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Research paper

Design, synthesis, and biological evaluation of novel miconazole analogues containing selenium as potent antifungal agents

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ABSTRACT

Herein, based on the theory of bioisosterism, a series of novel miconazole analogues containing selenium were designed, synthesized and their inhibitory effects on thirteen strains of pathogenic fungi were evaluated. It is especially encouraging that all the novel target compounds displayed significant antifungal activities against all tested strains. Furthermore, all the target compounds showed excellent inhibitory effects on fluconazole-resistant fungi. Subsequently, preliminary mechanistic studies indicated that the representative compound **A03** had a strong inhibitory effect on *C.alb*. CYP51. Moreover, the target compounds could prevent the formation of fungi biofilms. Further hemolysis test verified that potential compounds had higher safety than miconazole. In addition, molecular docking study provided the interaction modes between the target compounds and *C.alb.* CYP51. These results strongly suggested that some target compounds are promising as novel antifungal drugs.

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1. Introduction

Invasive fungal infection is closely correlated with high rates of morbidity and mortality in immunocompromised hosts, such as HIV-infected patients, transplant patients or patients who are receiving chemotherapeutic agents [1,2]. Although many agents have emerged to manage these infections over the past 30 years, treatment is still not optimal, because of the toxicities and drug interaction [3,4]. Therefore, it is urgent to develop new antifungal drugs.

Fungi are different from mammals, fungal cells rely mainly on ergosterol as the major membrane sterol while mammalian cells need cholesterol to function [5]. Therefore, most of the clinically used antifungal agents either target membrane ergosterol or the ergosterol biosynthetic pathway [6]. Azoles could prevent the conversion of lanosterol to ergosterol by inhibiting the activity of cytochrome P450 (CYP)-dependent 14-α-demethylase (CYP51), thus inhibiting the growth of the fungus [7,8]. The discovery of azole antifungals was a huge step in the war against superficial and invasive fungal infections, given the efficacy, safety and oral bioavailability of these drugs [9]. The clinically used azole antifungals (Fig. 1), characterized by core azole rings, include imidazoles, which contain two nitrogens, and triazoles, which have three nitrogens on the 5-member azole ring [10].

Recently, there are more and more reports about selenium-containing compounds because of their significant biological activities such as antifungal, antibacterial, anti-tumor and anti-inflammatory properties [11–13]. It is well-known that selenium-containing compounds show broad similarities with the corresponding sulphur-containing compounds, that is, they could be widely used in the research and development of pharmaceuticals [14]. Moreover, the bioisosteric replacement of the oxygen or sulphur atom with selenium in known bioactive compounds is usually an easy and efficient approach in medicinal chemistry [15]. In our previous studies, antifungal activities of compounds such as selenochroman-4-ones and 2,3-dihydro-4*H*-1-benzoselenin-4-one semicarbazone derivatives were strengthened obviously by replacing sulphur atom with selenium in these molecules [16,17]. (Fig. 2). These useful results have a great guiding significance for finding better selenium-containing antifungal agents. Therefore, we intended to continue to focus our efforts in this area in this study.

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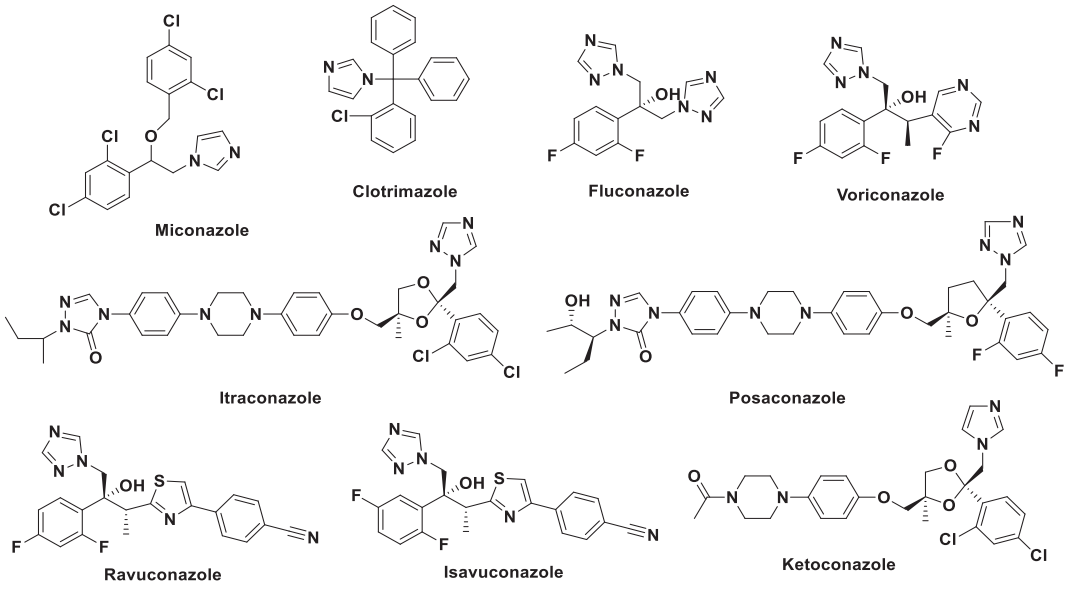


Fig. 1. Chemical structures of clinically used azole antifungal agents.

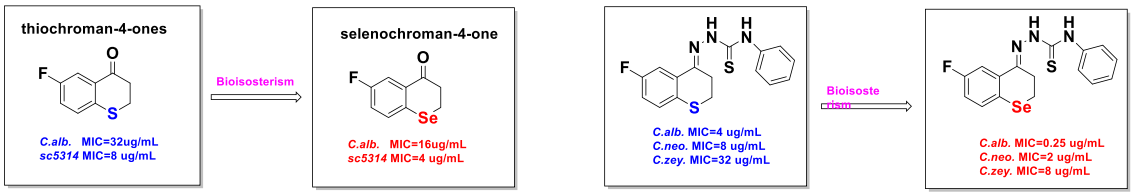


Fig. 2. Selenium-containing antifungal compounds obtained by bioisosterism.

Miconazole, an imidazole-containing compound, is a clinical widely used antifungal agent with low toxicity and excellent safety profile [18]. Meanwhile, miconazole possesses broad antimicrobial spectrum, for example, could be used to treat skin infections caused by Gram-positive bacteria both *in vitro* and *in vivo* [19]. At present, much effort has been devoted to the structural modification and development of miconazole, and many excellent achievements have been obtained [20,21]. For example, sulconazole, an imidazole broad-spectrum antifungal drug, was obtained by replacing oxygen atom of miconazole with sulphur atom [22] (Fig. 3). In view of the above observations and our previous work, herein we designed and synthesized thirty selenium-substituted miconazole derivatives according to the biosostere theory (Fig. 3). In order to verify the effectiveness of the design, then we carried out a series of experiments including *in vitro* antifungal activity, time-kill assays, biofilm inhibition experiment, and so on.

2. Results and discussion

2.1. Chemistry

The synthesis of the intermediates **5a**-**5p**, **11a**-**11b** and the target compounds **A01**-**A30** was presented at Scheme 1, Scheme 2, and Scheme 3, respectively. Firstly, disodium diselenide (**3**) was prepared from selenium powder and sodium borohydride under nitrogen condition through alkylation. Subsequently, the key intermediates dibenzyl selenide (**5a**-**5p**) were obtained from chlorobenzyl or substituted chlorobenzyl (**4a**-**4p**) by coupling reaction with disodium diselenide (Scheme 1).

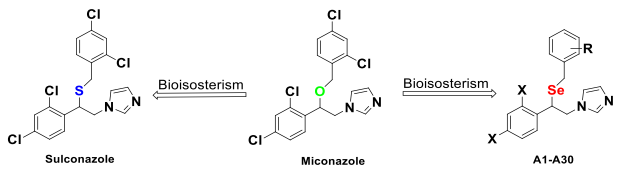


Fig. 3. Rational design of target compounds.

Reagents and conditions: (1) EtOH, 30 min; (2) CH_2Cl_2, 40 °C, 10 h.

The synthetic pathway of key intermediates **11a**-**11b** was depicted in Scheme 2. Intermediates **11a**-**11b** were synthesized from 1,3-dichlorobenzene (**6a**) or 1,3-difluorobenzene (**6b**). Firstly, compounds **6a** or **6b** reacted with 2-chloroacetyl chloride to form intermediates **8a**-**8b** by friedel-crafts acylation reaction. Subsequently, intermediates **9a**-**9b** were obtained by nucleophilic substitution with imidazole. Next, intermediates **9a**-**9b** could be reduced to intermediates **10a**-**10b** by sodium borohydride. Finally, intermediates **11a**-**11b** were obtained by chlorination reaction in the thionyl chloride.

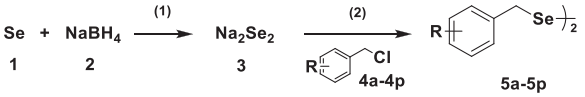
Reagents and conditions: (1) $AlCl_3$, 30 °C, 3 h; (2) imidazole, MeOH, 65 °C, 6 h; (3) $NaBH_4$, EtOH, 20 °C, 12 h; (4) $SOCl_2$, 20 °C, 12 h.

The general synthetic route of target compounds is shown in Scheme 3. The target compounds **A01**-**A30** were respectively obtained by the nucleophilic substitution of the intermediates **11a**-**11b** with intermediates **5a**-**5p** in good yields.

Reagents and conditions: 65 °C, 1 h.

