



Cite this: *RSC Adv.*, 2022, 12, 25633

Received 10th August 2022

Accepted 21st August 2022

DOI: 10.1039/d2ra05009a

rsc.li/rsc-advances

# 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 EC<sub>50</sub> values of these compounds were much better than that of the positive control, bismethiazol.

## Introduction

Plant diseases have been one of the main factors restricting food security.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3</sup> 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.<sup>4</sup> A large number of pesticides have been found in the market.<sup>5</sup> For example, fluopyram (Fig. 1) was developed and applied to control the plant pathogens in crops such as rice, citrus, and kiwi fruit.<sup>6</sup> Bismethiazol (Fig. 1) was used to control the effect of rice bacterial blight and bacterial leaf streak.<sup>7</sup> In 2012, fluxapyroxad (Fig. 1) was developed as a new pesticide for corresponding plant diseases.<sup>8</sup>

Isioxazole derivatives are important heterocyclic compounds that are widely used in pesticides.<sup>9</sup> For instance, 5-methylisoxazol-3-ol (hymexazol in Fig. 1) is considered as a soil disinfectant and plant growth regulator.<sup>10</sup> Isouron (Fig. 1), a selective herbicide, is mostly employed to control the weeds such as paspalum and white grass.<sup>11</sup> Isoxaflutole (Fig. 1) belongs

to early sulfone herbicides, and are mainly applied in corn and sugarcane fields.<sup>12</sup> Benzamizole, a herbicide cell division inhibitor (Fig. 1), is usually used in broad-leaved plants including cereal crops, broad beans, peas, trees, and grapes.<sup>13</sup> 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.<sup>14</sup> Among the synthetic methods of isoxazole compounds, the most efficient strategy is [3 + 2] cycloaddition.<sup>15</sup> Therefore, many chemists have used this method to synthesize isoxazole compounds and apply them to develop new pesticides.<sup>16</sup>

Our group has focused on the development of small molecules with various biological activities for several years.<sup>17</sup> Considering the urgent need for novel pesticides containing isoxazole rings and our previous research on heterocyclic compounds,<sup>18</sup> 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.

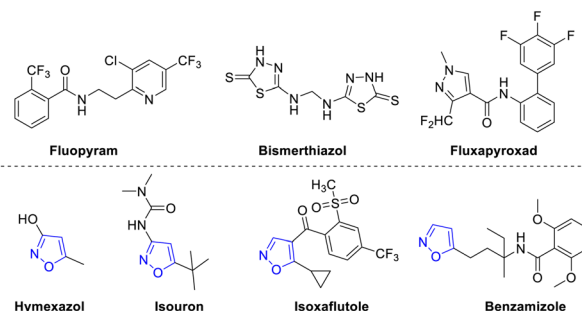


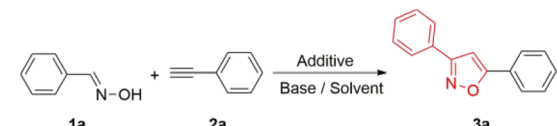
Fig. 1 Important antibacterial agents in market.

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† Electronic supplementary information (ESI) available. CCDC 2130131, 2130134 and 2153382. For ESI and crystallographic data in CIF or other electronic format see <https://doi.org/10.1039/d2ra05009a>



**Table 1** Reaction optimization of (*E*)-benzaldehyde oxime and phenylacetylene<sup>a,b</sup>


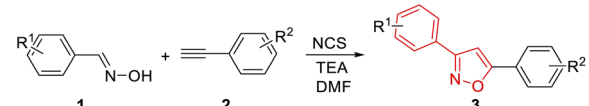
Entry	Additive	Base	Solvent	T (°C)	Yield (%)
1	NCS	DBU	DMF	25	65 (63) <sup>c</sup>
2	NCS	DBU	CH <sub>2</sub> Cl <sub>2</sub>	25	38
3	NCS	DBU	THF	25	31
4	NCS	DBU	Dioxane	25	None
5	NBS	DBU	DMF	25	17
6	NIS	DBU	DMF	25	19
7	PIFA	DBU	DMF	25	39
8	Chloramine	DBU	DMF	25	37
9	NCS	CS <sub>2</sub> CO <sub>3</sub>	DMF	25	43
10	NCS	K <sub>2</sub> CO <sub>3</sub>	DMF	25	51
11	NCS	NaO <sup>t</sup> Bu	DMF	25	51
12	NCS	Na <sub>2</sub> CO <sub>3</sub>	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

<sup>a</sup> Reagents and conditions: **1a** (1 mmol), **2a** (1.2 mmol), additive (2 mmol), base (1 mmol), solvent (6 mL), 6 h. <sup>b</sup> Isolated yields.

