} = 49 mg L^{-1}) *in vivo*, the activity was similar to that of the control Kresoxim-methyl (EC_{50} = 44 mg L^{-1}). However, the target of these compounds should not only be tyrosinase, and the mode of action needs to be further studied. In addition, the structure–activity relationship indicated that the trifluoromethyl group was more beneficial for antifungal activity. This is the first report that fluorine-containing *N*(3)-benzyl sydnone compounds have good fungicidal activity. These results will provide a basis for the development of sydnone mesoionic as new lead fungicidal agents.

Fungal diseases on crops have pose a serious threat to global food security. It has indicated that the global crop losses caused by crop pests and pathogens to wheat, rice, corn, potatoes and soybeans are range between 17% and 30%[1]. The use of fungicides is an important means to ensure agricultural production. But prolonged and excessive use of fungicides have caused serious resistance problems. Therefore, there is an urgent need to development of new fungicides with high efficiency, novel action mechanism and high safety.

Tyrosinase is a key enzyme in the synthesis of melanin[2], which is widely found in animals, plants, and microorganisms[3,4]. Melanin[5] is not only related to the pathogenic ability of fungi, but also enhances the fungal resistance to environmental stress (pH, temperature, radiation, poison, natural enemies, etc.). Therefore, the inhibition of tyrosinase can control the growth of fungi. In 2018, Lopes *et al.*[6] reported that piperonal 1,3,4-thiadiazolium-2-phenylamines mesoionic derivatives exhibited non-competitive tyrosinase inhibitory activity, and **PMI-5** (Fig. 1) was the most active compound.

The potential value as biologically active substances of mesoionic compounds is found in their relatively small size and the possibility of selecting different patterns of electron density by selecting different mesoionic systems[7]. Sydnone[8] (Fig. 1) is a kind of mesoionic[9], which has a wide range of biological activities[10], such as antibacterial[11], antimicrobial[12,13], anti-inflammatory[14,15], antitumor[16,17], antioxidant activities[18], antidiabetic[19], analgesic[20] and insecticidal[21] activities. But the fungicidal activity of sydnone is rarely reported. Through the modification of this kind of compound, excellent lead fungicide with high bioactivity and new mode of action is expected to be obtained.

Triflumezopyrim[22] (Fig. 1) is the first commercialized mesoionic pesticide. With the advent of triflumezopyrim, research on mesoionic compounds has received impetus. This series of compounds possess high insecticidal activity against brown planthopper and lepidoptera[23]. The activity of these compounds is considerably affected by two substituents on the mesoionic ring[24]. Novel pyrido[1,2-α]pyrimidinone mesoionic compounds containing vanillin moieties also showed excellent activity against *Xanthomonas oryzae pv. Oryzae*[25]. Mesoionic compounds can be used to design new insecticides or fungicides by modifying their substituents.

Here, encouraged by the structure of pyrido[1,2-α]pyrimidinone

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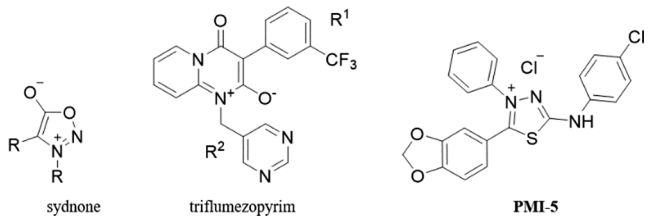


Fig. 1. The chemical structures of sydnone, triflumezopyrim, and **PMI-5**.

mesoionic and 1,3,4-thiadiazole-2-phenylamine mesoionic derivatives, sydnones were used as the lead structure in which the substituted benzyl group was introduced at the *N*(3) position and the substituted phenyl group at the *C*(4) position (Fig. 2). In addition, compounds containing trifluoromethyl are widely used in bioactive molecules because of their high electronegativity and bioavailability. Therefore, trifluoromethyl was introduced into the benzene ring in this article. In short, a series of novel 3-benzyl-4-phenyl-sydnones were designed and synthesized. Subsequently, antifungal activities were screened against *Pseudoperonospora cubensis* (CDM), *Colletotrichum orbiculare* (CA), *Puccinia sorghi* (CSR), *Blumeria graminis* (WPM), *Pyricularia grisea* (RB), *Botrytis cinerea Pers* (CGM), and *Sphaerotheca cucurbitae* (CPM).

