European Journal of Medicinal Chemistry 46 (2011) 364–369

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Antifungal agents. Part 4: Synthesis and antifungal activities of novel indole[1,2-*c*]-1,2,4-benzotriazine derivatives against phytopathogenic fungi *in vitro*

Hui Xu [a,b,*], Ling-ling Fan [a]

[a] Laboratory of Pharmaceutical Design and Synthesis, College of Sciences, Northwest A&F University, Xinong Road 22#, Yangling, Shaanxi 712100, China
[b] State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, China

ARTICLE INFO

Article history:
Received 21 July 2010
Received in revised form
19 October 2010
Accepted 20 October 2010
Available online 27 October 2010

Keywords:
Indole[1,2-*c*]-1,2,4-benzotriazine
Cyclization
Sandmeyer reaction
Antifungal activity
Phytopathogenic fungi

ABSTRACT

A series of novel indole[1,2-*c*]-1,2,4-benzotriazine derivatives were obtained by a modified Sandmeyer reaction in the presence of *tert*-butylnitrite (*t*-BuONO). As compared with hymexazol, a commercially available agricultural fungicide, at the concentration of 50 μg/mL, two indole[1,2-*c*]-1,2,4-benzotriazines, **5h** and **5k**, exhibited the more promising and pronounced antifungal activities *in vitro* against five phytopathogenic fungi. It clearly demonstrated that introduction of appropriate substituents on the indolyl ring of indole[1,2-*c*]-1,2,4-benzotriazine (**5a**) would lead to the more potent derivatives.

1. Introduction

Phytopathogenic fungi, which are hard to control, easily infect many crops, therefore development of new compounds that effectively inhibit those agricultural diseases is still highly desirable. The indole moiety (**I**, Fig. 1) represents an important structural component associated with a variety of alkaloids [1,2] and wide-ranging biological activities, such as antiviral activities [3–7], antitumor activities [8,9], antimicrobial activities [10,11], antituberculosis activities [12], and antifungal activities [13]. Meanwhile, the benzotriazine derivatives (**II**, Fig. 1) have also gained widespread interest due to their broad potential activities [14]. Recently, fragment-based lead discovery has emerged as a more rational and focused approach for molecular modification and drug design. As a part of our ongoing program aimed at the discovery and development of compounds with superior bioactivities [15–18], consequently, in this article we designed and prepared some novel indole[1,2-*c*]-1,2,4-benzotriazine derivatives (**5a**–**k**, Fig. 1) by combinatorial optimization of the indole unit with the benzotriazine moiety, and wanted to investigate their antifungal activities against phytopathogenic fungi.

2. Results and discussion

2.1. Chemistry

As shown in Scheme 1, 5-bromoindole (**1b**) was prepared from indole (**1a**) in the presence of ethanol and 27% aqueous sodium bisulfite, followed by reaction with acetic anhydride and bromine [19]. Then **1b** reacted with sodium methoxide in the presence of cuprous iodide (CuI) and *N*,*N*-dimethylformamide (DMF) to give 5-methoxyindole (**1c**). As outlined in Scheme 2, indole derivatives (**1a**–**h**) firstly reacted with 2-nitrophenyl halides (**2a** and **b**) by the S_NAr reaction mediated with cesium carbonate (Cs_2CO_3) to yield **3a**–**j** [20], which were subsequently reduced to **4a**–**j** in the presence of stannous chloride dihydrate ($SnCl_2{\cdot}2H_2O$). Finally, indole [1,2-*c*]-1,2,4-benzotriazine derivatives (**5a**–**j**) were obtained from **4a**–**j** by a modified Sandmeyer reaction *via* the intramolecular cyclization in the presence of *tert*-butylnitrite (*t*-BuONO). Compound **5k** was synthesized starting from indole as described in Scheme 3. At first, **1a** reacted with DMF in the presence of phosphorus chloride oxide ($POCl_3$) to produce 3-formylindole (**1i**) [21], which further reacted with 2-fluoronitrobenzene by the S_NAr

* Corresponding author. Laboratory of Pharmaceutical Design and Synthesis, College of Sciences, Northwest A&F University, Xinong Road 22#, Yangling, Shaanxi 712100, China. Tel./fax: +86 29 87091952.
E-mail address: orgxuhui@nwsuaf.edu.cn (H. Xu).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.10.022

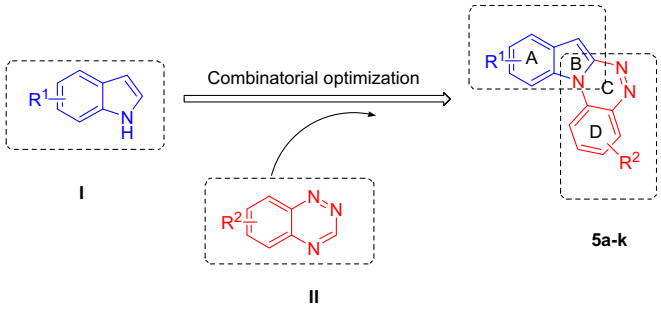


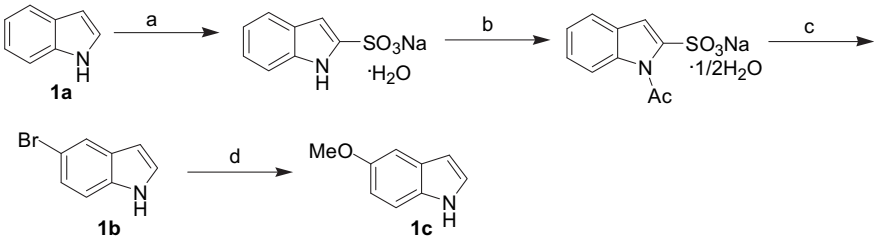
Fig. 1. Design strategy of the target compounds **5a**–**k**.

reaction to give **3k**. Then **3k** was reduced to **4k** by $SnCl_2 \cdot 2H_2O$, and **4k** was further reduced to **4l** with sodium borohydride ($NaBH_4$). At last, **4l** reacted with *t*-BuONO to give **5k** *via* the intramolecular cyclization. However, the diazonium salt of **4k** did not cyclize to produce **5l** at all. The structures of the target compounds were well characterized by ^1H NMR, ^{13}C NMR, m.p., HRMS or MS.

