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# Novel sulfonamides against *Botrytis cinerea* with no positive cross-resistance to commercial fungicides: design, synthesis and SAR study

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**Abstract**

Thirty-four novel compounds were synthesized using chesulfamide (*N*-(2-trifluoromethyl-4-chlorophenyl)-2-oxocyclohexyl sulfonamide), a high-profile fungicide, as the lead compound, and their structures were characterized by $^{1}$H NMR, $^{13}$C NMR, MS and elemental analysis. Additionally, the structure of (*1S,2R*)-2-((3-bromophenethyl)amino)-*N*-(4-chloro-2-trifluoromethylphenyl)cyclohexane-1-sulfonamide (**IV-9**) was confirmed by X-ray single crystal diffraction. The mycelium inhibition tests, spore germination inhibition tests, tomato pot tests and field trials were performed against strains of *B. cinerea*. Bioassay results showed that most of target compounds had good fungicidal activity against *B. cinerea*, in particular, **IV-9** exhibited similar or superior effects to procymidone, boscalid and pyrisoxazole in all *in vitro* and *in vivo* tests. Moreover, there was no positive cross-resistance found between the compound **IV-9** and eight commercial fungicides (azoxystrobin, boscalid, chlorothalonil, diethofencarb, fludioxonil, procymidone, pyrimethanil and pyrisoxazole) in the cross-resistance validation test performed by an innovative method.

**Key words:** cycloalkylsulfonamides; reductive amination; *Botrytis cinerea*; fungicidal activity; SAR; cross resistance

*Botrytis cinerea* (teleomorph: *Botryotinia fuckeliana*) is an airborne plant pathogen with a necrotrophic lifestyle attacking over 200 dicotyledonous crops worldwide, which main threaten the mature or senescent tissues and the products in

retail chain. [1,2] The fungus is considered as a typical necrotroph, which co-opts programmed cell death pathways in the host to accomplish infection. [2] Besides, *B. cinerea* has a high risk of development of resistance due to its genetic plasticity, and under selective pressure, the proportion of resistant strains inclines to increase in populations after the acquired benefits of resistant over the wild-type phenotype. [1,3,4] Moreover, during the past decades, many strains of *B. cinerea* with multiple-resistance to conventional fungicides have appeared on various crops worldwide. [5-9] Therefore, the risk of drug-free rotation drives us to search for new candidates to control this plant disease.

Sulfonamides have received extensive attention by chemists and biologists owing to their eminent biological activities, such as anti-cancer [10,11], antimicrobial [12-14], treatment of chronic pain [15] and inhibition of certain enzymes [16-18]. It is worth mentioning that the antimicrobial sulfonamides exhibit a broad-spectrum effect, covering all organisms that synthesize folic acid *de novo* through the folate pathway (they inhibit the enzyme dihydropteroate synthase (DHPS)). [19,20] Rather, the biosynthetic pathway exists in many pathogenic microorganisms but absent in mammals. Regrettably, commercial varieties of sulfonamide fungicides, like tolnifanide, cyazofamid and amisulbrom, are less used in the field owing to their action characteristics are sometimes not obvious enough. [21] Therefore, it is meaningful to develop the sulfonamide fungicides with more excellent properties.

Our team dedicated in the synthesis and fungicidal activities research of sulfonamides, especially the cycloalkylsulfonamides. Previously, our team reported several series of 2-oxyalkylsulfonamides (Figure 1, A-D) which showed excellent fungicidal activity [22-25], and the potential of chesulfamide as a fungicidal lead compound was found therein. Chesulfamide, a cycloalkylsulfonamide fungicide, can effectively control *B. cinerea* with strong preventive, therapeutic and osmotic activity. [26] Using chesulfamide as the lead structure, we reduced the oxo group to the hydroxyl group [27] to make relative esters [28,29] and replaced the hydroxyl group with amino group [30], which enabled the introduction of aromatic and heterocyclic rings [31] (Figure 2). Some of these compounds exhibited more pronounced fungicidal activities than chesulfamide. These achievements motivated us to further modify the structure of the lead compound to obtain the potential antifungal agents with superb properties. Hence, based on structure-activity relationship in our previous experiments, we designed to modify the lead compound with an active substituted aromatic ethylamine group, which contained in the commercial fungicides like fluopyram and mandipropamid (Figure 3). Moreover, the substituted ethylamine was attached to the lead structure with a flexible carbon chain, which allowed for abundant conformations and increased probability of binding to the targets. In this paper, we designed and synthesized a series of 2-substituted ethylamino-*N*-substituted phenyl cycloalkylsulfonamides in a simplified synthesis method compared to our previous studies. [30] The comprehensive bioassays against *B. cinerea* of all target

compounds were evaluated and the structure-activity relationship (SAR) was analyzed. In addition, we performed the cross-resistance tests between the optimal compound **IV-9** and eight commonly used commercial fungicides against *B. cinerea* with a novel method.

