Introduction

Plant diseases have been one of the main factors restricting food security. These diseases not only affect the yield of crops but also hamper the quality of food. The use of antibacterial agents and fungicides has greatly reduced the economic losses caused by plant diseases. However, there are also some negative effects along with the social benefits. Due to the unreasonable application of these agents, the resistance of pathogens has become increasingly serious, which also has a tremendous impact on non-target organisms. Additionally, these effects bring great pressure to the environment. With the extensive attention to health and increasing environmental awareness, the development of efficient, low toxic, and green antibacterial agents or fungicides has become a hot topic in the field of pesticide research. A large number of pesticides have been found in the market. For example, fluopyram (Fig. 1) was developed and applied to control the plant pathogens in crops such as rice, citrus, and kiwi fruit. Bismertiazol (Fig. 1) was used to control the effect of rice bacterial blight and bacterial leaf streak. In 2012, fluxapyroxad (Fig. 1) was developed as a new pesticide for corresponding plant diseases.

Isoxazole derivatives are important heterocyclic compounds that are widely used in pesticides. For instance, 5-methylisoxazol-3-ol (hymexazol in Fig. 1) is considered as a soil disinfectant and plant growth regulator. Isouron (Fig. 1), a selective herbicide, is mostly employed to control the weeds such as paspalum and white grass. Isoxaflutole (Fig. 1) belongs to early sulfone herbicides, and are mainly applied in corn and sugarcane fields. Benazimazole, a herbicide cell division inhibitor (Fig. 1), is usually used in broad-leaved plants including cereal crops, broad beans, peas, trees, and grapes. Based on the characteristics and wide application of isoxazole derivatives, the rapid and efficient construction of various isoxazole derivatives has become the focus of researchers. Among the synthetic methods of isoxazole compounds, the most efficient strategy is [3 + 2] cycloaddition. Therefore, many chemists have used this method to synthesize isoxazole compounds and apply them to develop new pesticides.

Our group has focused on the development of small molecules with various biological activities for several years. Considering the urgent need for novel pesticides containing isoxazole rings and our previous research on heterocyclic compounds, we designed and synthesized phenylisoxazole derivatives as a novel pesticide in agriculture. Furthermore, there are only a few reports on phenylisoxazoles as antibacterial agents in literature. In this study, we first developed a new synthetic method for constructing a phenylisoxazole ring with diverse substituent groups. Subsequently, we screened the biological activities of these compounds to obtain novel pesticides.

Discovery of 4-nitro-3-phenylisoxazole derivatives as potent antibacterial agents derived from the studies of [3 + 2] cycloaddition†

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Polysubstituted phenylisoxazoles were designed and synthesized to discover new antibacterial agents via [3 + 2] cycloaddition. Thirty-five compounds with a phenylisoxazole scaffold were characterized by NMR, HRMS, and X-ray techniques. After being evaluated against Xanthomonas oryzae (Xoo), Pseudomonas syringae (Psa), and Xanthomonas axonopodis (Xac), 4-nitro-3-phenylisoxazole derivatives were found to better antibacterial activities. Further studies have shown that the EC50 values of these compounds were much better than that of the positive control, bismertiazol.

Fig. 1 Important antibacterial agents in market.
Results and discussion

Mohammed et al. reported benzaldehyde oxime \((\text{1a})\) reacted with phenylacetylene \((\text{2a})\) to afford compound \((\text{3a})\) in the presence of NCS and DBU in 2015.\(^1\) Initially, we repeated the reaction and obtained the isolated yield of 63%. When the additive NCS was increased to two equivalents, the yield still remained at 65% (Table 1, entry 1). Later, we screened different solvents to improve the yield and identified that DMF is still the best solvent (Table 1, entries 1–4). The different additives such as NBS, NIS, PIFA, chloramine-T were also used to accelerate the reaction.\(^2\) Unfortunately, these additives were all ineffective in improving the yield (Table 1, entries 5–8). Subsequently, we found that different bases could severely influence the reaction rate (Table 1, entries 9–18). When triethylamine was used as the base, the separated yield reached 85% (Table 1, entry 18), indicating that the weak organic base was ideal for accelerating the yield. Interestingly, the yield decreased slowly with the increase in temperature (Table 1, entries 19–21). This may be due to the substrate side effects accompanied with the increase in temperature, which reduced the reaction yield. Finally, the optimal condition was determined in the presence of TEA and DMF at room temperature (Table 1, entry 18).

