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Research paper

Lead optimization generates selenium-containing miconazole CYP51 inhibitors with improved pharmacological profile for the treatment of fungal infections

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ABSTRACT

A series of selenium-containing miconazole derivatives were identified as potent antifungal drugs in our previous study. Representative compound **A03** (MIC = 0.01 μg/mL against *C.alb.* 5314) proved efficacious in inhibiting the growth of fungal pathogens. However, further study showed lead compound **A03** exhibited potential hemolysis, significant cytotoxic effect and unfavorable metabolic stability and was therefore modified to overcome these drawbacks. In this article, the further optimization of selenium-containing miconazole derivatives resulted in the discovery of similarly potent compound **B17** (MIC = 0.02 μg/mL against *C.alb.* 5314), exhibiting a superior pharmacological profile with decreased rate of metabolism, cytotoxic effect and hemolysis. Furthermore, compound **B17** showed fungicidal activity against *Candida albicans* and significant effects on the treatment of resistant *Candida albicans* infections. Meanwhile, compound **B17** not only could reduce the ergosterol biosynthesis pathway by inhibiting CYP51, but also inhibited biofilm formation. More importantly, compound **B17** also shows promising *in vivo* efficacy after intraperitoneal injection and the PK study of compound **B17** was evaluated. In addition, molecular docking studies provide a model for the interaction between the compound **B17** and the CYP51 protein. Overall, we believe that these selenium-containing miconazole compounds can be further developed for the potential treatment of fungal infections.

1. Introduction

Due to high incidence and high mortality, invasive fungal infections (IFIs) remain a major global public health problem [1–3]. The current situation of drug therapy for invasive fungal infections has the huge challenges: low efficacy, drug interaction, significant toxicity and drug resistance [4–6]. For example, fluconazole is used widely for treating fungal infections, but the use of fluconazole can be limited by hepatotoxicity and severe drug resistance, despite its low cost and fungistatic efficacy [7,8]. In a word, it is imperative to develop novel, effective and safe antifungal drugs [9]. Developing novel antifungal targets (such as AHAS) and modifying existing drugs (azole drugs) are effective means to develop effective antifungal drugs [10,11].

CYP51 (sterol 14α-demethylase) is a cytochrome P450 enzyme which is a major target for the treatment of fungal infection and protozoan infections [12–14]. CYP51 has been identified as a potential drug target for the reduction of fungal ergosterol (ergosterol plays a crucial role in the biosynthesis of fungal cell membranes) which is encoded by the ERG11 gene, and its inhibitors (Fig. 1) have demonstrated excellent antifungal activity both *in vivo* and *in vitro* [15–17]. Although some novel CYP51 inhibitors have been developed, it is still a huge challenge to convert *in vitro* activity to therapeutic efficacy due to unfavorable pharmacokinetic profiles and pharmacological properties [18,19].

In a previous paper, the lead finding process of a new class of selenium-containing miconazole CYP51 inhibitors was described and these derivatives exhibited excellent inhibitory activity against

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most clinically pathogenic fungus [20]. Among all synthesized compounds, compound **A03** (Fig. 2) showed more excellent antifungal activity than miconazole (Table 1). Meanwhile, our previous results suggested that the molecular target of compound **A03** is CYP51. In particular, compound **A03** could inhibit the growth of biofilms and kill the fungus. In view of its excellent antifungal activity and possible progression into a compound suitable for *in vivo* evaluation, compound **A03** was chosen for further pharmacological studies.

Firstly, cytotoxicity is a prerequisite for drug safety, so the cytotoxicity of compound **A03** was priority assessed. Our preliminary results showed compound **A03** displayed significant cytotoxicity (Table 2). The IC_{50} values of compound **A03** for inhibition of HL-60, MDA-MB-231 and PC-3 proliferation were 1.02, 9.06 and 13.10 μM, respectively. Secondly, the hemolytic activity is an important issue that should to be considered in drug design. Compound **A03** also showed hemolytic activity at moderate doses (4 μg/mL). Finally, the metabolic stability of compound **A03** was evaluated with mouse liver microsomes prior to the *in vivo* studies. However, compound **A03** was found to be labile in these liver fractions with 4.2 min half-life. Therefore, these suboptimal pharmacological properties limited the further study of compound **A03**.

In this article, we expected to avoid potential problems further down the road to a clinical candidate. Therefore, we thus proceeded to a lead optimization procedure with the aim of discovering novel derivatives of compound **A03** with comparable inhibitory potency but with an improved pharmacological profile. Therefore, 42 new analogues were synthesized and their structure-activity relationships were discussed. Much to our relief, among all the target compounds, compound **B17** displayed superior pharmacological profile than the previous best compound **A03**.

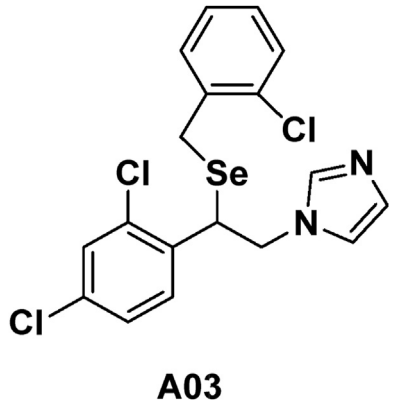


Fig. 2. Chemical structures of lead compound **A03**.

2. Results and discussion

2.1. Structural modification strategy of lead compound *A03*

In order to maintain the antifungal activity of lead compound **A03** and make some improvements in terms of cytotoxicity, hemolysis, and metabolic stability in liver microsomes, less drastic structural optimization will be performed in this study. Obviously, the chemical construction of compound **A03** fits structural characteristics of classical CYP51 inhibitors. The key pharmacophores of typical CYP51 inhibitors consist of three obvious sites for structural modification: (i) the dichlorophenyl (or difluorophenyl) ring, (ii) a side chain, (iii) the imidazole or 1,2,4-triazole ring. In our previous study, we had synthesized compounds **A17**-**A30** with difluorophenyl in place of dichlorophenyl, with a premise that the fluorine

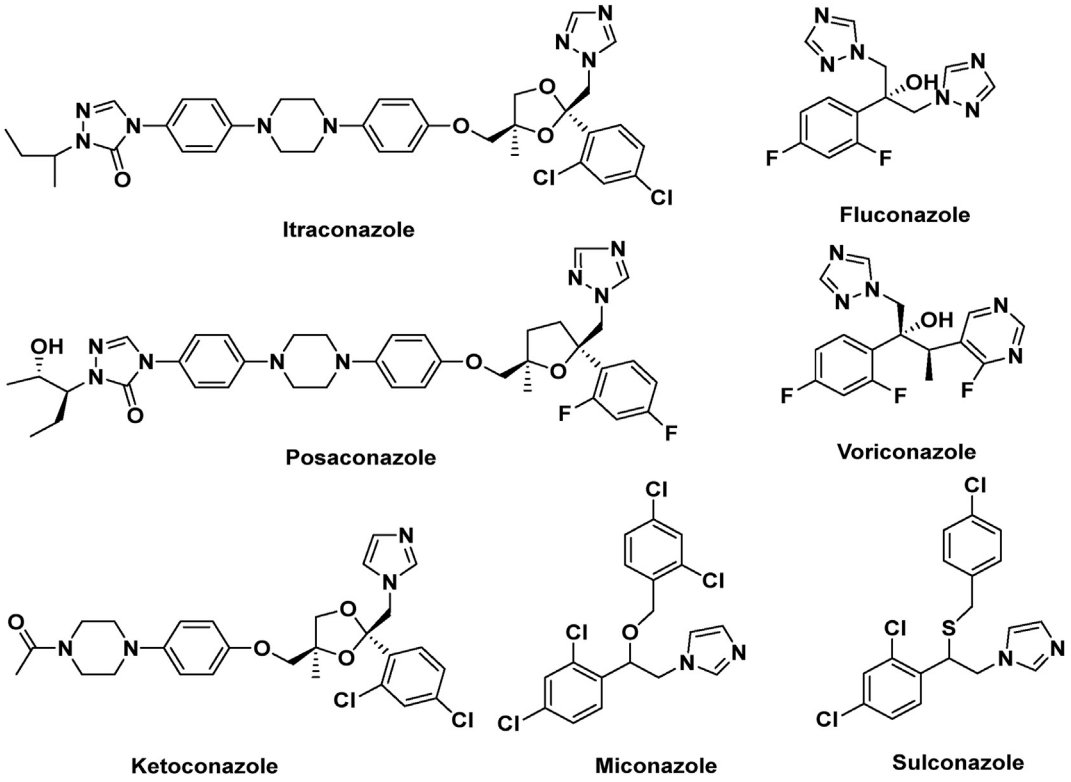


Fig. 1. Chemical structures of clinically used azole antifungal agents.

Table 1
The antifungal activity of the lead compound **A03**.

Compds	MIC/(μg/mL)	Compds	MIC/(μg/mL)	Compds	MIC/(μg/mL)	Compds	MIC/(μg/mL)	Compds
	C.alb		C.alb		C.alb		C.alb	
A03	0.02	0.01	1	0.25	0.13	0.5	0.13	2
Miconazole	0.25	0.03	1	0.5	0.5	0.5	0.25	2

Table 2
The physiological properties and pharmacokinetic profiles of the lead compound **A03**.

Compd.	Minimum hemolytic concentration (μg/mL)	IC_{50} μM			$T_{1/2}$ (min)	CL (mL/min/kg)
		HL-60	MDA-MB-231	PC-3		
A03	4	1.02	9.06	13.10	4.2	1311.5

atom(s) often enhances ADME properties. Indeed, metabolic stability has not been enhanced by this transformation. Therefore, we shifted our attention to the modification of imidazole group and side chain group. Based on the above analysis, derivatives **B01**—**B28** and **C01**—**C14** were designed and synthesized to observe whether these structural modifications could produce potent antifungal activity, combined with more safety and favorable metabolic stability (Fig. 3). Compounds **B01**—**B28** was designed by shorting benzyl to phenyl. In addition, the replacement of imidazole moiety with 1,2,4-triazole was carried out to obtain compounds **C01**—**C14**. Then further assay of *in vitro* pharmacological properties, cytotoxicity, hemolysis, and metabolic stability of the target compounds will be investigated.

2.2. Chemistry

The synthesis of reaction intermediates **3a**-**3n**, **8a**-**8p** and **14a**-**14d** is showed in Scheme 1, Scheme 2, and Scheme 3, respectively. The synthetic route of the contain-selenium miconazole analogues (**B01**—**B28** and **C01**—**C14**) is outlined in Scheme 4.

A series of analogues containing diselenide group (**3a**-**3n** and **8a**-**8p**) with various substitutions were first synthesized. The synthesis of intermediates diaryl selenide (**3a**-**3n**) commenced with the commercially available magnesium powder and a series of different bromobenzene by grignard reaction [21,22]. Phenyl magnesium bromide (**2a**-**2n**) were coupled under the action of selenium powder to give **3a**-**3n**. The disodium diselenide (**6**) were obtained by the alkylation of selenium powder with sodium borohydride under nitrogen condition. Next, we constructed the dibenzyl selenide (**8a**-**8p**) via chlorobenzyl or substituted chlorobenzyl (**7a**-**7p**) with disodium diselenide in dichloromethane by coupling reaction.

The general synthetic route of key intermediates **14a**-**14d** is shown in Scheme 3. Firstly, the intermediates **11a**-**11b** were synthesized by the acylation of 1,3-dichlorobenzene or 1,3-difluorobenzene with 2-chloroacetyl chloride under Friedel—Crafts acylation conditions. Subsequently, intermediates with an imidazole or 1,2,4-triazole group (**12a**-**12d**) were synthesized via a nucleophilic substitution reaction of **11a**-**11b**. Next, reduction reactions of intermediates **12a**-**12d** with sodium borohydride gave **13a**-**13d** under mild conditions. Finally, intermediates **13a**-**13d** were treated with thionyl chloride to yield the desired products **14a**-**14d**.

As shown in Scheme 4, intermediates **3a**-**3n** were dealt with sodium borohydride and the nucleophilic substitution occurred with **14a**-**14b** to afford target compounds **B01**—**B28**. By a similar method depicted in Scheme 4, intermediates **8a**-**8p** were treated with **14c**-**14d** in the presence of sodium borohydride to afford target compounds **C01**—**C14**.

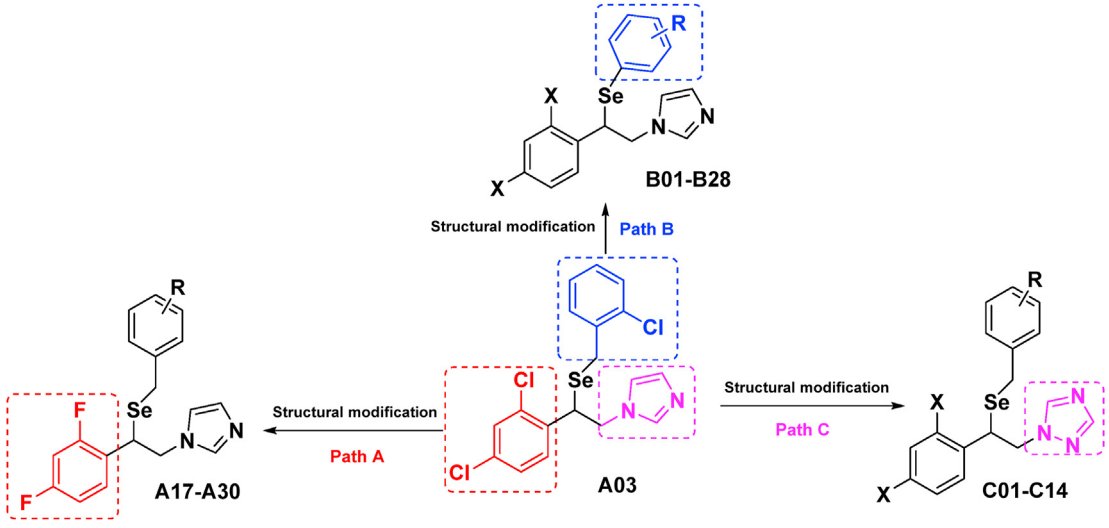
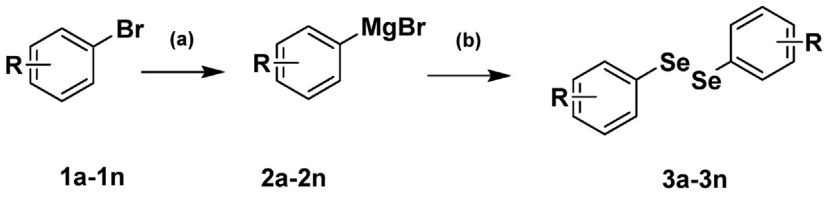
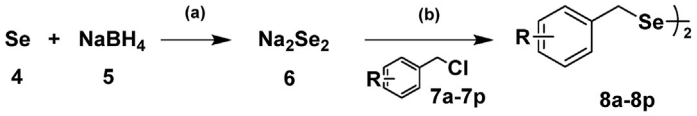


Fig. 3. General strategy for lead optimization.



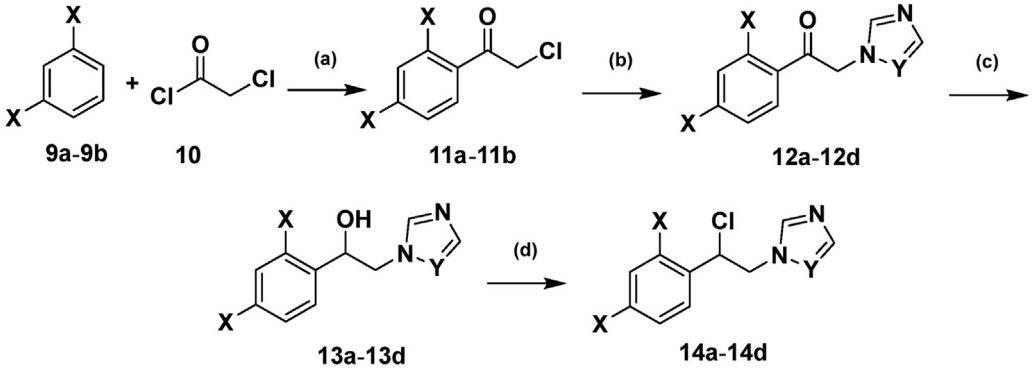
3a R=H; 3b R=4-F; 3c R=3-F; 3d R=4-CH_3; 3e R=3-CH_3; 3f R=2-CH_3; 3g R=4-OCH_3; 3h R=3-OCH_3; 3i R=2-OCH_3; 3j R=2,5-di-CH_3; 3k R=2,6-di-CH_3; 3l R=3,5-di-CH_3; 3m R=4-CH_2CH_3; 3n R=2-CH_2CH_3

Scheme 1. Synthesis of intermediates **3a**-**3n**
Reagents and conditions: (a) Mg, Et_2O, 30 °C, 30 min; (b) Se, NH_4Cl, 30 °C, 30 min.



