ORIGINAL ARTICLE

Design and synthesis of small molecular 2-aminobenzoxazoles as potential antifungal agents against phytopathogenic fungi

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Abstract

In order to discover novel antifungal agents, three series of simple 2-aminobenzoxazole derivatives were designed, synthesized and evaluated for their antifungal activities against eight phytopathogenic fungi. The in vitro antifungal results showed that most of the target compounds exhibited excellent and broad-spectrum antifungal activities to all the tested fungi. Particularly, the six compounds **3a**, **3b**, **3c**, **3e**, **3m** and **3v** displayed the most potent antifungal activity, with EC_{50} value of 1.48–16.6 µg/mL, which were much superior to the positive control hymexazol. The in vivo study further confirmed that compounds **3a**, **3c**, **3e** and **3m** displayed good preventative effect against *Botrytis cinerea* at the concentration of 100 µg/mL. The structure–activity relationships research provides significant reference for the further structural optimization of 2-aminobenzoxazole as potential fungicides.

Graphic abstract

Forty-four 2-aminobenzoxazole derivatives were designed and synthesized as agricultural antifungal agents, the in vitro and in vivo antifungal experiments showed that compounds **3a**, **3b**, **3c**, **3e**, **3m** and **3v** exhibited excellent and broad-spectrum antifungal activities compare with the commercial fungicide hymexazol.
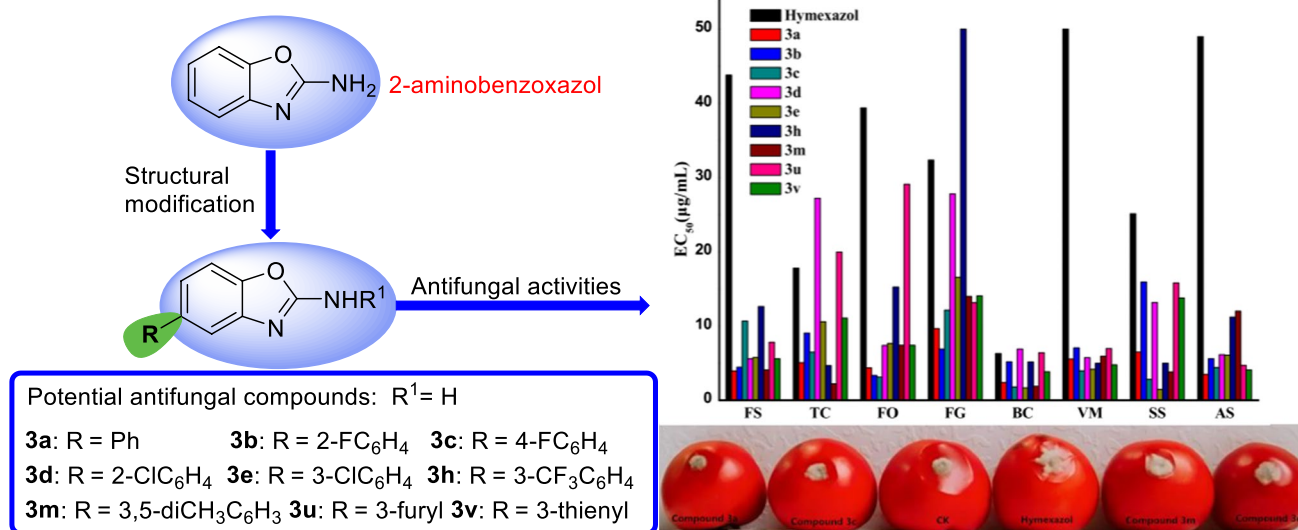
Keywords 2-Aminobenzoxazole derivatives · Pathogenic fungi · Antifungal activity · Structure–activity relationships

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Introduction

Phytopathogenic fungi can seriously interrupt the normal growth of crops, fruits and vegetables, which not only lead to serious economic losses of agriculture worldwide, but also some species can produce mycotoxins that are harmful to human and animal health. In the past decades, many fungicides that target different pathogens and diseases have been used to improve agricultural production and ensure food security, but some fungicides have been restricted due to toxic residues, disease resurgence and pathogen resistance [1]. Therefore, it is necessary to develop novel antifungal agents with low toxicity and high efficiency to control agricultural diseases.

Benzoxazole, an important class of heterocyclic scaffolds, exhibits broad biological activities in the fields of medicinal and agricultural, including antiproliferative [2], anticancer [3, 4], anti-HIV [5], antimicrobial [6–9], antiviral [10], herbicidal [11] and antifungal activity [12]. As shown in Fig. 1, some reported drugs containing benzoxazole skeleton were listed. For example, benoxaprofen and flunoxaprofen are non-steroidal anti-inflammatory drugs (NSAIDs), reportedly useful in the treatment of rheumatoid arthritis, myositis, synovitis and osteoarthritis [13, 14]. Calcimycin, an antibiotic and divalent cation ionophore, is a structural unique benzoxazole polyether produced by Streptomyces chartreusis NRRL3882 [15]. Oxazosulfyl, a novel benzoxazole insecticide containing ethyl sulfonyl pyridine fragment, was developed by Sumitomo Chemical Co., Ltd. Thus, benzoxazole is a magical and useful chemical group, which is worthy of being studied further.

To the best of our knowledge, 2-aminobenzoxazole as an important intermediate for the synthesis of antitumor drugs has rarely reported the antifungal activity against plant pathogenic fungi. In continuation of our program aimed at the discovery and development of novel antifungal candidates [16–21], herein we design and synthesize three series of 2-aminobenzoxazole derivatives and evaluated their antifungal activities against eight phytopathogenic fungi.

Results and discussion

Chemistry

As shown in Scheme 1, firstly, 2-nitrophenols were reduced by $SnCl_2 \cdot 2H_2O$ to afford 2-aminophenol derivatives (**1a–c**) [22], which were further cyclized with cyanogen bromide (BrCN) in methanol to give the key intermediates 5-substituted 2-aminobenzoxazoles (**2a–d**). Then, C-5-alkyl-substituted 2-aminobenzoxazole derivatives (**3a–y**) were smoothly obtained by Suzuki cross-coupling reaction. Meanwhile, amides (**4a–j**) and sulfonamides (**5a–i**) derivatives were conveniently prepared by acylation reaction. The structures of all the target compounds were characterized by M. p., ^1H NMR, ^{13}C NMR and HRMS.

