



Cite this: *RSC Adv.*, 2019, 9, 36204

### 3(2H)-pyridazinone derivatives: a new scaffold for novel plant activators†

Qinjie Shi,<sup>a</sup> Yanxia Shi,<sup>b</sup> Kang Chang,<sup>a</sup> Jianqin Chen,<sup>a</sup> Zhenjiang Zhao,<sup>c</sup> Weiping Zhu,<sup>a</sup> Yufang Xu,<sup>ID</sup>\*<sup>a</sup> BaoJu Li\*<sup>b</sup> and Xuhong Qian\*<sup>a</sup>

Due to the emergence of drug resistance, pesticide residue and environmental contamination, it is important to develop novel eco-friendly strategies to protect plants. Among them, plant activators have been gaining more and more attention. Herein, based on SHAFTS method, a new scaffold for novel plant activators was predicted and the discovery and structure–activity relationships of a series of 3(2H)-pyridazinone derivatives as novel plant activators were elucidated in detail. The vast majority of compounds exhibited excellent broad-spectrum induced resistance activity against tested diseases *in vivo* but no direct antimicrobial activity *in vitro*. Among them, compound **32** showed excellent efficacy against four pathogens and great potential as new plant activators in crop protection.

Received 30th August 2019  
 Accepted 24th October 2019

DOI: 10.1039/c9ra06892a

rsc.li/rsc-advances

Threatened by a large quantity of pathogens, such as fungi, bacteria, and viruses, the production of agricultural crops is still facing a big challenge.<sup>1,2</sup> Although the use of many traditional pesticides could directly control the diseases and improve the productivity, it simultaneously created serious problems, including pesticide residue, environmental contamination, and drug resistance.<sup>3</sup>

In order to deal with these issues, plant activator, a novel kind of agrochemical, has been gaining more and more attention.<sup>4</sup> It can protect crops from diseases by inducing plant immune responses without direct toxicity to pathogens and shows high efficacy, low toxicity, and no drug resistance.<sup>5</sup>

Several plant activators, such as PBZ,<sup>6</sup> BTH,<sup>7,8</sup> TDL,<sup>9</sup> and ISOTIANIL<sup>10</sup> that molecularly mimic salicylic acid (SA), could induce the plant's systemic acquired resistance<sup>4</sup> and have been commercially applied in crop protection.<sup>11–16</sup> Owing to the great advantages of plant activators, it is necessary to develop more efficient and eco-friendly plant activators to protect plants. In our previous work, a novel approach called SHaPe-FeaTure Similarity (SHAFTS),<sup>17,18</sup> which is a hybrid 3D similarity calculation algorithm considering both the molecular shape and pharmacophore features, was used for virtual screening to find novel scaffolds of plant activators.<sup>19,20</sup>

Pyridazinone scaffolds are a series of potent bioactive molecules applied in medicine and agriculture. Among them, 3(2H)-pyridazinone derivatives are extensively developed in crop protection, such as insecticide/acaricide,<sup>21</sup> herbicide,<sup>22</sup> plant growth regulator,<sup>23</sup> and fungicides,<sup>24</sup> while there is no application as plant activators.

In this communication, based on the structures of PBZ, BTH and plant hormones SA and jasmonic acid (JA), several 3(2H)-pyridazinone compounds with high similarity scores were discovered as promising plant activators by SHAFTS method against the MayBridge database. In order to verify the lead compound, we bought them from the commercial library and

<sup>a</sup>State Key Laboratory of Bioreactor Engineering, Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China. E-mail: yfxu@ecust.edu.cn

<sup>b</sup>Institute of Vegetables and Flowers, Chinese Academy of Agricultural Science, Beijing 100081, China. E-mail: libj@mail.caas.net.cn

<sup>c</sup>State Key Laboratory of Bioreactor Engineering, Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9ra06892a

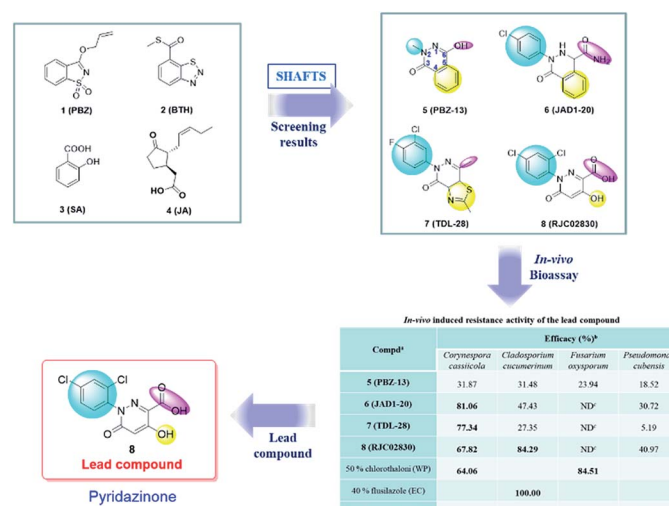


Fig. 1 The discovery of the lead compound **8**.