<sup>c</sup> Reagents and conditions: **1a** (1 mmol), **2a** (1.2 mmol), NCS (1.2 mmol), DBU (1 mmol), DMF (6 mL), 6 h.

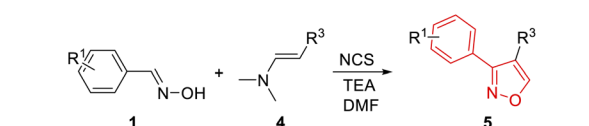
## Results and discussion

Mohammed *et al.* reported benzaldehyde oxime (**1a**) reacted with phenylacetylene (**2a**) to afford compound **3a** in the presence of NCS and DBU in 2015.<sup>19</sup> 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.<sup>20</sup> 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 2** Reaction examples of **1** and **2**<sup>a</sup>


<b>3a</b> (85%)	<b>3b</b> (74%)	<b>3c</b> (76%)
<b>3d</b> (86%)	<b>3e</b> (86%)	<b>3f</b> (65%)
<b>3g</b> (88%)	<b>3h</b> (71%)	<b>3i</b> (71%)
<b>3j</b> (76%)	<b>3k</b> (65%)	<b>3l</b> (68%)

<sup>a</sup> Reagents and conditions: **1** (1 mmol), **2** (1.2 mmol), NCS (2 mmol), TEA (1 mmol), DMF (6 mL), 25 °C, 6 h.

**Table 3** Reaction examples of **1** and **4**<sup>a</sup>


<b>5a</b> (61%)	<b>5b</b> (57%)	<b>5c</b> (53%)	<b>5d</b> (58%)	<b>5e</b> (62%)
<b>5f</b> (43%)	<b>5g</b> (48%)	<b>5h</b> (63%)	<b>5i</b> (67%)	<b>5j</b> (63%)
<b>5k</b> (60%)	<b>5l</b> (44%)	<b>5m</b> (63%)	<b>5n</b> (52%)	
<b>5o</b> (61%)	<b>5p</b> (57%)	<b>5q</b> (59%)	<b>5r</b> (63%)	
<b>5s</b> (53%)	<b>5t</b> (55%)	<b>5u</b> (56%)	<b>5v</b> (42%)	<b>5w</b> (36%)

<sup>a</sup> Reagents and conditions: **1** (1 mmol), **4** (1.2 mmol), NCS (2 mmol), TEA (1 mmol), DMF (6 mL), 25 °C, 6 h.



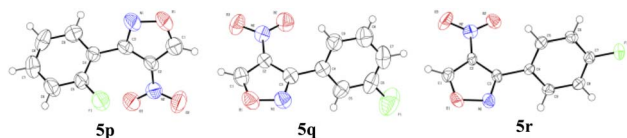


Fig. 2 X-ray structures of 5p, 5q, and 5r.

Having the optimal condition in hand (Table 1, entry 18), the scope was explored in different substituted phenyloxime derivatives and phenylacetylene derivatives (Table 2). Phenyloximes with electron-donating groups  $-\text{CH}_3$  and  $-\text{OCH}_3$  resulted in lower yields than **2a**, while electron-withdrawing groups  $-\text{F}$  and  $-\text{CF}_3$  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, phenyloxime 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 anti-bacterial agents.<sup>21</sup> 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.<sup>20</sup> Subsequently, a phenyl ring with electron-donating groups  $-\text{CH}_3$  (**5b**) and  $-\text{OCH}_3$  (**5c**) produced lower yields, while electron-withdrawing groups  $-\text{CF}_3$  (**5e**) and  $-\text{NO}_2$  (**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*<sup>a</sup>