Antifungal activity

According to the mycelium linear growth rate method [19], all the target compounds (**2a–d**, **3a–y**, **4a–j** and **5a–i**) were screened in vitro for their antifungal activities at 50 μg/mL against eight phytopathogenic fungi [e.g., *Fusarium sulphureum* (FS), *Thanatephorus cucumeris* (TC), *Fusarium*
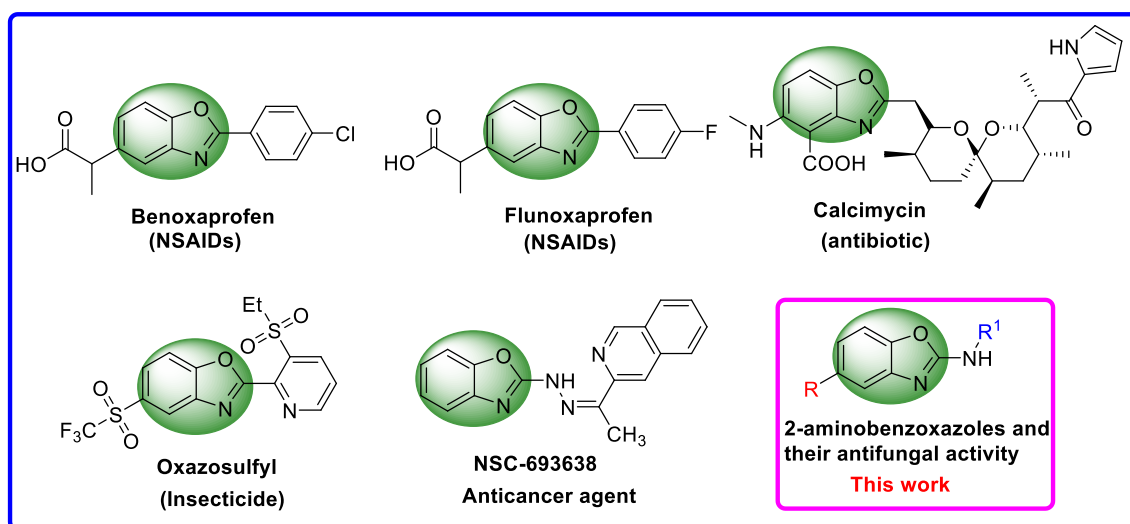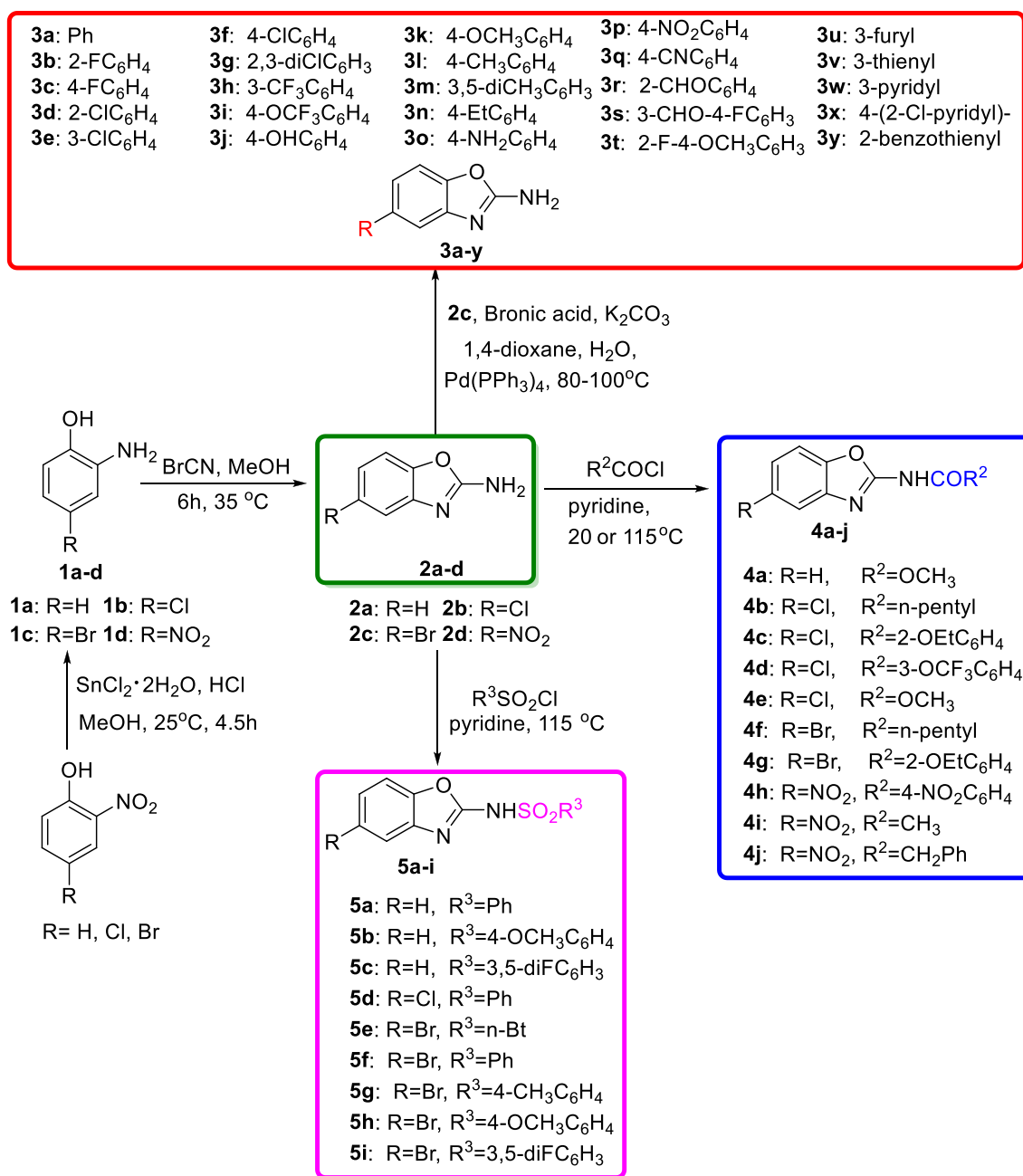

Fig. 1 Chemical structures of containing benzoxazole skeleton

Scheme 1 General synthetic rout of target compounds

oxysporum (FO), *Fusarium graminearum* (FG), *Botrytis cinerea* (BC), *Valsa mali* (VM), *Sclerotiua sclerotiorum* (SS) and *Alternaria solani* (AS)]. Hymexazol, a commercial agricultural fungicide, was used as the positive control.