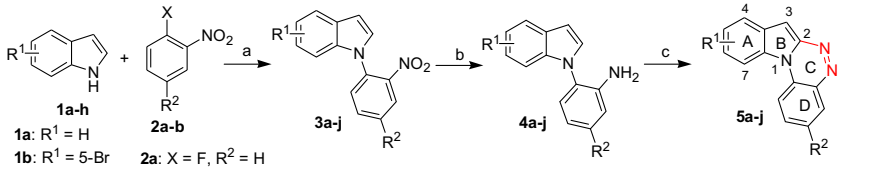
2.2. Antifungal activity

The antifungal activities of 11 novel indole[1,2-*c*]-1,2,4-benzotriazine derivatives (**5a**–**k**) against five phytopathogenic fungi (*i.e.*, *Fusarium graminearum*, *Alternaria alternata*, *Pyricularia oryzae*, *Fusarium oxysporum* f. sp. *vasinfectum*, and *Alternaria brassicae*) were investigated at the concentration of 50 μg/mL *in vitro* by poisoned food technique [22]. Hymexazol, a commercially available agricultural fungicide, was used as a positive control at 50 μg/mL.

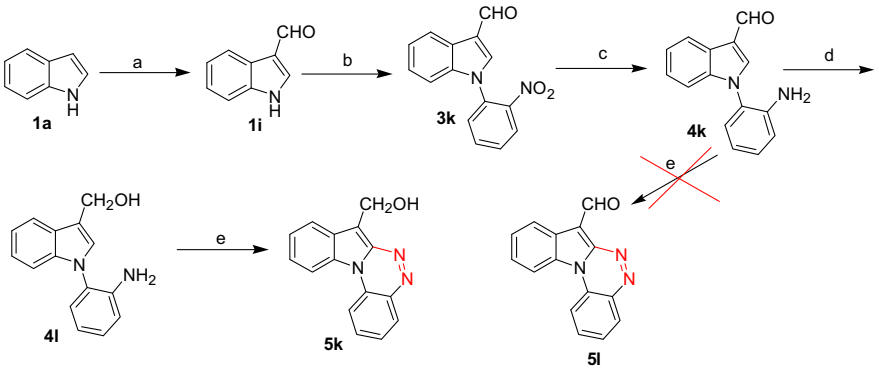
As outlined in Table 1, among all the derivatives, compounds **5h** and **5k** generally exhibited the more promising and pronounced antifungal activities than hymexazol. Through a comparative study on the relationship between the chemical structures and antifungal activities of **5a**–**k** (SAR), some interesting results were described as follows: (1) In general, introduction of the phenylaminocarbonyl substituent on the D-ring of **5a** or **5d** gave **5i** or **5j**, the corresponding antifungal activities of which were decreased as compared with **5a** and **5d**, respectively. For example, the inhibition



Scheme 1. Synthesis of compounds **1b** and **1c**. *Reagents and conditions*: (a) 27% aq. $NaHSO_3$, EtOH, rt, 20 h, 98%; (b) Ac_2O, 70 °C, 2 h, then 90 °C, 0.5 h, 89%; (c) Br_2, H_2O, 0–5 °C, 1 h, then rt, 1 h, 74%; (d) MeONa, DMF, CuI, reflux, 6 h, 98%.



Scheme 2. The synthetic route of compounds **5a**–**j**. *Reagents and conditions*: (a) Cs_2CO_3, DMSO, 40 °C, 2–8 h, 21–99%; (b) $SnCl_2 \cdot 2H_2O$, EtOAc, reflux, 1–4 h, 44–93%; (c) *t*-BuONO, MeCN, rt, 1/6–22 h, 24–99%.



Scheme 3. The synthetic route of compound **5k**. *Reagents and conditions*: (a) $POCl_3$, DMF, 0 °C, 20 min, then 40 °C, 1 h, then 20% aq. NaOH, 0 °C, then reflux, 6 h, 96%; (b) Cs_2CO_3, 2-fluoronitrobenzene, DMSO, 40 °C, 5 h; (c) $SnCl_2 \cdot 2H_2O$, EtOAc, reflux, 22 h, 29% (from **1i** to **4k**); (d) $NaBH_4$, MeOH, rt, 1 h, 78%; (e) *t*-BuONO, MeCN, rt, 0.5 h, 66%.

Table 1
Antifungal activities of compounds **5a**–**k** at 50 μg/mL.