8a R=H; 8b R=2,4-di-Cl; 8c R=2-Cl; 8d R=3-OCH_3; 8e R=3,5-di-OCH_3; 8f R=2-Br; 8g R=4-Br; 8h R=2-Br-4,5-di-OCH_3; 8i R=3-CH_3; 8j R=3,4-di-CH_3; 8k R=3-F; 8l R=2-Br-5-F; 8m R=4-Cl; 8n R=4-CH_3; 8o R=4-F; 8p R=4-OCH_3

Scheme 2. Synthetic routes of intermediates **8a**-**8p**
Reagents and conditions: (a) EtOH, 30 min; (b) CH_2Cl_2, 40 °C, 10 h.



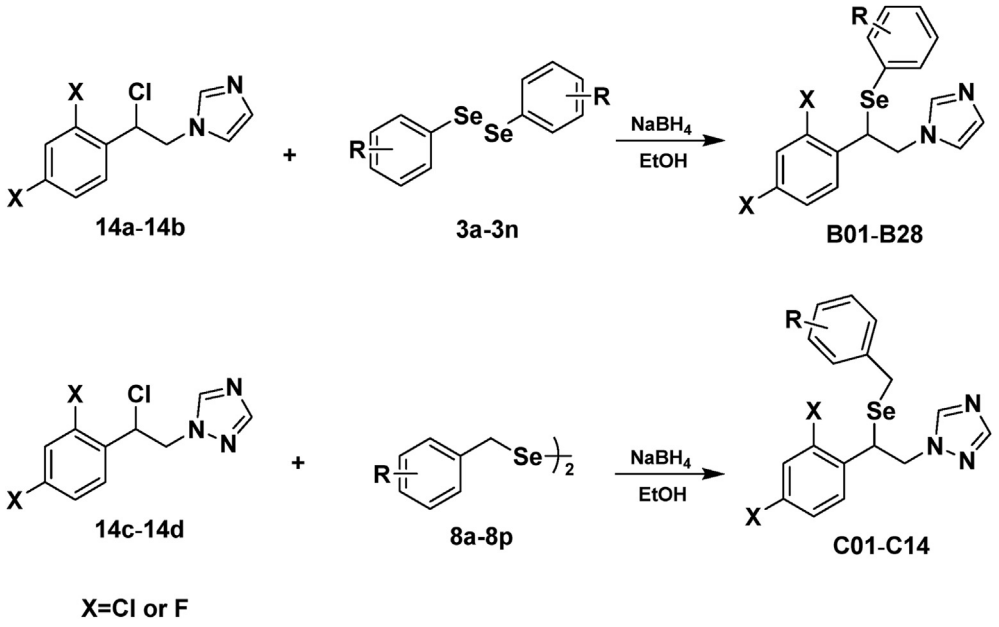
Scheme 3. Synthetic routes of intermediates **14a**-**14d**
Reagents and conditions: (a) $AlCl_3$, 30 °C, 3 h; (b) imidazole or 1,2,4-triazole, MeOH, 65 °C, 6 h; (c) $NaBH_4$, EtOH, 20 °C, 12 h; (d) $SOCl_2$, 20 °C, 12 h.

2.3. In vitro antifungal activity of synthesized compounds

The minimal inhibitory concentrations (MICs) of the target compounds were determined against *Candida albicans*, *Candida zeylanoides*, *Cryptococcus neoformans*, *Candida kruseii*, *Candida glabrata*, *Candida parapsilosis* and *Aspergillus fumigatus*using microbroth dilution method in 96-well plates according to the CLSI M27-A3 guidelines by using miconazole and fluconazole as the control drug. The antifungal activities are indicated as the MICs and obtained *in vitro* antifungal activity of the target compounds are summarized in Table 3.

The results showed that almost all series **B** compounds possessed broad-spectrum antifungal activity. However, derivatives **C01**—**C14** exhibited moderate to best antifungal activities against most strains. SAR revealed that the replacement of imidazole with 1,2,4-triazole resulted in substantial decrease of the antifungal activity. As for the antifungal inhibitory data in Table 3, the following points should be taken into account:

1) Compared with fluconazole (MIC: 4 μg/mL), series **B** compounds (MIC: 0.03—0.5 μg/mL) had an 8—32 folds improvement in antifungal activity in *Candida albicans* (CPCC 400616). Most of the compounds **B01**—**B28** showed inhibitory efficiency against *Candida albicans* (CPCC 400616) in comparison with the reference miconazole (MIC: 0.25 μg/mL). Among them, compound **B25** exhibited the best activity against *Candida albicans*, which was 8 folds more potent than the reference miconazole (MIC: 0.03 μg/mL).
2) Series **B** compounds showed promising activities against *Candida albicans* (ATCC SC5314) with MIC ranging from 0.02 to 0.13 μg/mL which was much more active than fluconazole (MIC: 1 μg/mL). Among them, compounds **B15**, **B17**, **B18**, **B21** and **B25** (MIC: 0.02 μg/mL) demonstrated slightly potent *in vitro* antifungal activity than miconazole (MIC: 0.03 μg/mL).



Scheme 4. Synthetic routes of target compoundsReagents and conditions: 65 °C, 1 h.

3) The *in vitro* antifungal activities of series **B** compounds (MIC: 0.03−1 μg/mL) were far more potent than fluconazole (MIC: 4 μg/mL) against *Cryptococcus neoformans* (CGMCC 2.3161), which was comparable to the reference miconazole (MIC: 0.5 μg/mL). Among them, compounds **B03** and **B28** possessed excellent potency against *C.neo.* (MIC: 0.03 μg/mL).
4) The results of MICs against other *Candida* spp. including *Candida zeylanoides*, *Candida kruseii, Candida glabrata* and *Candida parapsilosis* indicated that most series **B** compounds exhibited considerable inhibitory activity with MIC in the range of 0.13−4 μg/mL, 0.02−1 μg/mL, 0.06−1 μg/mL, 0.13−1 μg/mL, respectively.
5) Against *Aspergillus fumigatus* (CGMCC 3.7795), consistent with series **B** (MIC = 2−16 μg/mL), series **C** (MIC = 4−16 μg/mL) with imidazole substituents by 1, 2, 4-triazole were the same potent.
6) The imidazole group was essential for the antifungal spectrum, the change of X and R did not have amajor impact on the inhibitory activity. For series **B** compounds, the activity of *C. gla.* was slightly increased when the X was Cl. And the activity of *C. zey.* was slightly increased when the R was multiple methyl substitution. R was the electron-donating substituent, the activity of series **B** compounds against *A. f.* was slightly decreased, whereas the activity against *C. gla.* was slightly increased.
7) For series **C** compounds, the activities of *C.alb.*, *C. gla.* and *A.f.* were slightly increased when the X was F, meanwhile the activity of *C. zey.* was significantly increased. The antifungal activity and antifungal spectrum were better when R was 2-Cl. The antifungal activity decreased obviously when R was the electron-withdrawing group, however, the increase of electron-donating group did not enhance the antifungal activity.
8) As compared with **A03**, the shorten of the benzyl group caused a slight increase of the inhibitory activity against *C.zey.*, *C. neo.*, *C. kru.* and *C. par.*. Series **B** compounds had a smaller concentration range against *C.zey.*, *C. neo.*, *C. kru.* and *C. par.*. (0.13−4 μg/mL, 0.03−1 μg/mL, 0.02−1 μg/mL, 0.06−1 μg/mL, 0.13−1 μg/mL, respectively).

2.4. Determination of MIC against fluconazole-resistant strains of Candida albicans

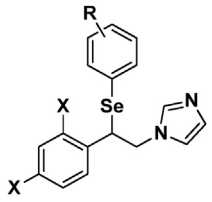
Due to the widespread use of antifungal drugs, the resistance is becoming widespread in clinic [23]. The clinical occurrence of drug-resistant strains lead to the significant impediment to the treatment of invasive fungal infection. As a consequence, the evaluation of antifungal activities of potent compounds against fluconazole-resistant strains is a validated strategy to combat with drug resistance. In this study, all target compounds were assessed for their *in vitro* antifungal activities against fluconazole-resistant clinical isolates (*C. albicans,* strains numbers 17#, CaR, 632, 901 and 904). The results are summarized in Table 4.

All series **B** compounds displayed potent inhibitory activity on fluconazole-resistant fungi, whereas fluconazole was completely inactive. Among all the target compounds, compounds **B02**, **B10**, **B17** and **B27** revealed most potent effects against fluconazole resistant *C. albicans* (MIC = 0.5−2, 0.13−0.5, 0.06−1 and 0.5−2 μg/mL, respectively). However, most of the analogues **C01**−**C14** showed significantly reduced inhibitory activity against fluconazole-resistant strains. These data indicated that the modification of side chain was helpful for promoting the inhibitory effect on fluconazole-resistant fungi while imidazole replacement with 1,2,4-triazole lead to the loss of the antifungal activity. Based on the results of *in vitro* antifungal activity against different strains of fungi, compounds **B02**, **B10**, **B17** and **B27** were chosen to carry out the further study.

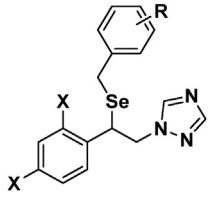
2.5. In vitro cytotoxicity assay

According to our previous study, lead compound **A03** exhibited significant cytotoxic effect and therefore further pharmaceutical research was limited. This prompted us to investigate whether compounds **B02**, **B10**, **B17** and **B27** are cytotoxic on these mammalian cell lines. The effects compounds **B02**, **B10**, **B17** and **B27** on the *in vitro* cytotoxicity of human leukemia cell line (HL-60), breast cancer cell line (MDA-MB-231), prostate cancer cell line

Table 3
The antifungal activity of the target compounds B01−B28 and C01−C14



B01-B28



C01-C14

			C.alb	*C.alb(sc5314)*	*C.zey*	*C.neo*	*C.kru*	*C.gla*	*C.par*	*A.f*
B01	Cl	H	0.25	0.13	0.25	0.13	0.13	1	0.25	8
B02	Cl	3-F	0.25	0.06	0.25	0.13	0.13	1	0.25	4
B03	Cl	4-F	0.25	0.03	0.25	0.03	0.02	0.5	0.25	8
B04	Cl	2-CH_3	0.13	0.03	0.5	0.5	0.13	0.25	0.5	16
B05	Cl	3-CH_3	0.25	0.03	0.25	0.25	0.06	1	0.25	4
B06	Cl	4-CH_3	0.25	0.03	0.25	0.13	0.5	0.25	0.5	8
B07	Cl	2-OCH_3	0.06	0.03	0.25	0.5	0.13	0.5	0.13	8
B08	Cl	3-OCH_3	0.25	0.03	0.5	0.25	0.06	0.5	0.5	8
B09	Cl	4-OCH_3	0.13	0.03	0.5	0.25	0.02	0.25	0.25	16
B10	Cl	2,5-di-CH_3	0.25	0.03	2	0.5	0.5	0.5	0.5	16
B11	Cl	2,6-di-CH_3	0.13	0.06	4	0.5	2	0.13	0.5	8
B12	Cl	3,5-di-CH_3	0.5	0.06	0.5	0.5	0.5	0.25	0.5	8
B13	Cl	2-CH_2CH_3	0.25	0.03	0.5	0.5	0.5	0.13	0.13	8
B14	Cl	4-CH_2CH_3	0.5	0.03	0.5	0.5	0.06	0.13	1	4
B15	F	H	0.5	0.02	0.25	0.5	0.25	1	0.06	2
B16	F	3-F	0.5	0.03	0.5	0.5	0.13	1	0.25	8
B17	F	4-F	0.25	0.02	0.25	0.5	0.5	1	0.03	4
B18	F	2-CH_3	0.13	0.02	0.5	0.5	0.02	1	0.13	8
B19	F	3-CH_3	0.25	0.03	0.5	0.25	0.06	1	0.25	8
B20	F	4-CH_3	0.5	0.03	0.5	0.13	0.13	0.5	0.25	8
B21	F	2-OCH_3	1	0.02	0.13	1	0.5	0.25	0.13	8
B22	F	3-OCH_3	0.5	0.13	1	0.5	0.13	0.5	0.25	4
B23	F	4-OCH_3	1	0.03	0.25	0.5	0.5	0.5	0.25	8
B24	F	2,5-di-CH_3	0.25	0.06	0.5	0.25	0.13	0.5	0.13	8
B25	F	2,6-di-CH_3	0.03	0.02	0.13	0.25	0.02	0.5	0.06	8
B26	F	3,5-di-CH_3	0.13	0.03	0.5	0.5	0.13	0.5	0.13	8
B27	F	2-CH_2CH_3	0.06	0.03	0.25	1	0.06	0.5	0.03	16
B28	F	4-CH_2CH_3	0.13	0.13	0.5	0.03	0.13	0.5	0.13	8
C01	Cl	2,4-di-Cl	0.25	0.25	128	64	128	0.5	64	16
C02	Cl	H	0.13	0.13	64	2	128	4	32	16
C03	Cl	2-Cl	0.03	0.13	16	2	0.06	4	8	16
C04	Cl	4-Cl	16	2	128	16	128	2	64	16
C05	Cl	4-F	2	0.13	64	8	128	4	64	16
C06	Cl	4-OCH_3	16	1	32	16	64	4	32	4
C07	Cl	4-CH_3	16	2	128	32	64	2	64	16
C08	F	2,4-di-Cl	0.13	0.06	64	4	128	2	>128	16
C09	F	H	0.5	0.06	8	4	4	2	16	8
C10	F	2-Cl	0.03	0.13	1	2	2	2	0.5	8
C11	F	4-Cl	0.13	0.13	4	4	128	0.5	64	8
C12	F	4-F	1	0.25	4	0.5	8	2	32	8
C13	F	4-OCH_3	1	2	32	32	32	2	>128	4
C14	F	4-CH_3	2	2	32	4	128	2	128	8
A03			0.02	0.01	1	0.25	0.13	0.5	0.13	2
Fluconazole			4	1	4	4	32	32	4	>128
Miconazole			0.25	0.03	1	0.5	0.5	0.5	0.25	2

Table 4
The antifungal activity of the target compounds **B01**−**B28** and **C01**−**C14**.