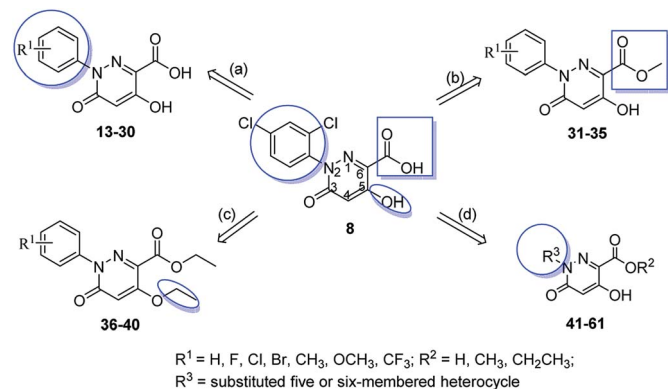


Fig. 2 The optimization strategy of the lead compound 8.

tested the *in vivo* induced resistance activity against four pathogens (Fig. 1, Table S1†). Results displayed that compound 8 had induced resistance against two pathogens. Therefore, compound 8 was chosen as the lead compound for the derivation of the new plant activator (Fig. 1). By comparing the activities of the four compounds, the preliminary structure–activity relationship was obtained as follows. The large steric hindrance in the 2-position and the electron-withdrawing property in the 6-position of compound 5 may be beneficial to the bioactivity (5 vs. 6). The aromatic ring in the position [4,5-*d*] maybe optional and the electron-donating group in the 6-position of compound 7 may reduce the bioactivity (6 vs. 7); hydrogen bond donor –OH and acceptor –COOH in the 5 and 6 positions of compound 8 may be necessary for broad-spectrum antimicrobial activity (7 vs. 8). Therefore, the optimization of

the lead compound was carried out through four aspects. (a) Introducing the substituents to the benzene ring; (b) changing the acid to ester; (c) modifying the hydroxyl group; (d) replacement of benzene ring by a five- or six-membered heterocyclic ring (Fig. 2). Hence, a series of 5-hydroxy-2-aryl-3-oxo-2,3-dihydropyridazine-6-carboxylic acid and its derivatives with broad-spectrum induced antimicrobial activity were designed and synthesized, and their structure–activity relationship was studied.

Initially, we introduced different substituents into *ortho*-, *meta*-, and *para*-positions of the phenyl ring to investigate the electronic and steric effect on the activity. Compounds 13–30 were synthesized, and their *in vivo* induced resistance activities are shown in Tables 1 and 2. According to the results, most of the compounds showed a strong induced resistance activity against two or more test pathogens. Comparing the compounds 13–18 (Table 1) and 19–24 (Table 2), when a mono-substituent was introduced to the *para*-position of the benzene ring, all compounds exhibited improved activities compared to the none substituted compound 13, which simultaneously showed resistance against two or three pathogens (compounds 14–18). The general trend in induced activity was as follows: 4- $\text{CF}_3 \geq 4\text{-F} \geq 4\text{-Cl} \geq 4\text{-OCF}_3 \geq 4\text{-OCH}_3$ .

The results indicated that the activity would be better with the introduction of a fluorine-containing group in the *para*-position of the benzene ring due to the electron absorption effect. However, when these groups were introduced into the *ortho*- or *meta*-position (compounds 19–24), the activity almost disappeared except for compound 24. We believe that there are two reasons for this. One is that the dihedral angle between the benzene ring and the pyridazinone ring became larger when the

Table 1 Structure and *in vivo* induced resistance activity of compounds 13–18, 25–30<sup>a</sup>