Compd	Antifungal activities (inhibition %)				
	F. graminearum	*P. oryzae*	*F. oxysporum* f. sp. *vasinfectum*	*A. alternata*	*A. brassicae*
5a	20.5 (±0.4)	4.4 (±1.2)	36.7 (±1.2)	13.4 (±1.2)	24.9 (±0.5)
5b	11.2 (±0.6)	2.7 (±0.7)	11.2 (±1.2)	10.9 (±1.0)	10.1 (±0.5)
5c	15.8 (±1.3)	13.8 (±2.2)	21.9 (±0.7)	18.1 (±1.7)	8.5 (±0)
5d	21.1 (±0.4)	7.3 (±0.7)	16.1 (±1.2)	15.5 (±1.2)	20.1 (±1.9)
5e	14.0 (±0.4)	0.2 (±0.7)	16.1 (±1.2)	16.8 (±0.6)	14.2 (±0.9)
5f	10.2 (±1.5)	3.0 (±0.7)	9.9 (±1.4)	9.2 (±0.6)	5.5 (±1.0)
5g	21.4 (±0.4)	16.3 (±1.3)	17.2 (±0.7)	18.5 (±1.7)	23.4 (±0.6)
5h	60.6 (±0.4)	72.6 (±1.4)	54.3 (±1.0)	75.3 (±0.6)	75.6 (±0.5)
5i	11.1 (±0.4)	3.8 (±1.3)	16.5 (±1.2)	1.1 (±0.6)	1.8 (±1.2)
5j	2.5 (±0.7)	0	10.1 (±0.7)	1.1 (±1.3)	4.1 (±1.6)
5k	80.1 (±0.6)	43.1 (±1.2)	74.2 (±1.2)	54.3 (±0.6)	57.2 (±0.5)
Hym	56.5 (±0.4)	56.4 (±1.2)	63.0 (±0.6)	64.8 (±0.6)	53.5 (±0.5)
Acetone	0	0	0	0	0

rates of **5a** and **5i** at 50 μg/mL against *F. graminearum*, *P. oryzae*, *F. oxysporum* f. sp. *vasinfectum*, *A. alternata*, and *A. brassicae* were 20.5%/11.1%, 4.4%/3.8%, 36.7%/16.5%, 13.4%/1.1%, and 24.9%/1.8%, respectively; the inhibition rates of **5d** and **5j** at 50 μg/mL against *F. graminearum*, *P. oryzae*, *F. oxysporum* f. sp. *vasinfectum*, *A. alternata*, and *A. brassicae* were 21.1%/2.5%, 7.3%/0%, 16.1%/10.1%, 15.5%/1.1%, and 20.1%/4.1%, respectively. (2) When the methyl group was introduced at the 7th position on the A-ring of **5a** to give **5h**, the corresponding antifungal activities of **5h** increased sharply as compared with **5a**. The inhibition rates of **5a** and **5h** at 50 μg/mL against *F. graminearum*, *P. oryzae*, *F. oxysporum* f. sp. *vasinfectum*, *A. alternata*, and *A. brassicae* were 20.5%/60.6%, 4.4%/72.6%, 36.7%/54.3%, 13.4%/75.3%, and 24.9%/75.6%, respectively. On the contrary, introduction of the methyl group, bromo atom or methoxy group at other position on the A- or B-ring of **5a** produced **5d**, **5f**, **5g**, **5b**, **5e**, or **5c**, the corresponding antifungal activities of which were not increased to some extent as compared with **5a**. (3) The hydroxymethyl group on the B-ring of **5k** was very crucial for the antifungal activities. For example, when the hydroxymethyl group was introduced on the B-ring of **5a** to give **5k**, the corresponding antifungal activities of **5k** increased obviously as compared with **5a**. The inhibition rates of **5a** and **5k** at 50 μg/mL against *F. graminearum*, *P. oryzae*, *F. oxysporum* f. sp. *vasinfectum*, *A. alternata*, and *A. brassicae* were 20.5%/80.1%, 4.4%/43.1%, 36.7%/74.2%, 13.4%/54.3%, and 24.9%/57.2%, respectively. While other functional group (*e.g.*, methyl group or bromo atom) was introduced on the B-ring of **5a**, the corresponding compounds did not display the potent antifungal activities (**5d** and **5e**).

3. Conclusion

In summary, 11 novel indole[1,2-*c*]-1,2,4-benzotriazine derivatives were synthesized by a modified Sandmeyer reaction in the presence of *tert*-butylnitrite, and evaluated *in vitro* for their antifungal activities against five phytopathogenic fungi at the concentration of 50 μg/mL. Among all the derivatives, compounds **5h** and **5k** generally exhibited the more promising and pronounced antifungal activities than hymexazol, a commercially available agricultural fungicide. It clearly demonstrated that introduction of appropriate substituents on the indolyl ring of indole[1,2-*c*]-1,2,4-benzotriazine (**5a**) would lead to the more potent derivatives. It implied that **5h** and **5k** might be considered as new promising lead candidates for further design and synthesis of agricultural fungicides.

4. Experimental

4.1. Chemistry

4.1.1. General remarks

All solvents and reagents (except **1b**, **1c**, and **1i**) were used as obtained from commercial sources without further purification. Thin-layer chromatography (TLC) and silica gel column chromatography were used with silica gel 60 GF_{254} and 200–300 mesh, respectively (Qingdao Haiyang Chemical Co., Ltd., China). Melting points were determined on a digital melting-point apparatus and were uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon-13 nuclear magnetic resonance spectra (^{13}C NMR) were recorded on Bruker Avance DMX 400 or 500 MHz and 125 MHz instruments, respectively, using TMS as the internal standard and $CDCl_3$ or DMSO-d_6 as the solvent. High-resolution mass spectra (HRMS) were carried out with APEX II Bruker 4.7T AS instrument. Electrospray ion trap mass spectrometry (ESI-MS) was carried out with Thermo Scientific LCQ Fleet mass spectrometer.