Results and discussion

Chemistry

The synthesis of sydnones **D1**–**D24** were shown in Fig. 3. Initially, intermediate **c** was obtained via reaction of compounds **a** and **b**, following which it was transformed into the corresponding target compound **D** via a one-pot reaction.

The first step is substitution reaction, which is more likely to occur when the substituents of amines contain stronger electron withdrawing groups. When the substituents of amines are more electron donating, the reaction time should be prolonged or the reaction solvent should be changed to ethyl acetate to increase the reaction temperature. The one-pot reaction involved nitritation of the secondary imine group and condensation reaction. The keys to the reaction are low temperature and long reaction time. The reaction works well in a nitrogen atmosphere. When isoamyl nitrite and trifluoroacetic anhydride are used, the reaction time is relatively short, and a good yield is obtained.

*Antifungal bioactivities of compounds **D1**–**D24** and analysis of structure-activity relationships (SAR)*

The antifungal activities of compounds **D1**–**D24** are shown in Tables 1, 2 and 3. Kresoxim-methyl (KSM) and azoxystrobin (AZS) were selected as positive controls at 400 mg L^{-1}, while pyrisoxazole (PSA) and tricyclazole (TCA) were selected as positive controls at 25 mg L^{-1} using the spore germination method.

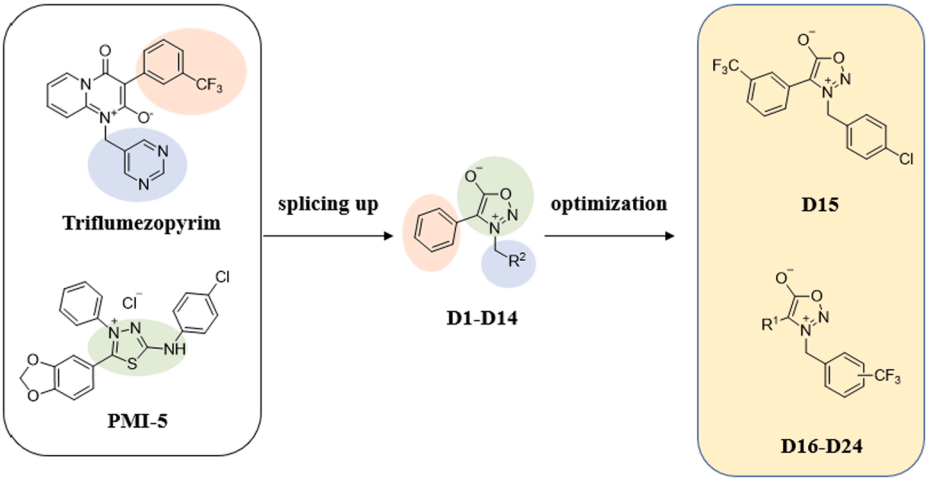
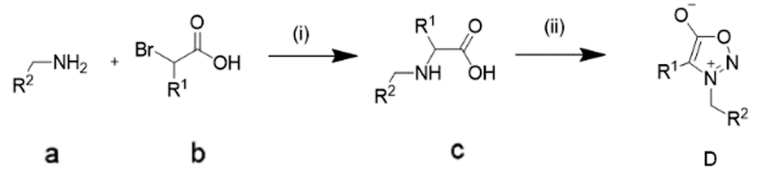


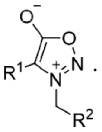
Fig. 2. Design of novel sydnone mesoionic compounds **D1**-**D24.**