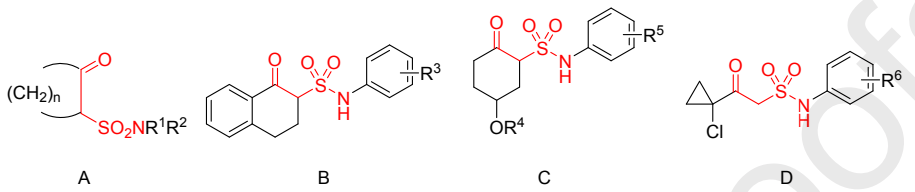



Figure 1. Reported 2-oxyalkylsulfonamide structures.

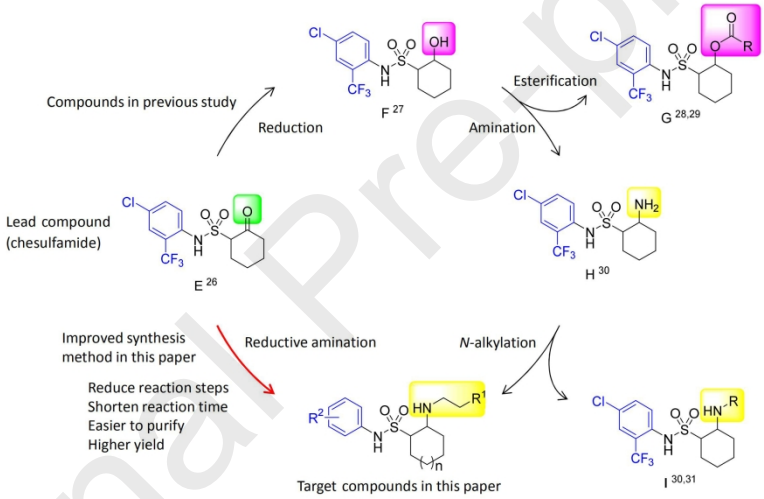



Figure 2. The designed strategy for the target compounds.

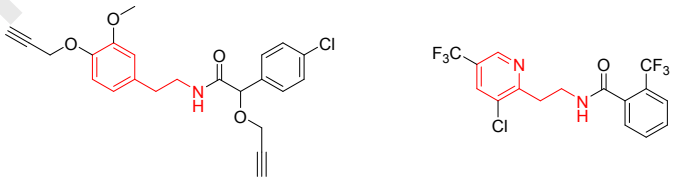
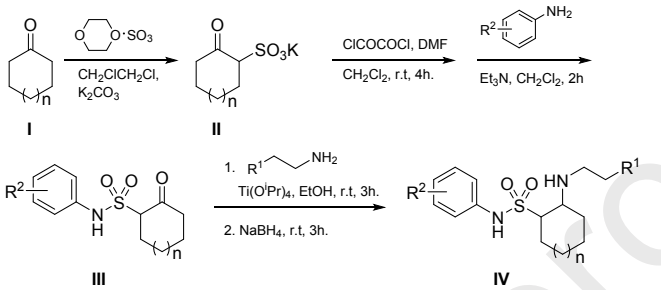



Figure 3. Commercial fungicides containing the structure of aromatic ethylamine.

The synthetic route of target compounds is shown in Scheme 1. The synthesis of key immediate **III** was done according to the reported method [32]. Then, the immediate **III** was subjected to reductive amination to give the target compounds

**IV-1** to **IV-22**. After analyzed the structure-activity relationship, it was determined the most optimal structure of $R^1$ as 3-Br-$C_6H_4$-. Afterwards, the structures of $R^2$ and the cyclohexane were tried to optimize and obtained the target compounds **IV-23** to **IV-34** by the same synthesis method.