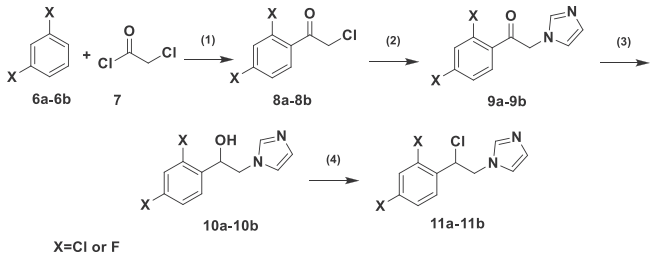
2.2. In vitro antifungal activity and structure-activity relationships

The *in vitro* antifungal activity of the target compounds was

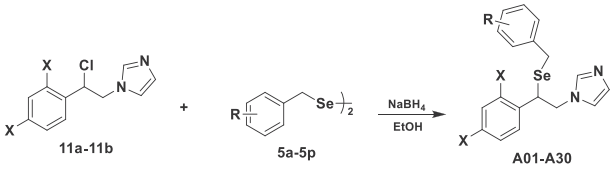


5a R=H; 5b R=2,4-di-Cl; 5c R=2-Cl; 5d R=3-OCH_3; 5e R=3,5-di-OCH_3; 5f R=2-Br; 5g R=4-Br; 5h R=2-Br-4,5-di-OCH_3; 5i R=3-CH_3; 5j R=3,4-di-CH_3; 5k R=3-F; 5l R=2-Br-5-F; 5m R=4-Cl; 5n R=4-CH_3; 5o R=4-F; 5p R=4-OCH_3

Scheme 1. Synthetic routes of intermediates **5a**-**5p**.



Scheme 2. Synthetic routes of intermediates **11a**-**11b**.



Scheme 3. Synthetic routes of target compounds.

determined by the minimal inhibitory concentrations (MICs) with fluconazole (FCZ) and miconazole (MCZ) as the control drug. The results expressed as MIC are shown in Table 1.

Overall, the result showed that almost all the compounds exhibited notable antifungal activities against all test strains. Notably, compounds **A03**, **A07**, **A09**, **A18** and **A23** showed excellent antifungal activity against various strains. The following should be noted regarding antifungal inhibitory data of Table 1:

1) Against *Candida albicans* (CPCC400616), all target compounds (MIC values of 0.02–0.5 μg/mL) showed appreciable higher *in vitro* antifungal activity than fluconazole (MIC = 4 μg/mL). And most of the target compounds (except **A20** and **A28**) showed *Candida albicans* (CPCC400616) inhibitory efficiency comparable to the reference miconazole (MIC = 0.25 μg/mL). Among them, compounds **A03**, **A07**, **A09** and **A23** showed the most potent inhibitory activity against *Candida albicans* (MIC = 0.02 μg/mL), which is 16 times better than miconazole.
2) Toward *Candida albicans* (ATCC SC5314), nearly all of the new compounds investigated here (except **A14**) showed excellent *in vitro* antifungal activity, with MIC values of 0.01–0.13 μg/mL, being much more effective as compared to fluconazole (MIC = 1 μg/mL). Compounds **A01**, **A03**, **A07**, **A09**, **A19** and **A23** possessed *in vitro* antifungal better activity than the control drug miconazole (MIC = 0.03 μg/mL).
3) *Cryptococcus neoformans* (CGMCC2.3161) was also strongly inhibited by the synthesized compounds (except **A25**), which showed MIC values in the range of 0.13–0.5 μg/mL. These compounds also possessed an similar or slightly improved inhibitory activity over miconazole (0.5 μg/mL).
4) Against *Candida glabrata* and *Aspergillus fumigatus*, all target compounds (MIC values of 0.13–1 μg/mL, 0.5–16 μg/mL, respectively) showed much better *in vitro* antifungal activity than fluconazole (MIC = 32 μg/mL, >128 μg/mL, respectively). Among them, compounds **A01**, **A02**, **A09** and **A18** (MIC = 0.5 μg/mL) showed high *in vitro* inhibitory activity against *Aspergillus fumigatus*, which is four times better than miconazole (MIC = 2 μg/mL).
5) Inhibitory efficiency against *Candida zeylanoides*, *Candida kruseii* and *Candida parapsilosis*, different compounds showed different *in vitro* antifungal activities, the MIC values were in the range of 0.25–128 μg/mL, 0.06–128 μg/mL and 0.02–128 μg/mL, respectively.

2.3. In vitro antifungal activity against fluconazole-resistant strains of Candida albicans

Azole drugs have been used broadly in the treatment of many kinds of fungal infection in clinic, so drug resistance is also becoming more and more serious. The clinical emergence of drug-resistant strains has created an enormous impediment to the cure of invasive fungal infection [23]. Therefore, the evaluation of antifungal activities of compounds against fluconazole-resistant strains is critically important in the discovery of innovative drugs. In this study, all the target compounds were evaluated for their antifungal activity against fluconazole-resistant strains of *C. albicans* including strain 17#, strain CaR, strain 632, strain 901 and strain 904. The experiment results are summarized in Table 2.

It could be observed that fluconazole was inactive against all test strains. Intriguingly, all target compounds showed potent inhibitory effect on fluconazole-resistant fungi. Notably, compounds **A03**, **A05**, **A07**, **A09**, **A18**, **A19**, **A21** and **A23** exhibited excellent antifungal activity against fluconazole-resistant strains.

2.4. Minimum fungicidal concentration of compounds *A03*, *A07*, *A09*, *A18*, *A21* and *A23* and time-kill assays of *A03*

As is well known, the determination of MIC values has been widely used in the research of azole antifungal drugs. However, fungicidal activity (minimum fungicidal concentration) of new compounds is rarely reported in the literature. As matter of fact, the killing of fungi is very important to the treatment of fungal infection, especially for the eradication of immune-mediated fungal infection. Therefore, we believe that fungicidal activity is also an important index in the research and development of antifungal drugs. So, in the follow-up experiment, the minimum fungicidal concentration (MFC) and the time-kill curve were used for evaluation of the *in vitro* fungicidal ability of the target compounds. Specifically, we evaluated MFC of potent compounds **A03**, **A07**, **A09**, **A18**, **A21** and **A23** in 48 h (Table 3). Meanwhile, we performed concentration-dependent time-kill assays against *Candida albicans* (CPCC400616) during a period of 24 h by potent compound **A03**. Related experimental result is shown in Table 3 and Fig. 4.

In fact, if the reduced amount of initial inoculum is greater than or equal to $3 \cdot \mathrm{Log}_{10}$ CFU/mL, the corresponding activity was

Table 1
The antifungal activity of the target compounds **A01**-**A30**.

Compd.	X	R	MIC/(μg/mL)							
			C.alb	*C.alb(sc5314)*	*C.zey*	*C.neo*	*C.kru*	*C.gla*	*C.par*	*A.f*
A01	Cl	H	0.06	0.02	0.5	0.5	0.5	0.5	0.25	0.5
A02	Cl	2,4-di-Cl	0.06	0.06	2	0.5	2	0.25	2	0.5
A03	Cl	2-Cl	0.02	0.01	1	0.25	0.13	0.5	0.13	2
A04	Cl	4-Cl	0.06	0.03	8	0.25	0.5	1	0.5	2
A05	Cl	3-F	0.03	0.03	1	0.25	2	0.5	0.02	2
A06	Cl	4-F	0.03	0.03	4	0.25	1	0.13	0.5	2
A07	Cl	2-Br	0.02	0.01	0.25	0.5	0.25	0.5	0.03	1
A08	Cl	4-Br	0.25	0.06	2	0.5	32	0.5	4	1
A09	Cl	2-Br-5-F	0.02	0.01	0.5	0.25	0.13	0.5	0.02	0.5
A10	Cl	2-Br-4,5-di-OCH3	0.25	0.03	>128	0.5	>128	0.5	>128	16
A11	Cl	3,5-di-OCH3	0.13	0.13	1	0.5	8	0.25	0.5	1
A12	Cl	3-OCH3	0.06	0.06	1	0.13	1	0.25	0.13	2
A13	Cl	4-OCH3	0.13	0.13	8	0.25	8	0.5	0.5	1
A14	Cl	3,4-di-CH3	0.25	4	>128	0.5	128	0.5	>128	4
A15	Cl	3-CH3	0.03	0.03	16	0.25	4	0.5	1	8
A16	Cl	4-CH3	0.03	0.13	16	0.25	4	1	1	2
A17	F	H	0.25	0.13	0.5	0.5	0.25	1	0.13	1
A18	F	2,4-di-Cl	0.03	0.13	0.5	0.25	0.06	1	0.03	0.5
A19	F	2-Cl	0.25	0.01	0.25	0.5	0.25	1	0.06	2
A20	F	4-Cl	0.5	0.03	1	0.5	0.5	1	0.13	1
A21	F	3-F	0.03	0.03	1	0.5	0.13	0.5	0.25	2
A22	F	4-F	0.06	0.03	1	0.5	1	1	0.25	2
A23	F	2-Br	0.02	0.01	0.25	0.25	0.06	1	0.02	1
A24	F	4-Br	0.25	0.13	0.25	0.5	1	2	0.25	1
A25	F	3,5-di-OCH3	0.5	0.13	1	8	8	0.5	8	8
A26	F	3-OCH3	0.06	0.13	1	0.5	2	1	0.5	2
A27	F	4-OCH3	0.25	0.13	2	0.5	2	0.5	0.25	1
A28	F	3,4-di-CH3	0.5	0.13	1	0.5	8	1	1	2
A29	F	3-CH3	0.13	0.06	1	0.5	1	1	0.25	2
A30	F	4-CH3	0.25	0.06	0.5	0.5	2	1	0.25	1
Fluconazole			4	1	4	4	32	32	4	>128
Miconazole			0.25	0.03	1	0.5	0.5	0.5	0.25	2

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); Values are the average of three independent determinations. Relative errors were generally in the range of 5−10%.

considered to be fungistatic. As shown in Fig. 4, the decrease of the amount of initial inoculum exceeded $3 \cdot \mathrm{Log}_{10}$ CFU/mL (99.9%) in 24 h from the time-kill curves of compound A03. This experiment once more proved that our compound had fungicidal effect. Moreover, the fungicidal activity of the compound A03 is dose-dependent. In addition, the results indicated that compound A03 displayed rapid fungicidal activity within 4 h at 8 × MIC in the viable colony counts. Therefore, we believe compound A03 will be able to show its advantages in reducing the treatment time of invasive infections and the probability of drug resistance.