Compd.	MIC/(μg/mL)				
	strain CaR	strain 17#	strain 632	strain 901	strain 904
B01	1	1	1	2	2
B02	2	1	0.5	1	1
B03	8	2	1	1	1
B04	4	2	0.5	0.5	0.5
B05	1	1	1	1	1
B06	32	4	4	8	16
B07	2	2	2	1	8
B08	8	2	16	16	4
B09	8	2	2	8	8
B10	0.25	0.13	0.5	0.25	0.25
B11	4	4	8	16	8
B12	8	16	16	4	16
B13	2	4	16	4	1
B14	8	8	4	4	15
B15	1	4	2	2	1
B16	4	4	1	2	2
B17	1	0.06	0.5	0.5	0.06
B18	8	4	4	2	1
B19	4	8	0.5	4	1
B20	2	16	1	8	4
B21	8	8	16	4	8
B22	2	4	1	4	8
B23	2	16	16	2	8
B24	8	4	2	4	8
B25	8	0.25	1	2	2
B26	2	4	8	4	8
B27	0.5	0.5	1	2	0.5
B28	1	4	1	2	8
C01	>128	>128	>128	>128	>128
C02	>128	64	>128	>128	>128
C03	>128	8	>128	>128	32
C04	>128	>128	>128	>128	>128
C05	>128	>128	>128	>128	>128
C06	128	32	>128	128	128
C07	>128	>128	>128	>128	>128
C08	>128	64	>128	>128	>128
C09	128	32	>128	16	16
C10	32	4	8	4	2
C11	128	16	>128	4	8
C12	128	32	>128	>128	64
C13	128	64	>128	>128	>128
C14	>128	64	>128	>128	>128
A03	0.06	0.13	1	0.5	0.25
FCZ	>128	128	>128	>128	>128

(PC3) and human normal mammary epithelial cells (MCF-10A) were investigated. Doxorubicin (DOX) and taxol were used as controls. All data were summarized in Table 5.

Remarkably, compounds **B02**, **B17** and **B27** significantly decreased the cytotoxicity of HL-60, MDA-MB-231 and PC-3 compared to lead compound **A03** and the inhibitory effect of compound **B17** on MCF-10A was one half of that of compound **A03**. Among them, compound **B17** exhibited relatively weak cytotoxicity against test cell lines with the IC_{50} values ranging from 24.8 to 59.4 μM (9.48−22.69 μg/mL), which were about 38−90 times higher than the MIC value against *C.albicans* (Table S1).

2.6. Hemolysis assays

Having several potent compounds in hand, we asked whether any of these compounds show ameliorated hemolysis effects in comparison to previous lead compound **A03**. In this study, rabbit red blood cells were used to for hemolysis assays of tested compounds **B02**, **B10**, **B17** and **B27** (Table 6).

All test compounds had different hemolysis effects at high concentrations (2−16 μg/mL). Miconazole displayed 87.34% hemolysis at 8 μg/mL (minimum hemolytic concentration, MHC),

Table 5
IC_{50} of tested samples in cancer cell lines.

Compd.	IC_{50} μM			
	HL-60	MDA-MB-231	PC-3	MCF-10A
B02	8.26	13.43	20.41	28.55
B10	6.95	6.61	7.25	27,20
B17	24.81	39.53	54.39	59.37
B27	16.95	26.24	30.25	28.11
A03	1.02	9.06	13.10	30.16
Miconazole	0.80	16.96	13.41	–
Taxol	–	<0.001	<0.025	–
Dox	<0.125	0.53	0.64	2.64

Values are the average of three independent experiments. Relative errors are generally within 5−10%.

whereas compounds **B17** was inactive (MHC/MIC = 64). After exposure, compounds **B27** displayed 93.59% hemolysis in the sample treated with 4 μg/mL (MHC/MIC = 64). Moreover, compound **B02** lysed 87.66% of red blood cells at concentrations of 4 μg/mL and compound **B10** exhibited 0.89% hemolysis at concentrations of 2 μg/mL. The MIC values of target compounds were much lower than their hemolytic concentrations and compound **B17** exhibited the lowest hemolytic activity. In addition, compound **B10** showed no significant improvement in cytotoxicity and hemolysis, further study of compound **B10** will not be considered.

2.7. Metabolic stabilities assay

The microsomal metabolic stabilities of three most potential compounds were tested before investigating *in vivo* antifungal activities (Table 7). Miconazole and lead compound **A03** were fast metabolic ($T_{1/2}$ = 8.3 min and 4.2 min, respectively) in the mouse liver microsomal stability test (Table S2). In contrast, compound **B17** ($T_{1/2}$ = 19.4 min) had a lower clearance rate than miconazole and compound **A03**, suggested that it might have good metabolic stability.

2.8. Minimum fungicidal concentration of compounds *B02*, *B17* and *B27* and time-kill curves of *B17*

Considering the excellent antifungal inhibitory activities, compounds **B02**, **B17** and **B27** were selected for further evaluations and compounds **B17** was also our focus. As is well known, fluconazole is a fungistatic clinical agent rather than fungicidal agent and it is therefore often used as maintenance therapy. The development of new fungicidal compounds to treat IFIs remains a major challenging. In our study, the discovery of effective fungicidal agents with high therapeutic index is of great importance. The minimum fungicidal activity (MFC), i.e., the lowest concentration that resulting in at least 99% killing of the inoculum, is used to characterize *in vitro* fungicidal activity. Kill of *C. albicans* (CPCC400616)

Table 6
The hemolysis ratio of the target compounds.

Compd.	Hemolysis ratio				
	1 μg/mL	2 μg/mL	4 μg/mL	8 μg/mL	16 μg/mL
B02	0	0	87.66	94,60	100
B10	0	0.89	84.36	100	100
B17	0	0	0	0.33	100
B27	0	0	93,59	100	100
A03	0.00	3.15	58.87	93.23	98.23
Miconazole	0.16	8.55	81.78	87.34	94.03

Values are the average of three independent experiments. Relative errors are generally within 5−10%.

Table 7
Metabolic stabilities of compounds **B02**, **B17** and **B27** in mouse liver microsomes.

Compd.	$T_{1/2}$ (min)	CL (mL/min/kg)
B02	10.3	530.7
B17	19.4	282.4
B27	14.9	368.2
A03	4.2	1311.5
Miconazole	8.3	660.1
Propafenone	2.3	2433.0

Considering *in vitro* antifungal activity, cytotoxicity, hemolytic and metabolic stability, **B17** was selected as the focus for further antifungal activity studies.

treated with compounds **B02**, **B17** and **B27** was investigated by determinating the minimum fungicidal concentration (MFC). Specifically, we evaluated MFC of potent compounds **B02**, **B17** and **B27** in 48 h (Table 8).

Meanwhile, time-Kill curves were assayed to further evaluate the fungicidal activity of compound **B17** during a period of 24 h and miconazole (2 μg/mL) was used as control drug. The gradient concentrations of compound **B17** (0.25 μg/mL, 0.5 μg/mL, 1 μg/mL and 2 μg/mL) were used in the time-Kill curve assay (Fig. 4).

As shown in Table 10, compounds **B17** and **B27** showed satisfactory fungicidal activity. As shown in Fig. 4, compound **B17** displayed rapid fungicidal activity against *C. albicans* (CPCC 400616), which could completely kill fungi cells at 2 μg/mL within 8 h in the viable colony counts (the CFU was reduced from 5 to 2 Log_{10} CFU/mL). Here it can be observed that compound **B17** could dose-dependently kill the *C. albicans* cells. And the fungicidal activity was much more effective than miconazole. Compound **B17** demonstrated potent fungicidal activity in this assay, therefore, it may have advantages in treating IFIs and drug resistance.

2.9. GC-MS analysis of sterol composition in Candida albicans

Clarifying the mechanism of action of compounds is an important step in drug discovery and development. In our previous study, the mechanism of lead compound **A03** had been clarified. Compound **A03** mediated ergosterol biosynthetic pathway by inhibiting sterol 14α-demethylase enzyme. In this study, we thus stared to determine whether compound **B17** possessed the same mechanism with lead compound **A03**. Sterol composition of fungal cells was detected using gas chromatography-mass spectrometry (GC-MS). Fluconazole and miconazole were employed as the controls [24]. *C. albicans* (ATCC SC5314) was used to be a model strain and pre-incubated together with test compounds for 16 h to investigate the biosynthesis of ergosterol. As shown in Table 9, the content of the lanosterol 14α-demethylase substrates (lanosterol, obtusifoliol and eburicol) was increasing. It's worth noting that eburicol displayed the largest amount of accumulation in all lanosterol 14α-demethylase substrates. Similar to miconazole, **B17** produced the accumulation of methylated sterols, and decreased the content of ergosterol whether at high or low doses. Together, these results suggested that compound **B17** inhibited lanosterol 14α-demethylase (CYP51) in a dose-dependent manner with the same targets and mechanism as lead compound **A03**.

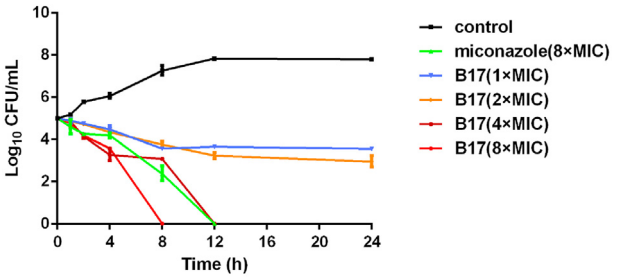


Fig. 4. Time-kill curve of compound **B17** against *Candida albicans* (CPCC400616). The detection limit of this assay was 100 CFU/mL.

2.10. Inhibition of the biofilm formation of FCZ-Resistant C. albicans

Biofilm is an important virulence factor contributing to recurrent fungal infection and high-level drug resistance in the clinic. Fungal cells are surrounded by biofilm, a self-produced extracellular polymeric substance, therefore antifungal drugs are difficult to enter the fungal cells [25,26]. So that biofilm could protect fungal cells against attack by antifungal agents. Therefore, it's a significant therapeutic meaning to develop novel derivatives with anti-biofilm activity. In this assay, the effect of compounds **B02**, **B17** and **B27** on biofilm formation of *C. albicans* (CPCC400616) was evaluated by using miconazole as control. The $SMIC_{50}$ and $SMIC_{80}$ values of compounds **B02**, **B17** and **B27** were shown in Table 10.

. In this assay, *C. albicans* (CPCC400616) were seeded on the 96-well plates for 1.5 h, 3 h, 6 h and 24 h to form biofilm (SMIC of FCZ >1024 μg/mL). The prepared biofilm was preincubated with different concentrations of the tested compounds for 24 h at 37 °C. Next, the effect of tested compounds on biofilm formation were investigated by the XTT reduction assay [27] (Fig. 5).

As showed in Fig. 5 and Table 12, treatment of 3 h-biofilms with 32 μg/mL miconazole inhibited 50% of 3 h-biofilms formation. At the lower concentration (16 μg/mL), **B17** inhibited 80% of 3 h-biofilms formation, indicating that **B17** is a more potent antifungal inhibitor. Notably, target compounds **B02**, **B17** and **B27** were shown to inhibit the formation of *C. alb.* biofilm in a dosage depended manner.

2.11. In vivo antifungal effects

Considering the good antifungal activity, better metabolic stability, lower hemolytic and cytotoxicity of compound **B17**, the *in vivo* efficacy was evaluated in infectious murine models by *C. albicans* ATCC SC5314. Compound **A03** (0.6 mg/kg/day) and fluconazole (0.6 mg/kg/day) were used as controls. Two different drug regimens of compound **B17** were investigated at the dosage of 0.4 and 0.6 mg/kg/day. Compound **B17** showed potent activity in a murine model of *C. albicans*. The results of tissue burdens obtained after 5 days of experiments in kidneys with **B17** was showed in Fig. 6. Experiments use CFU counts of the kidneys of mice infected intravenously with *C. albicans*. The results showed that compound **B17** significantly reduced the kidney fungal burden in a murine

Table 8
The MFC of the most potent compounds.

Compd.	MIC against *C.alb.* (μg/mL)	MFC against *C.alb.* (μg/mL)	MFC/MIC
B02	0.25	–	–
B17	0.25	1	4
B27	0.06	0.25	4
Miconazole	0.25	2	8

Table 9
Analysis of sterol composition in *Candida albicans* (ATCC SC5314) by GC-MS.

Compd.	concentration (μg/mL)	% of total sterols *Candida albicans* (ATCC SC5314)			
		Ergosterol	Obtusifoliol	Lanosterol	Eburicol
Fluconazole	0.03125	86.34	1.0	9.8	2.9
	0.125	80.8	1.6	12.4	5.3
	0.5	78.9	2.9	11.6	6.7
	2	19.8	9.5	7.2	63.5
	8	4.2	11.4	14.1	70.3
Miconazole	0.03125	12.2	7.2	10.0	70.6
	0.125	8.3	17.4	9.5	64.8
	0.5	7.1	9.0	8.9	75.1
	2	5.2	6.1	13.6	75.2
	8	4.1	7.8	7.4	80.7
B17	0.03125	8.7	17.0	4.1	70.2
	0.125	5.8	11.1	9.9	73.2
	0.5	4.9	9.9	10.1	75.1
	2	2.2	10.8	9.0	78.0
	8	2.1	10.2	5.7	82.0
Control	–	98.3	0	1.7	0

Table 10
SMIC values of tcompounds against FCZ-Resistant *C. albicans* CPCC400616 biofilms.

Compd.	$SMIC_{50}$ (μg/mL)				$SMIC_{80}$ (μg/mL)			
	1.5 h	3 h	6 h	24 h	1.5 h	3 h	6 h	24 h
B02	4	8	128	>128	16	128	>128	>128
B17	4	8	64	>128	8	16	128	>128
B27	8	16	>128	>128	128	>128	>128	>128
Miconazole	16	32	>128	>128	128	128	>128	>128

model of *C. albicans*.

The kidney fungal burden could be visually reduced by intraperitoneal injection of compound **B17** (0.4 mg/kg and 0.6 mg/kg once daily). The results highlighted compound **B17** has better antifungal activity *in vivo* than fluconazole.

2.12. Pharmacokinetic study

Since compound **B17** has good antifungal activity *in vivo*, we selected compound **B17** to evaluate its pharmacokinetic properties for further study. In order to study the pharmacokinetic characteristics of compound **B17** in SD rats, plasma concentration of compound **B17** was measured after single intravenous administration (2 mg/kg) and intragastric administration (5 mg/kg). The plasma concentrations of compound **B17** over time are presented in Fig. 7 and the pharmacokinetic parameters of compound **B17** are given in Table 11.

After intravenous administration of 2 mg/kg area under blood concentration curve (AUC_{0-t}) and the final half-life ($T_{1/2}$) of compound **B17** in rats were observed 2249 ± 112 h ng/mL and 1.89 ± 0.01 h, respectively. After intragastric administration of 5 mg/kg area under blood concentration curve (AUC_{0-t}), the final half-life ($T_{1/2}$) and bioavailability (%) of compound **B17** in rats were observed 1256 ± 135 h ng/mL, 1.93 ± 0.04 h and 22.3%, respectively.

2.13. Molecular docking analysis

To validate the design rationale and determine the binding modes, compound **B17** and miconazole were selected to be docked into the active site of *C.albicans* CYP51 (PDB ID: 5tz1) from Protein Data Bank (PDB). The results revealed that compound **B17** shared the similar binding modes with miconazole. The docking results are depicted in Fig. 8. The imidazole group of target compounds **B17** was coupled to the Fe^{2+} atom of the heme group of *C.albicans* CYP51. CYS470, GLY307, LEU376, SER378, MET508, HEM601 and THR311 were the main function residues. The side chain extended into the CYP51 channel to form favorable hydrophobic interactions and van der Waals with the surrounding residues such as THR311, LEU376, SER378 and MET508.

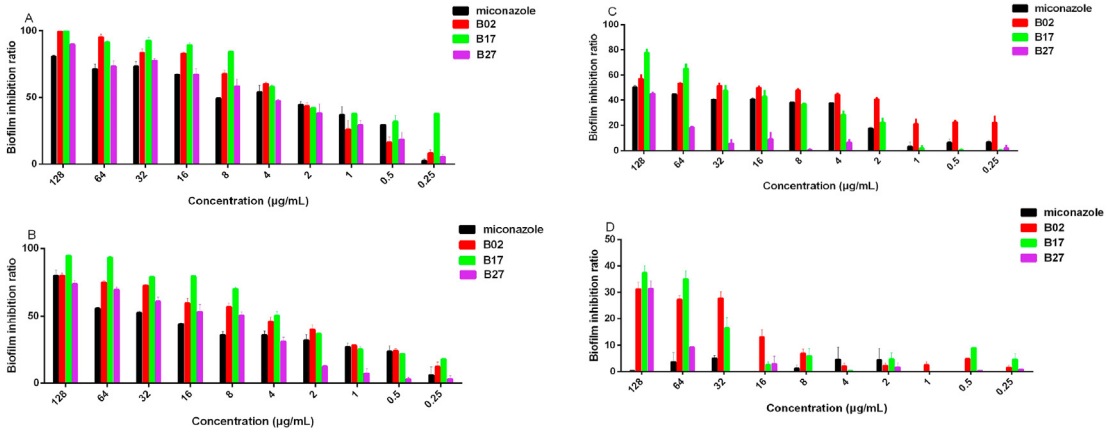


Fig. 5. Antibiofilm effect of compounds **B02**, **B17**, **B27**, and miconazole against FCZ-Resistant *C. albicans* CPCC400616 biofilms for 1.5 h (A), 3 h (B), 6 h (C) and 24 h (D).