Table 1 Reaction optimization of \((E)-\)benzaldehyde oxime and phenylacetylene\(^a\),\(^b\)

| Entry | Additive | Base | Solvent | \(T\) (°C) | Yield (%) |
|-------|----------|------|---------|-----------|-----------|
| 1     | NCS      | DBU  | DMF     | 25        | 65 (63)c  |
| 2     | NCS      | DBU  | CHCl\(_2\) | 25        | 38        |
| 3     | NCS      | DBU  | THF     | 25        | 31        |
| 4     | NCS      | DBU  | Dioxane | 25        | None      |
| 5     | NBS      | DBU  | DMF     | 25        | 17        |
| 6     | NIS      | DBU  | DMF     | 25        | 19        |
| 7     | PIFA     | DBU  | DMF     | 25        | 39        |
| 8     | Chloramine | DBU | DMF     | 25        | 37        |
| 9     | NCS      | Cs\(_2\)CO\(_3\) | DMF | 25        | 43        |
| 10    | NCS      | K\(_2\)CO\(_3\) | DMF | 25        | 51        |
| 11    | NCS      | NaO’Bu | DMF | 25        | 51        |
| 12    | NCS      | Na\(_2\)CO\(_3\) | DMF | 25        | 53        |
| 13    | NCS      | Pyrrolidine | DMF | 25        | 47        |
| 14    | NCS      | NaOH  | DMF     | 25        | 52        |
| 15    | NCS      | DMAP  | DMF     | 25        | 55        |
| 16    | NCS      | DIEA  | DMF     | 25        | 80        |
| 17    | NCS      | DABCO | DMF     | 25        | 63        |
| 18    | NCS      | TEA   | DMF     | 25        | 85        |
| 19    | NCS      | TEA   | DMF     | 50        | 84        |
| 20    | NCS      | TEA   | DMF     | 75        | 79        |
| 21    | NCS      | TEA   | DMF     | 100       | 74        |

\(^a\) Reagents and conditions: \(1\) (1 mmol), \(2\) (1.2 mmol), additive (2 mmol), base (1 mmol), solvent (6 mL), 6 h. \(^b\) Isolated yields.
Having the optimal condition in hand (Table 1, entry 18), the scope was explored in different substituted phenylxime derivatives and phenylacetylene derivatives (Table 2). Phenylximes with electron-donating groups –CH₃ and –OCH₃ resulted in lower yields than 2a, while electron-withdrawing groups –F and –CF₃ resulted in higher yields than 2a (3a–3g). Furthermore, the steric effect of substituents on the phenyl ring also decreased the yield of this [3+2] cycloaddition (3c–3e). In addition, phenylxime derivatives such as 3-methyl (2h), 4-methyl (2i), 4-methoxy (2j), 3-chloro (2k) benzaldehyde oximes produced the desired products in 65–76% yield (3h–3l).

Structural diversity is very crucial for screening new antibacterial agents. To ensure molecular diversity, 3,4-disubstituted isoxazoles were synthesized (Table 3), and benzaldehyde oxime (1a) and commercially available 4-dimethylamino-but-3-en-2-one (4a) were chosen as the starting materials. Under the identical conditions as mentioned previously, compound 5a was isolated with 61% yield (Table 3). The structure of 5a was confirmed by NMR and HRMS, which was consistent with that obtained from other cycloadditions using hypervalent iodine reagent as the catalyst. Subsequently, a phenyl ring with electron-donating groups –CH₃ (5b) and –OCH₃ (5e) produced lower yields, while electron-withdrawing groups –CF₃ (5e) and –NO₂ (5h) produced higher yields than 5a. The steric effect of substituents on the phenyl ring significantly decreased the yield of this cycloaddition (5f, 5g and 5h). Besides, 3-dimethylamino-