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

Target compounds are novel antifungal compounds which were obtained by replacing the oxygen atom in miconazole with a selenium atom. All of the target compounds may inhibit the activity of sterol 14α-demethylase enzyme in the ergosterol biosynthetic pathway. To study the antifungal mechanism of action of target compounds, gas chromatography-mass spectrometry (GC-MS) was applied to analyze the sterol composition of fungal cells by employing fluconazole and miconazole as the control drugs [25]. More specifically, we have chosen *Candida albicans* (ATCC SC5314) as model strain and then investigated the biosynthesis of ergosterol in this strain after representative compound and *Candida albicans* were preincubated together for 16 h.

If azole derivatives inhibit the activity of 14a-demethylase, it will lead to the reduction of ergosterol synthesis and the accumulation of methylated sterols such as eburicol, obtusifoliol and lanosterol. As shown in Table 4, compound A03 intensively inhibited the biosynthesis of ergosterol at both low and high doses. Meanwhile, the content of lanosterol, eburicol and obtusifoliol increases, especially eburicol showed the largest amount of accumulation. The results indicated that the antifungal mechanism of compound A03 was similar to fluconazole and miconazole that affect the biosynthesis of ergosterol by inhibiting the activity of lanosterol 14a-demethylase (CYP51).

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

Fungi biofilms are complex functional communities which are formed on implanted biomaterials and host surfaces to reduce susceptible to antifungal agents. Because of fungi biofilms, antifungal agents are difficult to reach the these difficult-to-treat networks embedded in fungal pathogens so that drug resistance and repeated infections occur frequently in antifungal treatment [26]. Therefore, the development of novel derivatives with anti-biofilm activity is an important issue for the treatment of fungal infections. For this reason, we focused on investigating the anti-biofilm activity of potential compounds A03 and A09 against *C. albicans* (CPCC400616) whose biofilm (SMIC of FCZ >1024 μg/mL) was incubated in a 96-well plate for 1.5 h, 3 h, 6 h and 24 h. Different concentrations of the tested compounds and prepared biofilm were preincubated together for 24 h and subsequent biofilm formation were investigated by the 2,3-bis (2-hydroxyethylthio) naphthalene-1,4-dione (XTT) reduction assay (Fig. 5). The $SMIC_{50}$ and $SMIC_{80}$ values for tested compounds were expressed in Table 5.

The results of the test showed that compounds A03 and A09 had

Table 2
The antifungal activity of the target compounds **A01**-**A30**.

Compd.	MIC/(μg/mL)				
	strain CaR	strain 17#	strain 632	strain 901	strain 904
A01	4	4	2	1	0.5
A02	128	16	64	32	8
A03	0.06	0.13	1	0.5	0.25
A04	16	8	64	8	>128
A05	1	0.5	1	0.25	0.25
A06	16	4	128	32	64
A07	0.5	0.5	1	1	0.25
A08	128	8	>128	64	128
A09	0.13	0.03	0.13	0.25	0.13
A10	128	>128	>128	128	32
A11	4	32	32	4	128
A12	2	4	2	1	1
A13	32	16	128	16	128
A14	128	8	16	32	128
A15	4	4	2	2	2
A16	16	32	128	64	>128
A17	4	8	4	2	64
A18	16	1	2	0.5	2
A19	2	1	0.5	1	0.5
A20	16	8	64	8	2
A21	4	0.06	8	8	1
A22	8	32	32	4	8
A23	0.13	0.06	0.25	0.25	0.06
A24	64	2	8	2	2
A25	128	8	8	2	4
A26	4	16	2	8	16
A27	8	8	8	8	2
A28	128	64	>128	128	>128
A29	2	8	16	4	1
A30	16	2	16	1	4
FCZ	>128	128	>128	>128	>128

Abbreviations: strain 17#, fluconazole-resistant strain of Candida albicans; strain CaR, fluconazole-resistant strain of Candida albicans; FCZ: Fluconazole. Strain 17# and strain CaR were provided by Institute of Microbiology, Chinese Academy of Sciences, which were isolated from AIDS patients. Strains 632, 901 and 904 were provided by the Second Military Medical University.

better inhibitory activity against biofilm than miconazole. Notably, target compounds A03 and A09 inhibited the formation of *C. albicans* biofilm in a dose-dependent manner. In addition, anti-biofilm effect was decreased when the ripening time of biofilm was increased.

$SMIC_{50}$ = 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%.

2.7. Hemolysis assays

Compounds A03, A09, A18 and A23 showed valuable antifungal activity, so further safety analysis was necessary for these compounds. We tested compounds A03, A09, A18 and A23 for their hemolytic activity against rabbit red blood cells (Table 6).

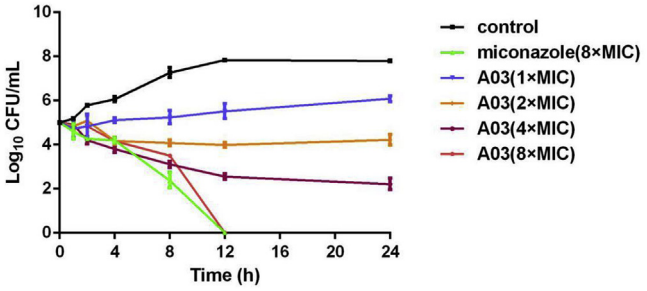


Fig. 4. Time-kill curve of compound A03 against *Candida albicans* (CPCC400616). The detection limit of this assay was 100 CFU/mL.

Miconazole displayed 81.78% hemolysis at concentrations of 4 μg/mL (8-fold of its overall MIC value). Similarly, compounds A03 and A09 showed 58.87% and 7.15% hemolysis at concentrations of 4 μg/mL (256-fold of its overall MIC value), respectively. In addition, compound A18 showed 12.10% hemolysis at concentrations of 2 μg/mL and compound A23 showed 93.47% hemolysis at concentrations of 8 μg/mL. The hemolytic concentrations of the target compounds were much higher than their MIC value. Overall, compound A23 displayed the lowest hemolytic activity. Compounds A03, A09 and A23 displayed less hemolytic effect than the control drug miconazole.

2.8. Molecular docking analysis

In order to determine the binding modes of the target compounds, molecular docking analysis were conducted for representative compounds A02, A03, A18 against CYP51 from *C.albicans* (PDB ID: 5tz1) by using discovery studio 3.0. The docking results are illustrated in Fig. 6. The target compounds have almost the same interaction mode as miconazole. The imidazole moiety of compounds A02, A03 and A18 was in coordination with the Fe^{2+} atom of the heme group. THR311, CYS470, ILE131, PHE126, MET508, LEU376 and PHE380 were the main function residues. Mutations in Y132 or F145 will lead to the disappear of internalization between the tertiary alcohol group of fluconazole and two important amimo acids (Y132 and F145) of CYP51 [27]. That's why some strains are resistant to fluconazole. Selenium atoms of the target compounds didn't interact with both of amino acid residues. Thus compared to compounds with a tertiary alcohol group, target compounds might be advantage for their antifungal activity against the fluconazole-resistance strains.

3. Conclusion

In this study, thirty new miconazole analogues were designed and synthesized based on the biosostere theory strategy. The

Table 3
The MFC of the most potent compounds.

Compd.	MIC against *C.alb.* (μg/mL)	MFC against *C.alb.* (μg/mL)	MFC/MIC
A03	0.02	0.125	8
A07	0.02	0.25	16
A09	0.02	0.0625	4
A18	0.03	0.125	4
A21	0.03	>128	–
A23	0.02	0.125	8
Miconazole	0.25	2	8

Abbreviations: C.alb., Candida albicans (CPCC400616); As shown in Table 3, all tested compounds (except A21) possessed satisfactory fungicidal activity. Then, compound A03 was selected to further study its fungicidal ability by the time-kill assay. The time-kill curves are shown in Fig. 4.

Table 4
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
A03	0.03125	11.3	17.7	6.4	64.6
	0.125	7.3	9.7	12.4	70.6
	0.5	8.3	8.1	9.4	74.2
	2	5.49	9.2	7.8	77.7
	8	3.4	14.6	7.4	74.8
Control	–	97.1	0	2.9	0

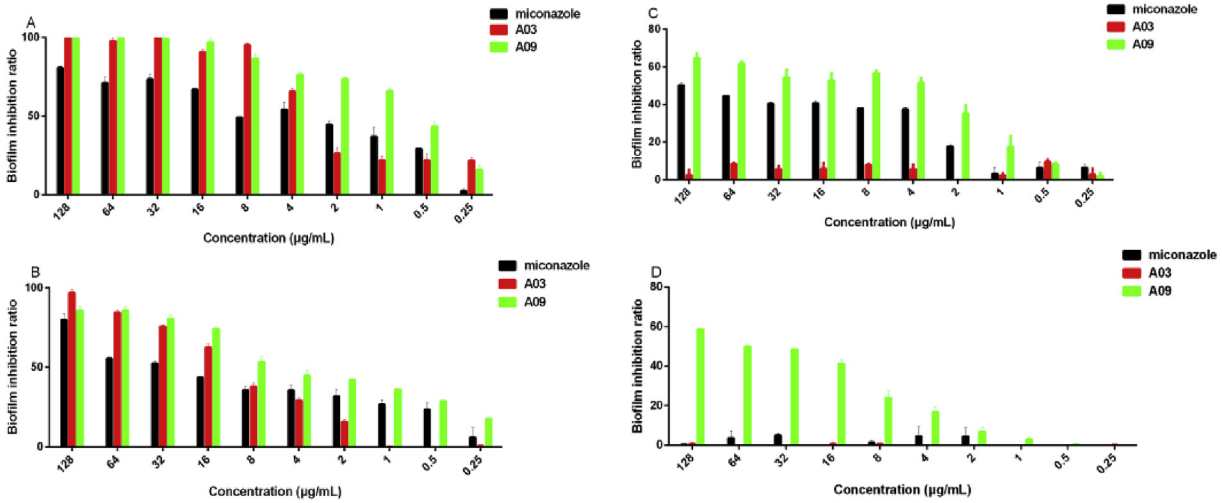


Fig. 5. Antibiofilm activity of compounds A03 and A09, as well as miconazole against FCZ-Resistant *C. albicans* CPCC400616 biofilms for 1.5 h (A), 3 h (B), 6 h (C) and 24 h (D).