4.1.2. Synthesis of 5-bromoindole (*1b*)

Compound **1b** was synthesized according to the literature [23].

Yield: 74%, white solid, m.p. 90–92 °C (lit. 88–90 °C [23]); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.49 (1H, s), 7.19 (1H, s), 7.23–7.28 (2H, m), 7.77 (1H, s), 8.14 (1H, s, NH); ESI-MS *m*/*z*: 194.16 ($[M-1]^-$, 100%,), 196.03 ($[M-1]^-$, 88%).

4.1.3. Synthesis of 5-methoxyindole (*1c*)

Firstly sodium (1.18 g, 51.3 mmol) reacted with absolute methanol (12 mL) to produce sodium methoxide, which was mixed directly with **1b** in the presence of cuprous iodide (CuI, 1.97 g, 103 mmol) and *N*,*N*-dimethylformamide (DMF, 21.2 mL), and the mixture was refluxed for 6 h. Then the mixture was filtered and the filtrate was concentrated *in vacuo*, followed by addition of 10% aq. NaOH (40 mL). The solution was extracted with EtOAc (30 mL × 3). Finally, the organic phases were combined, dried over Na_2SO_4, and purified by silica gel column chromatography to give 5-methoxyindole (**1c**).

Yield: 98%, white solid, m.p. 59–61 °C (56–57 °C [23]); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.86 (3H, s, OCH_3), 6.47 (1H, s), 6.85 (1H, dd, J = 8.5, 2.0 Hz), 7.11 (1H, s), 7.15 (1H, t, J = 2.5 Hz), 7.25 (1H, d, J = 9.0 Hz), 8.03 (1H, s, NH); ESI-MS *m*/*z*: 146.20 ($[M-1]^-$, 73%).

4.1.4. Synthesis of 3-formylindole (*1i*)

To DMF (7 mL), phosphorus oxychloride ($POCl_3$, 1 mL) was added dropwise at 0 °C. The mixture was stirred for 20 min, then a solution of indole (10 mmol) in DMF (3 mL) was added dropwise. After the mixture was stirred at 35 °C for 1 h, ice was added, followed by 20% aq. sodium hydroxide (NaOH), and the mixture was refluxed for 6 h. On cooling, the mixture was poured into ice water, and the precipitated product was collected, washed by water, and dried.

Yield: 96%, pink solid, m.p. 190–192 °C (190–192 °C [24]); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.93 (s, 1H, CHO), 8.29 (s, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.20–7.28 (m, 2H); ESI-MS, *m*/*z* (%) 145 (M^+, 96).

4.1.5. General procedures for the synthesis of N-(2-aminophenyl) indole derivatives (*4a*–*j*)

Compounds **3a**–**j** were prepared as described in our previous paper [20]. A mixture of **3a**–**j** (1 equiv.) and stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$, 5 equiv.) in ethyl acetate (EtOAc, 10 mL) was refluxed for 1–4 h under N_2. The reaction mixture was alkalinized to pH = 8 or 9 with the saturated $NaHCO_3$, and the solution was

filtered to remove the precipitate. Then the organic phase was separated from the water phase, and the latter was extracted with EtOAc (20 mL × 2). Finally, the organic phases were combined, dried over Na_2SO_4, and purified by silica gel column chromatography to give **4a**–**j**.

*4.1.5.1. N-(2-Aminophenyl)indole (**4a**).* Yield: 81%, colorless oil (lit. colorless oil [25]); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.43 (2H, br. s, NH_2), 6.68 (1H, d, J = 3.0 Hz), 6.82–6.87 (2H, m), 7.14–7.26 (6H, m), 7.68 (1H, d, J = 7.5 Hz); ESI-MS m/z: 209.15 ($[M + 1]^+$, 100%).

*4.1.5.2. N-(2-Aminophenyl)-5-bromoindole (**4b**).* Yield: 93%, colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.53 (2H, br. s, NH_2), 6.61 (1H, d, J = 3.0 Hz), 6.82–6.87 (2H, m), 7.00 (1H, d, J = 8.5 Hz), 7.15 (1H, dd, J = 7.5, 1.5 Hz), 7.19 (1H, d, J = 3.0 Hz), 7.23–7.27 (2H, m), 7.80 (1H, d, J = 8.0 Hz); ESI-MS m/z: 286.96 ($[M + 1]^+$, 100%), 289.03 ($[M + 1]^+$, 88%).

*4.1.5.3. N-(2-Aminophenyl)-5-methoxyindole (**4c**).* Yield: 64%, colorless oil [26]; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.36 (2H, br. s, NH_2), 3.85 (3H, s, OCH_3), 6.59 (1H, d, J = 3.0 Hz), 6.82–6.85 (3H, m), 7.02 (1H, d, J = 9.0 Hz), 7.14–7.23 (4H, m); ESI-MS m/z: 239.18 ($[M + 1]^+$, 100%).

*4.1.5.4. N-(2-Aminophenyl)-3-methylindole (**4d**).* Yield: 85%, colorless oil [27]; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.38 (3H, s, CH_3), 3.58 (2H, s, NH_2), 6.80–6.86 (2H, m), 6.98 (1H, d, J = 0.5 Hz), 7.10–7.23 (5H, m), 7.62 (1H, dd, J = 6.5, 1.5 Hz); ESI-MS m/z: 223.14 ($[M + 1]^+$, 100%).