Entry	Cpd	<i>Xoo</i> ( $\mu\text{g mL}^{-1}$ )		<i>Xac</i> ( $\mu\text{g mL}^{-1}$ )		<i>Psa</i> ( $\mu\text{g mL}^{-1}$ )	
		100	50	100	50	100	50
1	<b>3a</b>	—	—	—	—	9.6 $\pm$ 2.1	—
2	<b>3b</b>	—	—	—	—	10.7 $\pm$ 3.8	—
3	<b>3c</b>	—	—	38.2 $\pm$ 3.7	15.0 $\pm$ 2.4	15.8 $\pm$ 1.4	—
4	<b>3d</b>	—	—	35.5 $\pm$ 2.8	12.9 $\pm$ 1.7	12.6 $\pm$ 1.1	—
5	<b>3e</b>	—	—	36.1 $\pm$ 3.2	14.6 $\pm$ 2.5	14.9 $\pm$ 0.8	—
6	<b>3f</b>	—	—	—	—	14.5 $\pm$ 4.5	—
7	<b>3g</b>	—	—	43.5 $\pm$ 5.9	38.1 $\pm$ 2.1	19.6 $\pm$ 1.5	—
8	<b>3h</b>	—	—	35.1 $\pm$ 3.7	20.3 $\pm$ 5.0	18.6 $\pm$ 5.8	—
9	<b>3i</b>	—	—	44.8 $\pm$ 3.6	21.6 $\pm$ 2.5	19.3 $\pm$ 4.5	18.3 $\pm$ 7.4
10	<b>3j</b>	—	—	14.4 $\pm$ 2.7	—	16.8 $\pm$ 4.9	9.3 $\pm$ 4.1
11	<b>3k</b>	—	—	46.7 $\pm$ 3.6	36.1 $\pm$ 4.1	19.2 $\pm$ 4.2	—
12	<b>3l</b>	—	—	72.8 $\pm$ 2.5	35.1 $\pm$ 1.3	16.0 $\pm$ 0.9	5.8 $\pm$ 2.2
13	<b>5a</b>	29.6 $\pm$ 0.9	22.4 $\pm$ 3.6	34.4 $\pm$ 3.5	25.3 $\pm$ 2.6	27.4 $\pm$ 0.4	17.7 $\pm$ 0.2
14	<b>5b</b>	41.1 $\pm$ 5.7	28.7 $\pm$ 3.1	45.8 $\pm$ 1.7	29.5 $\pm$ 4.3	27.0 $\pm$ 5.7	14.9 $\pm$ 2.8
15	<b>5c</b>	32.9 $\pm$ 4.1	31.5 $\pm$ 2.2	39.0 $\pm$ 8.2	12.3 $\pm$ 6.0	27.6 $\pm$ 4.9	16.5 $\pm$ 2.0
16	<b>5d</b>	42.4 $\pm$ 5.2	27.6 $\pm$ 3.4	46.7 $\pm$ 1.8	28.6 $\pm$ 4.1	26.8 $\pm$ 5.3	13.7 $\pm$ 2.5
17	<b>5e</b>	42.2 $\pm$ 1.7	10.4 $\pm$ 5.9	64.0 $\pm$ 4.6	56.8 $\pm$ 5.1	29.6 $\pm$ 2.2	16.0 $\pm$ 3.0
18	<b>5f</b>	33.5 $\pm$ 1.1	24.1 $\pm$ 1.6	60.6 $\pm$ 7.4	46.2 $\pm$ 4.2	26.8 $\pm$ 2.7	14.3 $\pm$ 2.6
19	<b>5g</b>	37.9 $\pm$ 6.9	26.8 $\pm$ 8.3	51.9 $\pm$ 8.4	29.3 $\pm$ 6.6	25.3 $\pm$ 1.9	12.0 $\pm$ 1.4
20	<b>5h</b>	48.4 $\pm$ 2.8	19.3 $\pm$ 4.7	51.3 $\pm$ 2.5	26.0 $\pm$ 6.5	25.7 $\pm$ 1.5	13.4 $\pm$ 1.6