Table 5
SMIC values (μg/mL) of test compounds 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
A03	4	16	>128	>128	8	64	>128	>128
A09	1	8	8	128	8	64	>128	>128
Miconazole	16	32	>128	>128	128	128	>128	>128

biological assay results revealed that all of the target compounds exhibited potent *in vitro* antifungal efficacies against all test strains. Meanwhile, all the target compounds possessed excellent inhibitory effect on fluconazole-resistant strains. In addition, potent compounds A03, A09, A18 and A23 possessed more stronger fungicidal activities than miconazole. Subsequently, preliminary mechanistic of action study showed that the potent compound A03 targeted *C. albicans* CYP51. Furthermore, two active molecules A03

Table 6
The hemolysis ratio of the target compounds.

Compd.	Hemolysis ratio					Hemolysis ratio/MIC against *C.alb.*
	1 μg/mL	2 μg/mL	4 μg/mL	8 μg/mL	16 μg/mL	
A03	0.00	3.15	58.87	93.23	98.23	256
A09	0.49	2.18	7.15	12.58	16.70	256
A18	0.33	12.10	93.87	98.63	100	64
A23	0.00	0.00	0.41	93.47	97.83	512
Miconazole	0.16	8.55	81.78	87.34	94.03	8

Abbreviations: C.alb., Candida albicans (CPCC400616); It is generally considered that the hemolysis rate <5% can be determined as non-hemolysis.

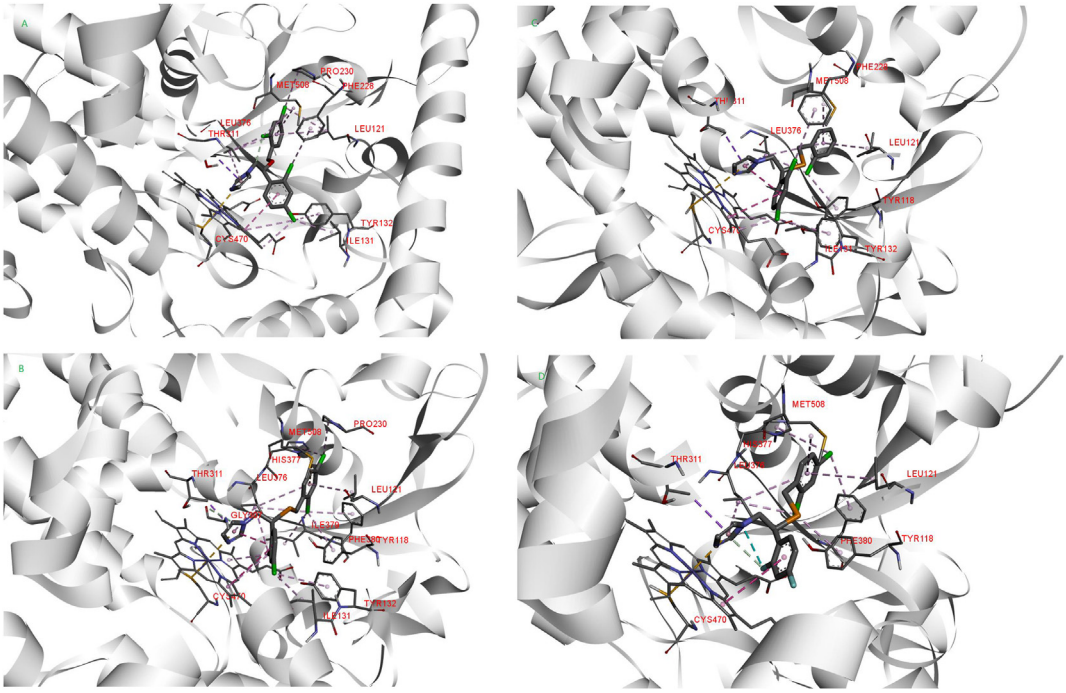


Fig. 6. Predicted binding mode of (A) miconazole, (B) A02, (C) A03 and (D) A18 in the active site of *Candida albicans* CYP51.

and A09 could effectively inhibited the formation of *C. albicans* biofilm in a dose-dependent manner. More importantly, further hemolysis test verified that potential compounds had higher safety than miconazole. At last, the docking study showed that the potent antifungal activities mainly caused by hydrogen and coordination bond interaction with the CYP51. Overall, compounds A03 and A09 were promising candidates for the development of drugs to fungal infection.

4. Experimental section

4.1. Chemistry

Unless otherwise stated, all starting materials and solvents were commercially available and used without further purification. Melting points were determined on a X-5 melting point apparatus (Beijing Tech Instrument Co., Ltd., Beijing, China) and are uncorrected. The silica gel precoated GF254 plates were selected for the TLC assay. All column chromatography was carried out on silica gel (200–300 mesh). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 (100)-MHz Bruker AV-400 spectrometer (Bruker Bioscience, Billerica, MA, USA) by using DMSO-d_6 as solvent. NMR spectra were processed by using MestReNova software. High-resolution mass spectra (HRMS) were recorded by Agilent Accurate-Mass Q-TOF 6530 (Agilent, Santa Clara, CA, USA) instrument in ESI mode. Mass spectrometry was determinated by Agilent 6120 equipped Agilent 1200 LC/MS in ESI mode (Agilent, Santa Clara, CA, USA). GC-MS analysis was performed on Agilent 6890N-5975 (Agilent, Santa Clara, CA, USA).

4.2. General procedure for the synthesis of compound 3

Ethanol (100 mL) was added to the mixture of selenium (3.95 g, 0.05 mol) and $NaBH_4$ (4 g, 0.1 mol) under N_2 (g). The mixture was stirred until the solution became colorless. Then, to this mixture were added selenium (3.95 g, 0.05 mol) again and was heated to boiling by using a hot air gun. The mixture was stirred for a further 30 min and then the solvent was evaporated to obtain Na_2Se_2 (10.0 g, 98%). The yellow microcrystalline solid was collected. m.p. 101–104 °C.

4.3. General procedure for the synthesis of compounds 5a-5p

Taking the synthesis of intermediate 1,2-dibenzyldiselane as an example (5a): The solution of chlorobenzyl (1.3 g, 0.01 mol) in dichloromethane (10 mL) was added Na_2Se_2 (2.45 g, 0.012 mol). The mixture was heated to 40 °C for 10 h. After the solvent was evaporated, the residual solids were dissolved as much as possible in hot hexane. Small amount of insoluble solids need to be separated by filtration. The filtrate was allowed to crystallize at room temperature for 3 h and then stored at 6 °C overnight. The white microcrystalline solids were gathered together and dried in the air (1.6 g, 95%). LC- MS(m/z):343.0[M+H]$^+$.

4.4. General procedure for the synthesis of compounds 8a-8b

To a mixture of m-dichlorobenzene (6a) (14.6 g, 0.1 mol) and anhydrous aluminum trichloride (21.3 g, 0.16 mol) was added 2-chloroacetyl chloride (7) (12.4 g, 0.11 mol) dropwise at room temperature and the dropping rate needed to be controlled so that the reaction temperature didn't exceed 30 °C. The mixture was stirred at 30 °C for 3 h. Then, the solution was carefully added to the mixture of ice and water (100 g) which contained concentrated hydrochloric acid (5 mL). The mixture was stirred to form a dark brown oil, then was extracted with dichloromethane (3 × 40 mL). The combined organic layers were washed with brine (40 mL), dried over anhydrous sodium carbonate overnight. The desiccant was filtered off and yellow solid was obtained under reduced pressure. The solid was purified by recrystallization with ethanol to give a white solid (6a). Yield 93.1%, LC-MS m/z: 222.9 [M+H]$^+$. According to the preparation method of 8a, m-difluorobenzene was used as raw material to prepare the intermediate 8b: white solid,

Yield 92.4%, LC-MS *m/z*: 191.01 $[M+H]^+$.

4.5. General procedure for the synthesis of compounds 9a-9b

Imidazole (10.36 g, 0.15 mol) was added to anhydrous methanol (40 mL) and the mixture was stirred to dissolve at room temperature. An amount of triethylamine (10.1 g, 0.1 mol) was then added dropwise and the reaction mixture was magnetically stirred at room temperature, then another solution containing 8a (22.3 g, 0.1 mol) and anhydrous methanol (20 mL) were added. The reaction was heated to reflux for 4 h and cooled to room temperature. Water (100 mL) and ethyl acetate (50 mL) were added to the mixture. The organic phase was separated and the water phase was extracted with ethyl acetate (2 × 50 mL). The combined organic phases were washed with water (20 mL) and saturated sodium chloride solution (20 mL) in order, and the organic layer was dried overnight with anhydrous sodium carbonate. The solution was moved by filtering and the yellow crystalline precipitate was dried in the air. The solid was purified by recrystallization from ethanol to obtain a white solid (9a). Yield 84.0% HRMS *m/z*: 255.0085 $[M+H]^+$, 276.9904 $[M+Na]^+$. According to the preparation method of 9a, 8b was used as raw material to prepare the intermediate 9b: white solid, Yield 92.1%, HRMS *m/z*: 223.0681 $[M+H]^+$.

4.6. General procedure for the synthesis of compounds 10a-10b

Sodium borohydride (12 g, 0.3 mol) was added to the ethanol solution (100 mL) of 9a (25.5 g, 0.1 mol). The mixture was stirred at 20 °C for 12 h. Then the water was carefully added to the mixture, the white solid was obtained by filtration and recrystallization (EtOH). Yield 98.0%, HRMS *m/z*: 257.0217$[M+H]^+$. According to the preparation method of 10a, 9b was used as raw material to prepare the intermediate 10b: white solid, Yield 97.4%, HRMS *m/z*: 225.0831 $[M+H]^+$.