*4.1.5.5. N-(2-Aminophenyl)-3-bromoindole (**4e**).* Yield: 87%, colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.45 (2H, br. s, NH_2), 6.83 (1H, t, J = 8.0 Hz), 6.87 (1H, d, J = 8.0 Hz), 7.12–7.14 (1H, m), 7.15 (1H, d, J = 7.5 Hz), 7.23–7.27 (4H, m), 7.62–7.64 (1H, m); ESI-MS m/z: 286.82 ($[M + 1]^+$, 100%), 288.86 ($[M + 1]^+$, 95%).

*4.1.5.6. N-(2-Aminophenyl)-4-methylindole (**4f**).* Yield: 85%, colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.60 (3H, s, CH_3), 3.40 (2H, br. s, NH_2), 6.70 (1H, d, J = 3.0 Hz), 6.82–6.87 (2H, m), 6.95–6.99 (2H, m), 7.08 (1H, t, J = 7.5 Hz), 7.17–7.25 (3H, m); ESI-MS m/z: 223.14 ($[M + 1]^+$, 100%).

*4.1.5.7. N-(2-Aminophenyl)-6-methylindole (**4g**).* Yield: 44%, white solid; m.p. 71–72 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.41 (3H, s, CH_3), 3.47 (2H, br. s, NH_2), 6.62 (1H, d, J = 2.0 Hz), 6.82–6.87 (2H, m), 6.93 (1H, s), 6.98 (1H, d, J = 8.0 Hz), 7.12 (1H, d, J = 3.0 Hz), 7.17–7.19 (1H, m), 7.22–7.25 (1H, m), 7.55 (1H, d, J = 8.0 Hz); ESI-MS m/z: 223.13 ($[M + 1]^+$, 100%).

*4.1.5.8. N-(2-Aminophenyl)-7-methylindole (**4h**).* Yield: 45%, white solid; m.p. 123–125 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.06 (3H, s, CH_3), 3.33 (2H, br. s, NH_2), 6.64 (1H, d, J = 3.5 Hz), 6.77–6.80 (2H, m), 6.90 (1H, d, J = 7.0 Hz), 7.03–7.06 (2H, m), 7.18 (1H, dd, J = 8.0, 3.0 Hz), 7.22–7.25 (1H, m), 7.52 (1H, d, J = 8.0 Hz); ESI-MS m/z: 223.18 ($[M + 1]^+$, 100%).

*4.1.5.9. N-(2-Amino-4-phenylaminocarbonylphenyl)indole (**4i**).* Yield: 63%, white solid; m.p. 158–160 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.32 (2H, br. s, NH_2), 6.73 (1H, d, J = 3.0 Hz), 7.14–7.30 (7H, m), 7.37 (1H, t, J = 8.0 Hz), 7.44 (1H, s), 7.64 (2H, d, J = 8.0 Hz), 7.70 (1H, d, J = 7.5 Hz), 7.86 (1H, d, J = 15 Hz, NH); ESI-MS m/z: 328.13 ($[M + 1]^+$, 100%).

*4.1.5.10. N-(2-Amino-4-phenylaminocarbonylphenyl)-3-methylindole (**4j**).* Yield: 53%, white solid; m.p. 144–146 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.39 (3H, s, CH_3), 3.83 (2H, br. s, NH_2), 6.99 (1H, s), 7.11–7.26 (6H, m), 7.37–7.42 (3H, m), 7.64–7.66 (3H, m), 7.90 (1H, s, NH); ESI-MS m/z: 342.11 ($[M + 1]^+$, 100%).

*4.1.6. Synthesis of N-(2-aminophenyl)-3-hydroxymethylindole (**4l**)*

N-(2-Nitrophenyl)-3-formylindole (**3k**) was prepared as described in our previous paper [20].

Yield: 98%, yellow solid; m.p. 126–128 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.05 (1H, d, J = 8.0 Hz), 7.28–7.34 (1H, m), 7.36–7.37 (1H, m), 7.60 (1H, dd, J = 8.0, 1.0 Hz), 7.70–7.74 (1H, m), 7.81–7.85 (2H, m), 8.14 (1H, dd, J = 8.0, 1.0 Hz), 8.36 (1H, d, J = 7.5 Hz), 10.11 (1H, s, CHO); ESI-MS m/z: 267.06 ($[M + 1]^+$, 100%).

Compound **4k** was prepared as the same procedure as **4a**–**j**. As **4k** was not very stable, it was used directly for the next step. Sodium borohydride ($NaBH_4$, 2.25 mmol) was added dropwise to a stirred solution of **4k** (1.5 mmol) in methanol (5 mL) at 0 °C. After adding, the mixture was stirred at room temperature for 1 h. Then the mixture was concentrated *in vacuo*, followed by addition of water (20 mL). The solution was extracted with EtOAc (30 mL × 3). Finally, the organic phases were combined, dried over Na_2SO_4, and purified by silica gel column chromatography to give **4l**.

Yield: 78%, colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.83 (2H, br. s, NH_2), 4.93 (2H, s, CH_2), 6.83–6.87 (2H, m), 7.14–7.24 (6H, m), 7.77–7.79 (1H, m); ESI-MS m/z: 221.14 ($[M + 1 - H_2O]^+$, 85%).