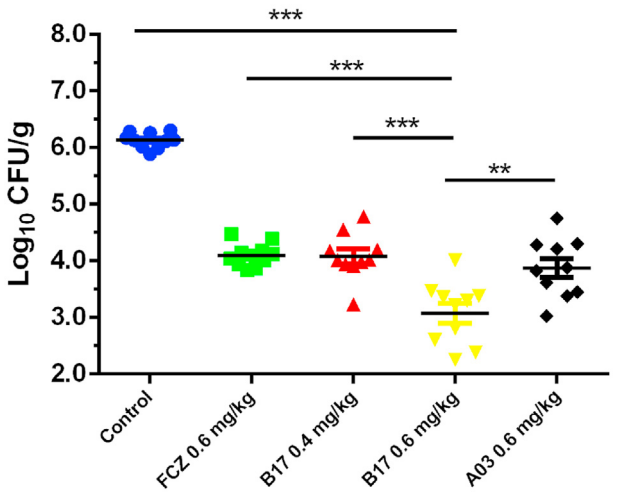


Fig. 6. Therapeutic efficacies of compound **A03** (0.6 mg/kg) **B17** (0.4 mg/kg and 0.6 mg/kg) and fluconazole in the *C.alb*. ATCC SC5314 model. (**$P < 0.01$, ***$P < 0.001$).

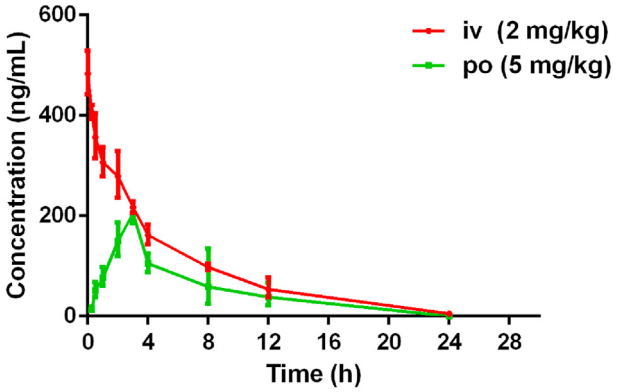


Fig. 7. Plasma concentration-time curves of compound **B17** intravenous administration and oral administration.

3. Conclusion

The selenium-containing miconazole **A03** we previously reported were identified and validated as the promising antifungal lead compound. The potent hemolysis, cytotoxicity and rapid metabolism, however, limit the widespread use as antifungal agents. To improve this disadvantage, we have synthesized a series of selenium-containing miconazole derivatives (**B01**–**B28** and **C01**–**C14**) in attempts to maintain their antifungal activity while reducing their hemolysis rate, cytotoxicity and enhancing metabolic stability. Due to the poor inhibitory activity against some clinically pathogenic fungi and fluconazole-resistant strains, analogues **C01**–**C14** don't meet the requirement for further stage studies on the activity and safety of these compounds. Series **B** compounds not only showed excellent inhibitory activities against various fungal pathogens, but also improved safety of hemolysis and cytotoxicity. Among them, compound **B17** and **B27** showed obvious improved metabolic stability. The time-kill curve assay revealed compound **B17** possess fungicidal activity and coluld completely killed *C. albicans* cells at 2 μg/mL in a concentration-dependent manner. *In vivo* efficacy was also demonstrated in a murine model of systemic *C. albicans* infection, which compound **B17** possess a significant fungal load reduction in kidneys. Generally, compound **B17** exhibited potent *in vitro* and *in vivo* antifungal activity in treating *C. albicans.* Herein, the potential applications and mechanism of action of compound **B17** for the treatment of IFIs were investigated. Like fluconazole and miconazole, compound **B17** produced the accumulation of methylated sterols and decrease ergosterol biosynthetic, which are symbol of the mechanism of inhibition of CYP51 by antifungal azoles. Moreover, compound **B17** showed excellent activity in inhibiting *C. albicans* biofilm formation (>80% inhibition at 16 μg/mL), highlighting its potential to treat biofilm-associated infections. In addition, docking analysis revealed the potential binding modes between the target compound **B17** and *C.alb.* CYP51. Considering the excellent antifungal activity, better metabolic stability, lower hemolytic and cytotoxicity, our results strongly suggest that compound **B17** are promising lead for the development of the antifungal azole analogues. The study of pharmacokinetics of compound **B17** also provides reference and foundation for our further work and subsequent structural modification. Our findings will facilitate further drug discovery efforts toward the identification of a preclinical candidate to treat fungal infections.

4. Experimental

4.1. Chemistry

All starting materials and analytically pure solvents, unless otherwise specified, were received from the vendors. Melting points were recorded on a X-5 melting point apparatus and are uncorrected. In all chemical reactions, TLC (thin-layer chromatography) monitor was performed by using silica gel precoated GF254 plates and then visualized with a UV lamp. All column chromatography separations were performed on silica gel. The ^{1}H NMR and ^{13}C NMR spectra date were collected on a Bruker AV-400 spectrometer machine and the solvent was chosen DMSO-d_6 with TMS. ^{1}H NMR spectra were run at 400 MHz and ^{13}C spectra were run at 100 MHz. The Low-resolution electrospray ionization mass spectrometry was measured by Agilent 6120 (quadrupole liquid chromatography) equipped Agilent 1200 LC/MS. High-resolution mass spectra experiments were measured on the Agilent Accurate-Mass Q-TOF 6530 instrument. GC-MS experiments were performed with the Agilent 6890N-5975 (Agilent, Santa Clara, CA, USA).

4.2. The preparation of compounds *3a-3n*

The synthesis of intermediate 1, 2-diphenyldiselane (**3a**) was chosen as the example: the solution of phenylmagnesium bromide was prepared from bromobenzene (5.8 mL), magnesium (1.2 g, 0.05 mol), and anhydrous diethyl ether (30 mL). The selenium power (3.5 g, 0.044 mol) was added in portions at a rate sufficient to maintain a vigorous reflux. After the mixture was stirred and heated at reflux for 30 min, saturated solution of ammonium

Table 11
Pharmacokinetic parameters after administration of rats.

route	dose (mg/kg)	AUC_{0-t} (h·ng/mL)	$T_{1/2}$ (h)	bioavailability (%)
intravenous	2	2249 ± 112	1.89 ± 0.01	–
per os	5	1256 ± 135	1.93 ± 0.04	22.3

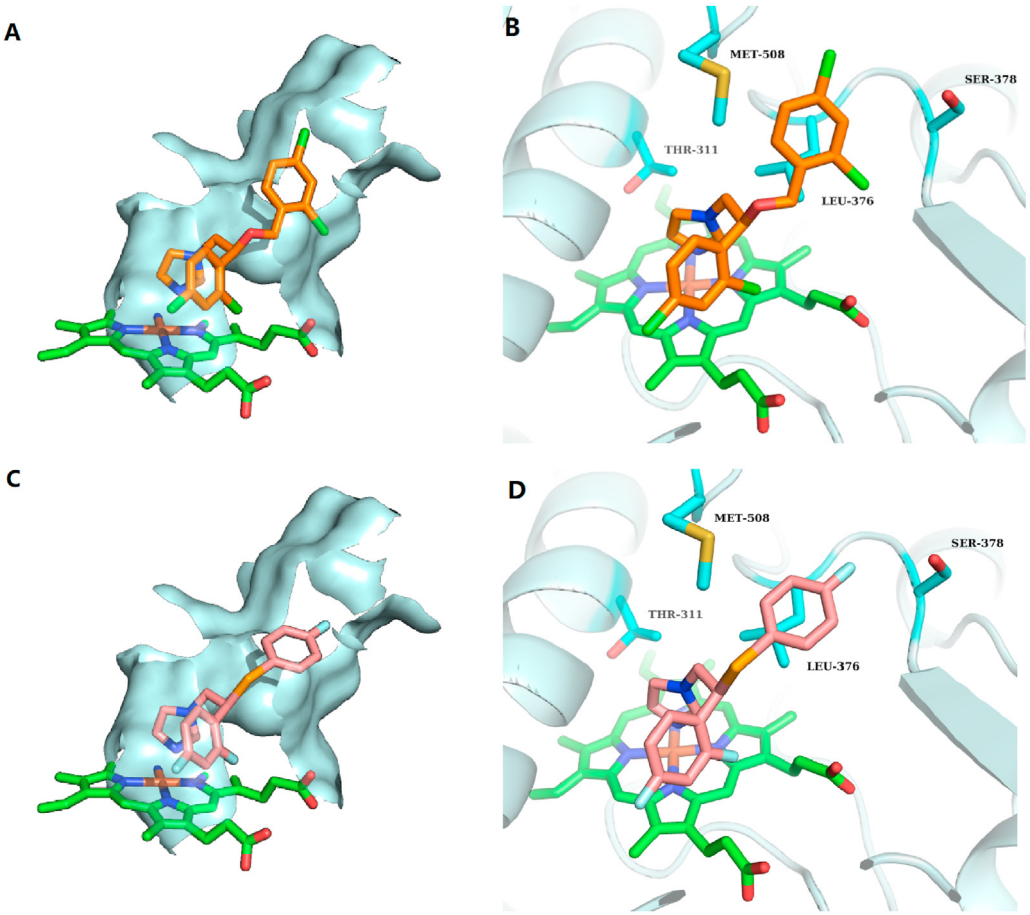


Fig. 8. Miconazole (A, B) and compound **B17** (C, D) were docked into the active site of *Candida albicans* CYP51.

chloride was then added (20 mL). The mixture was filtered and extracted with ether (3 × 20 mL). After evaporating the combined extracts to dryness, the residue was dissolved in hot hexane (10 mL) and a small amount of insoluble substance was separated by thermal filtration. Solids precipitate out of solution at 4 °C. The yellow solid was collected and dried in the air (2.8 g, 43%). m.p. 58–61 °C. LC- MS(*m*/*z*): 313.9 $[M + H]^+$.

4.3. The preparation of compound ***6***

Selenium power (7.9 g, 0.1 mol) and $NaBH_4$ (8 g, 0.2 mol) were added to a solution of ethanol (180 mL) under N_2 (g). The mixture was allowed to stir at 25 °C for 15 min. Then, another part of selenium power (7.9 g, 0.1 mol) was added into the solution and the mixture was heated to 80 °C. The reaction mixture was then stirred for another 30 min and filtered. The filtrate was concentrated under reduced pressure to afford yellow solid Na_2Se_2 (20.0 g, 98%). m.p. 101–104 °C.

4.4. The preparation of compounds ***8a-8p***

The Na_2Se_2 (**6**, 5.9 g, 0.024 mol) and chlorobenzyl (**7a-7p**) (2.6 g, 0.02 mol) were added into the solution of methanol (20 mL) with stirring. The mixture was stirred at 25 °C for 15 h under N_2. Then the solvent was evaporated to dryness, and the residue was purified by flash chromatography to afford the white solid (2.8 g, 83.13%). LC- MS(*m*/*z*): 343.0 $[M + H]^+$.

4.5. The preparation of compounds ***12a-12d***

The synthesis of intermediate 1-(2,4-difluorophenyl)-2-(1*H*-imidazole-1-yl) ethan-1-one (**12b**) was chosen as the example: The 2-chloroacetyl chloride (**10**) (6.7 g, 0.06 mol) was added dropwise to the mixture of 1,3-difluorobenzene (5.7 g, 0.05 mol) and anhydrous aluminum trichloride (10.7 g, 0.08 mol) at 25 °C. The reaction was stirred at room temperature for 4 h. Subsequently, the mixture was diluted with ice water mixture (100 g) containing concentrated hydrochloric acid (10 mL). The solution was extracted with dichloromethane (3 × 20 mL). The organic layers were combined and washed with saturated salt water (30 mL). The organic layer was dried with Na_2SO_4, then filtered and concentrated under reduced pressure. The residue was purified by recrystallization with ethanol to give **11b** (93.1%) as a yellow solid. To a stirred solution of intermediate **11b** (14.5 g, 0.08 mol) in 70 mL anhydrous methanol with triethylamine (8.1 g, 0.08 mol), imidazole (10 g, 0.15 mol) was then added at 25 °C. The mixture was magnetically stirred heated to reflux for 4 h and then concentrated under reduced pressure to remove methanol. The residue was purified by recrystallization from ethanol to obtain 1-(2,4-difluorophenyl)-2-(1*H*-imidazole-1-yl) ethan-1-one (**12b**, 90.6%) as a white solid. HRMS *m*/*z*: found 223.0675 [M + H]+(calcd. for $C_{11}H_8N_2F_2O$, 223.0683).

4.6. The preparation of compounds ***14a-14d***

The synthesis of intermediate 1-(2-chloro-2-(2,4-

difluorophenyl) ethyl)-1*H*-imidazole (**14b**) was chosen as the example: $NaBH_4$ (6 g, 0.1 mol) was in portions to a stirred solution of intermediate **12b** (11.1 g, 0.05 mol) in 70 mL ethanol. The solution was stirred at room temperature for 8 h. The reaction mixture was then poured into water and white solid was collected by filtration. The residue was purified by silica gel chromatography to obtain 1-(2,4-difluorophenyl)-2-(1*H*-imidazole-1-yl) ethan-1-ol (**13b**, 79.1%). Intermediate **13b** (2.2 g, 0.01 mol) was added to thionyl chloride (6 g, 0.05 mol) and the mixture was stirred at room temperature for 8 h. The reaction mixture was poured into the ice water mixture and the pH value of water was adjusted to 7–8 by using sodium hydroxide. After filtration, the residue was purified by silica gel chromatography to collect 1-(2-chloro-2-(2,4-difluorophenyl) ethyl)-1*H*-imidazole (**14b**, 62.1.0%) as a yellow solid HRMS *m*/*z*: found 243.0504 [M + H] $^+$(calcd. for $C_{11}H_9N_2F_2Cl$, 245.0501).

4.7. The preparation of target compounds

The synthesis of **B15** was chosen as the example: Add **3a** (3.14 g, 0.01 mol) into 30 mL ethanol at room temperature and add sodium borohydride successively (1 g, 0.04 mol). After stirring for 20 min, **14b** (4.8 g, 0.02 mol) was added. Then, the mixture was stirred for 1 h at 65 °C. After that, the solution was evaporated to dryness and the mixture was purified by column chromatography. Compounds **C01**–**C14** were prepared from intermediate **8a**-**8p** and **14c-14d** using a method which was similar to that of compounds **B01**–**B28**.

4.7.1. 1-(2-(2,4-dichlorophenyl)-2-(phenylselanyl) ethyl)-1H-imidazole (*B01*)

White solid; yield 75.40%; m.p.: 102.2.1–103.4 °C. ESI-HRMS(*m*/*z*): found 396.9776 [M + H]+(calcd. for $C_{17}H_{15}N_2Cl_2Se$, 396.9778); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.55 (d, J = 1.1 Hz, 1H), 7.51 (d, J = 2.2 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 7.41–7.38 (m, 2H), 7.37–7.34 (m, 1H), 7.31–7.27 (m, 3H), 7.10 (t, J = 1.3 Hz, 1H), 6.75 (t, J = 1.1 Hz, 1H), 5.12–5.07 (m, 1H), 4.74 (dd, J = 14.2, 9.9 Hz, 1H), 4.60 (dd, J = 14.2, 6.4 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 137.9, 135.9, 135.6, 134.2, 132.9, 129.7, 129.4, 129.0, 128.8, 127.8, 119.7, 49.6.