Structural optimization of $R^1$

$R^2$ = 2-$CF_3$-4-Cl, n = 1,

$R^1$ = 2-F-$C_6H_4$-, 3-F-$C_6H_4$-, 2-Cl-$C_6H_4$-, 3-Cl-$C_6H_4$-, 4-Cl-$C_6H_4$-, 2,5-Cl-$C_6H_3$-, 3,4-Cl-$C_6H_3$-, 2-Br-$C_6H_4$-, 3-Br-$C_6H_4$-, 4-Br-$C_6H_4$-, 4-$CH_3$-$C_6H_4$-, 3,4-$CH_3$-$C_6H_4$-, 3-$CF_3$-$C_6H_4$-, 4-$CF_3$-$C_6H_4$-, 2-$OCH_3$-$C_6H_4$-, 3-$OCH_3$-$C_6H_4$-, 4-$OCH_3$-$C_6H_4$-, 3,4-$OCH_3$-$C_6H_3$-, 4-$OCF_3$-$C_6H_4$-, 4-OH-$C_6H_4$-, 4-pyridine, 2-morpholine;

Structural optimization of $R^2$

$R^1$ = 3-Br-$C_6H_4$-, n =1,

$R^2$ = 2-F, 3-F, 4-F, 2-Cl, 3-Cl, 4-Cl, 2-Br, 3-Br, 4-Br, 2,4,5-Cl;

Structural optimization of n

$R^1$ = 3-Br-$C_6H_4$-, $R^2$ = 2-$CF_3$-4-Cl, n = 2, 3.

Scheme 1. The synthetic route of target compounds.

In our previous study, the carbonyl group was generally reduced to an amine group by introducing ammonia gas into the ethanol solution of immediate **III** and titanium (IV) isopropoxide, then, followed by a series of *N*-alkylation reactions for further structural derivation [30-33]. Rather, in this paper, we had optimized the synthetic route. This improved method reduced the reaction steps, shortened the reaction time, and easiness of product purification with higher yield.

The structure of target compounds were characterized by $^1$H NMR, $^{13}$C NMR, MS and elemental analysis. Additionally, the structure of **IV-9** was confirmed by the X-ray single crystal diffraction (CCDC No. 1867015, Figure 4). The spatial structure

of cyclohexane in **IV-9** was the chair conformation, the sulfonamide moiety on the equatorial bond position and the phenylethylamine moiety connected to the cyclohexane by an axial bond. The first chiral carbon atom (C1) existed as the *R* configuration and the second chiral carbon atom (C6) had the *S* configuration. Due to the structural similarity of this series of compounds, it was inferred that all target compounds had the same configuration as **IV-9**.

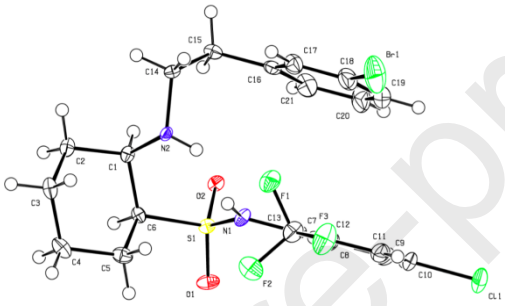



Figure 4. X-ray single crystal structure of **IV-9**.

The results for mycelium inhibition of all target compounds against three strains of *B. cinerea* (DL-11, KZ-9, 5055) are shown in Table 1. The results showed that **IV-2**, **IV-9** and **IV-16** had higher fungicidal activities against DL-11 with the $EC_{50}$ values of 1.96, 1.39 and 2.07 μg $mL^{-1}$ than procymidone, boscalid, pyrisoxazole and the lead compound chesulfamide with 14.02, 2.11, 2.08 and 8.27 μg $mL^{-1}$, respectively. In addition, 13 compounds had lower $EC_{50}$ values than 5 μg $mL^{-1}$ against KZ-9. Based on the results, 13 compounds with excellent fungicidal activities were tested on 5055, and the result also confirmed the high fungicidal activity.