*4.1.7. General procedures for the synthesis of indole[1,2-c]-1,2,4-benzotriazine derivatives (**5a**–**k**)*

To a stirred solution of **4a**–**k** (0.5 mmol) in MeCN (2 mL) at room temperature, a solution of *tert*-butylnitrite (*t*-BuONO, 0.75 mmol) in MeCN (3 mL) was added dropwise. When the reaction was complete according to TLC analysis, the solvent was evaporated under reduced pressure to give the residue, to which water (20 mL) was added. Then the above mixture was extracted by EtOAc (40 mL × 3). Subsequently, the combined organic phase was washed by brine (40 mL), dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo* and purified by silica gel column chromatography to give **5a**–**k**, which were well characterized by ^{1}H NMR, ^{13}C NMR, HRMS, and m.p.

*4.1.7.1. Indole[1,2-c]-1,2,4-benzotriazine (**5a**).* Yield: 71%, red solid, m.p. 220–222 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.50–7.57 (2H, m), 7.61–7.65 (1H, m), 7.70 (1H, s), 7.80–7.84 (1H, m), 8.06 (1H, d, J = 8.0 Hz), 8.32 (2H, dd, J = 8.0, 3.2 Hz), 8.49 (1H, d, J = 7.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 102.4, 114.2, 115.1, 123.6, 124.7, 126.0, 127.6, 128.8, 129.3, 131.8, 133.6, 136.8, 142.3; HRMS-ESI: Calcd. for $C_{14}H_9N_3$ ($[M + H]^+$): 220.0869; Found: 220.0873.

*4.1.7.2. 5-Bromoindole[1,2-c]-1,2,4-benzotriazine (**5b**).* Yield: 56%, red solid, m.p. 222–224 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.59–7.64 (2H, m), 7.69 (1H, dd, J = 8.8, 1.2 Hz), 7.85 (1H, t, J = 7.6 Hz), 8.22–8.31 (3H, m), 8.53 (1H, d, J = 7.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 101.5, 114.2, 116.5, 117.3, 125.2, 126.0, 127.2, 127.9, 128.9, 130.4, 132.2, 134.0, 137.1, 142.7; HRMS-ESI: Calcd. for $C_{14}H_8BrN_3$ ($[M + H]^+$): 297.9974; Found: 297.9980.

*4.1.7.3. 5-Methoxyindole[1,2-c]-1,2,4-benzotriazine (**5c**).* Yield: 44%, red solid, m.p. 184–186 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.95 (3H, s, CH_3), 7.28 (1H, s), 7.38 (1H, s), 7.53 (1H, t, J = 7.6 Hz), 7.62 (1H, s), 7.79 (1H, t, J = 8.0 Hz), 8.23–8.29 (2H, m), 8.48 (1H, d, J = 8.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 55.8, 101.7, 102.6, 114.0, 116.1, 118.1, 124.5, 124.7, 127.4, 130.2, 131.8, 133.5, 136.9, 143.0, 156.5; HRMS-ESI: Calcd. for $C_{15}H_{11}N_3O$ ($[M + H]^+$): 250.0975; Found: 250.0970.

*4.1.7.4. 3-Methylindole[1,2-c]-1,2,4-benzotriazine (**5d**).* Yield: 99%, red solid, m.p. 176–178 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.95 (3H, s,

CH_3), 7.48–7.54 (2H, m), 7.62–7.66 (1H, m), 7.74–7.78 (1H, m), 8.04 (1H, d, J = 8.0 Hz), 8.24 (1H, d, J = 8.4 Hz), 8.30 (1H, d, J = 8.4 Hz), 8.42 (1H, dd, J = 8.0, 1.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 8.3, 111.8, 113.9, 114.8, 121.7, 122.9, 124.3, 126.2, 127.9, 128.6, 129.0, 131.6, 133.2, 136.7, 139.7; HRMS-ESI: Calcd. for $C_{15}H_{11}N_3$ ($[M + H]^+$): 234.1026; Found: 234.1022.

*4.1.7.5. 3-Bromoindole[1,2-c]-1,2,4-benzotriazine (**5e**).* Yield: 64%, red solid, m.p. 254–256 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.71–7.79 (3H, m), 8.02–8.07 (2H, m), 8.54 (1H, d, J = 7.6 Hz), 8.73 (1H, d, J = 8.4 Hz), 8.80 (1H, d, J = 8.4 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 91.1, 115.4, 116.4, 121.1, 125.0, 126.0, 126.6, 127.4, 127.5, 128.3, 131.6, 135.4, 137.2, 138.2; HRMS-ESI: Calcd. for $C_{14}H_8BrN_3$ ($[M + H]^+$): 297.9974; Found: 297.9976.

*4.1.7.6. 4-Methylindole[1,2-c]-1,2,4-benzotriazine (**5f**).* Yield: 67%, red solid, m.p. 212–214 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.75 (3H, s, CH_3), 7.28 (1H, d, J = 6.8 Hz), 7.49–7.56 (2H, m), 7.69 (1H, s), 7.78 (1H, t, J = 8.0 Hz), 8.12 (1H, d, J = 8.8 Hz), 8.30 (1H, d, J = 7.2 Hz), 8.47 (1H, d, J = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 19.3, 100.9, 112.4, 114.2, 123.4, 124.6, 126.2, 127.6, 129.0, 129.1, 131.6, 133.2, 133.4, 136.9, 142.1; HRMS-ESI: Calcd. for $C_{15}H_{11}N_3$ ($[M + H]^+$): 234.1026; Found: 234.1022.