As depicted in Table 1, most of the target compounds displayed good-to-excellent inhibitory effects on the growth of the tested phytopathogenic fungi at 50 µg/mL. Generally, these compounds can be divided into three groups according to the average inhibition rates. Compounds **3a–e**, **3h**, **3m**, **3u** and **3v** showed the highest activity with average

inhibition rates of 80.7–97.5%, compounds **2b**, **2c**, **3f**, **3i**, **3k**, **3p**, **3q**, **3t**, **3x**, **4h** and **5e** exhibited moderate activity with average inhibition rates of 46.1–70.2%, and the other compounds displayed weak activity against the tested fungi. For FS strain, it was worth mentioning that thirty compounds exhibited more pronounced antifungal activity (> 52.2%) than the positive control hymexazol (50.4%); particularly, the inhibition rates of compounds **3a**, **3b**, **3d**, **3e** and **3v** reached 100% at the concentration of 50 µg/mL. For TC strain, seven compounds **3a**, **3b**, **3c**, **3e**, **3h**, **3m** and **3v**

Table 1 Antifungal activities of the target compounds against phytopathogenic fungi at 50 μg/mL

No	R	R¹	Average inhibition rate ± SD (%) (n = 3)[a]							
			FS	TC	FO	FG	BC	VM	SS	AS
2a	H	H	45.1±1.3	5.0±0.3	37.5±1.1	28.8±1.5	11.4±2.3	41.0±2.4	34.4±3.1	20.5±0.8
2b	Cl	H	57.3±1.4	87.5±1.0	42.4±2.3	57.6±1.7	91.3±0.1	66.9±0.8	91.8±0.1	46.4±2.3
2c	Br	H	74.8±1.4	91.9±1.8	45.1±0.3	53.4±1.4	95.6±2.3	63.1±3.1	84.3±0.3	53.4±1.9
2d	NO_2	H	24.4±0.5	7.1±1.1	16.8±3.8	5.1±0.4	21.8±1.1	15.3±1.2	13.8±0.9	2.4±3.4
3a	Ph	H	100±0.3	100±0.1	100±0.5	70.5±2.7	100±0.1	93.7±1.2	100±0.8	100±0.2
3b	$2\text{-}FC_6H_4$	H	100±0.7	100±0.2	98.4±0.7	83.8±0.4	100±0.4	100±0.5	77.6±3.8	81.0±2.4
3c	$4\text{-}FC_6H_4$	H	99.8±2.5	100±0.1	100±2.6	99.5±0.8	100±0.5	82.8±0.9	99.8±0.9	98.4±2.7
3d	$2\text{-}ClC_6H_4$	H	100±1.2	63.2±3.5	89.9±1.3	62.3±0.9	97.8±0.1	95.9±1.5	71.0±2.4	100±0.2
3e	$3\text{-}ClC_6H_4$	H	100±0.4	100±0.3	96.6±3.1	86.9±4.3	100±0.3	100±0.6	99.5±0.9	87.3±3.7
3f	$4\text{-}ClC_6H_4$	H	87.0±2.2	49.4±4.4	57.3±0.5	70.4±2.4	83.3±1.3	75.8±1.5	64.2±1.3	38.9±0.5
3g	$2,3\text{-}diClC_6H_3$	H	15.1±1.2	29.4±2.9	48.8±2.3	39.2±3.7	44.6±1.3	32.8±2.4	29.0±3.8	15.1±3.6
3h	$3\text{-}CF_3C_6H_4$	H	81.0±1.5	100±0.4	69.9±0.7	43.9±3.5	87.8±1.3	90.7±0.9	91.1±0.9	81.0±2.4
3i	$4\text{-}OCF_3C_6H_4$	H	85.5±1.5	14.1±1.1	52.1±0.9	70.4±2.8	38.6±3.9	73.7±0.8	36.1±4.3	34.1±3.6
3j	$4\text{-}OHC_6H_4$	H	36.5±4.0	25.9±0.8	43.4±0.7	10.8±1.3	51.1±2.0	57.1±1.7	60.1±0.9	36.5±0.9
3k	$4\text{-}OCH_3C_6H_4$	H	79.7±2.5	22.4±2.9	54.7±1.5	44.6±2.2	62.1±3.5	79.0±0.4	50.3±2.5	49.2±2.7
3l	$4\text{-}CH_3C_6H_4$	H	20.6±3.5	16.7±2.7	3.9±1.3	3.5±1.8	52.3±3.1	21.2±1.5	32.2±2.5	20.6±2.7
3m	$3,5\text{-}diCH_3C_6H_3$	H	93.4±0.2	100±0.2	68.1±0.1	77.3±2.7	97.7±0.5	90.2±1.7	96.5±0.2	78.3±2.7
3n	$4\text{-}EtC_6H_4$	H	52.2±2.2	10.3±2.9	6.8±3.9	10.3±1.6	14.4±3.2	29.5±0.8	11.5±2.8	9.5±4.8
3o	$4\text{-}NH_2C_6H_4$	H	7.1±2.4	7.9±0.8	25.2±1.8	14.0±0.6	23.1±2.4	36.1±0.4	25.7±1.9	7.1±2.4
3p	$3\text{-}NO_2C_6H_4$	H	76.1±2.2	86.5±3.8	52.1±3.4	32.9±2.2	76.5±1.3	49.0±2.3	66.1±2.5	89.7±0.6
3q	$4\text{-}CNC_6H_4$	H	76.1±2.3	65.4±3.2	21.8±1.3	41.8±2.9	51.5±4.7	53.5±2.3	35.0±2.5	23.8±0.8
3r	$2\text{-}CHOC_6H_4$	H	10.3±4.0	9.2±0.4	51.6±1.8	21.6±2.7	33.7±4.0	41.2±2.9	48.6±4.1	10.3±2.7
3s	$3\text{-}CHO\text{-}4FC_6H_3$	H	59.4±3.3	10.9±4.0	17.1±1.9	6.1±2.9	28.0±1.3	21.0±0.4	25.1±3.4	4.8±1.8
3t	$2\text{-}F\text{-}4\text{-}OCH_3C_6H_3$	H	88.4±1.3	19.2±0.9	55.6±3.9	34.3±2.2	56.8±2.3	65.9±0.8	72.1±1.6	39.7±1.4
3u	3-furyl	H	96.0±1.7	80.8±1.9	89.6±0.7	100±0.4	100±0.2	99.5±0.9	98.9±1.9	100±0.1
3v	3-thienyl	H	100±0.2	97.8±0.5	94.3±4.7	46.8±1.0	80.7±1.3	87.6±0.9	60.9±2.9	100±0.1
3w	3-pyridyl	H	14.3±4.0	29.8±3.3	27.1±1.3	28.7±2.7	33.0±3.0	34.8±1.3	36.1±1.7	14.3±4.1
3x	4-(2-Cl-pyridyl)-	H	79.7±4.5	69.2±0.7	33.3±2.5	40.4±4.3	87.1±3.0	50.0±1.5	88.0±3.2	38.1±2.4
3y	2-benzothienyl	H	6.3±3.1	0.9±1.3	3.1±1.3	6.4±1.0	38.6±0.4	11.6±0.9	12.0±4.1	6.3±2.7
4a	H	CH_3OCO	20.7±1.5	0	9.8±1.5	0	0	8.1±0.2	0	7.3±0.7
4b	Cl	n-penylCO	1.6±2.8	11.3±0.2	3.0±2.3	4.7±0.8	19.7±1.5	6.4±0.3	5.7±1.2	29.2±0.2
4c	Cl	$2\text{-}OEtC_6H_4CO$	8.1±1.2	17.3±0.4	9.1±1.3	4.2±1.4	7.7±2.6	13.3±0.9	20.2±1.1	27.8±2.1
4d	Cl	$3\text{-}OCF_3C_6H_4CO$	18.7±1.1	29.9±0.1	9.8±1.3	28.6±0.8	64.5±3.4	44.4±0.4	20.6±3.3	26.4±2.4
4e	Cl	CH_3OCO	68.3±3.4	0	8.2±2.3	3.8±0.6	3.8±1.3	8.9±1.2	0	3.7±1.7