Table 1. Inhibitory effect of target compounds on mycelium growth of *B. cinerea*.

| Compd. | Structure | | | $EC_{50}$ (95% Confidence Limit) (μg $mL^{-1}$) | | |
|---|---|---|---|---|---|---|
| | n | $R^1$ | $R^2$ | DL-11 | KZ-9 | 5055 |
| **IV-1** | 1 | 2-F-$C_6H_4$- | 2-$CF_3$-4-Cl- | 8.98(4.04-19.95) | 10.70(3.70-30.92) | / |
| **IV-2** | 1 | 3-F-$C_6H_4$- | 2-$CF_3$-4-Cl- | 1.96(0.77-5.01) | 2.03(0.82-5.00) | / |
| **IV-3** | 1 | 2-Cl-$C_6H_4$- | 2-$CF_3$-4-Cl- | 2.43(1.15-5.14) | 3.48(1.18-10.21) | 3.17(1.82-5.50) |
| **IV-4** | 1 | 3-Cl-$C_6H_4$- | 2-$CF_3$-4-Cl- | 3.76(1.85-7.62) | 4.05(2.16-7.59) | / |
| **IV-5** | 1 | 4-Cl-$C_6H_4$- | 2-$CF_3$-4-Cl- | 3.60(1.93-6.68) | 3.88(2.47-6.09) | 3.94(1.73-9.01) |
| **IV-6** | 1 | 2,5-Cl-$C_6H_3$- | 2-$CF_3$-4-Cl- | 3.39(2.03-5.66) | 3.99(2.62-6.08) | 2.90(1.24-6.75) |
| **IV-7** | 1 | 3.4-Cl-$C_6H_3$- | 2-$CF_3$-4-Cl- | 3.50(1.88-6.52) | 4.06(2.21-7.45) | / |
| **IV-8** | 1 | 2-Br-$C_6H_4$- | 2-$CF_3$-4-Cl- | 9.01(5.40-15.02) | 3.04(1.25-7.41) | / |
| **IV-9** | 1 | 3-Br-$C_6H_4$- | 2-$CF_3$-4-Cl- | 1.39(0.57-3.40) | 2.92(1.44-5.91) | 1.90(0.67-5.37) |
| **IV-10** | 1 | 4-Br-$C_6H_4$- | 2-$CF_3$-4-Cl- | 2.39(1.15-4.95) | 2.18(1.06-4.51) | / |
| **IV-11** | 1 | 4-$CH_3$-$C_6H_4$- | 2-$CF_3$-4-Cl- | 3.44(1.63-7.26) | 5.34(2.82-10.11) | 3.27(1.26-8.44) |
| **IV-12** | 1 | 3,4-$CH_3$-$C_6H_3$- | 2-$CF_3$-4-Cl- | 6.71(4.29-10.51) | 4.08(2.62-6.34) | / |
| **IV-13** | 1 | 3-$CF_3$-$C_6H_4$- | 2-$CF_3$-4-Cl- | 3.78(1.61-8.88) | 3.41(0.42-28.07) | 2.89(1.51-5.52) |
| **IV-14** | 1 | 4-$CF_3$-$C_6H_4$- | 2-$CF_3$-4-Cl- | 5.07(2.15-11.96) | 3.28(1.53-7.05) | 4.45(2.11-9.37) |
| **IV-15** | 1 | 2-$OCH_3$-$C_6H_4$- | 2-$CF_3$-4-Cl- | 9.68(4.90-19.15) | 6.69(3.73-11.98) | / |
| **IV-16** | 1 | 3-$OCH_3$-$C_6H_4$- | 2-$CF_3$-4-Cl- | 2.07(0.81-5.32) | 5.16(0.08-341.76) | 1.56(0.38-6.45) |
| **IV-17** | 1 | 4-$OCH_3$-$C_6H_4$- | 2-$CF_3$-4-Cl- | 4.68(2.52-8.70) | 5.10(2.74-9.50) | / |