Compound	R <sup>1</sup>	Efficacy (%)							
		MM	CC	RS	BC	FO	PI	PT	PL
13	H	<b>84.44</b>	<b>82.62</b>	–2.86	–8.40	5.94	33.17	ND	26.95
14	4-F	27.78	<b>75.41</b>	<b>50.05</b>	–1.41	–7.5	7.09	ND	<b>52.77</b>
15	4-Cl	<b>58.33</b>	<b>50.82</b>	–4.04	–9.06	11.31	32.46	ND	<b>69.58</b>
16	4-CF <sub>3</sub>	17.5	<b>82.3</b>	6.01	11.78	–2.13	<b>54.62</b>	ND	<b>64.98</b>
17	4-OCH <sub>3</sub>	<b>58.61</b>	44.26	46.35	24.19	2.58	2.08	ND	<b>51.89</b>
18	4-OCF <sub>3</sub>	ND	ND	<b>59.05</b>	ND	<b>76.92</b>	16.67	<b>66.15</b>	ND
25	3,5-Di-Cl	<b>91.67</b>	<b>72.31</b>	–2.86	–10.4	–1.46	23.53	ND	<b>60.38</b>
26	2,4-Di-Cl	39.17	<b>81.31</b>	12.51	–0.91	11.31	26.38	ND	48.7
27	3,4-Di-Cl	43.33	<b>65.66</b>	3.74	–15.1	1.9	19.95	ND	38.44
28	3,4-Di-F	ND	ND	<b>53.12</b>	ND	<b>82.05</b>	–9.72	<b>100</b>	ND
29	4-F-3-CF <sub>3</sub>	ND	ND	<b>53.12</b>	ND	10.26	2.78	<b>100</b>	ND
30	3,5-Di-OCH <sub>3</sub>	ND	ND	<b>59.05</b>	ND	<b>92.31</b>	–5.56	44.62	ND
BTH		<b>88.06</b>	–8.2	16.06	46.8	19.37	<b>64.98</b>	ND	<b>75.59</b>
50% kresoximmethyl (WG)		<b>98.43</b>							
75% chlorothalonil (WP)			<b>90.16</b>						
5% validamycin A (WP)				<b>83.38</b>					
50% procymidone (WP)					41.87				
70% mildothane (WP)						<b>75.14</b>			
50% dimethomorph (WP)							<b>96.78</b>		
20% bismethlazole (WP)								<b>68.55</b>	<b>67.8</b>

<sup>a</sup> ND-not detected; inducing activity > 50% are shown in bold.



Table 2 Structure and *in vivo* induced resistance activity of compounds 19–24<sup>a</sup>

Compound	R <sup>1</sup>	Efficacy (%)			
		RS	FO	PI	PT
19	2-F	40.36	<b>71.79</b>	13.89	4.62
20	2-CF <sub>3</sub>	<b>54.9</b>	23.08	25	44.62
21	2-OCH <sub>3</sub>	32.34	38.46	22.22	ND
22	3-F	30.17	35.9	6.94	4.62
23	3-CF <sub>3</sub>	<b>61.42</b>	−23.08	11.11	38.46
24	3-OCH <sub>3</sub>	<b>73</b>	<b>58.97</b>	0	<b>78.46</b>
5% validamycin A (WP)		<b>83.38</b>			
70% mildothane (WP)			<b>75.14</b>		
50% dimethomorph (WP)				<b>96.78</b>	
20% bismethlazol (WP)					<b>68.55</b>

<sup>a</sup> ND-not detected; inducing activity > 50% are shown in bold.

groups were introduced from the *para*-position to the *ortho*-position, leading to a reduction in the activity. The other is that the larger steric hindrance at the *para*-position was better than the *meta*-position for the activity. On the other hand, when two-substituents were introduced to the benzene ring, the induced activities of compounds against test pathogens were in the following order: 3,4-di-F ≥ 3,5-di-Cl ≥ 4-F-3-CF<sub>3</sub> ≥ 3,5-di-OCH<sub>3</sub> » 2,4-di-Cl, 3,4-di-Cl. It implied that the fluorine-containing group introduced to the 4-position or the 3,5-di-substituent of the benzene ring was beneficial to broad-spectrum disease resistance. To sum up, the stronger the electron absorption effect of the phenyl group, the higher the activity.

Next, we explored the importance of the withdrawing effect of the carboxylic group through esterification and compounds 31–35 were derived. The results showed that these compounds had an improved broad-spectrum activity (Table 3). Surprisingly, compound 32 showed excellent activity against four pathogens. Hence, the withdrawing effect of the carboxylic

group is not necessary in the existence of the hydrogen bond acceptor for broad-spectrum disease resistance.

Then, we investigated the necessity of the hydrogen bond donor –OH. The results are shown in Table S3;† when the hydroxyl group was replaced by ether, the activity was lost. It implied that the hydrogen bond donor hydroxyl group is very important for the induced activity.