Table 1 (continued)

No	R	R^1	Average inhibition rate ± SD (%) ($n=3$)[a]							
			FS	TC	FO	FG	BC	VM	SS	AS
4f	Br	n-penylCO	13.8±2.2	0.8±0.7	2.3±2.3	27.3±1.0	2.1±0.7	0	3.5±1.8	10.6±2.5
4g	Br	2-OEtC$_6$H$_4$CO	43.1±1.6	5.0±0.4	4.5±0.2	25.3±1.6	4.5±1.4	5.1±3.2	2.0±1.8	12.1±2.6
4h	NO$_2$	4-NO$_2$C$_6$H$_4$CO	51.2±0.2	75.4±3.4	44.6±1.5	35.6±1.4	84.2±0.9	45.2±4.7	43.4±1.9	23.2±2.5
4i	NO$_2$	CH$_3$CO	62.8±1.6	1.2±1.7	6.5±1.6	0	6.8±2.1	13.7±1.3	3.9±1.9	0
4j	NO$_2$	PhCH$_2$CO	71.3±2.6	86.5±1.1	39.1±1.5	37.8±1.8	54.1±1.1	42.7±1.2	3.9±0.6	25.6±1.7
5a	H	PhSO$_2$	71.5±2.9	0	16.7±2.6	29.8±2.3	55.3±3.7	22.1±0.9	39.6±1.8	19.7±0.6
5b	H	4-OCH$_3$C$_6$H$_4$SO$_2$	74.0±1.6	5.0±0.5	30.3±3.5	45.5±0.2	68.2±1.5	46.7±3.6	46.9±0.2	22.7±4.5
5c	H	3,5-diClC$_6$H$_3$SO$_2$	51.2±2.5	5.0±0.3	16.7±1.3	23.2±1.7	75.0±3.9	13.8±0.8	53.1±3.4	7.6±2.5
5d	Cl	PhSO$_2$	26.0±0.9	5.0±1.3	6.8±0.1	0.9±1.3	56.1±1.2	8.7±0.9	42.7±0.9	9.1±4.5
5e	Br	n-BtSO$_2$	67.5±1.6	20.4±0.7	47.7±2.3	37.9±1.6	86.4±0.5	25.6±3.9	72.9±0.9	24.2±2.6
5f	Br	PhSO$_2$	77.2±1.4	5.0±1.0	28.0±1.3	37.9±0.3	73.5±3.5	14.4±2.4	47.9±1.8	27.3±0.3
5g	Br	4-CH$_3$C$_6$H$_4$SO$_2$	20.3±0.7	17.5±1.3	18.2±0.3	6.6±1.4	71.2±1.3	20.5±3.9	77.1±1.6	25.8±1.4
5h	Br	4-OCH$_3$C$_6$H$_4$SO$_2$	68.3±3.7	33.3±2.5	39.4±1.3	26.3±0.3	68.2±3.9	17.9±3.9	65.6±1.1	31.8±1.7
5i	Br	3,5-diFC$_6$H$_3$SO$_2$	12.2±3.7	4.0±0.2	13.8±3.5	6.0±1.3	63.6±1.7	13.8±1.1	53.1±1.4	13.6±1.7
Hymexazol			**50.4±1.3**	**89.7±0.5**	**53.5±0.9**	**64.2±1.0**	**88.6±0.1**	**10.1±1.5**	**70.2±1.3**	**42.9±0.6**

[a]Values are the mean ± SE of three replicates

showed considerable antifungal activity with inhibition rates over 97.8%, which were much better than that of hymexazol (89.7%). Furthermore, compounds **3a–3f**, **3m** and **3v** were found to exhibit higher antifungal activity against FO and FG strains than hymexazol, and compounds **2b**, **2c**, **3a–3e**, **3h**, **3m** and **3u** possessed significant antifungal activity against BC, SS and AS (53.4–100%) in comparison with hymexazol (42.9–88.6%). To our delight, almost all the target compounds displayed better antifungal activity against VM than hymexazol (10.1%). In general, compounds **3a**, **3b**, **3c**, **3e** and **3m** presented more promising antifungal activity against a broad spectrum of phytopathogenic fungi than the commercial agricultural fungicide hymexazol.

Moreover, some interesting results of structure–activity relationships (SARs) were found as follows (Fig. 2): (1) Introduction of chlorine and bromine atoms is very beneficial to enhance the antifungal activity (**2b** and **2c** vs. **2a** and **2d**). (2) Amino group is very essential for the antifungal activity, acylation and sulfonylation which can obviously decrease the activity against some tested fungi. (3) Compared with the intermediate compound **2c**, selection of suitable aryl groups replacing bromine atom can significantly improve the antifungal effects, such as the inhibition rates of compounds **3a** (Ph), **3b** (2-FC$_6$H$_4$), **3c** (4-FC$_6$H$_4$), **3d** (2-ClC$_6$H$_4$), **3e** (3-ClC$_6$H$_4$), **3m** (3,5-diCH$_3$C$_6$H$_3$), **3u** (3-furyl) and **3v** (3-thienyl) against most of strains were over 90%. (4) It is noteworthy that introduction of electron-withdrawing groups (such as F, Cl, CF$_3$, NO$_2$ and CN) at the 5-position on phenyl ring of compound **3a** could result in more potent compounds than electron-donating groups (such as OH, CH$_3$, Et, NH$_2$) excepted for compounds **3g** and **3m** (**3b–3f**, **3h**, **3p** and **3q** vs. **3j**, **3l**, **3n** and **3o**). (5) The number and position of chlorine atom also have some influence on the antifungal activity. For instance, mono-chloro compounds (**3d**, **3e** and **3f**) exhibited much better antifungal activity than the corresponding bis-chloro compound (**3g**), and the effect

of chlorine atom position on antifungal activity was meta-(**3e**) > ortho-(**3d**) > para-(**3f**). (6) The target compounds bearing furan (**3u**) and thiophene (**3v**) ring at the 5-position of compound **2a** displayed better inhibition effects than those bearing a pyridine (**3w**), 2-chloropyridine (**3x**) and benzothiophene ring (**3y**). The aforementioned result demonstrates that the antifungal effect can be dramatically influenced by the substituents group on the NH$_2$ and 5-position of 2-aminobenzoxazole.