21	<b>5i</b>	34.9 $\pm$ 1.6	26.5 $\pm$ 5.1	70.4 $\pm$ 5.0	51.6 $\pm$ 3.4	26.0 $\pm$ 0.7	14.8 $\pm$ 5.1
22	<b>5j</b>	37.7 $\pm$ 1.4	28.9 $\pm$ 4.3	63.7 $\pm$ 4.5	46.2 $\pm$ 2.8	28.2 $\pm$ 0.5	16.4 $\pm$ 5.8
23	<b>5k</b>	36.8 $\pm$ 1.9	28.7 $\pm$ 6.7	72.9 $\pm$ 6.1	53.7 $\pm$ 4.9	27.1 $\pm$ 0.8	15.3 $\pm$ 6.6
24	<b>5l</b>	42.2 $\pm$ 1.7	10.4 $\pm$ 5.9	74.0 $\pm$ 4.6	56.8 $\pm$ 7.1	26.5 $\pm$ 0.7	16.6 $\pm$ 4.5
25	<b>5m</b>	33.5 $\pm$ 1.1	24.1 $\pm$ 1.6	60.6 $\pm$ 2.4	46.2 $\pm$ 5.2	27.1 $\pm$ 0.8	15.3 $\pm$ 3.6
26	<b>5n</b>	41.2 $\pm$ 1.8	26.7 $\pm$ 1.5	62.1 $\pm$ 2.1	42.0 $\pm$ 4.8	28.8 $\pm$ 1.2	16.8 $\pm$ 3.4
27	<b>5o</b>	97.7 $\pm$ 0.2	93.6 $\pm$ 0.3	99.5 $\pm$ 0.4	98.5 $\pm$ 0.1	60.8 $\pm$ 3.2	55.6 $\pm$ 6.5
28	<b>5p</b>	97.7 $\pm$ 1.0	96.8 $\pm$ 0.0	97.9 $\pm$ 1.0	96.4 $\pm$ 0.6	66.3 $\pm$ 5.6	63.7 $\pm$ 5.2
29	<b>5q</b>	97.7 $\pm$ 0.1	94.0 $\pm$ 0.3	97.8 $\pm$ 0.7	95.6 $\pm$ 0.1	65.4 $\pm$ 4.5	57.0 $\pm$ 4.6
30	<b>5r</b>	96.4 $\pm$ 0.1	93.5 $\pm$ 0.1	99.2 $\pm$ 0.2	97.7 $\pm$ 0.2	58.4 $\pm$ 1.4	41.7 $\pm$ 1.3
31	<b>5s</b>	97.8 $\pm$ 0.1	97.6 $\pm$ 0.1	97.9 $\pm$ 0.3	97.5 $\pm$ 0.0	55.0 $\pm$ 4.1	51.7 $\pm$ 5.3
32	<b>5t</b>	97.9 $\pm$ 0.1	96.6 $\pm$ 0.1	99.8 $\pm$ 0.4	97.6 $\pm$ 0.6	53.3 $\pm$ 0.5	48.0 $\pm$ 1.9
33	<b>5u</b>	96.0 $\pm$ 0.3	69.4 $\pm$ 6.5	99.6 $\pm$ 0.2	95.4 $\pm$ 0.0	42.7 $\pm$ 4.5	21.7 $\pm$ 0.7
34	<b>5v</b>	97.5 $\pm$ 0.1	94.1 $\pm$ 0.5	98.9 $\pm$ 0.0	98.7 $\pm$ 0.1	44.2 $\pm$ 5.0	40.8 $\pm$ 1.6
35	<b>5w</b>	97.5 $\pm$ 0.1	94.1 $\pm$ 0.5	98.9 $\pm$ 0.0	98.7 $\pm$ 0.1	44.2 $\pm$ 5.0	40.8 $\pm$ 1.6
	<b>Bismertiazol</b>	73.9 $\pm$ 1.1	29.3 $\pm$ 1.7	78.8 $\pm$ 6.6	46.7 $\pm$ 2.3	39.0 $\pm$ 3.5	15.6 $\pm$ 4.1