| **IV-18** | 1 | 3,4-$OCH_3$-$C_6H_3$- | 2-$CF_3$-4-Cl- | 6.84(3.36-13.92) | 6.60(4.08-10.69) | / |
| **IV-19** | 1 | 4-$OCF_3$-$C_6H_4$- | 2-$CF_3$-4-Cl- | 7.38(4.32-12.59) | 4.59(3.16-6.67) | / |
| **IV-20** | 1 | 4-OH-$C_6H_4$- | 2-$CF_3$-4-Cl- | 15.26(10.05-23.19) | 19.04(10.59-34.21) | / |
| **IV-21** | 1 | 4-pyridin- | 2-$CF_3$-4-Cl- | 31.95(10.27-99.36) | 20.71(5.41-79.32) | / |
| **IV-22** | 1 | 2-morpholino- | 2-$CF_3$-4-Cl- | 39.07(12.25-124.58) | 18.40(4.63-73.09) | / |
| **IV-23** | 2 | 3-Br-$C_6H_4$- | 2-$CF_3$-4-Cl- | 49.00(9.85-243.68) | 36.98(3.02-452.59) | / |
| **IV-24** | 3 | 3-Br-$C_6H_4$- | 2-$CF_3$-4-Cl- | 5.92(3.53-9.92) | 5.85(1.45-23.66) | 4.67(2.55-8.55) |
| **IV-25** | 1 | 3-Br-$C_6H_4$- | 2-F- | 17.17(7.99-36.90) | 13.23(2.67-65.58) | / |
| **IV-26** | 1 | 3-Br-$C_6H_4$- | 3-F- | 21.45(11.76-39.13) | 20.79(3.07-140.71) | / |
| **IV-27** | 1 | 3-Br-$C_6H_4$- | 4-F- | 21.07(11.49-38.64) | 20.54(6.66-63.41) | / |
| **IV-28** | 1 | 3-Br-$C_6H_4$- | 2-Cl- | 17.48(8.86-34.47) | 31.07(4.98-193.92) | / |
| **IV-29** | 1 | 3-Br-$C_6H_4$- | 3-Cl- | 20.40(9.40-44.25) | 15.30(3.18-73.64) | 9.57(4.59-19.94) |
| **IV-30** | 1 | 3-Br-$C_6H_4$- | 4-Cl- | 18.97(8.57-42.01) | 21.08(5.04-88.10) | / |
| **IV-31** | 1 | 3-Br-$C_6H_4$- | 2,4,5-Cl- | 15.80(7.93-31.47) | 10.30(4.50-23.59) | 5.83(3.85-8.82) |
| **IV-32** | 1 | 3-Br-$C_6H_4$- | 2-Br- | 19.02(7.52-48.07) | 21.84(5.26-90.66) | / |
| **IV-33** | 1 | 3-Br-$C_6H_4$- | 3-Br- | 20.70(8.82-48.60) | 9.84(4.96-19.51) | 7.12(4.30-11.79) |
| **IV-34** | 1 | 3-Br-$C_6H_4$- | 4-Br- | 18.79(8.24-42.84) | 13.75(5.20-36.35) | 9.95(5.45-18.17) |
| chesulfamide | / | / | / | 8.27(3.75-18.26) | 8.11(3.97-16.58) | 8.34(4.63-15.02) |
| procymidone | / | / | / | 14.02(6.00-32.74) | 7.39(2.10-26.05) | 1.66(1.08-2.54) |
| boscalid | / | / | / | 2.11(1.07-4.14) | 2.09(1.16-3.75) | 1.60(0.49-5.24) |
| pyrisoxazole | / | / | / | 2.08(1.41-3.06) | 1.81(1.20-2.75) | 1.13(0.73-1.73) |