4.7. General procedure for the synthesis of compounds 11a-11b

Intermediate 10a (25.7 g, 0.1 mol) was added to the solution of thionyl chloride (59.5 g, 0.5 mol). The mixture was stirred at 20 °C for 12 h. Then, the solution was added to the mixture of ice and water (300 g). The pH value of the mixture was adjusted to neutral with potassium hydroxide. The white solid was obtained by filtration and recrystallization (EtOH). Yield 74.3%, HRMS *m/z*: 275.9833.0217 $[M+Na]^+$. According to the preparation method of 11a, 10b was used as raw material to prepare the intermediate 11b: white solid, Yield 73.6%, HRMS *m/z*: 243.0504 $[M+H]^+$.

4.8. General procedure for the synthesis of target compounds

Taking A01 as an example: intermediate 5a (1.7 g, 0.005 mol) and intermediate 11a (2.8 g, 0.01 mol) were added to ethanol (20 mL) and the mixture was stirred until all the reactant dissolved in the solution. Sodium borohydride (0.5 g, 0.02 mol) was added to the system, then the mixture was heated to reflux for 1 h. The solution was removed by vacuum distillation and the target compound was obtained from the crude product by column chromatography.

4.8.1. 1-(2-(benzylselanyl)-2-(2,4-dichlorophenyl)ethyl)-1H-imidazole (A01)

White solid; yield 83.32%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 410.9900 $[M+H]^+$ 432.9723 $[M+Na]^+$ (calcd. for $C_{18}H_{16}N_2Cl_2Se$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.61 (d, J = 8.6 Hz, 1H), 7.51–7.45 (m, 2H), 7.38 (dd, J = 8.4, 2.2 Hz, 1H), 7.33–7.28 (m, 4H), 7.23 (dq, J = 5.7, 2.8 Hz, 1H), 6.98 (d, J = 1.5 Hz, 1H), 6.78 (s, 1H), 4.74 (dd, J = 12.8, 8.9 Hz, 1H), 4.55 (ddd, J = 18.8, 13.8, 6.0 Hz, 2H), 3.97 (d, J = 11.6 Hz, 1H), 3.85 (d, J = 11.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 139.02, 137.71, 136.54, 133.96, 132.86, 130.51, 129.49, 128.94, 128.79, 128.04, 127.30, 119.57, 49.94, 28.54.

4.8.2. 1-(2-((2,4-dichlorobenzyl)selanyl)-2-(2,4-dichlorophenyl) ethyl)-1H-imidazole (A02)

White solid; yield 93.16%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 478.9064 $[M+H]^+$ 502.8884 $[M+Na]^+$ (calcd. for $C_{18}H_{14}N_2Cl_4Se$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.63 (d, J = 8.5 Hz, 1H), 7.59 (d, J = 2.1 Hz, 1H), 7.53–7.47 (m, 3H), 7.39 (ddd, J = 8.4, 4.4, 2.2 Hz, 2H), 7.06 (t, J = 1.3 Hz, 1H), 6.78 (t, J = 1.1 Hz, 1H), 4.82–4.59 (m, 3H), 4.04 (d, J = 12.0 Hz, 1H), 3.91 (d, J = 11.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 137.77, 136.29, 136.24, 134.34, 133.94, 133.01, 132.83, 132.65, 130.47, 129.55, 129.52, 128.81, 128.08, 127.92, 119.63, 49.93, 25.43.

4.8.3. 1-(2-((2-chlorobenzyl)selanyl)-2-(2,4-dichlorophenyl)ethyl)-1H-imidazole (A03)

White solid; yield 91.59%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 444.9514 $[M+H]^+$ 466.9332 $[M+Na]^+$ (calcd. for $C_{18}H_{15}N_2Cl_3Se$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.64 (d, J = 8.5 Hz, 1H), 7.53–7.48 (m, 2H), 7.47–7.37 (m, 3H), 7.33–7.24 (m, 2H), 7.05 (t, J = 1.2 Hz, 1H), 6.78 (t, J = 1.1 Hz, 1H), 4.82–4.68 (m, 2H), 4.59 (dd, J = 13.1, 5.3 Hz, 1H), 4.04 (d, J = 11.8 Hz, 1H), 3.94 (d, J = 11.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 137.75, 136.92, 136.34, 133.99, 133.41, 132.95, 131.53, 130.46, 130.08, 129.53, 129.34, 128.81, 128.06, 127.80, 119.59, 49.96, 26.12.

4.8.4. 1-(2-((4-chlorobenzyl)selanyl)-2-(2,4-dichlorophenyl)ethyl)-1H-imidazole (A04)

White solid; yield 89.11%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 444.9533 $[M+H]^+$ (calcd. for $C_{18}H_{15}N_2Cl_3Se$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.61 (d, J = 8.5 Hz, 1H), 7.49 (t, J = 1.8 Hz, 2H), 7.40–7.31 (m, 5H), 7.01 (t, J = 1.3 Hz, 1H), 6.79 (d, J = 1.1 Hz, 1H), 4.74 (dd, J = 15.4, 11.5 Hz, 1H), 4.62–4.54 (m, 2H), 3.95 (d, J = 11.8 Hz, 1H), 3.84 (d, J = 11.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 138.38, 137.74, 136.43, 133.91, 132.91, 131.80, 131.27, 130.53, 129.49, 128.85, 128.81, 128.07, 119.62, 49.93, 27.62.

4.8.5. 1-(2-(2,4-dichlorophenyl)-2-((3-fluorobenzyl)selanyl)ethyl)-1H-imidazole (A05)

White solid; yield 83.19%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 428.9824 $[M+H]^+$ (calcd. for $C_{18}H_{15}N_2Cl_2FSe$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.61 (d, J = 8.5 Hz, 1H), 7.54–7.44 (m, 2H), 7.41–7.31 (m, 2H), 7.15 (ddt, J = 9.0, 7.7, 1.5 Hz, 2H), 7.09–6.98 (m, 2H), 6.79 (d, J = 1.1 Hz, 1H), 4.75 (dd, J = 15.5, 11.7 Hz, 1H), 4.61–4.54 (m, 2H), 3.97 (d, J = 11.8 Hz, 1H), 3.87 (d, J = 11.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.74, 161.32, 142.29, 142.22, 137.73, 136.39, 133.92, 132.93, 130.85, 130.77, 130.56, 129.49, 128.81, 128.08, 125.60, 125.57, 119.57, 116.20, 115.99, 114.18, 113.97, 49.93, 27.80.

4.8.6. 1-(2-(2,4-dichlorophenyl)-2-((4-fluorobenzyl)selanyl)ethyl)-1H-imidazole (A06)

White solid; yield 81.20%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 428.9823 $[M+H]^+$ (calcd. for $C_{18}H_{15}N_2Cl_2FSe$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.61 (d, J = 8.5 Hz, 1H), 7.52–7.45 (m, 2H), 7.40–7.33 (m, 3H), 7.17–7.11 (m, 2H), 7.00 (t, J = 1.3 Hz, 1H), 6.78 (d, J = 1.1 Hz, 1H), 4.74 (dd, J = 15.6, 11.4 Hz, 1H), 4.61–4.54 (m, 2H), 3.96 (d, J = 11.8 Hz, 1H), 3.84 (d, J = 11.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 162.74, 160.33, 137.73, 136.49, 135.42, 135.39, 133.90, 132.87, 131.40, 131.32, 130.52, 129.49, 128.80, 128.06, 119.60, 115.78, 115.57, 49.94, 27.58.

4.8.7. 1-(2-((2-bromobenzyl)selanyl)-2-(2,4-dichlorophenyl)ethyl)-1H-imidazole (A07)

White solid; yield 81.12%; m.p.: 225.1–228.3 °C. White solid; yield 91.59%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 490.9000 $[M+H]^+$ 512.8810 $[M+Na]^+$ (calcd. for $C_{18}H_{15}N_2Cl_2BrSe$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.66–7.58 (m, 2H), 7.54–7.49 (m, 2H), 7.46 (dd, J = 7.6, 1.8 Hz, 1H), 7.41–7.32 (m, 2H), 7.18 (td, J = 7.7, 1.7 Hz, 1H), 7.05 (t, J = 1.3 Hz, 1H), 6.78 (t, J = 1.1 Hz, 1H), 4.82–4.70 (m, 2H), 4.59 (dd, J = 12.5, 4.7 Hz, 1H), 4.05 (d, J = 11.7 Hz, 1H), 3.94 (d, J = 11.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 138.59, 137.76, 136.34, 134.00, 133.37, 132.96, 131.50, 130.48, 129.54, 129.52, 128.80, 128.39, 128.06, 124.27, 119.61, 49.97, 28.97.

4.8.8. 1-(2-((4-bromobenzyl)selanyl)-2-(2,4-dichlorophenyl)ethyl)-1H-imidazole (A08)

White solid; yield 83.10%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 490.8994 $[M+H]^+$ 512.8821 $[M+Na]^+$ (calcd. for $C_{18}H_{15}N_2Cl_2BrSe$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.60 (d, J = 8.4 Hz, 1H), 7.52–7.47 (m, 4H), 7.38 (dd, J = 8.4, 2.2 Hz, 1H), 7.30–7.26 (m, 2H), 7.01 (t, J = 1.2 Hz, 1H), 6.78 (t, J = 1.1 Hz, 1H), 4.74 (dd, J = 15.3, 11.6 Hz, 1H), 4.60–4.54 (m, 2H), 3.93 (d, J = 11.8 Hz, 1H), 3.82 (d, J = 11.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 138.81, 137.75, 136.43, 133.91, 132.91, 131.78, 131.63, 130.52, 129.49, 128.80, 128.07, 120.26, 119.63, 49.92, 27.68.