<sup>a</sup> The average of three trials.

Table 5 EC<sub>50</sub> values of compounds **5o–5w** against *Xoo*, *Xac*, and *Psa*<sup>a</sup>

Compound	Regression equation	Correlation coefficient ( <i>r</i> )	EC <sub>50</sub> (μg mL <sup>-1</sup> )
<i>Xanthomonas oryzae</i> ( <i>Xoo</i> )			
Bismethiazol	$y = 0.5792x + 2.3536$	0.9595	82.3 ± 5.1
<b>5o</b>	$y = 3.2815x + 0.658$	0.9856	15.0 ± 0.8
<b>5p</b>	$y = 3.6552x + 7.277$	0.9683	11.7 ± 0.4
<b>5q</b>	$y = 3.7164x + 3.068$	0.9883	12.6 ± 0.5
<b>5r</b>	$y = 3.5643x + 2.158$	0.9835	13.4 ± 0.4
<b>5s</b>	$y = 6.55167x + 0.248$	0.9530	7.6 ± 0.3
<b>5t</b>	$y = 5.3958x + 0.8359$	0.9838	9.1 ± 0.3
<b>5u</b>	$y = 3.1172x + 0.4496$	0.9667	15.9 ± 0.8
<b>5v</b>	$y = 1.6916x + 0.0594$	0.9870	29.5 ± 1.4
<b>5w</b>	$y = 6.0038x + 2.4873$	0.9861	7.9 ± 0.4
<i>Xanthomonas axonopodis</i> ( <i>Xac</i> )			
Bismethiazol	$y = 0.8141x + 0.3426$	0.9599	61.0 ± 4.4
<b>5o</b>	$y = 19.477x + 1.2731$	0.9394	2.5 ± 0.1
<b>5p</b>	$y = 15.73x + 6.1445$	0.9082	2.8 ± 0.2
<b>5q</b>	$y = 9.3762x + 5.5612$	0.9371	4.7 ± 0.2
<b>5r</b>	$y = 6.8912x + 5.5944$	0.9804	6.4 ± 0.3
<b>5s</b>	$y = 10.836x + 18.611$	0.9176	2.9 ± 0.2
<b>5t</b>	$y = 13.73x + 10.154$	0.9309	2.9 ± 0.2
<b>5u</b>	$y = 7.3972x + 5.8451$	0.9466	6.0 ± 0.3
<b>5v</b>	$y = 6.922x + 5.6802$	0.9762	6.4 ± 0.4
<b>5w</b>	$y = 12.884x + 14.465$	0.9782	2.8 ± 0.2
<i>Pseudomonas syringae</i> ( <i>Psa</i> )			
Bismethiazol			>100
<b>5o</b>	$y = 0.6055x + 23.34$	0.9104	44.0 ± 3.8
<b>5p</b>	$y = 1.1501x + 19.741$	0.9581	26.3 ± 2.2
<b>5q</b>	$y = 0.9974x + 13.765$	0.9199	36.3 ± 2.7
<b>5r</b>	$y = 0.6203x + 14.995$	0.9779	56.4 ± 5.1
<b>5s</b>	$y = 0.699x + 6.2785$	0.9784	62.6 ± 5.3
<b>5t</b>	$y = 0.9171x + 6.8663$	0.9650	47.0 ± 3.7
<b>5u</b>	$y = 0.6372x + 14.585$	0.9821	55.6 ± 4.6
<b>5v</b>			>100
<b>5w</b>			>100

<sup>a</sup> 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.<sup>22</sup> To investigate the antibacterial activities of nitroisoxazoles, 4-nitro-3-phenylisoxazole derivatives were prepared. Dimethyl-(2-nitrovinyl)-amine (**4c**) reacted with corresponding phenyl-oximes (**1**) to give the products **5o–5w**. The yield was moderate from 36% to 63%.