The target compounds exhibited mediocre inhibition effect on spore

germination at the concentrations of 10 and 50 μg $mL^{-1}$ (Table 2). But with increasing to 100 μg $mL^{-1}$ of specific compounds, they completely inhibited spore germination. Resultantly, the increase in concentration was accompanied by a proportional increase in the inhibition of spores. In comparison with the positive controls, the target compounds were not typical respiratory inhibitor like boscalid. While, part of compounds can almost completely inhibit spore germination under higher concentration conditions, and have the same or higher inhibition effect to procymidone and pyrisoxazole. Therefore, it can be preliminarily inferred that the mode of action of the target compounds should be inhibit the mycelium growth or the synthesis of certain substances.

Table 2. Inhibition rate of target compounds on spore germination of *B. cinerea.*

| Compd. | Inhibition rate (%) | | | Compd. | Inhibition rate (%) | | |
|---|---|---|---|---|---|---|---|
| | 10 μg $mL^{-1}$ | 50 μg $mL^{-1}$ | 100 μg $mL^{-1}$ | | 10 μg $mL^{-1}$ | 50 μg $mL^{-1}$ | 100 μg $mL^{-1}$ |
| **IV-1** | 8.74 | 52.33 | / | **IV-20** | 11.46 | 27.11 | / |
| **IV-2** | 2.86 | 51.06 | / | **IV-21** | 17.85 | 37.75 | / |
| **IV-3** | 17.97 | 38.40 | / | **IV-22** | 20.31 | 53.94 | / |
| **IV-4** | 10.29 | 70.76 | 98.25 | **IV-23** | 20.58 | 40.76 | / |
| **IV-5** | 1.87 | 49.65 | / | **IV-24** | 29.88 | 33.59 | 96.08 |
| **IV-6** | 0.30 | 55.49 | / | **IV-25** | 31.68 | 59.94 | 94.50 |
| **IV-7** | 1.68 | 23.24 | / | **IV-26** | 18.51 | 41.52 | / |
| **IV-8** | 5.54 | 40.17 | / | **IV-27** | 4.08 | 46.92 | / |
| **IV-9** | 3.25 | 52.61 | 96.67 | **IV-28** | 21.30 | 57.93 | 93.40 |
| **IV-10** | 2.34 | 24.84 | / | **IV-29** | -8.08 | 5.90 | / |
| **IV-11** | 1.54 | 56.86 | 92.65 | **IV-30** | 11.02 | 44.05 | / |
| **IV-12** | 1.49 | 29.82 | / | **IV-31** | 22.19 | 35.03 | / |
| **IV-13** | 8.71 | 49.77 | 95.21 | **IV-32** | 34.36 | 53.36 | / |
| **IV-14** | 2.18 | 34.64 | / | **IV-33** | 39.99 | 58.64 | 94.78 |
| **IV-15** | -1.27 | 22.19 | / | **IV-34** | 13.13 | 47.39 | / |
| **IV-16** | 10.39 | 56.29 | / | chesulfamide | 11.81 | 39.73 | 87.66 |
| **IV-17** | 31.73 | 22.19 | / | procymidone | 6.61 | 42.08 | 91.00 |
| **IV-18** | 6.07 | 40.28 | / | boscalid | 96.55 | 93.82 | 97.25 |
| **IV-19** | 10.62 | 36.64 | / | pyrisoxazole | 28.9 | 23.66 | 92.97 |

Hereafter, compounds **IV-1** to **IV-22** were tested for their *in vivo* fungicidal activity on tomato seedlings, the leading compound chesulfamide and the commercial fungicides were used as the positive control (Table 3). The obtained results from bioassay study demonstrated that the control efficacy of 4 compounds (**IV-5**, **IV-9**, **IV-16** and **IV-18**) on tomato seedlings reached over 80%. Moreover, **IV-9** was the best compound with the control efficacy of 90.27%, which were superior to all positive controls.

Table 3. *In vivo* fungicidal activities of target compounds in pot tests at 200 μg $mL^{-1}$.

| Compd. | Control efficacy % (On tomato leaves) | Compd. | Control efficacy % (On tomato leaves) |
|---|---|---|---|
| **IV-1** | 35.63 | **IV-20** | 35.32 |
| **IV-2** | 49.77 | **IV-21** | 54.24 |
| **IV-3** | 28.05 | **IV-22** | 78.35 |
| **IV-4** | 42.68 | **IV-23** | 29.81 |
| **IV-5** | 86.32 | **IV-24** | 30.52 |
| **IV-6** | 72.39 | **IV-25** | 30.18 |
| **IV-7** | 77.74 | **IV-26** | 40.70 |
| **IV-8** | 35.40 | **IV-27** | 66.44 |
| **IV-9** | 90.27 | **IV-28** | 1.46 |
| **IV-10** | 48.84 | **IV-29** | 11.14 |
| **IV-11** | 78.88 | **IV-30** | 43.61 |
| **IV-12** | 67.75 | **IV-31** | 51.09 |
| **IV-13** | 60.53 | **IV-32** | 14.01 |
| **IV-14** | 62.75 | **IV-33** | 75.33 |
| **IV-15** | 66.30 | **IV-34** | 60.91 |
| **IV-16** | 85.64 | chesulfamide | 59.98 |
| **IV-17** | 66.47 | procymidone | 52.44 |
| **IV-18** | 81.38 | boscalid | 71.70 |
| **IV-19** | 74.43 | pyrisoxazole | 66.73 |

After the *in vitro* and *in vivo* bioassay tests, the optimal structure of $R^1$ was determined to be 3-Br-$C_6H_4$-. In order to improve the fungicidal activity, $R^2$ and the carbon number of cyclohexane were modified respectively. But it was regrettable that

the change did not lead to an increase in activity in all *in vitro* tests (Table 1 and Table 2). The *in vivo* bioassays of compounds **IV-23** to **IV-34** were carried out in the same method described (Table 3). It was obvious that no satisfactory compounds had been screened out in these trials. This result could be explained by the fact that introduction of trifluoromethyl group improved the physicochemical properties of the compounds, such as lipophilicity, metabolic stability, activity, etc [34], which guided the direction for the further optimization.