4.8.9. 1-(2-((2-bromo-5-fluorobenzyl)selanyl)-2-(2,4-dichlorophenyl)ethyl)-1H-imidazole (A09)

White solid; yield 78.12%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 508.8910 $[M+H]^+$ (calcd. for $C_{18}H_{14}N_2Cl_2BrFSe$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.67–7.59 (m, 2H), 7.55–7.47 (m, 2H), 7.41–7.33 (m, 2H), 7.10–7.04 (m, 2H), 6.79 (d, J = 1.1 Hz, 1H), 4.84–4.72 (m, 2H), 4.62 (dd, J = 10.9, 3.1 Hz, 1H), 4.04 (d, J = 12.0 Hz, 1H), 3.92 (d, J = 12.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 162.84, 160.40, 141.26, 141.18, 137.77, 136.21, 134.94, 134.86, 133.97, 133.05, 130.54, 129.55, 128.81, 128.09, 119.58, 118.84, 118.81, 118.20, 117.97, 116.55, 116.33, 49.97, 28.64.

4.8.10. 1-(2-((2-bromo-4,5-dimethoxybenzyl)selanyl)-2-(2,4-dichlorophenyl)ethyl)-1H-imidazole (A10)

White solid; yield 75.29%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 550.9229 $[M+H]^+$ 572.9037 $[M+Na]^+$ (calcd. for $C_{20}H_{19}N_2Cl_2BrO_2Se$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.63 (d, J = 8.5 Hz, 1H), 7.52 (d, J = 2.2 Hz, 2H), 7.40 (dd, J = 8.4, 2.3 Hz, 1H), 7.11 (s, 1H), 7.06 (t, J = 1.2 Hz, 1H), 7.03 (s, 1H), 6.78 (t, J = 1.1 Hz, 1H), 4.81–4.73 (m, 2H), 4.64–4.55 (m, 1H), 3.99 (d, J = 11.7 Hz, 1H), 3.88 (d, J = 11.7 Hz, 1H), 3.74 (d, J = 3.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 149.04, 148.62, 137.77, 136.58, 133.97, 132.89, 130.51, 130.24, 129.52, 128.80, 128.06, 119.58, 116.26, 114.40, 114.21, 56.24, 50.13, 49.06, 28.92.

4.8.11. 1-(2-(2,4-dichlorophenyl)-2-((3,5-dimethoxybenzyl)selanyl)ethyl)-1H-imidazole (A11)

White solid; yield 76.24%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 471.0120 $[M+H]^+$ 492.9936 $[M+Na]^+$ (calcd. for $C_{20}H_{20}N_2Cl_2O_2Se$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.61 (d, J = 8.5 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.45 (t, J = 1.1 Hz, 1H), 7.39 (dd, J = 8.4, 2.2 Hz, 1H), 6.97 (t, J = 1.2 Hz, 1H), 6.78 (t, J = 1.1 Hz, 1H), 6.48 (d, J = 2.2 Hz, 2H), 6.36 (t, J = 2.3 Hz, 1H), 4.73 (dd, J = 13.3, 9.5 Hz, 1H), 4.63–4.50 (m, 2H), 3.90 (d, J = 11.6 Hz, 1H), 3.79 (d, J = 11.6 Hz, 1H), 3.72 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 160.88, 141.35, 137.69, 136.58, 133.96, 132.86, 130.58, 129.48, 128.78, 128.05, 119.51, 107.47, 99.29, 55.63, 49.98, 28.73.

4.8.12. 1-(2-(2,4-dichlorophenyl)-2-((3-methoxybenzyl)selanyl)ethyl)-1H-imidazole (A12)

White solid; yield 78.25%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 441.0011 $[M+H]^+$ (calcd. for $C_{19}H_{18}N_2Cl_2OSe$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.61 (d, J = 8.5 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.45 (t, J = 1.1 Hz, 1H), 7.38 (dd, J = 8.4, 2.2 Hz, 1H), 7.22 (dd, J = 8.2, 7.5 Hz, 1H), 6.97 (t, J = 1.3 Hz, 1H), 6.91–6.87 (m, 2H), 6.81–6.76 (m, 2H), 4.73 (dd, J = 13.2, 9.3 Hz, 1H), 4.62–4.49 (m, 2H), 3.94 (d, J = 11.6 Hz, 1H), 3.83 (d, J = 11.6 Hz, 1H), 3.73 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 159.75, 140.60, 137.70, 136.56, 133.96, 132.85, 130.54, 129.99, 129.49, 128.78, 128.05, 121.76, 119.53, 114.93, 112.94, 55.49, 49.95, 28.53.

4.8.13. 1-(2-(2,4-dichlorophenyl)-2-((4-methoxybenzyl)selanyl)ethyl)-1H-imidazole (A13)

White solid; yield 76.58%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 441.0026 $[M+H]^+$ (calcd. for $C_{19}H_{18}N_2Cl_2OSe$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.60 (d, J = 8.4 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.46 (d, J = 1.1 Hz, 1H), 7.38 (dd, J = 8.4, 2.2 Hz, 1H), 7.26–7.21 (m, 2H), 6.99 (t, J = 1.2 Hz, 1H), 6.89–6.85 (m, 2H), 6.77 (t, J = 1.0 Hz, 1H), 4.73 (dd, J = 13.0, 9.1 Hz, 1H), 4.55 (ddd, J = 18.9, 14.0, 5.9 Hz, 2H), 3.93 (d, J = 11.6 Hz, 1H), 3.80 (d, J = 11.6 Hz, 1H), 3.73 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 158.67, 137.71, 136.67, 133.95, 132.78, 130.66, 130.62, 129.47, 128.77, 128.02, 119.58, 114.37, 55.57, 49.96, 28.10.

4.8.14. 1-(2-(2,4-dichlorophenyl)-2-((3,4-dimethylbenzyl)selanyl)ethyl)-1H-imidazole (A14)

White solid; yield 73.06%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 439.0233 $[M+H]^+$ (calcd. for $C_{20}H_{20}N_2Cl_2Se$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.60 (d, J = 8.5 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.45 (t, J = 1.1 Hz, 1H), 7.38 (dd, J = 8.4, 2.2 Hz, 1H), 7.07–6.99 (m, 3H), 6.97 (t, J = 1.2 Hz, 1H), 6.77 (t, J = 1.1 Hz, 1H), 4.72 (dd, J = 13.4, 9.7 Hz, 1H), 4.63–4.48 (m, 2H), 3.90 (d, J = 11.4 Hz, 1H), 3.77 (d, J = 11.4 Hz, 1H), 2.17 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 137.70, 136.65, 136.62, 136.03, 135.20, 133.98, 132.79, 130.54, 129.97, 129.45, 128.76, 128.01, 126.82, 119.55, 49.95, 28.33, 19.76, 19.48.

4.8.15. 1-(2-(2,4-dichlorophenyl)-2-((3-methylbenzyl)selanyl)ethyl)-1H-imidazole (A15)

White solid; yield 71.20%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 425.0081 $[M+H]^+$ (calcd. for $C_{19}H_{18}N_2Cl_2Se$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.61 (d, J = 8.5 Hz, 1H), 7.49 (d, J = 2.2 Hz, 1H), 7.46 (t, J = 1.1 Hz, 1H), 7.38 (dd, J = 8.4, 2.2 Hz, 1H), 7.19 (td, J = 7.4, 0.8 Hz, 1H), 7.10 (dd, J = 7.9, 1.5 Hz, 2H), 7.05–7.01 (m, 1H), 6.97 (t, J = 1.3 Hz, 1H), 6.78 (t, J = 1.1 Hz, 1H), 4.73 (dd, J = 13.3, 9.4 Hz, 1H), 4.61–4.49 (m, 2H), 3.93 (d, J = 11.5 Hz, 1H), 3.81 (d, J = 11.5 Hz, 1H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 138.82, 138.02, 137.70, 136.57, 133.98, 132.84, 130.58, 130.05, 129.47, 128.83, 128.78, 128.03, 127.94, 126.57, 119.53, 49.95, 28.49, 21.37.

4.8.16. 1-(2-(2,4-dichlorophenyl)-2-((4-methylbenzyl)selanyl)ethyl)-1H-imidazole (A16)

White solid; yield 69.08%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 425.0075 $[M+H]^+$ (calcd. for $C_{19}H_{18}N_2Cl_2Se$); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.60 (d, J = 8.5 Hz, 1H), 7.50 (d, J = 2.2 Hz, 1H), 7.46 (t, J = 1.2 Hz, 1H), 7.38 (dd, J = 8.4, 2.2 Hz, 1H), 7.21–7.18 (m, 2H), 7.11 (d, J = 7.8 Hz, 2H), 6.98 (t, J = 1.3 Hz, 1H), 6.77 (t, J = 1.1 Hz, 1H), 4.73 (dd, J = 13.3, 9.5 Hz, 1H), 4.62–4.49 (m, 2H), 3.93 (d, J = 11.4 Hz, 1H), 3.81 (d, J = 11.5 Hz, 1H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 137.71, 136.62, 136.48, 135.81, 133.96, 132.81, 130.52, 129.48, 129.37, 128.77, 128.02, 119.57, 49.94, 28.33, 21.16.

4.8.17. 1-(2-(benzylselanyl)-2-(2,4-difluorophenyl)ethyl)-1H-imidazole (A17)

White solid; yield 81.33%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 379.0515 $[M+H]^+$ (calcd. for $C_{18}H_{16}N_2F_2Se$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.54–7.46 (m, 2H), 7.30 (d, J = 5.2 Hz, 4H), 7.24–7.20 (m, 1H), 7.13 (ddd, J = 10.8, 9.3, 2.6 Hz, 1H), 7.07–7.00 (m, 2H), 6.78 (d, J = 1.1 Hz, 1H), 4.67–4.58 (m, 1H), 4.54–4.45 (m, 2H), 3.92 (d, J = 11.5 Hz, 1H), 3.82 (d, J = 11.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.05, 161.34, 161.22, 160.60, 160.48, 158.88, 158.76, 139.11, 137.74, 130.76, 130.72, 130.67, 130.62, 129.34, 128.75, 127.27, 119.61, 112.26, 112.22, 112.05, 112.01, 104.76, 104.50, 104.24, 50.14, 35.58, 28.34.