Table 4 Antibacterial activities of compounds 3 and 5 against Xoo, Xac and Psa

| Entry | Cpd  | Xoo (µg mL⁻¹) | Xac (µg mL⁻¹) | Psa (µg mL⁻¹) |
|-------|------|---------------|---------------|---------------|
|       |      | 100 50        | 100 50        | 100 50        |
| 1     | 3a   | —             | —             | 9.6 ± 2.1     |
| 2     | 3b   | —             | —             | 10.7 ± 3.8    |
| 3     | 3c   | 38.2 ± 3.7    | 15.0 ± 2.4    | 15.8 ± 1.4    |
| 4     | 3d   | 35.5 ± 2.8    | 12.9 ± 1.7    | 12.6 ± 1.1    |
| 5     | 3e   | 36.1 ± 3.2    | 14.6 ± 2.5    | 14.9 ± 0.8    |
| 6     | 3f   | —             | —             | 14.5 ± 4.5    |
| 7     | 3g   | 43.5 ± 5.9    | 38.1 ± 2.1    | 19.6 ± 1.5    |
| 8     | 3h   | 35.1 ± 3.7    | 20.3 ± 5.0    | 18.6 ± 5.8    |
| 9     | 3i   | 44.8 ± 3.6    | 21.6 ± 2.5    | 19.5 ± 4.5    |
| 10    | 3j   | 14.4 ± 2.7    | —             | 16.8 ± 4.9    |
| 11    | 3k   | 46.7 ± 3.6    | 36.1 ± 4.1    | 19.2 ± 4.2    |
| 12    | 3l   | 72.8 ± 2.5    | 35.1 ± 1.3    | 16.0 ± 0.9    |
| 13    | 5a   | 29.6 ± 0.9    | 22.4 ± 3.6    | 27.4 ± 0.4    |
| 14    | 5b   | 41.1 ± 5.7    | 28.7 ± 3.1    | 27.0 ± 5.7    |
| 15    | 5c   | 32.9 ± 4.1    | 31.5 ± 2.2    | 27.6 ± 4.9    |
| 16    | 5d   | 42.4 ± 5.2    | 27.6 ± 3.4    | 26.8 ± 5.3    |
| 17    | 5e   | 42.2 ± 1.7    | 10.4 ± 5.9    | 29.6 ± 2.2    |
| 18    | 5f   | 33.5 ± 1.1    | 24.1 ± 1.6    | 26.8 ± 2.7    |
| 19    | 5g   | 37.9 ± 6.9    | 26.8 ± 8.3    | 25.3 ± 1.9    |
| 20    | 5h   | 48.4 ± 2.8    | 19.3 ± 4.7    | 25.7 ± 1.5    |
| 21    | 5i   | 34.9 ± 1.6    | 26.5 ± 5.1    | 26.0 ± 0.7    |
| 22    | 5j   | 37.7 ± 1.4    | 28.9 ± 4.3    | 28.2 ± 0.5    |
| 23    | 5k   | 36.8 ± 1.9    | 28.7 ± 6.7    | 27.1 ± 0.8    |
| 24    | 5l   | 42.2 ± 1.7    | 10.4 ± 5.9    | 26.5 ± 0.7    |
| 25    | 5m   | 33.5 ± 1.1    | 24.1 ± 1.6    | 27.1 ± 0.8    |
| 26    | 5n   | 41.2 ± 1.8    | 26.7 ± 1.5    | 28.8 ± 1.2    |
| 27    | 5o   | 97.7 ± 0.2    | 93.6 ± 0.0    | 60.8 ± 3.2    |
| 28    | 5p   | 97.7 ± 1.0    | 96.8 ± 0.0    | 66.3 ± 5.6    |
| 29    | 5q   | 97.7 ± 0.1    | 94.0 ± 0.3    | 65.4 ± 4.5    |
| 30    | 5r   | 96.4 ± 0.1    | 93.5 ± 0.1    | 58.4 ± 1.4    |
| 31    | 5s   | 97.8 ± 0.1    | 97.6 ± 0.1    | 55.0 ± 4.1    |
| 32    | 5t   | 97.9 ± 0.1    | 96.6 ± 0.1    | 51.7 ± 5.3    |
| 33    | 5u   | 96.0 ± 0.3    | 69.4 ± 6.5    | 42.7 ± 4.5    |
| 34    | 5v   | 97.5 ± 0.1    | 94.1 ± 0.5    | 42.4 ± 5.0    |
| 35    | 5w   | 97.5 ± 0.1    | 94.1 ± 0.5    | 42.4 ± 5.0    |
| Bismertiazol | 73.9 ± 1.1 | 29.3 ± 1.7 | 47.6 ± 2.3 | 39.0 ± 3.5 | 15.6 ± 4.1 |