To further evaluate the inhibitory of the most promising synthesized compounds, the median effective concentrations (EC$_{50}$) values of compounds **3a–e**, **3h**, **3m**, **3u** and **3v** against eight phytopathogenic fungi were tested. As shown in Table 2, it was noticed that the nine compounds exhibited impressive antifungal effects against FS, FO, VM, SS and AS, which were better than that of hymexazol. For example, compounds **3a**, **3b** and **3m** exhibited the best anti-FS effects in vitro, with the EC$_{50}$ values as low as 3.96 μg/mL, 4.47 μg/mL and 4.10 μg/mL, respectively; compounds **3a–e**, **3m** and **3v** exhibited 5.1–12.6 folds more potent activities than hymexazol against FO strain; five compounds **3a**, **3c**, **3e**, **3h** and **3m** exhibited remarkable antifungal activity against SS strains in vitro, with the corresponding EC$_{50}$ values of 6.50 μg/mL, 2.82 μg/mL, 1.48 μg/mL, 5.00 μg/mL and 3.82 μg/mL, much superior to hymexazol (25.12 μg/mL). Furthermore, all the target compounds (except **3d** and **3u**) possessed higher antifungal effects than hymexazol against TC and BC strains. Regarding FG strains, compounds **3a** (EC$_{50}$ = 9.68 μg/mL) and **3b** (EC$_{50}$ = 6.91 μg/mL) are identified with excellent antifungal competence, compounds **3m**, **3u**, **3v**, **3c** and **3e** displayed moderate activity (EC$_{50}$ = 12.16–16.60 μg/mL), and compound **3d** with the EC$_{50}$ value of 27.8 μg/mL, which was comparable with that of hymexazol (32.36 μg/mL). This result suggested that the tested fungal displayed high susceptibility to the nine compounds. Meanwhile, the effects of compound **3d** on the growth of FS and FO strains
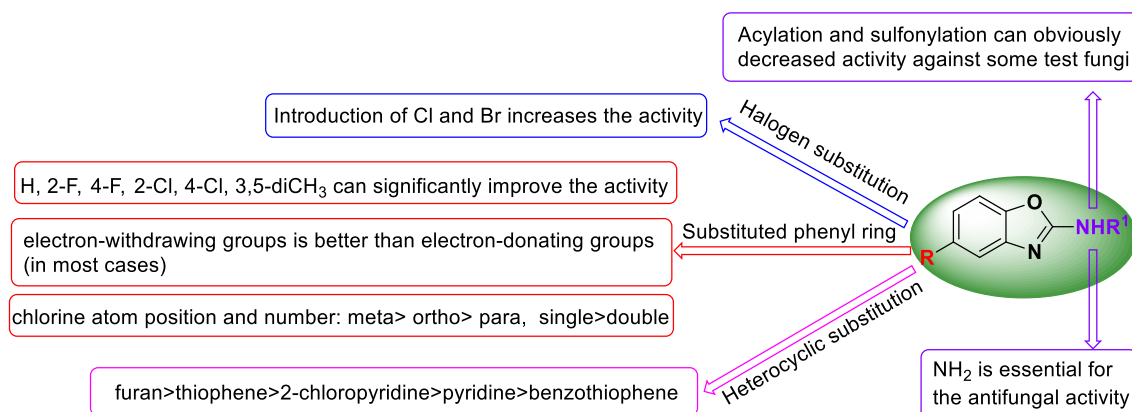

Fig. 2 Structure–activity relationship of the target compounds

Table 2 EC_{50} values of some selected compounds against eight phytopathogenic fungi

Compounds	$EC_{50} \pm SD$ values (µg/mL)[a]							
	FS	TC	FO	FG	BC	VM	SS	AS
3a	*3.96 ± 0.2*	5.08 ± 0.2	4.36 ± 0.4	9.68 ± 1.1	2.40 ± 0.5	5.56 ± 0.2	6.50 ± 0.7	*3.50 ± 0.2*
3b	*4.47 ± 0.1*	9.07 ± 1.2	*3.35 ± 0.5*	*6.91 ± 0.9*	5.18 ± 0.8	7.07 ± 0.3	15.95 ± 0.5	5.60 ± 0.1
3c	10.69 ± 0.3	6.49 ± 0.5	*3.13 ± 0.2*	12.16 ± 2.1	*1.81 ± 0.2*	3.97 ± 0.1	2.82 ± 0.2	4.43 ± 0.3
3d	5.60 ± 0.1	27.23 ± 0.4	7.40 ± 0.8	27.8 ± 1.9	6.91 ± 0.3	5.75 ± 1.1	13.20 ± 1.1	6.15 ± 0.6
3e	5.77 ± 0.2	10.60 ± 0.7	7.67 ± 0.2	16.60 ± 1.4	*1.69 ± 0.8*	4.19 ± 0.2	*1.48 ± 0.3*	6.10 ± 0.9
3h	12.65 ± 0.4	4.69 ± 0.5	15.27 ± 1.0	> 50	5.17 ± 0.7	4.99 ± 0.1	5.00 ± 1.0	11.22 ± 1.2
3m	*4.10 ± 0.3*	*2.23 ± 0.3*	7.41 ± 0.1	14.02 ± 0.2	1.89 ± 1.4	5.95 ± 0.2	3.82 ± 0.7	12.02 ± 1.3
3u	7.83 ± 0.1	19.95 ± 1.4	28.10 ± 0.7	13.18 ± 2.1	6.42 ± 0.5	6.98 ± 0.2	15.82 ± 0.6	4.71 ± 0.6
3v	5.60 ± 0.1	11.10 ± 1.1	7.41 ± 0.3	14.09 ± 0.4	3.85 ± 0.1	4.78 ± 1.2	13.81 ± 0.5	4.10 ± 0.2
Hymexazol	**43.8 ± 0.6**	**17.78 ± 2.1**	**39.40 ± 1.1**	**32.36 ± 2.0**	**6.30 ± 0.2**	**> 50**	**25.12 ± 0.5**	**48.98 ± 2.1**

[a]50% Effective concentration: concentration of compound that inhibits the fungi growth

at different concentrations are shown in Fig. 3. It's obviously that the antifungal efficiency significantly depended on the drug concentrations.