4.7.2. 1-(2-(2,4-dichlorophenyl)-2-((3-fluorophenyl) selanyl) ethyl)-1H-imidazole (*B02*)

White solid; yield 87.44%; m.p.: 107.2–108.8 °C. ESI-HRMS(*m*/*z*): found 414.9688 [M + H]+(calcd. for $C_{17}H_{14}N_2Cl_2FSe$, 414.9683); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.65 (d, J = 1.2 Hz, 1H), 7.62–7.56 (m, 2H), 7.44–7.38 (m, 2H), 7.32–7.23 (m, 3H), 7.21 (t, J = 1.3 Hz, 1H), 6.83 (t, J = 1.1 Hz, 1H), 5.24 (dd, J = 9.5, 6.7 Hz, 1H), 4.84 (dd, J = 14.2, 9.5 Hz, 1H), 4.72 (dd, J = 14.2, 6.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.4, 160.9, 137.9, 135.8, 134.3, 133.1, 131.4, 131.4, 131.2, 131.2, 130.9, 129.4, 128.8, 127.8, 121.8, 121.6, 119.7, 116.0, 115.8, 49.6.

4.7.3. 1-(2-(2,4-dichlorophenyl)-2-((4-fluorophenyl) selanyl) ethyl)-1H-imidazole (*B03*)

White solid; yield 85.60%; m.p.: 107.1–109.1 °C. ESI-HRMS(*m*/*z*): found 414.9678 [M + H]+(calcd. for $C_{17}H_{14}N_2Cl_2FSe$, 414.9683); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.56 (d, J = 1.0 Hz, 1H), 7.52 (d, J = 2.2 Hz, 1H), 7.42–7.37 (m, 3H), 7.30 (dd, J = 8.4, 2.2 Hz, 1H), 7.16–7.10 (m, 3H), 6.75 (d, J = 1.1 Hz, 1H), 5.06 (dd, J = 9.8, 6.5 Hz, 1H), 4.74–4.59 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.9, 162.2, 138.7, 138.7, 137.9, 135.8, 134.3, 132.9, 129.4, 128.8, 127.7, 119.7, 116.9, 116.8, 49.4.

4.7.4. 1-(2-(2,4-dichlorophenyl)-2-(o-tolylselanyl) ethyl)-1H-imidazole (*B04*)

White solid; yield 88.90%; m.p.: 98.4–100.1 °C. ESI-HRMS(*m*/*z*): found 410.9931 [M + H]+(calcd. for $C_{18}H_{17}N_2Cl_2Se$, 410.9934); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.52 (d, J = 1.3 Hz, 1H), 7.50–7.43 (m, 3H), 7.31 (dd, J = 8.5, 2.2 Hz, 1H), 7.27–7.23 (m, 2H), 7.12 (dt, J = 7.6, 2.5 Hz, 1H), 7.09–7.07 (m, 1H), 6.74 (d, J = 1.1 Hz, 1H), 5.08 (dd, J = 10.2, 6.1 Hz, 1H), 4.75 (dd, J = 14.1, 10.1 Hz, 1H), 4.57 (dd, J = 14.2, 6.2 Hz, 1H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 141.7, 137.9, 136.4, 135.9, 134.2, 133.0, 130.9, 130.6, 129.4, 129.3, 128.8, 127.8, 127.2, 119.6, 49.6, 22.9.

4.7.5. 1-(2-(2,4-dichlorophenyl)-2-(m-tolylselanyl) ethyl)-1H-imidazole (*B05*)

White solid; yield 81.20%; m.p.: 99.1–101.2 °C. ESI-HRMS(*m*/*z*): found 410.9922 [M + H]+(calcd. for $C_{18}H_{17}N_2Cl_2Se$, 410.9934); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.55 (t, J = 1.1 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 7.30 (dd, J = 8.5, 2.2 Hz, 1H), 7.23–7.15 (m, 3H), 7.12 (dq, J = 1.7, 0.9 Hz, 1H), 7.10 (t, J = 1.2 Hz, 1H), 6.76 (t, J = 1.1 Hz, 1H), 5.08 (dd, J = 9.6, 6.5 Hz, 1H), 4.72 (dd, J = 14.1, 9.7 Hz, 1H), 4.59 (dd, J = 14.1, 6.6 Hz, 1H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 139.1, 137.9, 136.0, 136.0, 134.3, 132.9, 132.6, 130.8, 129.7, 129.5, 129.3, 128.8, 127.7, 119.7, 49.6, 21.2.

4.7.6. 1-(2-(2,4-dichlorophenyl)-2-(p-tolylselanyl) ethyl)-1H-imidazole (*B06*)

White solid; yield 75.75%; m.p.: 101.3–102.8 °C. ESI-HRMS(*m*/*z*): found 410.9922 [M + H]+(calcd. for $C_{18}H_{17}N_2Cl_2Se$, 410.9934); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.54–7.49 (m, 2H), 7.40 (d, J = 8.4 Hz, 1H), 7.29 (dd, J = 9.5, 7.6 Hz, 3H), 7.13–7.07 (m, 3H), 6.74 (s, 1H), 5.02 (dd, J = 9.9, 6.3 Hz, 1H), 4.70 (dd, J = 14.1, 9.9 Hz, 1H), 4.57 (dd, J = 14.1, 6.4 Hz, 1H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 138.8, 137.9, 136.0, 136.0, 134.2, 132.90, 130.7, 130.4, 129.4, 128.8, 127.7, 119.7, 49.6, 21.2.

4.7.7. 1-(2-(2,4-dichlorophenyl)-2-((2-methoxyphenyl) selanyl) ethyl)-1H-imidazole (*B07*)

White solid; yield 81.69%; m.p.: 105.1–106.8 °C. ESI-HRMS(*m*/*z*): found 426.9881 [M + H]+(calcd. for $C_{18}H_{17}N_2Cl_2OSe$, 426.9883); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.53 (d, J = 8.5 Hz, 1H), 7.50 (t, J = 1.7 Hz, 2H), 7.37 (dd, J = 7.6, 1.7 Hz, 1H), 7.34–7.28 (m, 2H), 7.05 (t, J = 1.3 Hz, 1H), 7.00 (dd, J = 8.3, 1.2 Hz, 1H), 6.87 (td, J = 7.5, 1.2 Hz, 1H), 6.75 (t, J = 1.1 Hz, 1H), 5.19 (dd, J = 9.9, 6.0 Hz, 1H), 4.72 (dd, J = 14.2, 10.0 Hz, 1H), 4.53 (dd, J = 14.2, 6.1 Hz, 1H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 158.7, 137.8, 136.1, 134.7, 134.3, 132.9, 131.0, 130.3, 129.3, 128.8, 127.8, 121.7, 119.7, 117.1, 111.7, 56.2, 49.9.

4.7.8. 1-(2-(2,4-dichlorophenyl)-2-((3-methoxyphenyl) selanyl) ethyl)-1H-imidazole (*B08*)

White solid; yield 81.75%; m.p.: 107.8–109.3 °C. ESI-HRMS(*m*/*z*): found 426.9884 [M + H]+(calcd. for $C_{18}H_{17}N_2Cl_2OSe$, 426.9883); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.56–7.51 (m, 2H), 7.46 (d, J = 8.4 Hz, 1H), 7.32 (dd, J = 8.4, 2.2 Hz, 1H), 7.21 (t, J = 7.9 Hz, 1H), 7.10 (d, J = 1.3 Hz, 1H), 6.99 (dt, J = 7.5, 1.3 Hz, 1H), 6.92–6.88 (m, 1H), 6.87–6.85 (m, 1H), 6.76 (d, J = 1.1 Hz, 1H), 5.11 (dd, J = 9.6, 6.5 Hz, 1H), 4.72 (dd, J = 14.2, 9.7 Hz, 1H), 4.61 (dd, J = 14.2, 6.6 Hz, 1H), 3.70 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 159.7, 137.9, 136.0, 134.3, 133.0, 130.9, 130.5, 129.4, 128.8, 128.5, 127.8, 127.5, 120.3, 119.7, 115.1, 55.6, 49.6.

4.7.9. 1-(2-(2,4-dichlorophenyl)-2-((4-methoxyphenyl) selanyl) ethyl)-1H-imidazole (*B09*)

White solid; yield 79.77%; m.p.: 101.6.1–103.9 °C. ESI-HRMS(*m*/*z*): found 426.9903 [M + H]+(calcd. for $C_{18}H_{17}N_2Cl_2OSe$, 426.9883); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.54 (t, J = 1.2 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 7.30–7.26 (m, 3H), 7.10–7.07 (m, 1H), 6.87–6.83 (m, 2H), 6.75 (t, J = 1.0 Hz, 1H), 4.97 (dd, J = 9.9, 6.4 Hz, 1H), 4.68 (dd, J = 14.1, 9.9 Hz, 1H), 4.57 (dd,

J = 14.1, 6.4 Hz, 1H), 3.75 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 160.4, 138.4, 137.9, 136.0, 134.2, 132.7, 130.4, 129.3, 128.8, 127.6, 119.6, 115.4, 55.7, 49.5.

*4.7.10. 1-(2-(2,4-dichlorophenyl)-2-((2,5-dimethylphenyl) selanyl) ethyl)-1H-imidazole (**B10**)*

White solid; yield 74.54%; m.p.: 107.6–109.8 °C. ESI-HRMS(m/z): found 425.0043 [M + H]+(calcd. for $C_{19}H_{19}N_2Cl_2Se$, 425.0091); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.53 (s, 1H), 7.50–7.45 (m, 2H), 7.31 (dd, J = 8.5, 2.2 Hz, 1H), 7.14–7.08 (m, 3H), 7.07–7.04 (m, 1H), 6.75 (s, 1H), 5.07 (dd, J = 10.0, 6.2 Hz, 1H), 4.73 (dd, J = 14.1, 10.0 Hz, 1H), 4.58 (dd, J = 14.1, 6.3 Hz, 1H), 2.21 (d, J = 4.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 138.5, 137.9, 137.0, 136.2, 136.0, 134.3, 132.9, 130.9, 130.3, 130.0, 129.2, 128.8, 128.2, 127.7, 119.7, 49.7, 22.4, 20.7.

*4.7.11. 1-(2-(2,4-dichlorophenyl)-2-((2,6-dimethylphenyl) selanyl) ethyl)-1H-imidazole (**B11**)*

White solid; yield 75.19%; m.p.: 107.4–109.4 °C. ESI-HRMS(m/z): found 425.0044 [M + H]+(calcd. for $C_{19}H_{19}N_2Cl_2Se$, 425.0091); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.62 (s, 1H), 7.48 (s, 1H), 7.45–7.40 (m, 1H), 7.35 (d, J = 8.5 Hz, 1H), 7.18 (dd, J = 8.6, 6.0 Hz, 1H), 7.12 (d, J = 6.5 Hz, 2H), 7.04 (d, J = 1.3 Hz, 1H), 6.73 (d, J = 1.1 Hz, 1H), 4.95 (s, 1H), 4.77 (dd, J = 14.0, 10.4 Hz, 1H), 4.51–4.40 (m, 1H), 2.37 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 144.1, 137.8, 136.4, 134.1, 132.9, 129.8, 128.8, 128.1, 127.9, 119.6, 49.8, 24.6.

*4.7.12. 1-(2-(2,4-dichlorophenyl)-2-((3,5-dimethylphenyl) selanyl) ethyl)-1H-imidazole (**B12**)*

White solid; yield 80.56%; m.p.: 107.2–109.1 °C. ESI-HRMS(m/z): found 425.0085 [M + H]+(calcd. for $C_{19}H_{19}N_2Cl_2Se$, 425.0091); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.55 (d, J = 1.1 Hz, 1H), 7.51 (dd, J = 2.2, 1.3 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.30 (dd, J = 8.5, 2.2 Hz, 1H), 7.10 (d, J = 1.2 Hz, 1H), 6.95 (d, J = 5.7 Hz, 3H), 6.76 (d, J = 1.1 Hz, 1H), 5.06 (dd, J = 9.7, 6.5 Hz, 1H), 4.70 (dd, J = 14.2, 9.7 Hz, 1H), 4.58 (dd, J = 14.2, 6.6 Hz, 1H), 2.20 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 138.8, 137.9, 136.1, 134.3, 133.1, 132.9, 130.8, 130.4, 129.3, 128.8, 127.6, 119.7, 49.6, 21.1.

*4.7.13. 1-(2-(2,4-dichlorophenyl)-2-((2-ethylphenyl) selanyl) ethyl)-1H-imidazole (**B13**)*

White solid; yield 73.38%; m.p.: 99.5–101.7 °C. ESI-HRMS(m/z): found 425.0095 [M + H]+(calcd. for $C_{19}H_{19}N_2Cl_2Se$, 425.0091); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.53–7.45 (m, 4H), 7.33–7.23 (m, 3H), 7.13 (td, J = 7.3, 1.9 Hz, 1H), 7.07 (d, J = 1.2 Hz, 1H), 6.74 (d, J = 1.1 Hz, 1H), 5.08 (dd, J = 10.1, 6.1 Hz, 1H), 4.76 (dd, J = 14.1, 10.2 Hz, 1H), 4.57 (dd, J = 14.2, 6.2 Hz, 1H), 2.61 (p, J = 7.3 Hz, 2H), 1.04 (t, J = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 147.4, 137.8, 136.5, 135.9, 134.2, 133.0, 130.9, 129.5, 129.3, 129.2, 128.8, 127.8, 127.2, 119.6, 49.6, 29.1, 16.1.

*4.7.14. 1-(2-(2,4-dichlorophenyl)-2-((4-ethylphenyl) selanyl) ethyl)-1H-imidazole (**B14**)*

White solid; yield 69.28%; m.p.: 99.5–101.7 °C. ESI-HRMS(m/z): found 425.0092 [M + H]+(calcd. for $C_{19}H_{19}N_2Cl_2Se$, 425.0091); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.54 (t, J = 1.1 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 7.31–7.26 (m, 3H), 7.14–7.11 (m, 2H), 7.09 (t, J = 1.3 Hz, 1H), 6.75 (t, J = 1.1 Hz, 1H), 5.04 (dd, J = 9.9, 6.3 Hz, 1H), 4.72 (dd, J = 14.2, 9.9 Hz, 1H), 4.57 (dd, J = 14.2, 6.4 Hz, 1H), 2.58 (q, J = 7.6 Hz, 2H), 1.15 (t, J = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 145.0, 137.9, 136.0, 136.0, 134.2, 132.8, 130.7, 129.3, 129.2, 128.8, 127.7, 119.7, 49.6, 28.3, 15.9.

*4.7.15. 1-(2-(2,4-difluorophenyl)-2-(phenylselanyl) ethyl)-1H-imidazole (**B15**)*

White solid; yield 73.15%; m.p.: 83.9–86.0 °C. ESI-HRMS(m/z): found 365.0362 [M + H]+(calcd. for $C_{17}H_{15}N_2F_2Se$, 365.0369); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.56 (d, J = 1.1 Hz, 1H), 7.42–7.25 (m, 6H), 7.14–7.07 (m, 2H), 6.99–6.93 (m, 1H), 6.77 (d, J = 1.1 Hz, 1H), 5.00 (dd, J = 9.5, 6.9 Hz, 1H), 4.67 (dd, J = 14.1, 9.5 Hz, 1H), 4.57 (dd, J = 14.1, 6.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 163.0, 161.6, 161.5, 160.6, 160.5, 159.1, 159.0, 137.9, 135.2, 131.0, 131.0, 130.9, 130.9, 129.7, 128.8, 128.0, 123.2, 123.2, 123.1, 123.1, 119.7, 112.0, 112.0, 111.8, 111.8, 104.6, 104.3, 104.1, 49.6.

