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Studies on the biological activity of gem-difluorinated 3,3′-spirocyclic indole derivatives

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ABSTRACT

The biological activities of a series of 3,3′-spirocyclic indole derivatives containing CF_2, phosphine oxide, indole, and cyano functional groups were evaluated, and these derivatives were found to exhibit anti-TMV, fungicidal, and insecticidal activities.

Here we evaluated the biological activities of a series of designed 3,3′-spirocyclic indole derivatives, which contain privileged moieties and functional groups, such as CF_2, phosphine oxide, indole, and cyano. The bioassay results show that the target compounds possess moderate to good anti-TMV activities, in which compound **5h** exhibits the highest antiviral activity (51.2, 49.0, 53.6 %, 500 mg/L) *in vivo*. This indicates compound **5h** could be a promising candidate for anti-TMV development. 3,3′-Spiroindoline derivatives bearing a cyano group have exhibited broad spectrum fungicidal activities against 14 kinds of phytopathogenic fungi and selective fungicidal activities against *Sclerotinia sclerotiorum*. Additionally, many target compounds have shown potent insecticidal activity against *Mythimna separata*. Some of them also show good activities against *Helicoverpa armigera*, and *Pyrausta nubilalis*, as well as *Culex pipiens pallens*.

The *gem*-difluoromethylene group (CF_2) has attracted significant attentions in medicinal [1] and agricultural chemistry [2] because of its specific steric and electronic properties [3-5]. It can function as a mimic for hydroxyl [6] and carbonyl [7] groups, as well as replace oxygen atoms in phosphates [8,9], sulfates [10,11] and aryl ethers [12], thereby enhancing lipophilicity, bioavailability and binding affinity. Nevertheless, the application of CF_2 group in agrochemicals is actually far less than that of fluoro and trifluoromethyl functional groups [2]. This is because of the limitations of synthetic method of CF_2-containing compounds [13]. In addition, for all existing difluoroalkylated agrochemicals, the CF_2 unit is always directly connected with aromatic rings or heteroatoms, such as oxygen and nitrogen, resulting in a lack of structural diversity [2].

The importance of nitrogen heterocycles in medicinal chemistry has been demonstrated by their presence in a considerable number of FDA-approved drugs [14]. Rigid, three-dimensional molecular scaffolds have also attracted great attentions in recent research of drug discovery [15]. Therefore, the 3,3′-spirocyclic indole derivatives, which fall into both of these categories, are particularly important. For example, 3,3′-spirooxindole and 3,3′-spiroindoline are both core skeleton widely existing in natural products [16]. They have shown useful biological activities and have been extensively used for drug discovery (Fig. 1a) [17,18]. However, compared with their tremendous applications in the field of pharmaceuticals, there are few reports for their pesticide activities [19-22]. Our group recently discovered that a series of 3,3′-spirooxindole and 3,3′-spiroindoline compounds exhibited potent antiviral, fungicidal and insecticidal activities (Fig. 1b) [19-21]. These studies mainly focused on the derivatization of the spiro ring at indole C-3 position. In contrast, the derivatization of the C-2 position of 3,3′-spirocyclic indole derivatives and the corresponding biological activities have not been extensively explored. As far as we know, only our group reported the fungicidal activities of a limited number of such compounds [23,24].

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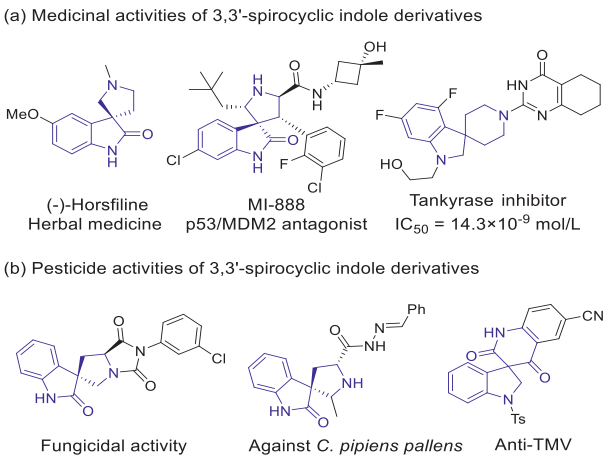


Fig. 1. Examples of bioactive molecules containing 3,3′-spirooxindole and 3,3′-spiroindoline skeleton.