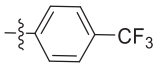
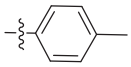
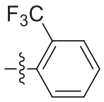
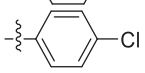
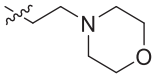
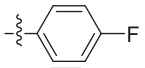
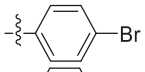
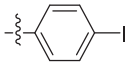
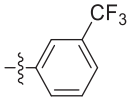
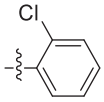
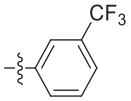
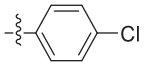
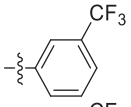
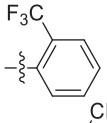
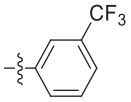
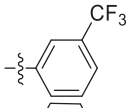
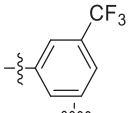
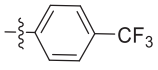
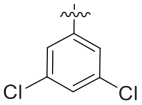
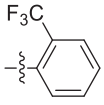
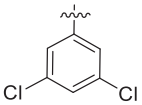
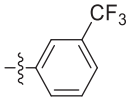


1 R^1=Ph R^2=4-CF_3-Ph
2 R^1=Ph R^2=4-CH_3-Ph
3 R^1=Ph R^2=Tetrahydrofuranyl-2-
4 R^1=Ph R^2=2-CF_3-Ph
5 R^1=Ph R^2=4-Cl-Ph
6 R^1=Ph R^2=Thienyl-2-
7 R^1=Ph R^2=Cyclopropyl
8 R^1=Ph R^2=3-morpholinoethyl
9 R^1=Ph R^2=4-F-Ph
10 R^1=Ph R^2=4-Br-Ph
11 R^1=Ph R^2=4-I-Ph
12 R^1=Ph R^2=Ph
13 R^1=Ph R^2=3-CF_3-Ph
14 R^1=Ph R^2=2-Cl-Ph
15 R^1=3-CF_3-Ph R^2=4-Cl-Ph
16 R^1=3-CF_3-Ph R^2=2-CF_3-Ph
17 R^1=3-CF_3-Ph R^2=3-CF_3-Ph
18 R^1=3-CF_3-Ph R^2=4-CF_3-Ph
19 R^1=3,5-dichlorophenyl R^2=2-CF_3-Ph
20 R^1=3,5-dichlorophenyl R^2=3-CF_3-Ph
21 R^1=3,5-dichlorophenyl R^2=4-CF_3-Ph
22 R^1=H R^2=2-CF_3-Ph
23 R^1=H R^2=3-CF_3-Ph
24 R^1=H R^2=4-CF_3-Ph

Fig. 3. General route of synthesis of the target compounds **D1**–**D24**. Reagents: (i) ether, reflux; (ii) isoamyl nitrite, THF, TFAA, r.t.

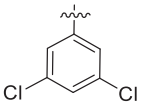
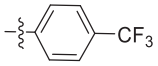
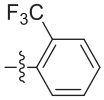
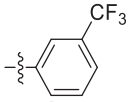
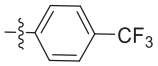
Table 1

In vitro antifungal activities of the target compounds **D1**–**D24**  .

NO.	R^1	R^2	inhibition rate(%, 25 mg L^{-1}) RB	CGM
D1	Ph		0	50
D2	Ph		0	0
D3	Ph		0	0
D4	Ph		50	50
D5	Ph		80	0
D6	Ph		0	0
D7	Ph		0	0
D8	Ph		0	0
D9	Ph		0	0
D10	Ph		0	0
D11	Ph		0	0
D12	Ph	Ph	0	0
D13	Ph		0	50
D14	Ph		0	0
D15			0	60
D16			50	0
D17			0	0
D18			0	0
D19			50	0
D20			0	0

(*continued on next page*)

Table 1 (*continued*)

NO.	R^1	R^2	inhibition rate(%, 25 mg L^{-1}) RB	CGM
D21			50	0
D22	H		0	0
D23	H		0	0
D24	H		0	0
PSA	–	–	/	100
TCA	–	–	100	/

RB, *Phyricularia grisea*; CGM, *Botrytis cinerea Pers*; PSA, Pyrisoxazole; TCA, Tricyclazole; /, not measured.