*4.1.7.7. 6-Methylindole[1,2-c]-1,2,4-benzotriazine (**5g**).* Yield: 54%, red solid, m.p. 144–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.66 (3H, s, CH_3), 7.33 (1H, d, J = 8.0 Hz), 7.51 (1H, t, J = 7.6 Hz), 7.64 (1H, s), 7.77 (1H, t, J = 8.4 Hz), 7.92 (1H, d, J = 8.4 Hz), 8.09 (1H, s), 8.29 (1H, d, J = 8.4 Hz), 8.45 (1H, d, J = 8.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 22.9, 102.4, 114.2, 114.5, 123.2, 124.5, 125.8, 126.8, 127.6, 129.7, 131.5, 133.3, 136.5, 136.8, 142.3; HRMS-ESI: Calcd. for $C_{15}H_{11}N_3$ ($[M + H]^+$): 234.1026; Found: 234.1033.

*4.1.7.8. 7-Methylindole[1,2-c]-1,2,4-benzotriazine (**5h**).* Yield: 24%, red solid, m.p. 126–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.82 (3H, s, CH_3), 7.39 (1H, d, J = 6.8 Hz), 7.45 (1H, t, J = 7.6 Hz), 7.51 (1H, d, J = 7.6 Hz), 7.62 (1H, s), 7.68 (1H, t, J = 8.0 Hz), 7.83–7.89 (2H, m), 8.44 (1H, d, J = 8.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.2, 103.6, 118.2, 120.6, 124.4, 124.5, 124.8, 128.4, 129.0, 129.8, 130.5, 130.6, 132.2, 137.9, 143.2; HRMS-ESI: Calcd. for $C_{15}H_{11}N_3$ ($[M + H]^+$): 234.1026; Found: 234.1031.

*4.1.7.9. Indole[1,2-c]-7-phenylaminocarbonyl-1,2,4-benzotriazine (**5i**).* Yield: 70%, red solid, m.p. 310–312 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.14–7.18 (1H, m), 7.39–7.43 (2H, m), 7.62–7.65 (1H, m), 7.74 (1H, t, J = 8.0 Hz), 7.86 (2H, d, J = 8.0 Hz), 7.92 (1H, s), 8.19 (1H, d, J = 7.6 Hz), 8.52 (1H, d, J = 8.8 Hz), 8.79–8.85 (2H, m), 9.16 (1H, s), 10.64 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 102.5, 115.0, 115.5, 120.3, 120.4, 123.2, 123.8, 123.9, 126.3, 128.1, 128.4, 128.5, 128.6, 129.8, 130.5, 133.3, 135.2, 138.8, 141.5, 163.5; HRMS-ESI: Calcd. for $C_{21}H_{14}N_4O$ ($[M + H]^+$): 339.1240; Found: 339.1245.

*4.1.7.10. 3-Methylindole[1,2-c]-7-phenylaminocarbonyl-1,2,4-benzotriazine (**5j**).* Yield: 80%, red solid, m.p. 302–304 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.89 (3H, s, CH_3), 7.15 (1H, s), 7.40 (2H, s), 7.62 (2H, d, J = 9.2 Hz), 7.86 (2H, d, J = 6.0 Hz), 8.18 (1H, d, J = 6.4 Hz), 8.46 (1H, s), 8.70 (2H, d, J = 6.8 Hz), 9.08 (1H, s), 10.59 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 8.3, 112.0, 115.2, 115.8, 120.9, 121.0, 121.9, 123.9, 124.3, 127.3, 128.4, 128.8, 129.1, 129.3, 130.1, 130.6, 133.6, 135.6, 139.3, 139.5, 164.1; HRMS-ESI: Calcd. for $C_{22}H_{16}N_4O$ ($[M + H]^+$): 353.1397; Found: 353.1393.

*4.1.7.11. 3-Hydroxymethylindole[1,2-c]-1,2,4-benzotriazine (**5k**).* Yield: 66%, red solid, m.p. 202–204 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.59 (2H, s, CH_2), 7.54–7.58 (2H, m), 7.64–7.68 (1H, m), 7.81–7.85 (1H, m), 8.19 (1H, d, J = 7.6 Hz), 8.30 (2H, t, J = 8.4 Hz), 8.46 (1H, dd, J = 8.0, 1.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 53.2, 114.8, 115.5, 116.0, 122.9, 123.9, 125.5, 126.9, 127.1, 128.2, 128.7, 131.5, 135.0, 136.8, 138.9; HRMS-ESI: Calcd. for $C_{15}H_{11}N_3O$ ($[M + H]^+$): 250.0975; Found: 250.0970.

4.2. Biological assay

Eleven 3-acylindole derivatives (**5a**–**k**) were screened *in vitro* for their antifungal activities against five phytopathogenic fungi (*i.e.*, *F. graminearum*, *A. alternata*, *P. oryzae*, *F. oxysporum* f. sp. *vasinfectum*, and *A. brassicae*). Potato dextrose agar (PDA) medium was prepared in the flasks and sterilized. Compounds **5a**–**k** were dissolved in acetone before mixing with PDA, and the concentration of test compounds in the medium was fixed at 50 μg/mL. The medium was then poured into sterilized Petri dishes. All types of fungi were incubated in PDA at 28 ± 1 °C for 5 d to get new mycelium for the antifungal assays, and a mycelia disk of approximately 5 mm diameter cut from culture medium was picked up with a sterilized inoculation needle and inoculated in the center of the PDA Petri dishes. The inoculated Petri dishes were incubated at 28 ± 1 °C for 4 d. Acetone without any compounds mixed with PDA was served as the control, while hymexazol, a commercially available agricultural fungicide, was used as a positive control at 50 μg/mL. For each treatment, three replicates were conducted. The radial growths of the fungal colonies were measured and the data were statistically analyzed. The inhibitory effects of the test compounds on these fungi *in vitro* were calculated by the formula:

$$\text{Inhibition rate}\ (\%) = (C - T) \times 100/C$$

where C represents the diameter of fungi growth on untreated PDA, and T represents the diameter of fungi on treated PDA.