* The average of three trials.
acrylic acid ethyl ester (4b) was also used in this reaction instead of compound 4a. Fortunately, the final targets 5i–5n were successfully synthesized with 44–67% yields. The effect of substituents on the yield was similar to 5a–5h. Nitroisoxazole derivatives have a wide range of biological activities, such as antitumor, antibacterial, and anti-inflammatory. To investigate the antibacterial activities of nitroisoxazoles, 4-nitro-3-phenylisoxazole derivatives were prepared. Dimethyl-(2-nitrovinyl)-amine (4c) reacted with corresponding phenylisoxazoles (1) to give the products 5o–5w. The yield was moderate from 36% to 63%.

In addition, the NMR spectra of compound 5o was consistent with the data in the literature. However, the test result of HRMS was inconsistent with the actual values. This phenomenon was observed in all the 4-nitro-3-phenylisoxazole derivatives. To ensure the structures of this series of products, compounds 5p, 5q, and 5r were characterized by X-ray diffraction analysis. As shown in Fig. 2, the deposition numbers in CCDC (Cambridge Crystallographic Data Centre) were 2130131 (5p), 2130134 (5q), and 2153382 (5r). These results indirectly indicate that the structures of compounds 5o–5w were appropriate.

To investigate the mechanism, the benzaldehyde oxime 1a and compound 4c were subjected to undergo [3 + 2] cycloaddition. Based on the observed results and the reports in the literature, the regioselectivity of the [3 + 2] cycloaddition reaction between nitrile N-oxides and conjugated nitroalkenes was determined by the nucleophilic attack of oxygen atom from the CNO moiety on the activated electrophilic 2-position of nitroalkene. Considering several mechanisms related to the 32CA reaction, we described a plausible mechanism in Scheme 1. Initially, the NCS chlorinated oxime 1a afforded an intermediate 6. Then, compound 6 was dechlorinated under base DIEA to form nitrile oxide 7. Finally, it was reacted with compound 4c to afford the isoxazole compound 5o through [3 + 2] cycloaddition with 55% yield.

In order to explain the pathway of this mechanism, we conducted two complementary experiments. As shown in Scheme 2, under the optimized conditions, the benzaldehyde oxime 1a was converted to compound 6 with 85% yield in the