Table 2
In vivo antifungal activities and tyrosinase inhibitory activities of the target compounds **D1**–**D24**.

NO.	inhibition rate(%) 400 mg L^{-1}				50 mg L^{-1}
	CDM	CA	WPM	CSR	tyrosinase
D1	50	0	20	0	47
D2	0	0	0	0	61
D3	0	0	0	0	14
D4	10	0	20	0	48
D5	0	0	0	0	52
D6	0	0	0	0	40
D7	0	0	0	0	28
D8	0	0	0	0	33
D9	0	0	0	0	49
D10	0	0	0	0	40
D11	0	0	0	0	59
D12	0	0	0	0	60
D13	20	0	20	0	18
D14	0	0	0	0	55
D15	0	0	0	0	47
D16	20	0	95	0	28
D17	90	70	90	0	44
D18	90	0	80	0	33
D19	0	0	90	0	54
D20	0	20	80	50	53
D21	0	0	80	0	52
D22	0	0	85	0	52
D23	0	80	60	0	20
D24	0	0	0	0	23
KSM	100	/	/	/	/
AZS	/	100	100	100	/
KA	/	/	/	/	92

CDM, *Pseudoperonospora cubensis*; CA, *Colletotrichum orbiculare*; CSR, *Puccinia sorghi*; WPM, *Blumeria graminis*; KSM, Kresoxim-methyl; AZS, Azoxystrobin; KA, Kojic acid; /, not measured.

In the spore germination experiments (25 mg L^{-1}), compound **D5** (80%) showed good antifungal activity against RB. The introduction of a chlorine atom at the 4-position of *N*(3) aryl was conducive for the activity. However, the activity of the compound was low at 10 mg L^{-1}, and hence, no further studies have been conducted.

As shown in Table 2, compounds **D1**–**D14** showed low *in vivo* antifungal activities against the tested fungi (400 mg L^{-1}). But the retention of trifluoromethyl substituted benzene ring on R^2 could increase the activity. Based on the structure of commercial pyrido[1,2-α]pyrimidinone mesoionic compounds and advantages of fluorinated pesticides, a halogen atom was introduced to the benzene ring on R^1 in the design of **D15**–**D24**.

Table 3
EC_{50} Values of the Target Compounds **D16**, **D17** and **D19**.

NO.	EC_{50} (mg L^{-1}) CPM	WPM	CDM
D16	138	564	/
D19	147	224	/
TDM	1.86	1.20	/
D17	/	/	49
KSM	/	/	44

CDM, *Pseudoperonospora cubensis*; WPM, *Blumeria graminis*; CPM, *Erysiphe cichoracearum*; TDM, Triadimenol; KSM, Kresoxim-methyl; /, not measured.

Inhibition of Mushroom Tyrosinase

Therefore, we designed and synthesized compounds **D15**–**D24** with trifluoromethyl substituted benzene ring and chloro-substituted benzene ring. Some of these compounds displayed good antifungal activities. For example, compounds **D17** (90%) and **D18** (90%) displayed identical antifungal activities against CDM. In addition, compounds **D16** (95%), **D17** (90%), **D19** (90%), and **D22** (85%) displayed antifungal activities against WPM.

To analyze SARs of the target compounds **D1**–**D24**, high bioactivity compounds **D16**, **D17**, and **D19** were selected for further studies and their EC_{50} values are listed in Table 3.