*4.7.16. 1-(2-(2,4-difluorophenyl)-2-((3-fluorophenyl) selanyl) ethyl)-1H-imidazole (**B16**)*

White solid; yield 75.09%; m.p.: 85.2.1–86.9 °C. ESI-HRMS(m/z): found 383.0264 [M + H]+(calcd. for $C_{17}H_{14}N_2F_3Se$, 383.0274); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.57 (t, J = 1.1 Hz, 1H), 7.45 (td, J = 8.7, 6.5 Hz, 1H), 7.32 (td, J = 8.0, 6.1 Hz, 1H), 7.26–7.20 (m, 2H), 7.20–7.11 (m, 3H), 7.03–6.96 (m, 1H), 6.77 (d, J = 1.1 Hz, 1H), 5.09 (dd, J = 9.3, 7.1 Hz, 1H), 4.72–4.57 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.4, 163.2, 163.1, 161.7, 161.5, 161.0, 160.8, 160.6, 159.2, 159.1, 137.9, 131.4, 131.3, 131.1, 131.1, 131.0, 131.0, 130.8, 130.8, 130.0, 129.9, 128.8, 123.0, 123.0, 122.9, 122.8, 121.4, 121.2, 119.8, 115.8, 115.6, 112.1, 112.1, 111.9, 111.9, 104.7, 104.4, 104.2, 49.5.

*4.7.17. 1-(2-(2,4-difluorophenyl)-2-((4-fluorophenyl) selanyl) ethyl)-1H-imidazole (**B17**)*

White solid; yield 88.56%; m.p.: 88.2–100.2 °C. ESI-HRMS(m/z): found 383.0271 [M + H]+(calcd. for $C_{17}H_{14}N_2F_3Se$, 383.0274); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.57 (t, J = 1.2 Hz, 1H), 7.42–7.32 (m, 3H), 7.16–7.08 (m, 4H), 7.00–6.93 (m, 1H), 6.77 (d, J = 1.1 Hz, 1H), 4.97 (dd, J = 9.3, 7.1 Hz, 1H), 4.70–4.54 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 164.2, 163.1, 163.0, 161.7, 161.6, 161.5, 160.7, 160.5, 159.1, 159.0, 138.4, 138.3, 137.9, 130.9, 130.8, 130.8, 130.8, 128.8, 123.1, 123.0, 122.9, 122.9, 122.7, 122.7, 119.7, 116.9, 116.7, 112.0, 112.0, 111.8, 111.8, 104.6, 104.4, 104.1, 49.4.

*4.7.18. 1-(2-(2,4-difluorophenyl)-2-(o-tolylselanyl) ethyl)-1H-imidazole (**B18**)*

White solid; yield 81.98%; m.p.: 87.6–89.4 °C. ESI-HRMS(m/z): found 379.0529 [M + H]+(calcd. for $C_{18}H_{17}N_2F_2Se$, 379.0525); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (t, J = 1.1 Hz, 1H), 7.46–7.39 (m, 2H), 7.25–7.21 (m, 2H), 7.13–7.06 (m, 3H), 6.97 (tdd, J = 8.6, 2.7, 1.0 Hz, 1H), 6.76 (d, J = 1.1 Hz, 1H), 4.96 (dd, J = 9.8, 6.7 Hz, 1H), 4.69 (dd, J = 14.1, 9.8 Hz, 1H), 4.55 (dd, J = 14.1, 6.7 Hz, 1H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 163.0, 161.6, 161.5, 160.7, 160.5, 159.2, 159.1, 141.3, 138.0, 136.0, 131.1, 131.0, 131.0, 131.0, 130.5, 129.1, 129.0, 128.8, 127.1, 123.2, 123.1, 123.1, 123.0, 119.7, 112.1, 112.0, 111.9, 111.8, 104.6, 104.3, 104.0, 49.6, 22.8.

*4.7.19. 1-(2-(2,4-difluorophenyl)-2-(m-tolylselanyl) ethyl)-1H-imidazole (**B19**)*

White solid; yield 82.66%; m.p.: 86.8–89.0 °C. ESI-HRMS(m/z): found 379.0518 [M + H]+(calcd. for $C_{18}H_{17}N_2F_2Se$, 379.0525); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.56 (d, J = 1.2 Hz, 1H), 7.39 (td, J = 8.7, 6.5 Hz, 1H), 7.21–7.09 (m, 6H), 6.97 (tdd, J = 8.5, 2.6, 1.0 Hz, 1H), 6.77 (t, J = 1.1 Hz, 1H), 4.97 (dd, J = 9.4, 6.9 Hz, 1H), 4.66 (dd, J = 14.1, 9.5 Hz, 1H), 4.55 (dd, J = 14.1, 6.9 Hz, 1H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 163.0, 161.6, 161.5, 160.6, 160.5, 159.2, 159.0, 139.0, 137.9, 135.7, 132.2, 131.0, 131.0, 130.9, 130.9, 129.5, 128.8, 127.7, 123.3, 123.2, 123.1, 123.1, 119.7, 112.0, 111.9, 111.8, 111.7, 104.6, 104.3, 104.1, 49.6, 21.2.

*4.7.20. 1-(2-(2,4-difluorophenyl)-2-(p-tolylselanyl) ethyl)-1H-imidazole (**B20**)*

Colourless oil; yield 76.14%. ESI-HRMS(m/z): found 379.0520 [M + H]+(calcd. for $C_{18}H_{17}N_2F_2Se$, 379.0525); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.55 (s, 1H), 7.36 (td, J = 8.7, 6.5 Hz, 1H), 7.27 (d, J = 8.1 Hz, 2H), 7.14–7.08 (m, 4H), 6.99–6.93 (m, 1H), 6.76 (d, J = 1.1 Hz, 1H), 4.92 (dd, J = 9.6, 6.8 Hz, 1H), 4.67–4.60 (m, 1H), 4.54 (dd, J = 14.1, 6.8 Hz, 1H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 162.9, 161.6, 161.5, 160.6, 160.5, 159.1, 159.0, 138.6, 137.9, 135.7, 130.4, 128.8, 124.1, 120.0, 119.71, 112.0, 111.9, 111.8, 111.7, 104.9, 104.6, 104.3, 104.1, 49.5, 21.2.

*4.7.21. 1-(2-(2,4-difluorophenyl)-2-((2-methoxyphenyl) selanyl) ethyl)-1H-imidazole (**B21**)*

Colourless oil; yield 74.36%. ESI-HRMS(m/z): found 395.0463 [M + H]+(calcd. for $C_{18}H_{17}N_2F_2OSe$, 395.0474); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.52 (s, 1H), 7.47 (td, J = 8.7, 6.5 Hz, 1H), 7.39 (dd, J = 7.6, 1.6 Hz, 1H), 7.29 (ddd, J = 8.2, 7.4, 1.7 Hz, 1H), 7.14–7.08 (m, 2H), 7.00–6.94 (m, 2H), 6.87 (td, J = 7.5, 1.2 Hz, 1H), 6.77 (d, J = 1.3 Hz, 1H), 5.06 (dd, J = 9.8, 6.4 Hz, 1H), 4.67 (dd, J = 14.1, 9.9 Hz, 1H), 4.53 (dd, J = 14.1, 6.4 Hz, 1H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 162.9, 161.7, 161.5, 160.6, 160.5, 159.2, 159.06, 158.5, 137.8, 134.2, 131.2, 131.2, 131.1, 131.1, 123.0, 128.7, 123.2, 123.1, 123.0, 123.0, 121.7, 119.7, 117.4, 112.0, 112.0, 111.8, 111.8, 111.6, 104.5, 104.3, 104.0, 49.9, 37.2.

*4.7.22. 1-(2-(2,4-difluorophenyl)-2-((3-methoxyphenyl) selanyl) ethyl)-1H-imidazole (**B22**)*

Colourless oil; yield 81.20%. ESI-HRMS(m/z): found 395.0371 [M + H]+(calcd. for $C_{18}H_{17}N_2F_2OSe$, 395.0474); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.56 (t, J = 10 Hz, 1H), 7.42 (td, J = 8.7, 6.5 Hz, 1H), 7.22–7.17 (m, 1H), 7.16–7.10 (m, 2H), 7.00–6.95 (m, 2H), 6.89 (dtt, J = 5.1, 2.6, 1.3 Hz, 2H), 6.77 (t, J = 1.1 Hz, 1H), 5.02 (dd, J = 9.4, 7.0 Hz, 1H), 4.66 (dd, J = 14.1, 9.4 Hz, 1H), 4.57 (dd, J = 14.1, 7.0 Hz, 1H), 3.71 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.0, 161.6, 161.5, 160.7, 160.6, 159.7, 159.2, 159.1, 137.9, 131.1, 131.0, 131.0, 131.0, 130.1, 128.9, 128.8, 127.1, 123.2, 123.1, 119.9, 119.8, 114.8, 112.0, 112.0, 111.8, 111.8, 104.6, 104.4, 104.1, 55.6, 49.6, 49.1.

*4.7.23. 1-(2-(2,4-difluorophenyl)-2-((4-methoxyphenyl) selanyl) ethyl)-1H-imidazole (**B23**)*

Colourless oil; yield 73.39%. ESI-HRMS(m/z): found 395.0466 [M + H]+(calcd. for $C_{18}H_{17}N_2F_2OSe$, 395.0474); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.56 (t, J = 1.1 Hz, 1H), 7.32–7.25 (m, 3H), 7.13–7.07 (m, 2H), 6.95 (tdd, J = 8.5, 2.7, 1.0 Hz, 1H), 6.86–6.83 (m, 2H), 6.76 (t, J = 1.1 Hz, 1H), 4.86 (dd, J = 9.5, 6.9 Hz, 1H), 4.63 (dd, J = 14.1, 9.5 Hz, 1H), 4.53 (dd, J = 14.1, 6.9 Hz, 1H), 3.74 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.0, 162.9, 161.6, 161.4, 160.6, 160.4, 160.3, 159.1, 159.0, 138.2, 138.1, 138.0, 137.9, 130.8, 130.7, 130.7, 130.6, 128.9, 128.8, 123.3, 123.3, 123.2, 123.1, 120.1, 119.7, 117.5, 115.3, 111.9, 111.8, 111.7, 111.6, 104.6, 104.3, 104.0, 55.7, 49.4.

*4.7.24. 1-(2-(2,4-difluorophenyl)-2-((2,5-dimethylphenyl) selanyl) ethyl)-1H-imidazole (**B24**)*

Colourless oil; yield 81.37%. ESI-HRMS(m/z): found 393.0677 [M + H]+(calcd. for $C_{19}H_{19}N_2F_2Se$, 393.0682); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.56 (t, J = 1.1 Hz, 1H), 7.42 (td, J = 8.7, 6.5 Hz, 1H), 7.17 (d, J = 1.9 Hz, 1H), 7.14–7.07 (m, 3H), 7.05–6.93 (m, 2H), 6.77 (t, J = 1.1 Hz, 1H), 4.95 (dd, J = 9.7, 6.8 Hz, 1H), 4.68 (dd, J = 14.1, 9.7 Hz, 1H), 4.56 (dd, J = 14.1, 6.8 Hz, 1H), 2.20 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 163.0, 161.7, 161.6, 160.7, 160.5, 159.2, 159.1, 138.1, 137.8, 136.5, 136.1, 131.1, 131.0, 131.0, 130.9, 130.2, 129.8, 128.8, 128.7, 123.2, 123.2, 123.1, 123.0, 119.7, 112.0, 112.0, 111.8, 111.7, 104.5, 104.2, 104,0, 49.6, 22.3, 20.7.

*4.7.25. 1-(2-(2,4-difluorophenyl)-2-((2,6-dimethylphenyl) selanyl) ethyl)-1H-imidazole (**B25**)*

Colourless oil; yield 65.38%. ESI-HRMS(m/z): found 393.0674 [M + H]+(calcd. for $C_{19}H_{19}N_2F_2Se$, 393.0682); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.89–7.77 (m, 1H), 7.55 (d, J = 1.1 Hz, 1H), 7.44 (td, J = 8.7, 6.5 Hz, 1H), 7.17–7.10 (m, 4H), 7.00–6.94 (m, 1H), 6.75 (t, J = 1.1 Hz, 1H), 4.76–4.68 (m, 2H), 4.54–4.45 (m, 1H), 2.34 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 163.0, 162.9, 161.7, 161.5, 161.2, 161.0, 160.6, 160.5, 159.2, 159.1, 143.9, 137.9, 137.7, 131.2, 131.1, 131.1, 130.4, 129.7, 129.6, 128.8, 128.0, 126.4, 126.3, 126.3, 126.3, 123.4, 123.3, 123.2, 123.2, 119.7, 117.0, 112.0, 112.0, 111.8, 111.8, 109.1, 104.8, 104.4, 104.2, 103.9, 49.6, 24.5.

*4.7.26. 1-(2-(2,4-difluorophenyl)-2-((3,5-dimethylphenyl) selanyl) ethyl)-1H-imidazole (**B26**)*

Colourless oil; yield 68.20%. ESI-HRMS(m/z): found 393.0682 [M + H]+(calcd. for $C_{19}H_{19}N_2F_2Se$, 393.0682); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.08 (s, 1H), 7.70 (td, J = 8.8, 6.5 Hz, 1H), 7.31 (ddd, J = 11.5, 9.2, 2.6 Hz, 1H), 7.16–7.12 (m, 2H), 6.96 (d, J = 7.9 Hz, 3H), 6.78 (t, J = 1.1 Hz, 1H), 4.96 (dd, J = 9.4, 6.9 Hz, 1H), 4.65 (dd, J = 14.1, 9.4 Hz, 1H), 4.55 (dd, J = 14.1, 6.9 Hz, 1H), 2.20 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 163.08, 161.7, 161.2, 161.0, 160.7, 160.5, 159.2, 159.1, 158.7, 158.6, 138.8, 137.9, 137.7, 132.7, 132.7, 131.0, 131.0, 130.9, 130.9, 130.4, 130.3, 129.3, 129.2, 129.2, 129.1, 128.8, 127.6, 126.4, 126.3, 126.3, 126.3, 119.8, 119.8, 117.0, 112.7, 112.7, 112.5, 112.5, 111.9, 111.9, 109.1, 105.1, 104.8, 104.6, 104.3, 104.0, 49.6, 21.1.

*4.7.27. 1-(2-(2,4-difluorophenyl)-2-((2-ethylphenyl) selanyl) ethyl)-1H-imidazole (**B27**)*

Colourless oil; yield 86.41%. ESI-HRMS(m/z): found 393.0673 [M + H]+(calcd. for $C_{19}H_{19}N_2F_2Se$, 393.0682); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.54 (d, J = 1.1 Hz, 1H), 7.48–7.39 (m, 2H), 7.29–7.22 (m, 2H), 7.14–7.05 (m, 3H), 6.97 (tdd, J = 8.6, 2.7, 1.0 Hz, 1H), 6.76 (t, J = 1.1 Hz, 1H), 4.95 (dd, J = 9.8, 6.7 Hz, 1H), 4.69 (dd, J = 14.0, 9.8 Hz, 1H), 4.56 (dd, J = 14.1, 6.7 Hz, 1H), 2.60 (qd, J = 7.4, 3.4 Hz, 2H), 1.04 (t, J = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 163.0, 161.6, 161.5, 160.6, 160.5, 159.2, 159.0, 147.1, 137.9, 136.2, 131.1, 131.1, 131.0, 131.0, 129.3, 129.1, 128.8, 128.5, 127.2, 123.2, 123.1, 123.0, 123.0, 119.7, 112.1, 112.0, 111.9, 111.8, 104.5, 104.3, 104.0, 49.6, 29.1, 16.0.

*4.7.28. 1-(2-(2,4-difluorophenyl)-2-((4-ethylphenyl) selanyl) ethyl)-1H-imidazole (**B28**)*

Colourless oil; yield 85.29%. ESI-HRMS(m/z): found 393.0671 [M + H]+(calcd. for $C_{19}H_{19}N_2F_2Se$, 393.0682); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.55 (d, J = 1.1 Hz, 1H), 7.36 (td, J = 8.7, 6.5 Hz, 1H), 7.31–7.28 (m, 2H), 7.15–7.09 (m, 4H), 6.98–6.92 (m, 1H), 6.76 (t, J = 1.1 Hz, 1H), 4.94 (dd, J = 9.6, 6.8 Hz, 1H), 4.65 (dd, J = 14.1, 9.6 Hz, 1H), 4.55 (dd, J = 14.1, 6.8 Hz, 1H), 2.58 (q, J = 7.6 Hz, 2H), 1.15 (t, J = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 162.7, 162.6, 161.2, 161.1, 161.0, 160.9, 159.5, 159.4, 144.8, 137.9, 135.7, 130.9, 130.9, 130.9, 129.2, 128.8, 124.5, 119.7, 111.9, 111.8, 104.5, 104.3, 49.6, 28.3, 15.9.