Based on the above research and the structures of the commercial plant activators, a strategy of bioisosteric replacement of benzene ring with a heterocyclic ring was carried out for the discussion of the effect of aromaticity and polarity of 2-substituent of the pyridazinone ring on activity. The results are shown in Table S4;† when the benzene ring was replaced by a five- or six-membered heterocyclic ring with a nitrogen atom, the induced resistance activity was weakened. When the benzene ring was replaced by a pyridin-4-yl group, it was helpful to maintain the activity by introducing one electron-withdrawing group to the pyridine ring than an electron-donating group, and the higher the polarity of the substituents, the better the activity (compounds 41–45). Whereas, the ester compounds exhibited a mild decrease in the activity than their acid products (compounds 47–51). A similar conclusion could be obtained when introducing other five-membered heterocycles with a nitrogen atom into the pyridazine ring (compounds 53–61).

For plant activators, one of the remarkable characteristics is that they are effective *in vivo* but not *in vitro*. Therefore, some representative compounds with good *in vivo* activity were chosen to test the direct antimicrobial activity. As we can see (Table 4), all compounds had no direct antimicrobial activity *in vitro* compared to the positive controls. The results demonstrated their potency to be used as novel plant activators. Therefore, 3(2*H*)-pyridazinone derivatives have great potential as new plant activators in crop protection.

In summary, based on the SHAFTS method, a new scaffold for novel plant activators was predicted and a series of 3(2*H*)-pyridazinone derivatives as novel plant activators were designed

Table 3 Structure and *in vivo* induced resistance activity of compounds 31–35<sup>a</sup>

Compound	R <sup>1</sup>	Efficacy (%)							
		MM	CC	RS	BC	FO	PI	PT	PL
31	4-F	<b>71.53</b>	<b>62.55</b>	32.02	20.2	−2.8	9.23	ND	<b>50.83</b>
32	4-OCH <sub>3</sub>	<b>55.83</b>	<b>67.9</b>	<b>64.53</b>	29.18	−5.49	22.1	ND	<b>54.36</b>
33	2-OCH <sub>3</sub>	ND	ND	<b>68.55</b>	ND	<b>87.18</b>	5.56	<b>63.08</b>	ND
34	3-F	ND	ND	24.04	ND	17.95	29.86	−66.2	ND
35	3,4-Di-Cl	10	<b>74.43</b>	39.7	−11.9	−0.11	14.95	ND	<b>71.61</b>
BTH		<b>88.06</b>	−8.2	16.06	46.8	19.37	<b>64.98</b>	ND	<b>75.59</b>
50% kresoximmethyl (WG)		<b>98.43</b>							
75% chlorothalonil (WP)			<b>90.16</b>						
5% validamycin A (WP)				<b>83.38</b>					
50% procymidone (WP)					41.87				
70% mildothane (WP)						<b>75.14</b>			
50% dimethomorph (WP)							<b>96.78</b>		
20% bismethlazol (WP)								<b>68.55</b>	<b>67.8</b>

<sup>a</sup> ND-not detected; inducing activity > 50% are shown in bold.



Table 4 *In vitro* anti-microbial activity of the representative compounds

Compound	Efficacy (%)			
	MM	CC	RS	PI
14	0.00	9.80	4.75	5.96
15	15.12	13.14	0.59	1.99
16	4.26	11.58	4.15	7.62
17	5.62	10.24	0.89	6.29
25	10.47	9.35	0.89	3.64
31	1.36	18.26	0.00	2.98
32	9.69	11.58	8.90	0.33
35	4.56	20.04	-1.48	7.28
CK	10	12	19	11
50% kresoximmethyl (WG)	71			
75% chlorothalonil (WP)		21		
5% validamycin A (WP)			50	
50% dimethomorph (WP)				80

and synthesized. Their induced resistance activities were evaluated through *in vivo* and *in vitro* assays using a broad spectrum of pathogens, including fungi, bacteria, and oomycete. The results showed that most 2-phenyl or heterocyclic rings with electron-withdrawing group substituted 3(2*H*)-pyridazinone derivatives are potent to be plant activators, which exhibited high efficacy *in vivo* inducing activity but no direct antibiotic activity *in vitro*. The hydrogen bond donor -OH in the 3-position and hydrogen bond acceptor groups in the 6-position of 3(2*H*)-pyridazinone may be vital for broad-spectrum disease resistance. Among them, compound 32 showed good broad-spectrum efficacy against four pathogens. Altogether, 3(2*H*)-pyridazinone derivatives have great potential as new plant activators in crop protection.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

This work was financially supported by the National Research and Development Program of China (2018YFD0200100), and the Fundamental Research Funds for the Central Universities.