The morphological changing of *Fusarium solani* (FS) and *Alternaria solani* (AS) was then viewed under the light microscope. From Fig. 4, it can be seen that the FS control group mycelium had an eel shape, smooth surface, uniform size and much-branched, but the compound **3a** treatment group mycelium appeared invagination, shriveling and few-branched (FS-CK vs. FS-**3a**). Meanwhile, the AS mycelium of treatment with compound **3a** could produce more less oval-shaped spores than that of control group (AS-CK vs. AS-**3a**). This phenomenon indicated that these compounds might exert antifungal effect by significantly inhibiting the growth and differentiation of fungal.

Finally, the in vivo antifungal effects of the promising compounds were conducted against *Botrytis cinerea* on tomato at 100 µg/mL. As shown in Fig. 5, compounds **3a**, **3c**, **3e** and **3m** exhibited much better preventative effect than hymexazol (23.1%), with the corresponding preventative rates of 46.7%, 48.3%, 55.1% and 53.3%. The results verified

that 2-aminobenzoxazole derivatives could be used as a lead compounds for the discovery of novel agrichemicals.

Molecular docking studies

Studies in the literature have reported that lipid transfer protein Sec14p (*Saccharomyces cerevisiae*) might be a potential target of inducing fungicidal activity by benzoxazole derivatives [12]. In an effort to elucidate the hypothesis that our compounds acted on sec14p, molecular docking of compounds **3a**, **3b**, **3d**, **3h**, **3m** and **3u** into the homology model according to the binding site of benzoxazoles on reported lipid binding pocket of Sec14p was performed, respectively. As shown in Fig. 6, the six-test compounds shared very similar binding modes with the literature reported. Benzoxazole ring was oriented pi-pi stacked interaction with Tyr 151 and pi-alkyl interaction with Arg208. The substituted phenyl ring made pi-pi stacked, pi-donor hydrogen bond and pi-alkyl interactions with Phe212 and Thr175 and Met209, respectively. This result suggested that the tested compounds were compatible with the active site of sec14p.

Fig. 3 Effects of compound **3d** on the growth of FS and FO at different concentrations (CK: blank control group)
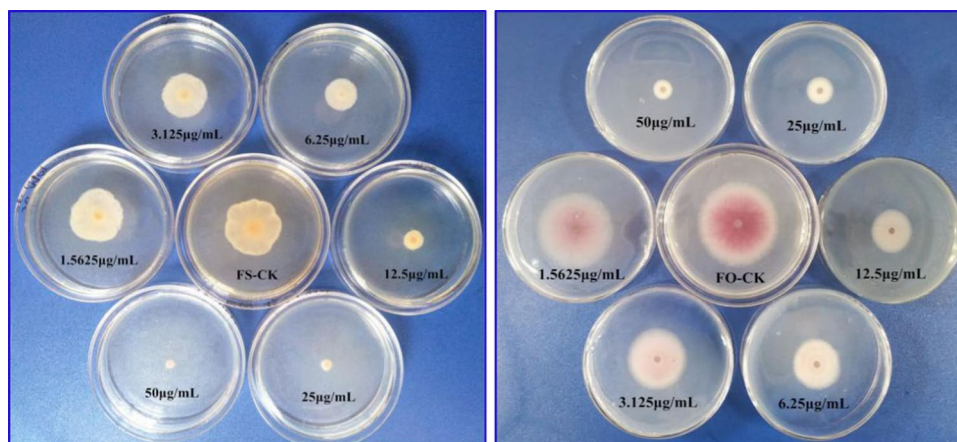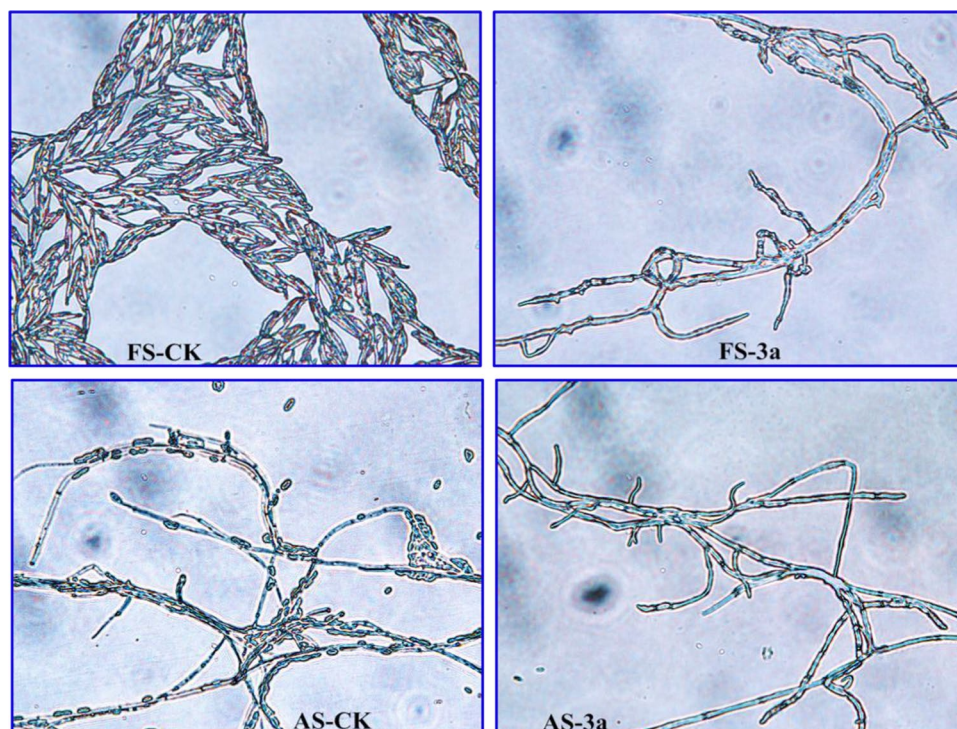
Fig. 4 The effects of compound **3a** on the growth of FS and AS at 25 µg/mL (FS-CK and AS-CK represented the normal mycelium morphology of FS and AS, and FS-**3a** and AS-**3a** represented the mycelium morphology of FS and AS after treatment with compound **3a**)

Compounds (100 µg/mL)	Hymexazol	**3a**	**3c**	**3e**	**3m**	CK
Preventative Rate (%)	23.1 ± 2.3	46.7 ± 1.1	48.3 ± 0.1	55.1 ± 1.3	53.3 ± 0.5	—