Based on the *in vitro* and *in vivo* bioassay results, the relationship between chemical structures and fungicidal activity was determined. As for the $R^1$ moiety, we summarized the following rules as (1) the activity of the benzene ring is superior to that of the heterocyclic rings; (2) in general, the activity of the electron-withdrawing group on the benzene ring is superior to that of the electron-donating group; (3) the chlorine or bromine atoms on the benzene ring show better activity than that of fluorine atoms; (4) Comparing the compound **IV-3** to **IV-5** with the compounds **IV-6** and **IV-7**, it can be seen that there is no significant difference in activity between the mono- and di-chlorine atom substitutions on the benzene ring. For the $R^2$ moiety, the trifluromethyl group is the functional group, and the loss of it leads to a significant decrease in activity. The activities of **IV-9** containing 6-membered ring is superior to that of compounds **IV-23** and **IV-24** containing 7- and 8-membered ring respectively.

Based on all experiment results, we selected the best performing compound **IV-9** for field trials in tomato greenhouses. We conducted three trials at different times and test pilots in Liaozhong District, Shenyang City, Liaoning Province, China. The

overall control efficacy of **IV-9** (80%) was better than that of the positive control boscalid (66%), and the combination of the two showed the best performance (86%).

Table 4. Control efficacy of compound **IV-9** in field.

| Compd. | Control efficacy (%) | | | |
|---|---|---|---|---|
| | 1st trial | 2nd trial | 3rd trial | average |
| **IV-9** | 91 | 74 | 75 | 80 |
| boscalid | 69 | 86 | 43 | 66 |
| **IV-9**:boscalid (1:1) | 100 | 87 | 72 | 86 |

For the purpose of conducting further meaningful research in practical application and determining the cross-resistance relationship between compound **IV-9** and commercial fungicides more accurately, we cultured the resistance of the *B. cinerea* strain using the compound **IV-9** instead of the conventional method [35]. The strain B0510, which is sensitive to the compound **IV-9** ($EC_{50}$ = 0.21 μg $mL^{-1}$, MIC (Minimum Inhibitory Concentration) = 75 μg $mL^{-1}$), was selected as the test strain. Inoculated 5 mm mycelium dishes in the center of fresh molten PDA medium were amended with low concentration of compound **IV-9**. When the mycelium growth reached to a diameter of about 5 cm, the mycelium were punched and transferred to a medium with a higher concentration of compound **IV-9**. The strain B0510R ($EC_{50}$ = 8.52 μg $mL^{-1}$, MIC = 400 μg $mL^{-1}$) was obtained by culturing the B0510 for 30 generations under the condition of the concentration of compound **IV-9** was gradually increased. Additionally, the sensitive strain B0510 and the resistant strain B0510R both had strong pathogenicity on cucumber leaves in the infection test. Compound **IV-9** and eight commercial fungicides (azoxystrobin, boscalid, chlorothalonil, diethofencarb, fludioxonil, procymidone, pyrimethanil and pyrisoxazole) were tested

for concentration gradient tests on B0510 and B0510R at concentrations of 10, 2.5, 0.625 and 0.15625 μg $mL^{-1}$ on PDA, respectively. The plates were sealed with parafilm and incubated under a regular 12:12 h light/dark regimen at 23℃ for 72 h. The radical growth diameters were measured and $EC_{50}$ values were calculated. These fungicides contain different mechanism of action, such as respiration inhibitor, single sterol biosynthesis inhibitor, methionine biosynthesis inhibitor, acting on signal transduction or tubulin assembly and multisite inhibitor. As can be seen from Table 5, the resistance factor of two strains to compound **IV-9** was 40.57 (Resistance Factor = $EC_{50resistant}$ / $EC_{50sensitive}$). Rather, there was no significant difference between the two strains in $EC_{50}$ values of all fungicides.