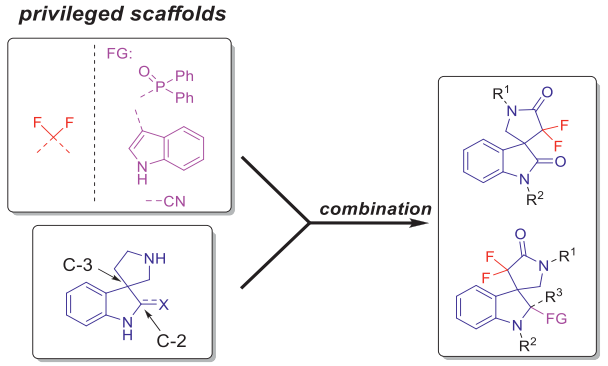


Fig. 2. Design of target compounds.

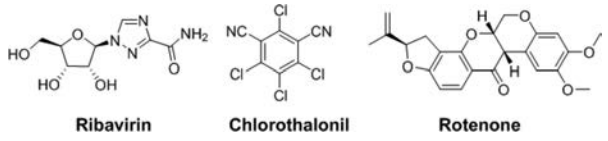


Fig. 3. Control drugs of biological activities tests.

Considering of the importance of CF_2 group and 3,3′-spirocyclic indole skeleton, as well as their insufficient applications in the field of pesticides, we here decided to combine these two moieties and designed a series of multi-functionalized 3,3′-spirocyclic indole derivatives. In order to increase the diversity of the existence of CF_2 group in pesticide active molecules, we designed to install it into the spiro ring at indole C-3 position to obtain the *gem*-difluorinated lactam moiety (Fig. 2). In addition, to further increase the molecular diversity and complexity, we designed to introduce other functional groups at the indole C-2 position (Fig. 2), such as phosphine oxide, indole, and cyano groups. This kind of functional groups are also widely used in bioactive molecules [14,25,26]. Then, we evaluated the anti-tobacco mosaic virus (TMV), fungicidal and insecticidal activities of these designed compounds.

We first synthesized four types of 3,3′-spirocyclic indole derivatives *via* visible light mediated radical cascade reactions by using indole-derived bromodifluoroacetamide as starting material (Scheme 1) [23,24,27]. The biological activities of these synthesized compounds were tested using previously reported method [19-21], Ribavirin, chlorothalonil, and rotenone were used as controls (Fig. 3). The operating steps and physical data in detail of intermediates, target compounds and the detail biological assays methods were given in Supporting information.

Table 1
In vivo anti-TMV activity of selected synthesized compounds.

Compd.	Inhibition rate (%)/500 mg/L		
	Inactivation	Curative	Protection
2a	36.3	-	-
2b	48.1	46.4	42.0
2c	36.3	-	-
2d	21.0	-	-
2e	34.0	-	-
2h	45.7	42.5	38.9
2i	39.0	-	-
3a	33.0	-	-
3b	41.6	30.2	38.1
3c	37.2	-	-
3d	34.6	-	-
3e	45.8	39.1	47.0
3f	43.2	46.1	40.0
4a	35.4	-	-
4b	42.3	40.7	44.5
4d	30.1	-	-
5a	46.0	50.6	43.1
5b	21.4	-	-
5d	27.1	-	-
5e	42.3	47.7	40.8
5f	49.5	45.5	41.5
5g	48.9	43.3	46.4
5h	51.2 (19.3[a])	49.0 (10.1[a])	53.6 (21.2[a])
5i	35.8	-	-
Ribavirin	38.7	37.1	39.0