Fig. 5 In vivo antifungal activity of compounds against *Botrytis cinerea*

Conclusion

In summary, three series of simple 2-aminobenzoxazole derivatives were synthesized and firstly evaluated for their antifungal activities. To our delight, most of the target compounds demonstrated moderate-to-excellent antifungal activities in vitro; particularly, compounds **3a**, **3b**, **3c**, **3e**, **3m** and **3v** presented more promising and comprehensive antifungal capacity against the corresponding phytopathogenic fungi than the commercialized fungicide hymexazol, with the EC_{50} values of 2.40–9.68 µg/mL, 3.35–15.95 µg/mL, 1.81–10.69 µg/mL, 1.48–16.6 µg/mL, 1.89–14.02 µg/mL and 3.85–14.09 µg/mL, respectively. Moreover, the in vivo experiments against *Botrytis cinerea* on tomato bioassay also demonstrated that compounds **3a**, **3c**, **3e** and **3m** had much better preventative effect than that of hymexazol. Thus, these compounds can be used as potential agricultural fungicides to protect many crops, vegetable and fruit. In addition, the SARs demonstrated that introduction of appropriate substituents on the 5-position of 2-aminobenzoxazole would lead to more potent derivatives. This study will lay a significant foundation for further preparation and application of 2-aminobenzoxazole derivatives as potential small molecular agrochemicals.
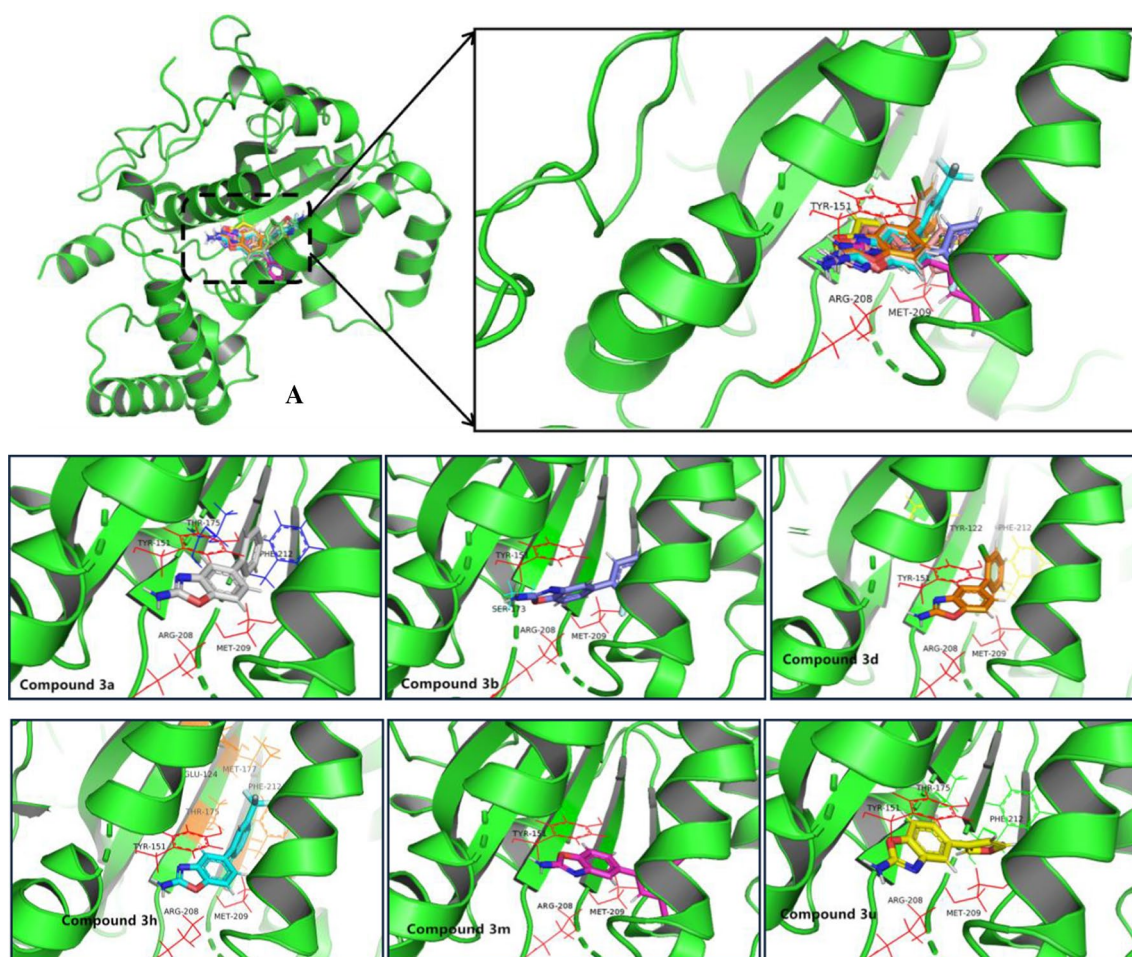
Fig. 6 The binding models of the test compounds into the lipid binding pocket of Sec14p from *S. cerevisiae*. **a** Overlay of the six test compounds into the active site of Sec14p: **3a** (gray), **3b** (blue), **3d** (orange), **3h** (light blue), **3m** (peach) and **3u** (yellow)

Experimental

All reagents and solvents were of reagent grade or purified according to standard methods before use. Thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were used with silica gel 60 GF254 (Qingdao Haiyang Chemical Co., Ltd., China). Melting points were determined by XY-4 melting point meter (Beijing Taike Instrument Co., Ltd., China). Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon-13 nuclear magnetic resonance spectra (^{13}C NMR) were recorded on Bruker Avance NEO 600/400 MHz and 150/100 MHz instruments, respectively, using TMS as the internal standard and CDCl$_3$ or DMSO-d_6 as the solvent. High-resolution mass spectra (HR-MS) were carried out with APEX II Bruker 4.7TAS instrument.

Synthesis of compounds 1a–c [22]

A solution of Stannous chloride dihydrate (SnCl$_2$·2H$_2$O, 40 g, 177.3 mmol) and HCl (80 mL) in methanol (150 mL) was cooled to 0 °C, and 2-nitrophenol derivatives (36.7 mmoL) was added. The mixture was stirred for 4.5 h at room temperature. Afterwards, the solution was diluted with ethyl acetate (30 mL) and neutralized with NaHCO$_3$ solution (to PH = 7). Subsequently, the solution was filtered to remove the white solid precipitated, then the organic phase was separated, and the water phase was extracted with ethyl acetate (50 mL × 3). Finally, the resulting organic phases was washed with brine, dried over anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure. The crude material was then purified by silica gel column chromatography to give the compounds **1a–c** in 85–90% yield as a white or fawn solid.