4.8.18. 1-(2-((2,4-dichlorobenzyl)selanyl)-2-(2,4-difluorophenyl) ethyl)-1H-imidazole (A18)

White solid; yield 88.66%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 446.9732 $[M+H]^+$ (calcd. for $C_{18}H_{14}N_2F_2Cl_2Se$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.61–7.48 (m, 3H), 7.45 (d, J = 8.3 Hz, 1H), 7.38 (dd, J = 8.3, 2.2 Hz, 1H), 7.14 (ddd, J = 10.8, 9.3, 2.6 Hz, 1H), 7.09–7.00 (m, 2H), 6.79 (t, J = 1.1 Hz, 1H), 4.69–4.61 (m, 2H), 4.61–4.51 (m, 1H), 3.98 (d, J = 11.9 Hz, 1H), 3.90 (d, J = 11.9 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.14, 163.02, 161.37, 161.25, 160.69, 160.57, 158.90, 158.78, 137.81, 136.37, 134.22, 132.77, 132.48, 130.77, 130.72, 130.67, 130.62, 129.49, 128.77, 127.93, 123.51, 119.67, 112.26, 112.23, 112.05, 112.02, 104.80, 104.55, 104.29, 50.12, 36.09, 25.23.

4.8.19. 1-(2-((2-chlorobenzyl)selanyl)-2-(2,4-difluorophenyl) ethyl)-1H-imidazole (A19)

White solid; yield 86.25%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 413.0121 $[M+H]^+$ (calcd. for $C_{18}H_{15}N_2F_2ClSe$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.58–7.49 (m, 2H), 7.42 (dd, J = 7.1, 2.2 Hz, 2H), 7.31–7.23 (m, 2H), 7.15 (ddd, J = 10.8, 9.3, 2.7 Hz, 1H), 7.08–7.00 (m, 2H), 6.79 (d, J = 1.2 Hz, 1H), 4.71–4.61 (m, 2H), 4.58–4.49 (m, 1H), 3.99 (d, J = 11.6 Hz, 1H), 3.92 (d, J = 11.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.12, 162.99, 161.39, 161.27, 160.67, 160.54, 158.92, 158.80, 137.79, 137.02, 133.30, 131.35, 130.77, 130.72, 130.67, 130.62, 130.05, 129.30, 128.77, 127.80, 123.62, 123.59, 123.48, 123.45, 119.64, 112.26, 112.22, 112.05, 112.01, 104.79, 104.53, 104.27, 50.16, 35.98, 25.91.

4.8.20. 1-(2-((4-chlorobenzyl)selanyl)-2-(2,4-difluorophenyl) ethyl)-1H-imidazole (A20)

White solid; yield 86.68%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 413.0123 $[M+H]^+$ (calcd. for $C_{18}H_{15}N_2F_2ClSe$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.53–7.47 (m, 2H), 7.37–7.29 (m, 4H), 7.13 (ddd, J = 10.8, 9.3, 2.6 Hz, 1H), 7.06–7.01 (m, 2H), 6.79 (t, J = 1.1 Hz, 1H), 4.63 (dd, J = 15.3, 11.4 Hz, 1H), 4.56–4.49 (m, 2H), 3.91 (d, J = 11.8 Hz, 1H), 3.81 (d, J = 11.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.08, 161.32, 161.20, 160.63, 160.50, 158.85, 158.73, 138.44, 137.78, 131.76, 131.12, 130.75, 130.70, 130.66, 130.61, 128.85, 128.77, 123.69, 123.65, 123.55, 123.51, 119.66, 112.27, 112.23, 112.06, 112.02, 104.77, 104.51, 104.25, 50.12, 35.65, 27.44.

4.8.21. 1-(2-(2,4-difluorophenyl)-2-((3-fluorobenzyl)selanyl)ethyl)-1H-imidazole (A21)

White solid; yield 86.46%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 397.0423 $[M+H]^+$ (calcd. for $C_{18}H_{15}N_2F_3Se$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.54–7.47 (m, 2H), 7.33 (td, J = 8.1, 6.2 Hz, 1H), 7.17–7.09 (m, 3H), 7.08–7.00 (m, 3H), 6.79 (d, J = 1.1 Hz, 1H), 4.63 (dd, J = 15.8, 11.7 Hz, 1H), 4.56–4.48 (m, 2H), 3.93 (d, J = 11.8 Hz, 1H), 3.83 (d, J = 11.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.71, 162.96, 161.29, 161.21, 160.63, 160.51, 158.74, 142.38, 142.31, 137.77, 130.84, 130.76, 130.67, 130.62, 128.76, 125.45, 125.42, 123.68, 123.65, 123.51, 119.62, 116.05, 115.83, 114.16, 113.95, 112.28, 112.24, 112.06, 112.03, 104.76, 104.50, 104.24, 50.11, 35.67, 27.64, 27.62.

4.8.22. 1-(2-(2,4-difluorophenyl)-2-((4-fluorobenzyl)selanyl) ethyl)-1H-imidazole (A22)

White solid; yield 79.47%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 397.0421 $[M+H]^+$ (calcd. for $C_{18}H_{15}N_2F_3Se$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.54–7.46 (m, 2H), 7.35–7.30 (m, 2H), 7.16–7.09 (m, 3H), 7.06–7.00 (m, 2H), 6.79 (d, J = 1.1 Hz, 1H), 4.67–4.59 (m, 1H), 4.56–4.47 (m, 2H), 3.91 (d, J = 11.8 Hz, 1H), 3.81 (d, J = 11.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.06, 162.93, 162.69, 161.32, 161.19, 160.61, 160.48, 160.27, 158.85, 158.73, 137.77, 135.47, 135.44, 131.25, 131.17, 130.75, 130.70, 130.65, 130.60, 128.76, 123.75, 123.71, 123.61, 123.58, 119.65, 115.78, 115.57, 112.26, 112.23, 112.05, 112.02, 104.76, 104.50, 104.24, 50.14, 35.57, 27.40.

4.8.23. 1-(2-((2-bromobenzyl)selanyl)-2-(2,4-difluorophenyl) ethyl)-1H-imidazole (A23)

White solid; yield 84.47%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 456.9618 $[M+H]^+$ 478.9434 $[M+Na]^+$ (calcd. for $C_{18}H_{15}N_2F_2BrSe$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.61–7.50 (m, 3H), 7.43 (dd, J = 7.6, 1.8 Hz, 1H), 7.33 (td, J = 7.5, 1.3 Hz, 1H), 7.19–7.12 (m, 2H), 7.09–7.01 (m, 2H), 6.79 (t, J = 1.1 Hz, 1H), 4.67 (d, J = 8.8 Hz, 2H), 4.58–4.49 (m, 1H), 4.00 (d, J = 11.7 Hz, 1H), 3.93 (d, J = 11.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.12, 162.99, 161.40, 161.28, 160.67, 160.54, 158.93, 158.81, 138.71, 137.79, 133.33, 131.31, 130.79, 130.74, 130.69, 130.64, 129.49, 128.77, 128.40, 124.16, 123.61, 123.58, 123.48, 123.44, 119.65, 112.27, 112.23, 112.06, 112.02, 104.80, 104.54, 104.28, 50.16, 36.01, 28.77.

4.8.24. 1-(2-((4-bromobenzyl)selanyl)-2-(2,4-difluorophenyl) ethyl)-1H-imidazole (A24)

White solid; yield 82.15%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 456.9616 $[M+H]^+$ (calcd. for $C_{18}H_{15}N_2F_2BrSe$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.54–7.46 (m, 4H), 7.27–7.22 (m, 2H), 7.13 (ddd, J = 10.8, 9.3, 2.6 Hz, 1H), 7.06–7.00 (m, 2H), 6.79 (t, J = 1.1 Hz, 1H), 4.63 (dd, J = 15.3, 11.4 Hz, 1H), 4.57–4.48 (m, 2H), 3.89 (d, J = 11.8 Hz, 1H), 3.79 (d, J = 11.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.08, 162.96, 161.31, 161.19, 160.63, 160.51, 158.85, 158.73, 138.87, 137.78, 131.78, 131.48, 130.76, 130.70, 130.66, 130.61, 128.76, 123.68, 123.64, 123.54, 123.50, 120.23, 119.67, 112.27, 112.24, 112.06, 112.03, 104.77, 104.51, 104.25, 50.11, 35.66, 27.49.

4.8.25. 1-(2-(2,4-difluorophenyl)-2-((3,5-dimethoxybenzyl)selanyl) ethyl)-1H-imidazole (A25)

White solid; yield 85.25%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 439.0686 $[M+H]^+$ 461.0508 $[M+Na]^+$ (calcd. for $C_{20}H_{20}N_2F_2O_2Se$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.56–7.43 (m, 2H), 7.19–7.09 (m, 1H), 7.09–6.97 (m, 2H), 6.78 (q, J = 1.3 Hz, 1H), 6.47–6.34 (m, 3H), 4.62 (dd, J = 12.2, 8.7 Hz, 1H), 4.57–4.45 (m, 2H), 3.90–3.84 (m, 1H), 3.75 (d, J = 11.6 Hz, 1H), 3.72 (d, J = 2.3 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 160.87, 160.82, 160.60, 141.47, 137.80, 137.74, 130.80, 130.75, 130.70, 128.73, 123.76, 123.66, 123.62, 119.57, 112.26, 112.02, 107.30, 104.75, 104.49, 104.23, 99.27, 55.60, 50.17, 35.64, 28.55.

4.8.26. 1-(2-(2,4-difluorophenyl)-2-((3-methoxybenzyl)selanyl) ethyl)-1H-imidazole (A26)

White solid; yield 76.18%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m/z*): 409.0619 $[M+H]^+$ (calcd. for $C_{19}H_{18}N_2F_2OSe$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.54–7.45 (m, 2H), 7.21 (dd, J = 8.4, 7.4 Hz, 1H), 7.14 (ddd, J = 10.6, 9.2, 2.6 Hz, 1H), 7.07–7.00 (m, 2H), 6.90–6.83 (m, 2H), 6.82–6.75 (m, 2H), 4.67–4.57 (m, 1H), 4.55–4.44 (m, 2H), 3.90 (d, J = 11.5 Hz, 1H), 3.79 (d, J = 11.5 Hz, 1H), 3.73 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.05, 161.23, 160.60, 160.48, 159.72, 158.88, 158.76, 140.70, 137.74, 130.78, 130.73, 130.68, 130.63, 129.99,

128.74, 123.78, 123.74, 123.60, 121.60, 119.59, 114.78, 112.89, 112.26, 112.23, 112.05, 112.02, 104.76, 104.50, 104.24, 55.45, 50.14, 35.59, 28.34.