[a] 100 mg/L

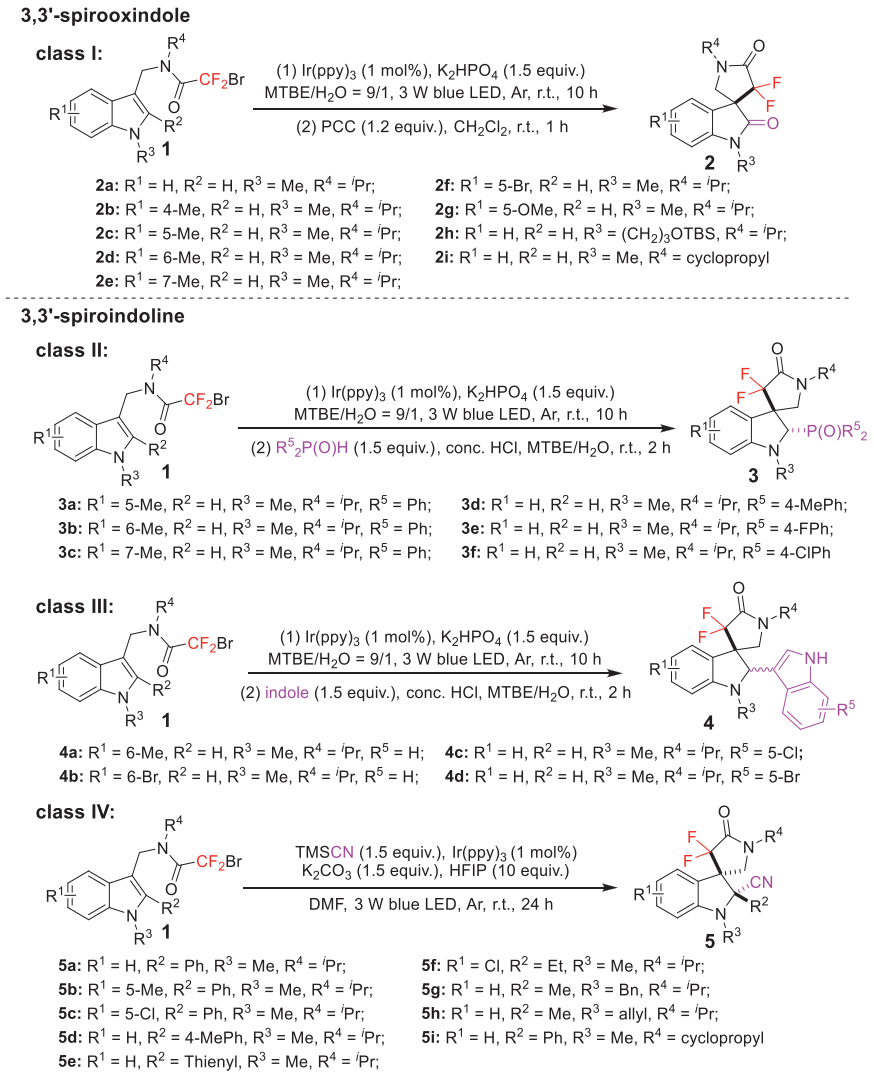
As shown in Table 1, the anti-TMV activities *in vivo*, including inactivation, curative and protection effects were evaluated for selected compounds. As we can see, all four types of synthesized compounds exhibit moderate to good anti-TMV activities, which suggests that the idea of introducing CF_2 group and functional groups such as phosphine oxide, indole and cyano into the 3,3′-spirocyclic indole skeleton is successful. Part of the compounds, such as compounds **2b**, **2h**, **3e**, **3f**, **4b**, **5a** and **5e**-**5h**, show significantly higher activities than that of commercialized anti-plan virus agent ribavirin.

For 3,3′-spirooxindole derivatives (class I), compound with a methyl substituent at C-4 position (**2b**) has significantly higher activity than compounds with the same substituent at other positions (**2c**-**2e**). Compound with a long carbon chain substituent on indole nitrogen (**2h**) has higher activity than compound with a methyl group (**2a**). The change of substituent on the amide group (**2i**) does not improve the activity.

For phosphine-oxide substituted 3,3′-spiroindoline derivatives (class II), the methyl substitution at indole C-6 position (**3b**) is beneficial for the anti-TMV activity. The study of electronic effects on the phosphine moiety shows that electron-withdrawing substituent (**3e** and **3f**) on the phenyl ring improves the anti-TMV activity.

For indole substituted 3,3′-spiroindoline derivatives (class III), the indole C-6 bromo-substituted compound **4b** give the best result.

For compounds containing a cyano group and two consecutive quaternary carbon centers (class IV), their structural modifications are most successful for anti-TMV activity. Compounds with alkyl substituents at indole C-2 position (**5f**-**5h**) all give higher anti-TMV activity than ribavirin. Especially for compound **5h**, it exhibits the best activity (51.2%, 49.0%, 53.6%, 500 mg/L) among all the selected compounds. This indicates compound **5h** could be a promising candidate for anti-TMV development. In addition, compound **5a** exhibits better anti-TMV activity than its substituted analogues **5b** and **5c**. Interestingly, compound **5e** with a thiophene moiety also shows good result.



Scheme 1. Synthesis of 3,3′-spirocyclic indole derivatives **2a**-**2i**, **3a**-**3f**, **4a**-**4d**, **5a**-**5i**.

Table 2
Fungicidal activity of selected compounds **5a**-**5c**.