*4.7.29. 1-(2-((2,4-dichlorobenzyl) selanyl)-2-(2,4-dichlorophenyl) ethyl)-1H-1,2,4-triazole (**C01**)*

White solid; yield 79.30%; m.p.: 91.3–92.8 °C. ESI-HRMS(m/z): found 481.9058 [M + H]+(calcd. for $C_{17}H_{14}N_3Cl_4Se$, 481.9078); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.43 (s, 1H), 7.92 (s, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.56 (d, J = 2.2 Hz, 1H), 7.51 (d, J = 2.2 Hz, 1H), 7.44 (d, J = 8.3 Hz, 1H), 7.38 (td, J = 6.2, 3.0 Hz, 2H), 4.97 (dd, J = 12.4, 4.8 Hz, 1H), 4.90–4.81 (m, 2H), 4.00 (d, J = 11.9 Hz, 1H), 3.88 (d, J = 11.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 152.0, 144.9, 136.0, 136.0, 134.3, 133.9, 133.1, 132.8, 132.6, 130.52, 129.6, 129.5, 128.1, 127.9,

52.4, 25.4.

*4.7.30. 1-(2-(benzylselanyl)-2-(2,4-dichlorophenyl) ethyl)-1H-1,2,4-triazole (**C02**)*

White solid; yield 73.31%; m.p.: 88.3–90.3 °C. ESI-HRMS(*m*/*z*): found 411.9870 [M + H]+(calcd. for $C_{17}H_{16}N_3Cl_2Se$, 411.9886); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.40 (s, 1H), 7.91 (s, 1H), 7.58 (d, J = 8.5 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.38 (dd, J = 8.4, 2.3 Hz, 1H), 7.29 (d, J = 4.4 Hz, 4H), 7.24–7.18 (m, 1H), 4.95 (dd, J = 13.7, 9.0 Hz, 1H), 4.84–4.73 (m, 2H), 3.92 (d, J = 11.6 Hz, 1H), 3.81 (d, J = 11.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 152.0, 144.8, 138.9, 136.4, 133.9, 132.9, 130.5, 129.5, 129.4, 128.9, 128.0, 127.3, 52.4, 28.5.

*4.7.31. 1-(2-((2-chlorobenzyl) selanyl)-2-(2,4-dichlorophenyl) ethyl)-1H-1,2,4-triazole (**C03**)*

White solid; yield 85.62%; m.p.: 82.8–84.9 °C. ESI-HRMS(*m*/*z*): found 445.9478 [M + H]+(calcd. for $C_{17}H_{15}N_3Cl_3Se$, 445.9497); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.42 (s, 1H), 7.92 (s, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.51 (d, J = 2.2 Hz, 1H), 7.43–7.37 (m, 3H), 7.31–7.25 (m, 2H), 5.00 (dd, J = 15.5, 11.1 Hz, 1H), 4.89–4.82 (m, 2H), 4.00 (d, J = 11.7 Hz, 1H), 3.89 (d, J = 11.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 152.0, 144.8, 136.7, 136.1, 133.9, 133.4, 133.0, 131.5, 130.5, 130.1, 129.6, 129.1, 128.1, 127.8, 52.4, 26.1.

*4.7.32. 1-(2-((4-chlorobenzyl) selanyl)-2-(2,4-dichlorophenyl) ethyl)-1H-1,2,4-triazole (**C04**)*

White solid; yield 82.32%; m.p.: 80.1–82.1 °C. ESI-HRMS(*m*/*z*): found 445.9473 [M + H]+(calcd. for $C_{17}H_{15}N_3Cl_3Se$, 445.9497); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.41 (s, 1H), 7.92 (s, 1H), 7.59–7.55 (m, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.40–7.29 (m, 5H), 5.00–4.79 (m, 2H), 4.72 (dd, J = 9.4, 6.5 Hz, 1H), 3.92 (d, J = 11.9 Hz, 1H), 3.82 (d, J = 11.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 152.1, 144.8, 138.2, 136.2, 133.8, 133.0, 131.8, 131.2, 130.5, 129.5, 128.8, 128.1, 52.3, 27.5.

*4.7.33. 1-(2-(2,4-dichlorophenyl)-2-((4-fluorobenzyl) selanyl) ethyl)-1H-1,2,4-triazole (**C05**)*

White solid; yield 72.70%; m.p.: 72.2–74.0 °C. ESI-HRMS(*m*/*z*): found 429.9759 [M + H]+(calcd. for $C_{17}H_{15}N_3Cl_2FSe$, 429.9792); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.41 (s, 1H), 7.92 (s, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.38 (dd, J = 8.4, 2.3 Hz, 1H), 7.35–7.30 (m, 2H), 7.15–7.08 (m, 2H), 5.00–4.79 (m, 2H), 4.72 (dd, J = 9.4, 6.5 Hz, 1H), 3.92 (d, J = 11.9 Hz, 1H), 3.82 (d, J = 11.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 162.8, 160.3, 152.0, 144.8, 136.3, 135.2, 135.2, 133.8, 132.9, 131.3, 131.3, 130.5, 129.5, 128.1, 115.8, 115.5, 52.4, 27.5.

*4.7.34. 1-(2-(2,4-dichlorophenyl)-2-((4-methoxybenzyl) selanyl) ethyl)-1H-1,2,4-triazole (**C06**)*

White solid; yield 83.43%; m.p.: 75.7–77.9 °C. ESI-HRMS(*m*/*z*): found 441.9867 [M + H]+(calcd. for $C_{18}H_{18}N_3Cl_2OSe$, 441.9892); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.41 (s, 1H), 7.92 (s, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.51 (d, J = 2.2 Hz, 1H), 7.38 (dd, J = 8.4, 2.2 Hz, 1H), 7.22–7.18 (m, 2H), 6.87–6.83 (m, 2H), 4.95 (dd, J = 13.8, 9.2 Hz, 1H), 4.84–4.71 (m, 2H), 3.88 (d, J = 11.6 Hz, 1H), 3.76 (d, J = 11.6 Hz, 1H), 3.72 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 158.7, 152.0, 144.8, 136.5, 133.9, 132.8, 130.5, 130.5, 129.5, 128.03, 114.4, 55.6, 52.4, 28.0.

*4.7.35. 1-(2-(2,4-dichlorophenyl)-2-((4-methylbenzyl) selanyl) ethyl)-1H-1,2,4-triazole (**C07**)*

Colourless oil; yield 75.40%. ESI-HRMS(*m*/*z*): found 426.0016 [M + H]+(calcd. for $C_{18}H_{18}N_3Cl_2Se$, 426.0043); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.39 (s, 1H), 7.91 (s, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.38 (dd, J = 8.4, 2.2 Hz, 1H), 7.16 (d, J = 8.1 Hz, 2H), 7.11–7.07 (m, 2H), 4.97–4.89 (m, 1H), 4.83–4.74 (m, 2H), 3.89 (d, J = 11.5 Hz, 1H), 3.76 (d, J = 11.5 Hz, 1H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 152.0, 144.8, 136.4, 136.4, 135.7, 133.9, 132.9, 130.5, 129.5, 129.5, 129.3, 128.0, 52.4, 28.3, 21.2.

*4.7.36. 1-(2-((2,4-dichlorobenzyl) selanyl)-2-(2,4-difluorophenyl) ethyl)-1H-1,2,4-triazole (**C08**)*

White solid; yield 75.83%; m.p.: 77.1–79.3 °C. ESI-HRMS(*m*/*z*): found 447.9673 [M + H]+(calcd. for $C_{17}H_{14}N_3Cl_2F_2Se$, 447.9698); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.41 (s, 1H), 7.91 (s, 1H), 7.57 (d, J = 2.1 Hz, 1H), 7.50 (td, J = 8.7, 6.4 Hz, 1H), 7.42 (d, J = 8.3 Hz, 1H), 7.36 (dd, J = 8.3, 2.1 Hz, 1H), 7.15 (ddd, J = 10.8, 9.3, 2.6 Hz, 1H), 7.03 (tdd, J = 8.5, 2.6, 1.1 Hz, 1H), 4.91–4.71 (m, 3H), 3.96 (d, J = 12.0 Hz, 1H), 3.87 (d, J = 11.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.2, 163.1, 161.4, 161.2, 160.8, 160.6, 158.9, 158.8, 152.0, 144.9, 136.2, 134.2, 132.8, 132.5, 130.8, 130.8, 130.7, 130.7, 129.5, 127.9, 123.4, 123.3, 123.2, 123.2, 112.3, 112.2, 112.0, 112.0, 104.8, 104.6, 104.3, 52.5, 35.2, 25.2.

*4.7.37. 1-(2-(benzylselanyl)-2-(2,4-difluorophenyl) ethyl)-1H-1,2,4-triazole (**C09**)*

Colourless oil; yield 71.34%. ESI-HRMS(*m*/*z*): found 380.0469 [M + H]+(calcd. for $C_{17}H_{16}N_3F_2Se$, 380.0478); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.39 (s, 1H), 7.91 (s, 1H), 7.47 (td, J = 8.7, 6.5 Hz, 1H), 7.31–7.25 (m, 4H), 7.24–7.18 (m, 1H), 7.14 (ddd, J = 10.8, 9.3, 2.6 Hz, 1H), 7.03 (tdd, J = 8.5, 2.6, 1.0 Hz, 1H), 4.90–4.72 (m, 2H), 4.63 (dd, J = 9.4, 6.7 Hz, 1H), 3.90 (d, J = 11.6 Hz, 1H), 3.80 (d, J = 11.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 163.0, 161.3, 161.2, 160.7, 160.5, 158.9, 158.7, 152.0, 144.8, 139.0, 130.8, 130.8, 130.7, 129.3, 128.9, 127.3, 112.3, 112.2, 112.0, 112.0, 104.8, 104.5, 52.5, 34.6, 28.3.

*4.7.38. 1-(2-((2-chlorobenzyl) selanyl)-2-(2,4-difluorophenyl) ethyl)-1H-1,2,4-triazole (**C10**)*

Colourless oil; yield 71.04%. ESI-HRMS(*m*/*z*): found 414.0068 [M + H]+(calcd. for $C_{17}H_{15}N_3ClF_2Se$, 14.0088); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.41 (s, 1H), 7.91 (s, 1H), 7.51 (td, J = 8.7, 6.4 Hz, 1H), 7.43–7.35 (m, 2H), 7.30–7.24 (m, 2H), 7.15 (ddd, J = 10.8, 9.2, 2.6 Hz, 1H), 7.03 (tdd, J = 8.5, 2.6, 1.0 Hz, 1H), 4.92–4.84 (m, 1H), 4.82–4.73 (m, 2H), 3.97 (d, J = 11.8 Hz, 1H), 3.89 (d, J = 11.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.2, 161.4, 161.3, 160.7, 160.6, 158.9, 158.8, 152.0, 144.8, 136.8, 133.3, 131.3, 130.8, 130.8, 130.7, 130.7, 130.0, 129.3, 127.8, 123.4, 123.4, 123.3, 123.3, 112.3, 112.2, 112.0, 112.0, 104.8, 104.6, 104.3, 52.6, 35.1, 25.9.

*4.7.39. 1-(2-((4-chlorobenzyl) selanyl)-2-(2,4-difluorophenyl) ethyl)-1H-1,2,4-triazole (**C11**)*

Colourless oil; yield 73.03%. ESI-HRMS(*m*/*z*): found 414.0088 [M + H]+(calcd. for $C_{17}H_{15}N_3ClF_2Se$, 14.0088); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.40 (s, 1H), 7.91 (s, 1H), 7.47 (td, J = 8.7, 6.5 Hz, 1H), 7.36–7.32 (m, 2H), 7.31–7.27 (m, 2H), 7.14 (ddd, J = 10.8, 9.3, 2.7 Hz, 1H), 7.02 (tdd, J = 8.4, 2.6, 1.0 Hz, 1H), 4.90–4.74 (m, 2H), 4.62 (dd, J = 9.3, 6.7 Hz, 1H), 3.90 (d, J = 11.9 Hz, 1H), 3.80 (d, J = 11.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 163.0, 161.3, 161.2, 160.7, 160.6, 158.8, 158.7, 152.0, 144.8, 138.3, 131.8, 131.1, 130.8, 130.8, 130.7, 130.7, 128.8, 123.5, 123.4, 123.3, 123.3, 112.3, 112.2, 112.1, 112.0, 104.8, 104.5, 104.3, 52.5, 34.6, 27.4.

*4.7.40. 1-(2-(2,4-difluorophenyl)-2-((4-fluorobenzyl) selanyl) ethyl)-1H-1,2,4-triazole (**C12**)*

Colourless oil; yield 61.48%. ESI-HRMS(*m*/*z*): found 398.0374 [M + H]+(calcd. for $C_{17}H_{15}N_3F_3Se$, 398.0383); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.40 (s, 1H), 7.92 (s, 1H), 7.47 (td, J = 8.7, 6.4 Hz, 1H), 7.3–7.28 (m, 2H), 7.17–7.08 (m, 3H), 7.03 (tdd, J = 8.5, 2.6, 1.0 Hz, 1H), 4.90–4.73 (m, 2H), 4.61 (dd, J = 9.4, 6.8 Hz, 1H), 3.90 (d, J = 11.8 Hz, 1H), 3.80 (d, J = 11.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO)

δ (ppm): 162.7, 160.3, 158.8, 158.7, 152.0, 144.8, 135.3, 135.3, 131.2, 131.1, 130.8, 130.7, 130.7, 130.6, 123.5, 123.5, 123.4, 123.4, 115.8, 115.5, 112.3, 112.2, 112.1, 112.0, 104.8, 104.5, 104.3, 52.5, 34.5, 27.4.

4.7.41. 1-(2-(2,4-difluorophenyl)-2-((4-methoxybenzyl) selanyl) ethyl)-1H-1,2,4-triazole (**C13**)

Colourless oil; yield 73.24%. ESI-HRMS(*m*/*z*): found 432.0392 [M + Na]+(calcd. for $C_{18}H_{17}N_3F_2OSeNa$, 432.0403); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.39 (s, 1H), 7.90 (s, 1H), 7.46 (td, *J* = 8.7, 6.5 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 3H), 7.03 (tdd, *J* = 8.5, 2.7, 1.1 Hz, 1H), 6.87–6.82 (m, 2H), 4.88–4.72 (m, 2H), 4.61 (dd, *J* = 9.4, 6.7 Hz, 1H), 3.86 (d, *J* = 11.6 Hz, 1H), 3.75 (d, *J* = 11.8 Hz, 1H), 3.73 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 162.9, 161.3, 161.2, 160.6, 160.5, 158.9, 158.7, 158.6, 152.0, 144.8, 130.8, 130.7, 130.7, 130.6, 130.6, 130.4, 114.4, 112.2, 112.2, 112.0, 112.0, 104.8, 104.5, 104.3, 55.5, 52.6, 34.5, 27.8.

4.7.42. 1-(2-(2,4-difluorophenyl)-2-((4-methylbenzyl) selanyl) ethyl)-1H-1,2,4-triazole (**C14**)

Colourless oil; yield 51.55%. ESI-HRMS(*m*/*z*): found 394.0608 [M + H]+(calcd. for $C_{18}H_{18}N_3F_2Se$, 394.0634); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.38 (s, 1H), 7.90 (s, 1H), 7.47 (td, *J* = 8.7, 6.5 Hz, 1H), 7.17–7.07 (m, 5H), 7.03 (tdd, *J* = 8.5, 2.6, 1.1 Hz, 1H), 4.90–4.71 (m, 2H), 4.62 (dd, *J* = 9.5, 6.7 Hz, 1H), 3.87 (d, *J* = 11.6 Hz, 1H), 3.75 (d, *J* = 11.6 Hz, 1H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.1, 163.0, 161.3, 161.2, 160.6, 160.5, 158.9, 158.7, 152.0, 144.8, 136.4, 135.8, 130.8, 130.8, 130.7, 130.7, 129.5, 129.2, 123.6, 123.6, 123.5, 123.4, 112.2, 112.2, 112.0, 112.0, 104.8, 104.5, 104.3, 52.5, 34.6, 28.1, 21.1.