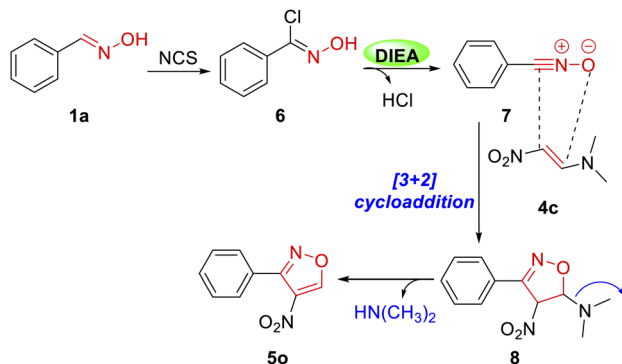
In addition, the NMR spectra of compound **5o** was consistent with the data in the literature.<sup>23</sup> However, the test result of HRMS was inconsistent with the actual values. This phenomenon was observed in all the 4-nitro-3-phenylisoxazole derivatives. To ensure the structures of this series 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.

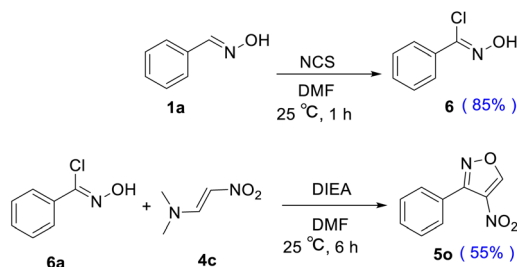
To investigate the mechanism, the benzaldehyde oxime **1a** and compound **4c** were subjected to undergo [3 + 2] cycloaddition. Based on the observed results and the reports in the literature,<sup>24</sup> the regioselectivity of the [3 + 2] cycloaddition reaction between nitrile *N*-oxides and conjugated 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,<sup>25</sup> 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





Scheme 1 Proposed mechanism.



Scheme 2 Complementary experiments.

presence of NCS. The NMR spectra of **6** was consistent with the literature.<sup>26</sup> 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  $\mu\text{g mL}^{-1}$  and 50  $\mu\text{g mL}^{-1}$  according to the previous report.<sup>27</sup> The results are listed in Table 4. The activities of compounds **3a–3l** and **5a–5n** were lower than those of bismethiazol and fluopyram.<sup>28</sup> 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  $\mu\text{g mL}^{-1}$  and 50  $\mu\text{g mL}^{-1}$ . Therefore, compounds **5o–5w** were selected for further studies as novel antibacterial agents.

As shown in Table 5, compounds **5o–5w** exhibited much better  $\text{EC}_{50}$  values against *Xoo*, *Xac*, and *Psa* than bismethiazol and fluopyram.<sup>28</sup> 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  $\mu\text{g mL}^{-1}$ ) and **5w** (–NO<sub>2</sub>, 7.9  $\mu\text{g mL}^{-1}$ ) have lower  $\text{EC}_{50}$  than small-steric substituted compound **5q** (–F, 12.6  $\mu\text{g mL}^{-1}$ ). A similar phenomenon was observed against *Xac*. However, against *Psa*, small-steric substituted compound **5q** (12.6  $\mu\text{g mL}^{-1}$ )

showed a better effect than large-steric substituted compound **5t** (47.0  $\mu\text{g mL}^{-1}$ ).

## 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  $\mu\text{g mL}^{-1}$  and 50  $\mu\text{g mL}^{-1}$  against *Xoo*, *Xac*, and *Psa*. The results suggested that 4-nitro-3-phenylisoxazole derivatives performed excellent antibacterial activities. Further studies on the  $\text{EC}_{50}$  values have shown that these compounds were much better than bismethiazol. 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.

## Acknowledgements

This work was financially supported by Guizhou University Found for Newly Enrolled Talent ([2019]15), Guizhou University Found for Cultivation ([2019]65), and the Guizhou Provincial Science and Technology Foundation ([2020]1Y09).

## Notes and references

- 1 J. L. Dangel, 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.