| Compound                     | Regression equation | Correlation coefficient ($r$) | EC50 ($\mu$g mL$^{-1}$) |
|------------------------------|---------------------|--------------------------------|--------------------------|
| **Xanthomonas oryzae (Xoo)** |                     |                                |                          |
| Bismethiazol                 | $y = 0.5792x + 2.3536$ | 0.9595                         | 82.3 ± 5.1               |
| 5o                           | $y = 3.2815x + 0.658$  | 0.9856                         | 15.0 ± 0.8               |
| 5p                           | $y = 3.6552x + 7.277$  | 0.9683                         | 11.7 ± 0.4               |
| 5q                           | $y = 3.7164x + 3.068$  | 0.9883                         | 12.6 ± 0.5               |
| 5r                           | $y = 3.5643x + 2.158$  | 0.9835                         | 13.4 ± 0.4               |
| 5s                           | $y = 6.5516x + 0.248$  | 0.9530                         | 7.6 ± 0.3                |
| 5t                           | $y = 5.3958x + 0.8359$ | 0.9838                         | 9.1 ± 0.3                |
| 5u                           | $y = 3.1172x + 0.4496$ | 0.9667                         | 15.9 ± 0.8               |
| 5v                           | $y = 1.6916x + 0.0594$ | 0.9870                         | 29.5 ± 1.4               |
| 5w                           | $y = 6.0038x + 2.4873$ | 0.9861                         | 7.9 ± 0.4                |
| **Xanthomonas axonopodis (Xac)** |                   |                                |                          |
| Bismethiazol                 | $y = 0.8141x + 0.3426$ | 0.9599                         | 61.0 ± 4.4               |
| 5o                           | $y = 19.4772x + 1.2731$ | 0.9394                         | 2.5 ± 0.1                |
| 5p                           | $y = 15.73x + 6.1445$  | 0.9082                         | 2.8 ± 0.2                |
| 5q                           | $y = 9.3762x + 5.5612$ | 0.9371                         | 4.7 ± 0.2                |
| 5r                           | $y = 6.8912x + 5.5944$ | 0.9804                         | 6.4 ± 0.3                |
| 5s                           | $y = 10.836x + 18.611$ | 0.9176                         | 2.9 ± 0.2                |
| 5t                           | $y = 13.73x + 10.154$  | 0.9309                         | 2.9 ± 0.2                |
| 5u                           | $y = 7.3972x + 5.8451$ | 0.9466                         | 6.0 ± 0.3                |
| 5v                           | $y = 6.922x + 5.6802$  | 0.9762                         | 6.4 ± 0.4                |
| 5w                           | $y = 12.884x + 14.465$ | 0.9782                         | 2.8 ± 0.2                |
| **Pseudomonas syringae (Psa)** |                   |                                |                          |
| Bismethiazol                 | $y = 0.6055x + 23.34$  | 0.9104                         | >100                     |
| 5o                           | $y = 1.1501x + 19.741$ | 0.9581                         | 44.0 ± 3.8               |
| 5p                           | $y = 0.9974x + 13.765$ | 0.9199                         | 26.3 ± 2.2               |
| 5q                           | $y = 0.6203x + 14.995$ | 0.9779                         | 36.3 ± 2.7               |
| 5r                           | $y = 0.699x + 6.2785$  | 0.9784                         | 56.4 ± 5.1               |
| 5s                           | $y = 0.9171x + 6.8663$ | 0.9650                         | 62.5 ± 5.3               |
| 5t                           | $y = 0.6372x + 14.585$ | 0.9821                         | 47.0 ± 3.7               |
| 5u                           | $y = 0.6372x + 14.585$ | 0.9821                         | 55.6 ± 4.6               |
| 5v                           | $y = 0.6372x + 14.585$ | 0.9821                         | >100                     |
| 5w                           | $y = 0.6372x + 14.585$ | 0.9821                         | >100                     |

* The average of three trials.
presence of NCS. The NMR spectra of 6 was consistent with the literature. At last, compound 6 reacted with (E)-N,N-dimethyl-2-nitroethen-1-amine 4c at 25 °C to afford the required product 5o in 55% yield. These complementary experiments successfully proved our proposed mechanism in Scheme 1.