## Notes and references

- M. Narusaka, T. Minami, C. Iwabuchi, T. Hamasaki, S. Takasaki, K. Kawamura and Y. Narusaka, *PLoS One*, 2015, **10**, e0115864.
- K. Jiang, T. Kurimoto, E. K. Seo, S. Miyazaki, M. Nakajima, H. Nakamura and T. Asami, *J. Agric. Food Chem.*, 2015, **63**, 7124–7133.
- A. L. S. Rangel de Souza, S. A. De Souza, M. V. V. De Oliveira, T. M. Ferraz, F. A. M. M. A. Figueiredo, N. D. Da Silva, P. L. Rangel, C. R. S. Panisset, F. L. Olivares, E. Campostrini and G. A. De Souza Filho, *Plant Soil*, 2015, **399**, 257–270.
- Y. Bektas and T. Eulgem, *Front. Plant Sci.*, 2014, **5**, 804.
- H. J. Lee, Y. J. Park, P. J. Seo, J. H. Kim, H. J. Sim, S. G. Kim and C. M. Park, *Plant Cell*, 2015, **27**, 3425–3438.
- M. Uchiyama, H. Abe, R. Sato, M. Shimura and T. Watanabe, *Agric. Biol. Chem.*, 1973, **37**, 737–745.
- L. Friedrich, K. Lawton, W. Ruess, P. Masner, N. Specker, M. G. Rella, B. Meier, S. Dincher, T. Staub, S. Uknes, J.-P. Métraux, H. Kessmann and J. Ryals, *Plant J.*, 1996, **10**, 61–70.
- W. Kunz, R. Schurter and T. Maetzke, *Pestic. Sci.*, 1997, **50**, 275–282.
- K. Tsubata, K. Kuroda, Y. Yamamoto and N. Yasokawa, *J. Pestic. Sci.*, 2006, **31**, 161–162.
- M. Ogawa, A. Kadowaki, T. Yamada, and O. Kadooka, R&D Report, 2011, **1**, 1–16.
- Y. Xu, Z. Zhao, X. Qian, Z. Qian, W. Tian and J. Zhong, *J. Agric. Food Chem.*, 2006, **54**, 8793–8798.
- Q. Du, W. Zhu, Z. Zhao, X. Qian and Y. Xu, *J. Agric. Food Chem.*, 2012, **60**, 346–353.
- M. Yasuda, H. Nakashita, S. Hasegawa, M. Nishioka, Y. Arai, M. Uramoto, I. Yamaguchi and S. Yoshida, *Biosci., Biotechnol., Biochem.*, 2003, **67**, 322–328.
- M. Nishioka, H. Nakashita, H. Suzuki, S. Akiyama, S. Yoshida and I. Yamaguchi, *J. Pestic. Sci.*, 2003, **28**, 416–421.
- P. Stanetty, M. Turner and M. D. Mihovilovic, *Journal*, 2005, **10**, 367–375.
- Z. Fan, Z. Shi, H. Zhang, X. Liu, L. Bao, L. Ma, X. Zuo, Q. Zheng and N. Mi, *J. Agric. Food Chem.*, 2009, **57**, 4279–4286.
- X. Liu, H. Jiang and H. Li, *J. Chem. Inf. Model.*, 2011, **51**, 2372–2385.
- W. Lu, X. Liu, X. Cao, M. Xue, K. Liu, Z. Zhao, X. Shen, H. Jiang, Y. Xu, J. Huang and H. Li, *J. Med. Chem.*, 2011, **54**, 3564–3574.
- K. Chang, Y. Shi, J. Chen, Z. He, Z. Xu, Z. Zhao, W. Zhu, H. Li, Y. Xu, B. Li and X. Qian, *MedChemComm*, 2016, **7**, 1849–1857.
- K. Chang, J. Q. Chen, Y. X. Shi, M. J. Sun, P. F. Li, Z. J. Zhao, W. P. Zhu, H. L. Li, Y. F. Xu, B. J. Li and X. H. Qian, *Chin. Chem. Lett.*, 2017, **28**, 919–926.
- K. Hirata, Y. Kawamura, M. Kudo and H. Igarashi, *J. Pestic. Sci.*, 1995, **20**, 213–221.
- B. Riggle and B. Dunbar, *J. Agric. Food Chem.*, 1990, **38**, 1922–1925.
- D. H. Schoene and O. L. Hoffmann, *Science*, 1949, **109**, 588–590.
- S. Tsuboi, S. Sasaki and Y. Hattori, *US Pat.*, 4731385, 1988.