Table 5. Inhibition effect of commercial fungicides on *B. cinerea* B0510 and B0510R.

| Compd. | $EC_{50}$ (95% Confidence Limit) (μg $mL^{-1}$) | |
|---|---|---|
| | B0510 | B0510R |
| **IV-9** | 0.21(0.05-0.92) | 8.52(2.13-30.45) |
| azoxystrobin | 2.70(1.00-7.29) | 3.37(1.00-11.30) |
| boscalid | 1.15(0.48-2.75) | 1.41(0.52-3.82) |
| chlorothalonil | 0.36 (0.13-1.32) | 1.12(0.64-1.95) |
| diethofencarb | 3.30(0.67-16.32) | 3.54(1.60-7.86) |
| fludioxonil | 0.19(0.12-0.30) | 0.26(0.14-0.47) |
| procymidone | 2.54(0.91-7.09) | 2.47(1.09-5.62) |
| pyrimethanil | 0.36(0.22-0.62) | 0.41(0.18-0.91) |
| pyrisoxazole | 0.66(0.37-1.17) | 0.38(0.18-0.80) |

In conclusion, 34 novel compounds were designed and synthesized, and their *in vitro* and *in vivo* fungicidal activities were evaluated against strains of *B. cinerea*. **IV-9** performed best in this series of compounds, which was similar or superior to all the positive control procymidone, boscalid and pyrisoxazole in both *in vitro* and *in*

*vivo* bioassays. The control efficacy of **IV-9** in field trials was 80%, which showed higher activity than boscalid (66%). Moreover, there was no positive cross-resistance relationship between compound **IV-9** and the eight commonly used commercial fungicides, which provided references for the development of sulfonamides and the creation of new pesticides.

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**Declaration of interests**

√ ☐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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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# Novel sulfonamides against *Botrytis cinerea* with no positive cross-resistance to commercial fungicides: design, synthesis and SAR study

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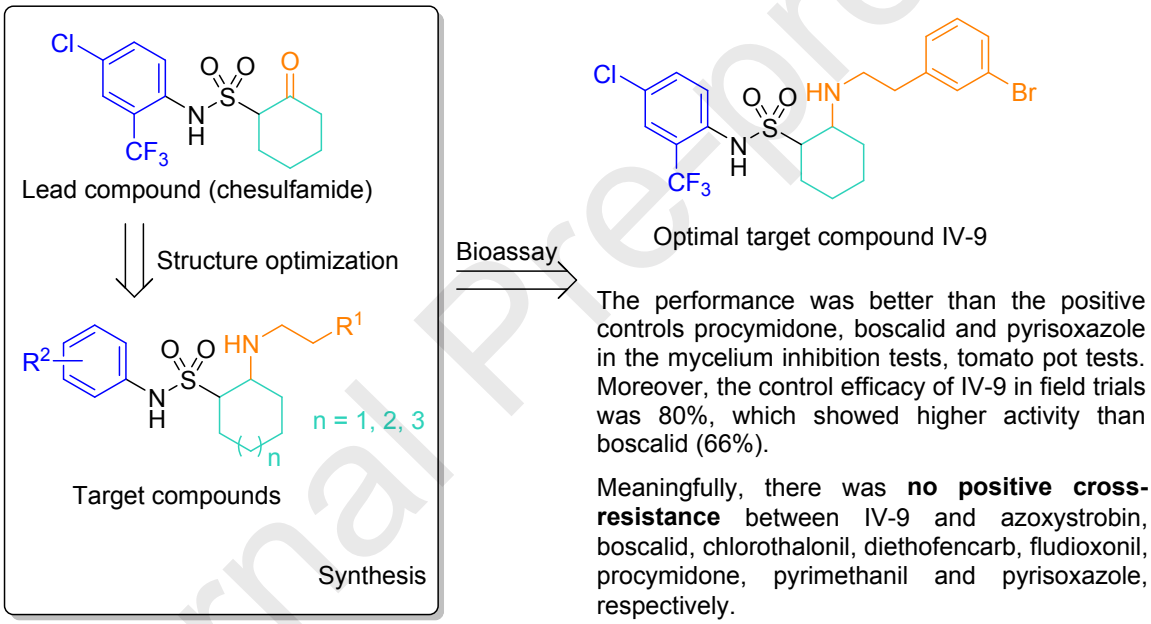


The performance was better than the positive controls procymidone, boscalid and pyrisoxazole in the mycelium inhibition tests, tomato pot tests. Moreover, the control efficacy of IV-9 in field trials was 80%, which showed higher activity than boscalid (66%).

Meaningfully, there was **no positive cross-resistance** between IV-9 and azoxystrobin, boscalid, chlorothalonil, diethofencarb, fludioxonil, procymidone, pyrimethanil and pyrisoxazole, respectively.