- 5 (a) D. Aboushady, S. S. Rasheed, J. Herrmann, A. Maher, E. M. El-Hossary, E. S. Ibrahim, A. H. Abadi, M. Engel, R. Müller, M. Abdel-Halim and M. M. Hamed, *Bioorg. Chem.*, 2021, **117**, 105422; (b) J. Wang, P. L. Zhang, M. F. Ansari, S. Li and C. H. Zhou, *Bioorg. Chem.*, 2021, **113**, 105039.
- 6 A. Harsanyi, A. Lückener, H. Pasztor, Z. Yilmaz, L. Tam, D. S. Yufit and G. Sandford, *Eur. J. Org. Chem.*, 2020, 3872–3878.
- 7 C. Shen, W. Lu, Y. Huang, J. Wu and H. Zhang, *Chem. Eng. J.*, 2015, **260**, 411–418.
- 8 J. V. Mercader, A. Abad-Somovilla, C. Agulló and A. Abad-Fuentes, *J. Agric. Food Chem.*, 2017, **65**, 9333–9341.
- 9 (a) A. Sysak and B. Obmińska-Mrukowicz, *Eur. J. Med. Chem.*, 2017, **137**, 292–309; (b) Y. Y. Zhang, S. Gao, Y. X. Liu, C. Wang, W. Jiang, L. X. Zhao, Y. Fu and F. Ye, *J. Agric. Food Chem.*, 2020, **68**, 3403–3414; (c) H. Z. Zhang, Z. L. Zhao and C. H. Zhou, *Eur. J. Med. Chem.*, 2018, **144**, 444–492.
- 10 X. Ma, J. Chen and X.-H. Du, *Org. Process Res. Dev.*, 2019, **23**, 1152–1158.
- 11 S. C. Cheng, R. H. Lee, J. Y. Jeng, C. W. Lee and J. Shiea, *Anal. Chim. Acta*, 2020, **1102**, 63–71.
- 12 F. Li, Z. Ye, Z. Huang, X. Chen, W. Sun, W. Gao, S. Zhang, F. Cao, J. Wang, Z. Hu and Y. Zhang, *Bioorg. Chem.*, 2021, **117**, 105452.
- 13 R. López-Ruiz, R. Romero-González, S. Martín-Torres, A. M. Jimenez-Carvelo and L. Cuadros-Rodríguez, *J. Chromatogr. A*, 2022, **1664**, 462791.
- 14 (a) O. B. Bondarenko and N. V. Zyk, *Chem. Heterocycl. Compd.*, 2020, **56**, 694–707; (b) A. Sysak and B. Obmińska-Mrukowicz, *Eur. J. Med. Chem.*, 2017, **137**, 292–309; (c) G. N. Pairas, F. Perperopoulou, P. G. Tsoungas and G. Varvounis, *ChemMedChem*, 2017, **12**, 408–419; (d) J. Zhu, J. Mo, H.-z. Lin, Y. Chen and H.-p. Sun, *Bioorg. Med. Chem.*, 2018, **26**, 3065–3075.
- 15 (a) C. Rajasekhar, S. Durgamma and A. Padmaja, *J. Heterocycl. Chem.*, 2014, **51**, 1727–1734; (b) T. V. Hansen, P. Wu and V. V. Fokin, *J. Org. Chem.*, 2005, **70**, 7761–7764; (c) J. E. Grob, J. Nunez, M. A. Dechantsreiter and L. G. Hamann, *J. Org. Chem.*, 2011, **76**, 10241–10248; (d) S. Kankala, R. Vadde and C. S. Vasam, *Org. Biomol. Chem.*, 2011, **9**, 7869–7876; (e) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599.
- 16 (a) S. Guo, J. Wang, X. Zhang, S. Cojean, P. M. Loiseau and X. Fan, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 2617–2620; (b) D. C. B. da Silva-Alves, J. V. dos Anjos, N. N. M. Cavalcante, G. K. N. Santos, D. M. d. A. F. Navarro and R. M. Srivastava, *Bioorg. Med. Chem.*, 2013, **21**, 940–947.
- 17 (a) Y. Zhang, Q. Wang, L. Li, Y. Le, L. Liu, J. Yang, Y. Li, G. Bao and L. Yan, *J. Enzyme Inhib. Med. Chem.*, 2021, **36**, 1205–1216; (b) L. Yan, Q. Wang, L. Liu and Y. Le, *J. Enzyme Inhib. Med. Chem.*, 2022, **37**, 832–843.