Compd.	Fungicidal activity (%)/50 mg/L													
	A.S	F.G	P.I	P.C	S.S	B.C	R.S	F.C	C.H	P.P	R.C	B.M	W.A	F.M
5a	29.6	27.5	29.0	26.7	59.1	37.5	45.7	15.6	28.0	18.8	37.9	25.0	35.3	25.0
5b	37.0	33.3	29.0	33.3	52.3	34.4	51.4	18.8	28.0	18.8	54.5	22.2	29.4	25.0
5c	37.0	23.5	29.0	30.0	75.0	43.8	41.4	21.9	36.0	20.8	59.1	33.3	41.2	29.2
Chlorothalonil	44.4	49.0	64.5	93.3	97.7	90.6	100	81.3	40.0	77.1	71.2	61.1	79.4	79.2

A.S, *Alternaria solani*; F.G, *Fusarium graminearum*; P.I, *Phytophthora infestans*; P.C, *Phytophthora capsici*; S.S, *Sclerotinia sclerotiorum*; B.C, *Botrytis cinerea*; R.S, *Rhizoctonia solani*; F.C, *Fusarium oxysporium f. sp. cucumeris*; C.H, *Cercospora arachidicola Hori*; P.P, *Physalospora piricola*; R.C, *Rhizoctonia cerealis*; B.M, *Bipolaris maydis*; W.A, watermelon anthracnose; F.M, *Fusarium moniliforme*.

Next, we investigated the fungicidal activities of our synthesized compounds against 14 kinds of phytopathogenic fungi by using chlorothalonil as a standard. As we mentioned above, the fungicidal activities of certain compounds have been reported [23,24], here we only focused on the cyano-substituted 3,3′-spiroindoline derivatives (Table 2). We found all the tested compounds exhibited broad-spectrum fungicidal activities, and the growth inhibitory rates against *Sclerotinia sclerotiorum* exceeded 50% for all compounds. In addition, compounds **5b** and **5c** also show good inhibitory activity against *Rhizoctonia cerealis*.

We also investigated the insecticidal activities of our synthesized compounds against Lepidoptera pests, such as *Mythimna separata, Helicoverpa armigera*, and *Pyrausta nubilalis*, as well as *Culex pipiens pallens* (Table 3). In general, most of the selected compounds exhibit complete insecticidal activity against *M. separata* at concentration of 600 mg/L. Especially for compounds **2d**, **2a** and **5a**, they still show 100% inhibition rate at the concentration of 200 mg/L. In addition, the phosphine-oxide substituted compound **3a** has shown potent activities against all three Lepidoptera pests. For *Culex pipiens pallens*, the spirooxindole compounds **2g** and **2h** both exhibit 100% larvicidal activity at 10 mg/L (Table 3).

In summary, a series of 3,3′-spirocyclic indole derivatives containing CF_2, phosphine oxide, indole, and cyano moieties were designed and synthesized using our previously reported methods on

Table 3
Insecticidal activity of selected synthesized compounds.

Compound	Insecticidal activity (%) at concentration (mg/L)								
	M. separata		*H. armigera*		*P. nubilalis*		*C. pipiens pallens*		
	600	200	100	600	200	600	200	10	5
2d	100	100	40	80	-	70	-	35	-
2f	100	40	-	50	-	45	-	65	-
2g	40	-	-	30	-	10	-	100	-
2h	40	-	-	15	-	15	-	100	-
3a	100	100	30	100	75	100	65	10	-
3d	100	30	-	75	-	70	-	5	-
3e	100	65	-	80	-	75	-	20	-
4b	65	-	-	30	-	35	-	5	-
4c	55	-	-	35	-	25	-	85	-
5a	100	100	30	100	50	60	-	35	-
5b	100	20	-	30	-	30	-	60	-
5c	100	50	-	40	-	55	-	35	-
5d	100	40	-	20	-	50	-	65	-
5e	40	-	-	25	-	25	-	40	-
Rotenone	65	-	-	65	-	55	-	100	100

the basis of the widely used privileged scaffolds in both drug and pesticide design. The bioassays results showed that the target compounds possessed moderate to good activities against TMV, among which compound **5h** showed the highest antiviral activity. In the meantime, cyano-substituted 3,3′-spiroindoline derivatives have exhibited broad spectrum fungicidal activities against 14 kinds of phytopathogenic fungi and selective fungicidal activities against *Sclerotinia sclerotiorum*. In addition, many target compounds have shown potent insecticidal activity against *M. separata.* Some of them also showed good activities against *H. armigera*, and *P. nubilalis*, as well as mosquito. Further investigation on structural optimization and mode of action are in progress in our laboratory.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2021.08.005.

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