The EC_{50} of **D16** and **D19** for WPM were inferior (**D16**, 564 mg L^{-1}; **D19**, 224 mg L^{-1}) to that for CPM (**D16**, 138 mg L^{-1}; **D19**, 147 mg L^{-1}). Compared to **D16**, **D19** and **D22**, possessed the same R^2 substituents, but not the same R^1 substituents. Results showed that 2-trifluoromethylbenzene at R^2 significantly improved its anti-powdery mildew activity. The inhibitory activities of compounds against powdery mildew were lower than that of the control, indicating that further improvement was required.

Compound **D17**, in which R^1 and R^2 both contain 3-trifluoromethylbenzene ring, showed good antifungal activity against CDM (EC_{50} = 49 mg L^{-1}). Compounds **D17**, **D20**, and **D23** have the same R^2 substituents, but not the same R^1 substituents. The antifungal activities of compounds **D20** and **D23** against CDM were low. Therefore, the presence of the 3-trifluoromethylbenzene ring at the R^1 position considerably affected CDM inhibition. The inhibitory activity of **D17** was slightly lower than that of the control KSM (EC_{50} = 44 mg L^{-1}), such compounds have the value of continuing research.

Compounds **D17** and **D18** showed considerable activity against CDM at 400 mg L^{-1}; however, at lower concentration of 100 mg L^{-1}, **D18** lost its activity.

A previous report showed that 1,3,4-thiadiazolium-2-phenylamine mesoionic compounds inhibited tyrosinase, which is a key enzyme of melanin synthesis, and catalyzes the hydroxylation of L-tyrosine to L-DOPA and the oxidation of L-DOPA to dopaquinone. Therefore, we tested the inhibitory activity of sydnone compounds on tyrosine using Kojic Acid (KA) as the positive control (Table 2).

All the sydnones had more or less inhibitory effect on tyrosinase. Among them, fourteen compounds with inhibition rates greater than 40% at 50 mg L^{-1} showed inhibition comparable to that of the most promising mesoionic tyrosinase inhibitor, **PMI-5** (44 mg L^{-1}-37%). But the inhibitory activities were lower than KA.

In terms of the structures of these compounds, it is beneficial to inhibition activity when both substituents of sydnones contain benzene ring. The inhibition rate of phenyl (60%) was better than that of cyclopropane (28%), morpholine (33%), thiophene (40%), and tetrahydrofuran (14%). When the phenyl group contains electron-withdrawing groups, the inhibition rate will be reduced to different degrees (**D12** higher than **D1, D4, D5, D9-D11**, **D13-D24**), but when the phenyl group contains electron-donating group (**D2**), the inhibition rate will be improved (61%).

However, there is no obvious correlation between the fungicidal activity and the inhibition rate of tyrosinase. These compounds do not produce activity only by inhibiting tyrosinase, there may be other acting sites. The mode of action needs to be further explored.

In summary, a series of mesoionic sydnones were designed and synthesized, and their fungicidal activities against seven phytopathogenic fungi were evaluated. This is a new attempt to use *N*(3) benzyl sydnones to combat plant diseases. The fungicidal activity of these compounds has not been reported yet. Most of the trifluoromethyl mesoionic sydnones showed good tyrosinase inhibitory activity. Among them, **D5** also showed antifungal activities against RB *in vitro*, and **D16**–**D21** were active against WPM *in vivo*. Extraordinary, **D17** (EC_{50} = 49 mg L^{-1}) showed significant antifungal activities against CDM, slightly lower than the control KSM (EC_{50} = 44 mg L^{-1}). The structure–activity relationship of these compounds points out that the introduction of trifluoromethyl groups is beneficial for activity. The mode of action needs to be further explored. Our results reveal the lead structure of mesoionic sydnone and substituent trifluoromethyl group, which can be used to develop fungicidal products against CDM and related fungi. The results of the current studies could support the development of new potent and antifungal candidates. Further synthesis optimization is currently in progress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128114.

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