- 18 Y. Le, Y. Zhang, Q. Wang, N. Rao, D. Li, L. Liu, G. Ouyang and L. Yan, *Tetrahedron Lett.*, 2021, **68**, 152903.
- 19 S. Mohammed, R. A. Vishwakarma and S. B. Bharate, *RSC Adv.*, 2015, **5**, 3470–3473.
- 20 A. Yoshimura, M. E. Jarvi, M. T. Shea, C. L. Makitalo, G. T. Rohde, M. S. Yusubov, A. Saito and V. V. Zhdankin, *Eur. J. Org. Chem.*, 2019, 6682–6689.
- 21 (a) M. Xu, P. Wu, F. Shen, J. Ji and K. P. Rakesh, *Bioorg. Chem.*, 2019, **91**, 103133; (b) M. Nazir, M. Saleem, M. I. Tousif, M. A. Anwar, F. Surup, I. Ali, D. Wang, N. Z. Mamadalieva, E. Alshammari, M. L. Ashour, A. M. Ashour, I. Ahmed, Elizbit, I. R. Green and H. Hussain, *Biomolecules*, 2021, **11**, 957; (c) T. Wood, K. Bertheussen and N. Martin, *Org. Biomol. Chem.*, 2019, **18**, 514–517.
- 22 (a) S. J. Liu, Q. Zhao, C. Peng, Q. Mao, F. Wu, F. H. Zhang, Q. S. Feng, G. He and B. Han, *Eur. J. Med. Chem.*, 2021, **217**, 113359; (b) N. Muthineni, N. S. Kumar, L. C. Rao, V. D. Kumar, S. Misra, L. R. Chowhan and H. M. Meshram, *ChemistrySelect*, 2016, **1**, 4197–4202; (c) V. Dočekal, S. Petrželová, I. Císařová and J. Veselý, *Adv. Synth. Catal.*, 2020, **362**, 2597–2603; (d) X. M. Hu, H. Dong, Y. D. Li, P. Huang, Z. Tian and P.-A. Wang, *RSC Adv.*, 2019, **9**, 27883–27887.
- 23 H. Hopf, A. F. Mourad and P. G. Jones, *Beilstein J. Org. Chem.*, 2010, **6**, 68–71.
- 24 (a) K. Zawadzińska, M. Ríos-Gutiérrez, K. Kula, P. Woliński, B. Mirosław, T. Krawczyk and R. Jasiński, *Molecules*, 2021, **26**, 6774–6792; (b) R. Jasiński, E. Jasińska and E. Dresler, *J. Mol. Model.*, 2016, **23**, 13–21; (c) K. Kula and K. Zawadzińska, *Curr. Chem. Lett.*, 2021, **10**, 6–19; (d) P. Wolinski, A. Kacka-Zych, O. M. Demchuk, A. Lapczuk-Krygier, B. Mirosław and R. Jasinski, *J. Clean. Prod.*, 2020, **275**, 122086–122099.
- 25 (a) G. Mloston, K. Urbaniak, A. Linden and H. Heimgartner, *Helv. Chim. Acta*, 2015, **98**, 453–461; (b) A. Fryzlewicz, A. Kacka-Zych, O. M. Demchuk, B. Mirosław, P. Wolinski and R. Jasinski, *J. Clean. Prod.*, 2021, **292**, 126079–126094; (c) R. Jasinski, *Comput. Theor. Chem.*, 2018, **1125**, 77–85; (d) R. Jasinski, *RSC Adv.*, 2015, **5**, 101045–101048; (e) R. Jasinski, *Tetrahedron Lett.*, 2015, **56**, 532–535; (f) R. Jasinski, *Monatsh. Chem.*, 2015, **146**, 591–599.
- 26 T. Chau, H. Dhondt, M. Flipo, B. Déprez and N. Willand, *Tetrahedron Lett.*, 2015, **56**, 4119–4123.
- 27 J. Shi, M. Ding, N. Luo, S. Wan, P. Li, J. Li and X. Bao, *J. Agric. Food Chem.*, 2020, **68**, 9613–9623.
- 28 J. Chen, C. Yi, S. Wang, S. Wu, S. Li, D. Hu and B. Song, *Bioorg. Med. Chem. Lett.*, 2019, **29**, 1203–1210.

