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 EC50 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 SnCl₂·2H₂O 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, ¹³C 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*...
oxysporum (FO), Fusarium graminearum (FG), Botrytis cinerea (BC), Valsa mali (VM), Sclerotinia 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

Scheme 1  General synthetic rout of target compounds
### Table 1  Antifungal activities of the target compounds against phytopathogenic fungi at 50 μg/mL.

| Compounds | Average inhibition rate ± SD (%) \( (n = 3) \) |
|-----------|------------------------------------------------|
|           | FS  | TC  | FO  | FG  | BC  | VM  | SS  | AS  |
| 2a        | R   | R'  |     |     |     |     |     |     |
|           |     |     |     |     |     |     |     |     |
| 3a        |     |     |     |     |     |     |     |     |
| 3b        |     |     |     |     |     |     |     |     |
| 3c        |     |     |     |     |     |     |     |     |
| 3d        |     |     |     |     |     |     |     |     |
| 3e        |     |     |     |     |     |     |     |     |
| 3f        |     |     |     |     |     |     |     |     |
| 3g        |     |     |     |     |     |     |     |     |
| 3h        |     |     |     |     |     |     |     |     |
| 3i        |     |     |     |     |     |     |     |     |
| 3j        |     |     |     |     |     |     |     |     |
| 3k        |     |     |     |     |     |     |     |     |
| 3l        |     |     |     |     |     |     |     |     |
| 3m        |     |     |     |     |     |     |     |     |
| 3n        |     |     |     |     |     |     |     |     |
| 3o        |     |     |     |     |     |     |     |     |
| 3p        |     |     |     |     |     |     |     |     |
| 3q        |     |     |     |     |     |     |     |     |
| 3r        |     |     |     |     |     |     |     |     |
| 3s        |     |     |     |     |     |     |     |     |
| 3t        |     |     |     |     |     |     |     |     |
| 3u        |     |     |     |     |     |     |     |     |
| 3v        |     |     |     |     |     |     |     |     |
| 3w        |     |     |     |     |     |     |     |     |
| 3x        |     |     |     |     |     |     |     |     |
| 3y        |     |     |     |     |     |     |     |     |
| 4a        |     |     |     |     |     |     |     |     |
| 4b        |     |     |     |     |     |     |     |     |
| 4c        |     |     |     |     |     |     |     |     |
| 4d        |     |     |     |     |     |     |     |     |
| 4e        |     |     |     |     |     |     |     |     |
Table 1 (continued)

| Compounds | Average inhibition rate ± SD (%) (n = 3)a |
|------------|------------------------------------------|
|            | FS  | TC  | FO  | FG  | BC  | VM  | SS  | AS  |
| No         |     |     |     |     |     |     |     |     |
| 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-OEtC₆H₄CO | 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         | NO₂ | 4-NO₂C₆H₄CO | 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         | NO₂ | CH₃CO | 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         | NO₂ | PhCH₃CO | 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   | PhSO₂ | 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-OCH₃C₆H₄SO₂ | 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-diClC₆H₃SO₂ | 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  | PhSO₂ | 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-BtSO₂ | 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  | PhSO₂ | 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-CH₃C₆H₄SO₂ | 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-OCH₃C₆H₄SO₂ | 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-diFC₆H₃SO₂ | 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 |

aValues 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-FC₆H₄), 3c (4-FC₆H₄), 3d (2-CIC₆H₄), 3e (3-CIC₆H₄), 3m (3,5-diCH₃C₆H₄), 3u (3-furanyl) 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, CF₃, NO₂ 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, CH₃, Et, NH₂) 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 NH₂ and 5-position of 2-aminobenzoazole.

To further evaluate the inhibitory of the most promising synthesized compounds, the median effective concentrations (EC₅₀) 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 EC₅₀ 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 EC₅₀ 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 (EC₅₀ = 9.68 μg/mL) and 3b (EC₅₀ = 6.91 μg/mL) are identified with excellent antifungal competence, compounds 3m, 3u, 3v, 3c and 3e displayed moderate activity (EC₅₀ = 12.16–16.60 μg/mL), and compound 3d with the EC₅₀ 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
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.

### Table 2

| Compounds | **EC_{50}± 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 | > 50 | 25.12 ± 0.5 | 48.98 ± 2.1 |

^a50% Effective concentration: concentration of compound that inhibits the fungi growth
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. 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)

**Fig. 5** In vivo antifungal activity of compounds against Botrytis cinerea
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 (\(^1\)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.

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)
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 NaHCO₃ and then extracted with EtOAc (3 × 50 mL). Finally, the resulting 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.

**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 (EC₅₀) 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 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 \(B.\, \text{cinerea}\) in vivo was calculated by the formula: Therapeutic effect (%) = \((C - T) \times 100/ (C - 4\, \text{mm})\), where \(C\) represents the diameter of \(B.\, \text{cinerea}\) growth on CK, and \(T\) represents the diameter of \(B.\, \text{cinerea}\) 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 \(Saccharomyces\, \text{cerevisiae}\) 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 \(^1\text{H}\)-NMR, \(^13\text{C}\)-NMR and HRMS are provided in the Supporting Information Section.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11030-021-10213-7.

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**Declarations**

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

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