Acknowledgments

This work was financially in part supported by the National Natural Science Foundation of China (no. 31071737), the Program for New Century Excellent University Talents, State Education Ministry of China (NCET-06-0868), the Fok Ying Tong Education Foundation for Young Talents (no. 121032), and the State Key Laboratory of Applied Organic Chemistry, Lanzhou University. We also thank Prof. H.L. Zhang for the NMR experiments.

References

[1] M. Somei, F. Yamada, Nat. Prod. Rep. 20 (2003) 216–242.
[2] L. Gupta, A. Talwar, P.M.S. Chauhan, Curr. Med. Chem. 14 (2007) 1789–1803.
[3] H. Xu, M. Lv, Curr. Pharm. Des. 15 (2009) 2120–2148.
[4] J. Ran, N. Huang, H. Xu, L. Yang, M. Lv, Y.T. Zheng, Bioorg. Med. Chem. Lett. 20 (2010) 3534–3536.
[5] A.K. Ghosh, G. Gong, V. Grum-Tokars, D.C. Mulhearn, S.C. Baker, M. Coughlin, B.S. Prabhakar, K. Sleeman, M.E. Johnson, A.D. Mesecar, Bioorg. Med. Chem. Lett. 18 (2008) 5684–5688.
[6] J.D. Williams, J.J. Chen, J.C. Drach, L.B. Townsend, J. Med. Chem. 47 (2004) 5766–5772.
[7] J.D. Williams, J.J. Chen, J.C. Drach, L.B. Townsend, J. Med. Chem. 47 (2004) 5753–5765.
[8] A. Andreani, S. Burnelli, M. Granaiola, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, L. Varoli, L. Landi, C. Prata, M.V. Berridge, C. Grasso, H.H. Fiebig, G. Kelter, A.M. Burger, M.W. Kunkel, J. Med. Chem. 51 (2008) 4563–4570.
[9] M.J. Slater, R. Baxter, R.W. Bonser, S. Cockerill, K. Gohil, N. Parry, E. Robinson, R. Randall, C. Yeates, W. Snowden, A. Walters, Bioorg. Med. Chem. Lett. 11 (2001) 1993–1995.
[10] B.S.D. Mathada, M.B.H. Mathada, Chem. Pharm. Bull. 57 (2009) 557–560.
[11] G. Gurkok, N. Altanlar, S. Suzen, Chemotherapy 55 (2009) 15–19.
[12] N. Karah, A. Gursoy, F. Kandemirli, N. Shvets, F.B. Kaynak, S. Ozbey, V. Kovalishyn, A. Dimoglo, Bioorg. Med. Chem. 15 (2007) 5888–5891.
[13] W.H. Dekker, H.A. Selling, J.C. Overeem, J. Agric. Food Chem. 23 (1975) 785–791.

[14] J. Cao, R. Fine, C. Gritzen, J. Hood, X. Kang, B. Llebansky, D. Lohse, C.C. Mak, A. McPherson, G. Noronha, M.S.S. Palanki, V.P. Pathak, J. Renick, R. Soll, B. Zeng, H. Zhu, Bioorg. Med. Chem. Lett. 17 (2007) 5812–5818.
[15] H. Xu, X. Xiao, Bioorg. Med. Chem. Lett. 19 (2009) 5415–5418.
[16] H. Xu, J.J. Wang, Bioorg. Med. Chem. Lett. 20 (2010) 2500–2502.
[17] L.L. Fan, W.Q. Liu, H. Xu, L.M. Yang, M. Lv, Y.T. Zheng, Chem. Pharm. Bull. 57 (2009) 797–800.
[18] H. Xu, K.Z. Jian, Q. Guan, F. Ye, M. Lv, Chem. Pharm. Bull. 55 (2007) 1755–1777.
[19] H.F. Russell, B.J. Harris, D.B. Hood, E.G. Thompson, A.D. Watkins, R.D. Williams, Org. Prep. Proced. Int. 17 (1985) 391–399.
[20] H. Xu, W.Q. Liu, L.L. Fan, Y. Chen, L.M. Yang, L. Lv, Y.T. Zheng, Chem. Pharm. Bull. 56 (2008) 720–722.
[21] D. Coowar, J. Bouissac, M. Hanbali, M. Paschaki, E. Mohier, B. Luu, J. Med. Chem. 47 (2004) 6270–6282.
[22] D.C. Erwin, J.J. Sims, D.E. Borum, J.R. Childers, Phytopathology 61 (1971) 964–967.
[23] M.L. Ji, C. Zhang, H. Liu, Jingxi Huagong Zhongjianti 35 (2005) 27–28, 40.
[24] G.W. Gribble, J. Jiang, Y. Liu, J. Org. Chem. 67 (2002) 1001–1003.
[25] P.K. Agarwal, D. Sawant, S. Sharma, B. Kundu, Eur. J. Org. Chem. (2009) 292–303.
[26] C.S. Yi, S.Y. Yun, J. Am. Chem. Soc. 127 (2005) 17000–17006.
[27] N.T. Patil, R.D. Kavthe, V.S. Shinde, B. Sridhar, J. Org. Chem. 75 (2010) 3371–3380.