4.8. In vitro antifungal testing

The target compounds were tested for their inhibitory potential against eight kinds of fungus strains by the micro-broth dilution assay according to the CLSI M27-A3 guidelines. The RPMI 1640 test medium (Gibco, USA) was buffered with morpholinepropanesulfonic acid (MOPS, Genyiew, USA). The Fungi cells which was in growing stage were gathered and inoculated in RPMI 1640 medium to an initial concentration of 1–5 × 10^3 CFU/mL. FCZ, MCZ and test compounds were dissolved in DMSO respectively as the stock solution. After inoculation, the plates inoculated fungal cells were at 35 °C for 24 h, while those *C. neo*. and *A, f.* were inoculated at 35 °C for 72 h. The MICs were defined as the lowest concentration of a compound that prevents visible growth. All measurements were made using biological repetition.

4.9. Evaluation of MFC

The pH of RPMI 1640 medium (Gibco, USA) was adjusted to 7.0 by employing NaOH, $NaHCO_3$ and morpholinepropanesulfonic acid (MOPS, Genyiew, USA). *C. albicans* (CPCC400616) cells which was in growing stage were gathered and inoculated in fresh RPMI 1640 medium to an initial concentration of 1–5 × 10^3 CFU/mL. Miconazole and test compounds were dissolved in DMSO respectively as the stock solution. The samples and fungal suspension were cultured at 35 °C for 48 h. Next, PDA plates were plated with fungal suspension (100 μL) from the 96-well plates which were invisible growth. Then the plates were incubated at 35 °C for 24 h. MFCs corresponds to the concentration of colonies less than 5 on the SDA plate.

4.10. Time-kill study

Fungi suspension of *C. albicans* (1 × 10^5 CFU/mL) were prepared in RPMI 1640 medium. Taken fungal suspension and different concentrations of **B17** and miconazole together to incubate at 35 °C for 24 h. The control group was not treated with compound. At designated time points, fungal suspension (10 μL) was obtained from the RPMI 1640 medium and then was10-fold serial diluted by using 0.9% saline. Then, the diluted fungal suspension was inoculated into PDA plate culture and incubated at 35 °C for 24 h. At last, the colony forming units (CFU) were counted. The Log_{10} CFU/mL was drawn on a chart as a time function. The detection limit of this method was 100 CFU/mL. The experiment was carried out in triplicate.

4.11. GC-MS analysis of sterol composition

C. albicans ATCC SC5314 cells were gathered and inoculated in fresh YEPD (60 mL) with various concentrations of compounds for 16 h at 35 °C. Cells were harvested by centrifuging at 3000 g for 10 min. Subsequently, the cells were washed by using PBS (3 × 10 mL) and saponified at 80 °C for 60 min by using saponification medium (3 mL, 15% NaOH solution in 90% ethanol). And the residue was extracted by petroleum ether (3 × 6 mL). Petroleum ether was concentrated under reduced pressure, and the residue was dissolved in hexamethylene for the next determination. The sterol composition analysis experiment was carried out by GC-MS. Analyses were carried out in a non-splitting mode by selecting helium as carrier gas (constant rate of 1.0 mL/min). The GC oven was programmed as follows: the injector temperature was 250 °C. Initial temperature was 100 °C, hold for 1 min, ramp to 300 °C at 10 min^{-1}, hold for 10 min.

4.12. In vitro biofilm formation assay

Fungal suspension of *C. albicans* (1 × 10^6 CFU/mL) was prepared in RPMI 1640 medium. Then the fungal suspension was moved to 96-well plates and incubated at 37 °C. The non-adherent cells and culture medium were removed after1.5 h, 3 h, 6 h and 24 h. Each well of 96-well plates was washed with PBS (3 × 0.1 mL) and different concentrations of compounds in fresh RPMI 1640 medium were added to the 96-well plates. The plates were continuously incubated at 37 °C for 24 h. XTT reduction method was used to calculate the semi-quantitative determination of the formed biofilms.

4.13. Hemolytic assays

Fresh white rabbit the red blood cells were used in this assay. Test compounds were dissolved in DMSO as the stock solution. The red blood cells suspension was prepared by washing the rabbit red blood cells with PBS buffer solution (three times). After diluting the RBCs to 2% concentration with PBS buffer solution (2%), the stock solution was diluted to double volume by employing PBS. Subsequently, the diluted solution of test compounds was mixed with the 2% the red blood cells suspension in the 8-well plates. The plates should be incubated at 37 °C for 3 h, and the mixtures were centrifuged at 600 g for 5 min. Subsequently, the supernatant was moved into 96-well plates. The absorbance was tested on a microplate reader at 575 nm. Meanwhile, the red blood cells with PBS served as the negative control (0% lysis), and the red blood cells which was treated with 2% Triton X-100 were used as controls (100% lysis).

4.14. Cytotoxicity assay

After recovery, the cells were cultured at 37 °C in a humidified atmosphere with 95% air and 5% CO_2. The MDA-MB-231 cells were grown in DMEM replenished with 10% fetal bovine serum, 100 IU/

mL of penicillin G sodium and 100 μg/mL of streptomycin sulphate. The HL-60 and PC3 cell lines were grown in RPMI 1640 medium replenished with 10% fetal bovine serum, 100 IU/mL of penicillin G sodium and 100 μg/mL of streptomycin sulphate. In this experiment, different cell lines were seeded at a density of $1.5-3 \times 10^3$ cells/well in 96-well microtiter plate, and incubated for 24 h. Cells grew for 96 h, after treating with 100 μL different concentrations of tested compounds. Subsequently, MTT was added at 5 mg/mL in each well and the plates were incubated for 4 h. The residual medium was removed and DMSO (100 μL) was added into each well to solubilise the formazan crystals. Absorbance was taken at 540 nm with a microplate reader. The IC_{50} value is defined as the concentration of the inhibitor that caused 50% inhibition.

4.15. Microsomal stability assays

The microsomal stability was performed by WuXi AppTec (https://www.wuxiapptec.com/zh-cn).

4.16. In vivo antifungal assay

Mice were assigned into four groups: the control group, the fluconazole treatment group (0.6 mg/kg/day), the **A03** treatment group (0.6 mg/kg/day) and two **B17** treatment group (0.4 and 0.6 mg/kg/day, respectively). Mice in each group were given 200 μL *C. albicans* ATCC SC5314 (5×106 CFU/mL) of by the tail vein. Fluconazole, **A03** and **B17** treatment were initiated by intraperitoneal injection at 2 h after infection. The treatments were administered daily for 4 days. The kidneys of the mice were removed, weighed and homogenized. To determine the efficacy of therapy in murine models, a determination of renal fungal burden as CFU is commonly used.

4.17. Pharmacokinetic assay

Six rats fasted for 12 h before the experiment, and had free access to water. Orbital blood samples were collected in heparinized tubes before dosing and at 5 min (only iv), 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 8 h, 12 h and 24 h after administration. Plasma samples were harvested immediately by centrifugation of the rat blood at 6000 rpm for 10 min and stored at −20 °C until analysis. LC-MS/MS method was used for quantitative determination, and the data were analyzed by DAS 2.0 (Chinese Pharmacological Society).

4.18. Docking analysis

The crystal structure of CYP51 (PDB code 5tz1) was obtained from the Protein Data Bank. The 3D structures of the test compounds were generated in ChemBio 3D Ultra 14.0. Test compounds were docked into the target proteins after removing hetero atoms, water molecules and co-factors by using Discovery Studio 3.0.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, "**Lead optimization generates selenium-containing miconazole CYP51 inhibitors with improved pharmacological profile for the treatment of fungal infections"** for possible publication in "***European Journal of Medicinal Chemistry***".

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113337.

Abbreviations

C.alb.	Candida albicans (CPCC400616)
C.alb (SC5314)	*Candida albicans* (ATCC SC5314)
C.zey.	*Candida zeylanoides* (CGMCC2.3739)
C.neo.	*Cryptococcus neoformans* (CGMCC2.3161)
C.kru.	*Candida kruseii* (AS 2.1045)
C.gla.	*Candida glabrata* (Clinical isolation)
C.par.	*Candida parapsilosis* (ATCC 22019)
A.f.	*Aspergillus fumigatus* (CGMCC 3.7795)
strain 17#	fluconazole-resistant strain of *C. albicans*
strain CaR	fluconazole-resistant strain of *C. albicans*
strain 632	fluconazole-resistant strain of *C. albicans*
strain 901	fluconazole-resistant strain of *C. albicans*
strain 904	fluconazole-resistant strain of *C. albicans*
FCZ	Fluconazole
MCZ	Miconazole
$T_{1/2}$	Half-life
CL	Clearance
$SMIC_{50}$	sessile minimum inhibitory concentration that reduced the metabolic activity of biofilms by 50%
$SMIC_{80}$	sessile minimum inhibitory concentration that reduced the metabolic activity of biofilms by 80%
XTT	3-bis (2-hydroxyethylthio) naphthalene-1,4-dione.

References

[1] T. Roemer, D.J. Krysan, Antifungal drug development: challenges, unmet clinical needs, and new approaches, Csh. Perspect. Med. 4 (2014) a019703-a019703.
[2] S. Dominique, Emerging Threats in antifungal-resistant fungal pathogens, Front. Med. 3 (2016) 11.
[3] S. Antinori, L. Milazzo, S. Sollima, M. Galli, M. Corbellino, Candidemia and invasive candidiasis in adults: a narrative review, Eur. J. Intern. Med. 34 (2016) 21–28.
[4] N.P. Wiederhold, Antifungal resistance: current trends and future strategies to combat, Infect. Drug Resist. 10 (2017) 249–259.
[5] M.W. Mccarthy, T.J. Walsh, Drug development challenges and strategies to address emerging and resistant fungal pathogens, Expert Rev. Anti Infect. Ther. 15 (2017) 577–584.
[6] D.S. Perlin, E. Shor, Y. Zhao, Update on antifungal drug resistance, Curr. Clin. Micro. Rpt. 2 (2015) 84–95.
[7] E.L. Berkow, S. R Lockhart, Fluconazole resistance in Candida species: a current perspective, Infect. Drug Resist. 10 (2017) 237–245.
[8] M.K. Kathiravan, A.B. Salake, A.S. Chothe, P.B. Dudhe, R.P. Watode, M.S. Mukta, S. Gadhwe, The biology and chemistry of antifungal agents: a review, Bioorg. Med. Chem. 20 (2012) 5678–5698.
[9] N. Liu, J. Tu, G. Dong, Y. Wang, C. Sheng, Emerging new targets for the treatment of resistant fungal infections, J. Med. Chem. 61 (2018) 5484–5511.
[10] R. Wu, T. Ren, J. Gao, L. Wang, Q. Yu, Z. Yao, G. Song, W. Ruan, C. Niu, F. Song, L. Zhang, M. Li, J. Wang, Chemical preparation, biological evaluation and 3D-QSAR of ethoxysulfuron derivatives as novel antifungal agents targeting acetohydroxyacid synthase, Eur. J. Med. Chem. 162 (2019) 348–363.
[11] K.A. Agnew-Francis, Y. Tang, X. Lin, Y. Low, S. Wun, A. Kuo, S.M.A.S.I. Elias, T. Lonhienne, N.D. Condon, B.N.A.S. Pimentel, C.E. Vergani, M.e T. Smith, J.A. Fraser, C.M. Williams, L.W. Guddat, Herbicides that target acetohydroxyacid synthase are potent inhibitors of the growth of drug-resistant Candida auris, ACS Infect. Dis. 6 (2020) 2901–2912.

[12] G.I. Lepesheva, L. Friggeri, M.R. Waterman, CYP51 as drug targets for fungi and protozoan parasites: past, present and future, Parasitology 145 (2018) 1–17.
[13] B.C. Monk, A.A. Sagatova, P. Hosseini, Y.N. Ruma, R.K. Wilson, M.V. Keniya, Fungal Lanosterol 14α-demethylase: a target for next-generation antifungal design, Biochim. Biophys. Acta 1868 (2020), 140206.
[14] R.C. Heel, R.N. Brogden, G.E. Pakes, T.M. Speight, G.S. Avery, Miconazole: a preliminary review of its therapeutic efficacy in systemic fungal infections, Drugs 19 (1980) 7–30.
[15] J. Zhang, L. Li, Q. Lv, L. Yan, Y. Wang, Y. Jiang, The fungal CYP51s: their functions, structures, related drug resistance, and inhibitors, Front. Microbiol. 10 (2019).
[16] J. Song, S. Zhang, L. Lu, Fungal cytochrome P450 protein Cyp51: what we can learn from its evolution, regulons and Cyp51-based azole resistance, Fungal Biol. Rev. 32 (2018) 131–142.
[17] Y. Zhang, G.L.V. Damu, S. Cui, J. Mi, V.K.R. Tangadanchu, C. Zhou, Discovery of potential antifungal triazoles: design, synthesis, biological evaluation, and preliminary antifungal mechanism exploration, Medchemcomm 8 (2017) 1631–1639.
[18] P. Nenoff, D. Koch, C. Krüger, C. Drechsel, P. Mayser, New insights on the antibacterial efficacy of miconazole in vitro, Mycoses (2017) 1–6.
[19] D. Allen, D. Wilson, R. Drew, J. Perfect, Azole antifungals: 35 years of invasive fungal infection management, Expert Rev. Anti-Infe 13 (2015) 787–798.
[20] H. Xu, X. Su, M. Guo, R. An, Y.M.Z. Hou, C. Guo, Design, synthesis, and biological evaluation of novel miconazole analogues containing selenium as potent antifungal agents, Eur. J. Med. Chem. 198 (2020), 112360.
[21] H. Xu, Y. Hu, M. Guo, A. Huang, X. Su, C. Guo, Design, synthesis and antifungal activity of novel selenochroman-4-one derivatives, Chem. Pap. 71 (2017) 2455–2463.
[22] H. Xu, X. Su, X. Liu, K. Zhang, Z. Hou, C. Guo, Design, synthesis and biological evaluation of novel semicarbazone selenochroman-4-ones hybrids as potent antifungal agents, Bioorg. Med. Chem. Lett 29 (2019), 126726.
[23] C. Ji, N. Liu, J. Tu, Z. Li, G. Han, J. Li, C. Sheng, Drug repurposing of haloperidol: discovery of new benzocyclane derivatives as potent antifungal agents against cryptococcosis and candidiasis, ACS Infect. Dis. 6 (2020) 768–786.
[24] L. Zhao, L. Tian, N. Sun, Y. Sun, Y. Chen, X. Wang, S. Zhao, X. Su, D. Zhao, M. Cheng, Design, synthesis, and structure-activity relationship studies of L-amino alcohol derivatives as broad-spectrum antifungal agents, Eur. J. Med. Chem. 177 (2019) 374–385.
[25] J. Tessier, M. Golmohamadi, K.J. Wilkinson, A.R. Schmitzer, Anti-staphylococcal biofilm activity of miconazoctylium bromide, Org. Biomol. Chem. 16 (2018) 4288–4294.
[26] S. Wu, Y. Wang, N. Liu, G. Dong, C. Sheng, Tackling fungal resistance by biofilm inhibitors, J. Med. Chem. 60 (2017) 2193–2221.
[27] I.B. Rosseti, J.B. Rocha, M.S. Costa, Diphenyl diselenide $(PhSe)_2$ inhibits biofilm formation by *Candida albicans*, increasing both ROS production and membrane permeability, J. Trace Elem. Med. Biol. 29 (2015) 289–295.