With the target compounds 3a–3l and 5a–5w in hand, we screened in vitro screening of antibacterial activities against representative plant diseases including Xanthomonas oryzae (Xoo), Xanthomonas axonopodis (Xac), and Pseudomonas syringae (Psa) at 100 μg mL⁻¹ and 50 μg mL⁻¹ according to the previous report. The results are listed in Table 4. The activities of compounds 3a–3l and 5a–5n were lower than those of bismertiazol and fluopyram. It was interesting to note that the activities of compounds 5o–5w were much better than the positive controls. Surprisingly, the inhibitions against Xoo and Xac were more than 90% at the concentration of 100 μg mL⁻¹ and 50 μg mL⁻¹. Therefore, compounds 5o–5w were selected for further studies as novel antibacterial agents.

As shown in Table 5, compounds 5o–5w exhibited much better EC₅₀ values against Xoo, Xac, and Psa than bismertiazol and fluopyram. Preliminary structure–activity relationship can also be found in Table 5. The ortho-substituted derivatives (5p, 5s) have better activities than meso-substituted derivatives (5q, 5t) and para-substituted derivatives (5r, 5u, and 5v). Against Xoo, the large-steric substituted compounds 5t (~Br, 9.1 μg mL⁻¹) and 5w (~NO₂, 7.9 μg mL⁻¹) have lower EC₅₀ than small-steric substituted compound 5q (~F, 12.6 μg mL⁻¹). A similar phenomenon was observed against Xac. However, against Psa, small-steric substituted compound 5q (12.6 μg mL⁻¹) showed a better effect than large-steric substituted compound 5t (47.0 μg mL⁻¹).

Conclusions

In summary, an efficient method was developed to prepare polysubstituted phenylisoxazoles via [3 + 2] cycloaddition. This series of phenylisoxazole derivatives were characterized and evaluated at 100 μg mL⁻¹ and 50 μg mL⁻¹ against Xoo, Xac, and Psa. The results suggested that 4-nitro-3-phenylisoxazole derivatives performed excellent antibacterial activities. Further studies on the EC₅₀ values have shown that these compounds were much better than bismertiazol. However, the antibacterial mechanism of these 4-nitro-3-phenylisoxazole derivatives is ongoing in our lab.

Author contributions

Yan Zhang and Zhiwu Long synthesized the compounds and performed the spectral studies; Longjia Yan and Li Liu contributed to the design and implementation of the research; Lan Yang and Yi Le tested bioactive activities, and wrote the manuscript with input from all authors.

Conflicts of interest

The authors declare no conflicts of interest.

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Notes and references

1 J. L. Dangl, D. M. Horvath and B. J. Staskawicz, Science, 2013, 341, 746–751.
2 (a) M. Liu, Z. Shi, X. Zhang, M. Wang, L. Zhang, K. Zheng, J. Liu, X. Hu, C. Di, Q. Qian, Z. He and D. L. Yang, Nat. Plants, 2019, 5, 389–400; (b) Y. Sun, Y. X. Zhu, P. J. Balint-Kurti and G. F. Wang, Trends Plant Sci., 2020, 25, 695–713; (c) S. Chakraborty and A. C. Newton, Plant Pathol., 2011, 60, 2–14.
3 (a) F. Gao, T. Wang, J. Xiao and G. Huang, Eur. J. Med. Chem., 2019, 173, 274–281; (b) P. E. Busby, K. G. Peay and G. Newcombe, New Phytol., 2016, 209, 1681–1692; (c) T. Zhou, R. Hu, L. Wang, Y. Qiu, G. Zhang, Q. Deng, H. Zhang, P. Yin, B. Situ, C. Zhan, A. Qin and B. Z. Tang, Angew. Chem., Int. Ed. Engl., 2020, 59, 9952–9956.
4 (a) S. Lehmann, M. Serrano, F. L’Haridon, S. E. Tjamos and J. P. Metraux, Phytochemistry, 2015, 112, 54–62; (b) J. G. Schaart, C. C. M. van de Wiel, L. A. P. Lotz and M. J. M. Smulders, Trends Plant Sci., 2016, 21, 438–449; (c) H. Derksen, C. Rampitsch and F. Daayf, Plant Sci., 2013, 207, 79–87.