1a: ESI–MS (m/z): 110.05 $(M+H)^+$; **1b**: ESI–MS (m/z): 144.02 $(M+H)^+$; **1c**: ESI–MS (m/z): 187.94 $(M+H)^+$.

Synthesis of compounds 2a–d

Cyanogen bromide (35.9 mmol) was added to a solution of 2-aminophenol derivatives (26.5 mmol) in 25 mL methanol, and the mixture was reacted for 6 h at the 35 °C. Subsequently, the organic solvent was removed and the resulting residue was diluted with cold water, and the pH was adjusted to 8–9 with aqueous $NaHCO_3$ and then extracted with EtOAc (3 × 50 mL). Finally, the resulting organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude material was then purified by silica gel column chromatography to give the compound **2a–d**.

2-Aminobenzoxazole (2a)

Yield: 88.0%; brown solid; m.p. 121–122 °C; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.56 (s, 2H), 7.36 (d, 2H, $J = 8.0$ Hz), 7.16 (t, 2H, $J = 8.0$ Hz). ESI–MS (m/z): 135.05 $(M+H)^+$.

2-Amino-5-chlorobenzoxazole (2b)

Yield: 85.2%; white solid; m.p. 159–161 °C; ^1H NMR (400 MHz, DMSO-d_6) δ: 7.61 (s, 2H), 7.34 (d, 1H, $J = 8.4$ Hz), 7.23 (d, 1H, $J = 2.2$ Hz), 6.97 (dd, 1H, $J = 8.4$, 2.2 Hz). ESI–MS (m/z): 169.01 $(M+H)^+$.

2-Amino-5-bromobenzoxazole (2c)

Yield: 75.3%; brown solid; m.p. 175–177 °C; ^1H NMR (400 MHz, DMSO-d_6) δ: 7.59 (s, 1H), 7.34 (s, 1H), 7.29 (d, 1H, $J = 7.6$ Hz), 7.10 (d, 1H, $J = 7.6$ Hz); ESI–MS (m/z): 212.90 $(M+H)^+$.

5-Nitrobenao[*d*]oxazol-2-amine (2d)

Yield: 89.3%; yellow solid; m.p. 275–277 °C; ^1H NMR (400 MHz, DMSO-d_6) δ: 8.02 (s, 2H), 7.92 (m, 2H), 7.53 (d, 1H, $J = 8.4$ Hz); ESI–MS (m/z): 180.02 $(M+H)^+$.

The general procedures for synthesis of compounds 3a–y

In a 25-mL round-bottom flask containing dioxane/H_2O (V:$V = 5$:1) (3 mL), K_2CO_3 (110 mg, 0.752 mmol), 2-amino-5-bromobenzoxazole (**2c**, 83 mg, 0.376 mmol), substituted boronic acid (0.41 mmol) and Pd(PPh$_3$)$_4$ (14 mg, 0.01 mmol) were added and reacted at 80 °C under N_2. When the reaction was complete (TLC control), the organic solvent was removed. The crude material was then purified by silica gel column chromatography to give desired products **3a–y**. The data of compounds **3a**–**y** can be found in the Supporting information.

The general procedures for synthesis of compounds 4a–j and 5a–i

In a 25-mL round-bottom flask containing pyridine (1 mL), 2-aminobenzoxazole derivatives (0.5 mmol) and substituted acyl chloride/sulfonyl chloride (0.55 mmol) were added and reacted at 20 °C (**4a** and **4e**) or 115 °C under N_2. The reaction progress was checked by TLC analysis. When the reaction was complete, the mixture was poured into the water and adjusted to the pH = 3–4 by 6 N HCl. Then, the mixture was extracted with EtOAc (3 × 20 mL), and the combined organic phase was dried over anhydrous sodium sulfate (Na_2SO_4), concentrated *in vacuo* and purified by silica gel column chromatography to afford compounds **4a–j** and **5a–i**. The data of compounds **4a–j** and **5a–i** can be found in the Supporting information.

Antifungal assay

Antifungal activities assay in vitro: three series of benzoxazole derivatives were screened in vitro for their antifungal activities against eight phytopathogenic fungi by the mycelial growth inhibitory rate method according to previously reported approaches [19]. Eight phytopathogenic fungi such as *Fusarium sulphureum* (FS), *Thanatephorus cucumeris* (TC), *Fusarium oxysporum* (FO), *Fusarium graminearum* (FG), *Botrytis cinerea* (BC), *Valsa mali* (VM), *Alternaria alternata* (AA) and *Alternaria solani* (AS) were used for the assays. Potato dextrose agar (PDA) medium was prepared in the flasks and sterilized. All target compounds were dissolved in acetone before mixing with PDA, and the concentration of test compounds in the medium was fixed at 50 μg/mL. Subsequently, 50% effective concentration (EC_{50}) values of some selected compounds were further calculated. The medium was then poured into sterilized Petri dishes. All types of fungi were incubated in PDA at 28 ± 1 °C for 5 days to get new mycelium for the antifungal assays, and a mycelia disk of approximately 4 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 days. Acetone without any compounds mixed with PDA was served as a control, while hymexazol, a commercial agricultural fungicide, was used as positive controls. 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: Inhibition rate $(\%) = (C - T) \times 100/(C - 4 \text{ mm})$, where C represents the diameter of fungi growth on untreated PDA, and T represents the diameter of fungi on treated PDA. Finally, the linear regressions of inhibition rates (%) versus seven concentrations of some selected compounds and hymexazol were obtained, and the EC_{50} values were calculated. The therapeutic effect of the test compounds on *B. cinerea* in vivo was calculated by the formula: Therapeutic effect $(\%) = (C - T) \times 100/(C - 4 \text{ mm})$, where C represents the diameter of *B. cinerea* growth on CK, and T represents the diameter of *B. cinerea* on treated group [23]. Statistical analysis was processed by the SPSS 21.0 (SPSS Inc., Chicago, USA) software.

Molecular docking studies

The crystal structure the lipid transfer protein sec14p from *Saccharomyces cerevisiae* was provided from the Brookhaven protein data bank (PDB 6F0E; http://www.rcsb.org/pdb). Docking studies were performed by using SYBYL-X 2.0 software. Geometric and energy optimization of these compounds was conducted with Gasteiger–Huckel charges in the Tripos force field and an energy convergence gradient of 0.005 kcal/mol and a maximum of 10,000 iterations. The receptor–ligand interactions on 2D diagram was calculated by using Discovery Studio visualizer v21.1. The analysis of docking poses was carried out by molecular graphics system PYMOL v2.2.1 (DeLano Scientific LLC, USA).

Supporting Information

Spectral images of ^{1}H-NMR, ^{13}C-NMR and HRMS are provided in the Supporting Information Section.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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