4.8.27. 1-(2-(2,4-difluorophenyl)-2-((4-methoxybenzyl)selanyl) ethyl)-1H-imidazole (A27)

White solid; yield 75.53%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 409.0621 $[M+H]^+$ (calcd. for $C_{19}H_{18}N_2F_2OSe$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.53–7.46 (m, 2H), 7.23–7.20 (m, 2H), 7.14 (ddd, J = 10.7, 9.3, 2.6 Hz, 1H), 7.06–7.01 (m, 2H), 6.88–6.84 (m, 2H), 6.78 (t, J = 1.1 Hz, 1H), 4.62 (dd, J = 15.6, 11.7 Hz, 1H), 4.49 (q, J = 5.2, 4.2 Hz, 2H), 3.88 (d, J = 11.5 Hz, 1H), 3.77 (d, J = 11.5 Hz, 1H), 3.73 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 162.89, 161.33, 161.21, 160.56, 160.44, 158.86, 158.74, 158.60, 137.75, 130.76, 130.74, 130.66, 130.61, 130.48, 128.73, 123.86, 123.72, 119.63, 114.36, 112.24, 112.20, 112.03, 111.99, 104.75, 104.49, 104.23, 55.53, 50.17, 35.48, 27.88.

4.8.28. 1-(2-(2,4-difluorophenyl)-2-((3,4-dimethylbenzyl)selanyl) ethyl)-1H-imidazole (A28)

White solid; yield 77.03%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 407.0825 $[M+H]^+$ (calcd. for $C_{20}H_{20}N_2F_2Se$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.54–7.45 (m, 2H), 7.14 (ddd, J = 10.8, 9.3, 2.6 Hz, 1H), 7.06–6.97 (m, 5H), 6.78 (t, J = 1.1 Hz, 1H), 4.61 (dd, J = 11.9, 8.4 Hz, 1H), 4.55–4.45 (m, 2H), 3.86 (d, J = 11.4 Hz, 1H), 3.74 (d, J = 11.4 Hz, 1H), 2.17 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.03, 162.91, 161.37, 161.25, 160.58, 160.46, 158.90, 158.78, 137.74, 136.64, 136.15, 135.15, 130.81, 130.76, 130.71, 130.66, 130.42, 129.98, 128.72, 126.68, 123.85, 123.81, 123.71, 123.67, 119.60, 112.22, 112.19, 112.01, 111.97, 104.73, 104.47, 104.21, 50.14, 35.63, 28.14, 19.75, 19.47.

4.8.29. 1-(2-(2,4-difluorophenyl)-2-((3-methylbenzyl)selanyl) ethyl)-1H-imidazole (A29)

White solid; yield 71.11%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 393.0662 $[M+H]^+$ (calcd. for $C_{19}H_{18}N_2F_2Se$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.54–7.46 (m, 2H), 7.20–7.11 (m, 2H), 7.09–7.00 (m, 5H), 6.79 (d, J = 1.1 Hz, 1H), 4.62 (dd, J = 11.7, 8.0 Hz, 1H), 4.56–4.45 (m, 2H), 3.89 (d, J = 11.5 Hz, 1H), 3.78 (d, J = 11.5 Hz, 1H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 163.05, 162.93, 161.38, 161.25, 160.60, 160.48, 158.91, 158.79, 138.92, 138.02, 137.74, 130.81, 130.76, 130.71, 130.66, 129.92, 128.83, 128.75, 127.92, 126.42, 123.81, 123.77, 123.67, 123.63, 119.59, 112.24, 112.20, 112.03, 111.99, 104.74, 104.48, 104.22, 50.14, 35.64, 28.31, 21.36.

4.8.30. 1-(2-(2,4-difluorophenyl)-2-((4-methylbenzyl)selanyl) ethyl)-1H-imidazole (A30)

White solid; yield 79.33%; m.p.: 225.1–228.3 °C. ESI-HRMS(*m*/*z*): 393.0670 $[M+H]^+$ (calcd. for $C_{19}H_{18}N_2F_2Se$); ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.53–7.45 (m, 2H), 7.19–7.09 (m, 5H), 7.07–7.00 (m, 2H), 6.78 (t, J = 1.1 Hz, 1H), 4.67–4.58 (m, 1H), 4.53–4.45 (m, 2H), 3.88 (d, J = 11.5 Hz, 1H), 3.78 (d, J = 11.5 Hz, 1H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ (ppm): 161.35, 137.74, 136.42, 135.91, 130.77, 130.72, 130.67, 130.62, 129.50, 129.24, 128.73, 123.78, 119.62, 112.25, 112.21, 112.04, 112.00, 104.75, 104.49, 104.23, 50.14, 35.54, 28.12, 21.15.

4.9. In vitro antifungal testing

The *in vitro* antifungal activity of target compounds was determined by the micro-broth dilution assay according to the CLSI M27-A3 guidelines. The RPMI 1640 medium (Gibco, USA) was prepared with 3-[N-morpholino]-propanesulfonic acid (MOPS, Genyiew, USA). Tested compounds and the control drug were dissolved in DMSO respectively and then Tween-20 was added as a stabilizer. The initial concentration of the fungal suspension in RPMI 1640 medium was adjusted to 1–5 × 10^3 CFU/mL. The prepared samples were serially two-fold diluted in growth medium. After inoculation, the incubation was performed at 35 °C. The MICs at 24 h for *C. alb., C. zey., C.gla., C.par.,* and *C.kru.,*and the MICs of *C. neo*. and *A, f.* were determined at 72 h. The MICs were defined as the lowest compound concentration which prevented visible growth. All determinations were implemented with biological replicates.

4.10. Determination of MFC

C. albicans (CPCC400616) was grown in RPMI 1640 medium which was suitable for fungal growth at 35 °C and then was diluted in respective media. The RPMI 1640 medium (Gibco, USA) was adjusted to pH 7.0 by using 3-[N-morpholino]-propanesulfonic acid (MOPS, Genyiew, USA). Tested compounds and the control drug were dissolved in DMSO respectively and Tween-20 was added as a stabilizer. The initial concentration of the fungal suspension in RPMI 1640 medium was adjusted to 1–5 × 10^3 CFU/mL.The MFCs were determined at 48 h. Then 100 μL fungal suspension was plated on PDA plates and incubated at 35 °C for 24 h. MFCs correspond to the concentration where were the number of colonies on the SDA plate is less than 5.

4.11. Time-kill assays

A fungal suspension of *C. albicans* (1 × 10^5 CFU/mL) were incubated with various concentrations of A03 (1 × , 2 × , 4 × , and 8 × MIC) at 35 °C. At different time intervals (1, 2, 4, 8, 12, and 24 h), aliquots (10 μL) were fetched out from the solution and then was carried out 10-fold serial dilution with 0.9% saline. The diluted fungal suspension was inoculated on PDA plates and incubated at 35 °C for 24 h, then the colony forming units (CFU) were counted. The Log_{10} CFU/mL was plotted on a graph as a time function. The detection limit of this assay was 100 CFU/mL. The experiment was performed in triplicate.

4.12. GC-MS analysis of sterol composition

Candida albicans ATCC SC5314 cells were incubated in YEPD (60 mL) for 16 h at 35 °C. Compounds were introduced into the culture medium before incubation. Cells were collected by centrifugation at 3000*g* for 10 min. Then, the cells were washed with PBS three times and suspended in saponification medium (3 mL, 15% NaOH solution in 90% ethanol). Then suspension was saponified at 80 °C for 60 min and the nonsaponifiable sterols were then extracted three times with petroleum ether (6 mL). The extract of petroleum ether was then evaporated, the residues were dissolved in hexamethylene. The sterols were analyzed by GC-MS (model 6890 N/5975i, Agilent Technologies, Palo Alto, CA, USA). The GC-MS data were analyzed by employing Agilent software with the NIST Spectrum Database (NIST MS search 2.0). Analyses were performed in a splitless mode by choosing 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.

4.13. In vitro biofilm formation assay

Fungal suspensions (1 × 10^6 fungal cells per milliliter) were grown in 96-well plates in RPMI 1640 medium and incubated at 37 °C. The RPMI 1640 medium and non-adherent cells were removed after1.5 h, 3 h, 6 h and 24 h for adhesion,. Then different concentration of test compounds were added, and the plates were further incubated at 37 °C for 24 h. A semiquantitative determination of the formed biofilms was calculated by XTT reduction

assay.

4.14. Hemolytic assays

The assays were performed by using fresh white rabbit RBCs. The RBCs were washed by using PBS buffer solution for three times and diluted with PBS buffer solution to obtain fungal cell suspension. The test compounds was diluted to double volume every time by using PBS and then the diluted solution were mixed with the 2% RBCs suspension. After incubation at 37 °C for 3 h, the mixtures were centrifuged at 600 g for 5 min. Then the supernatant were moved to a 96-well plate. The absorbance of the liquor was determined on a microplate reader to monitor the release of hemoglobin at 575 nm. In addition, RBCs with PBS served as the negative control (0% lysis), and RBCs treated with 2% Triton X-100 were used as positive control (100% lysis).

4.15. Molecular docking studies

The crystal structure of CYP51 (PDB code 5tz1) in the docking analysis was obtained from the Protein Data Bank. After removing hetero atoms, water molecules and co-factors, the docking analysis of target compounds and target proteins were accomplished 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, “Design, synthesis, and biological evaluation of novel miconazole analogues containing selenium as potent antifungal agents”.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2020.112360.

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