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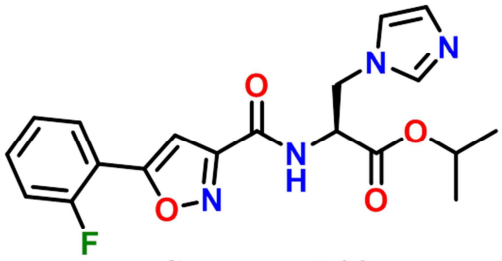
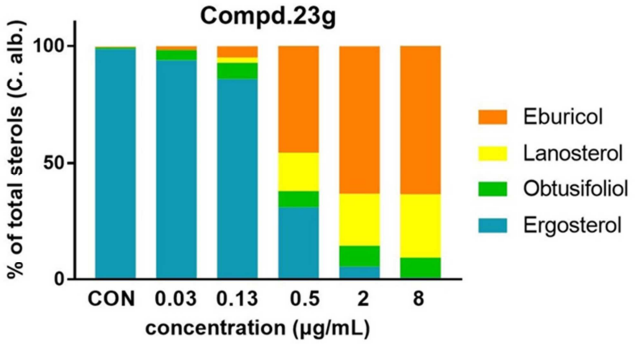


Compound 23g




| Compd. | MIC(μg/mL) | | | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|---|---|
| | *C. alb.* | *C. neo.* | *A. fum.* | CYP1A2 | CYP2C9 | CYP2C19 | CYP2D6 | CYP3A4-M |
| 23g | 0.0625 | 0.5 | 4 | >50 | >50 | >50 | >50 | 5.81 |

# Design, synthesis and evaluation of aromatic heterocyclic derivatives as potent antifungal agents

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## ABSTRACT:

To further enhance the anti-*Aspergillus* efficacy of our previously discovered antifungal lead compounds (**1**), a series of aromatic heterocyclic derivatives were designed, synthesized and evaluated for in vitro antifungal activity. Many of the target compounds showed good inhibitory activity against *Candida albicans* and *Cryptococcus neoformans*. In particular, the isoxazole nuclei were more suited for improving the activity against *Aspergillus spp*. Among these compounds, 2-F substituted analogues **23g** and **23h** displayed the most remarkable in vitro activity against *Candida spp.*, *C. neoformans*, *A. fumigatus* and fluconazole-resistant *C.alb.* strains, which is superior or comparable to the activity of the reference drugs fluconazole and voriconazole. Notably, the compounds **23g** and **23h** exhibited low inhibition profiles for various isoforms of human cytochrome P450 and excellent blood plasma stability.

## Keywords:

Antifungal activity, Azole antifungals, CYP51, Structure-activity relationship

## 1. Introduction

The incidence of fungal infections has steadily increased over the past few decades and presents a serious threat to human health, especially in immunocompromised patients, such as those undergoing organ transplants or anticancer chemotherapy and patients with AIDS[1-3].

*Candida spp., Cryptococcus neoformans and Aspergillus spp.* are still the three main pathogens of fungal infections[4]. The clinically available antifungal agents can be divided into four different classes based on their mode of action, including the polyenes (e.g., amphotericin B and nystatin)[4], echinocandins (e.g., caspofungin and micafungin)[5], azoles(e.g., fluconazole, voriconazole and itraconazole)[6], and antimetabolites (e.g., 5-fluorocytosine)[7].

Azole antifungal agents are currently most widely used in first-line antifungal therapy. They prevent the synthesis of ergosterol biosynthesis in the cell by inhibiting the activity of lanosterol 14-demethylase (CYP51), a member of the CYP51 class of cytochrome P450 enzymes[8, 9]. The imidazole or triazole ring of azole antifungals agents is an essential pharmacophoric portion, which can coordinate with the heme iron atom cofactor of the CYP51[10]. During the past 35 years, azole drugs, such as fluconazole, itraconazole, voriconazole and posaconazole, have made a significant impact on the management of systemic fungal infections[11]. However, several factors have limited their practical applications, such as drug resistance, narrow antifungal spectrum, and low bioavailability[12, 13]. Therefore, there is still an urgent need for developing novel azole antifungal agents with potent activity, broad spectrum, low toxicity, and low resistance.

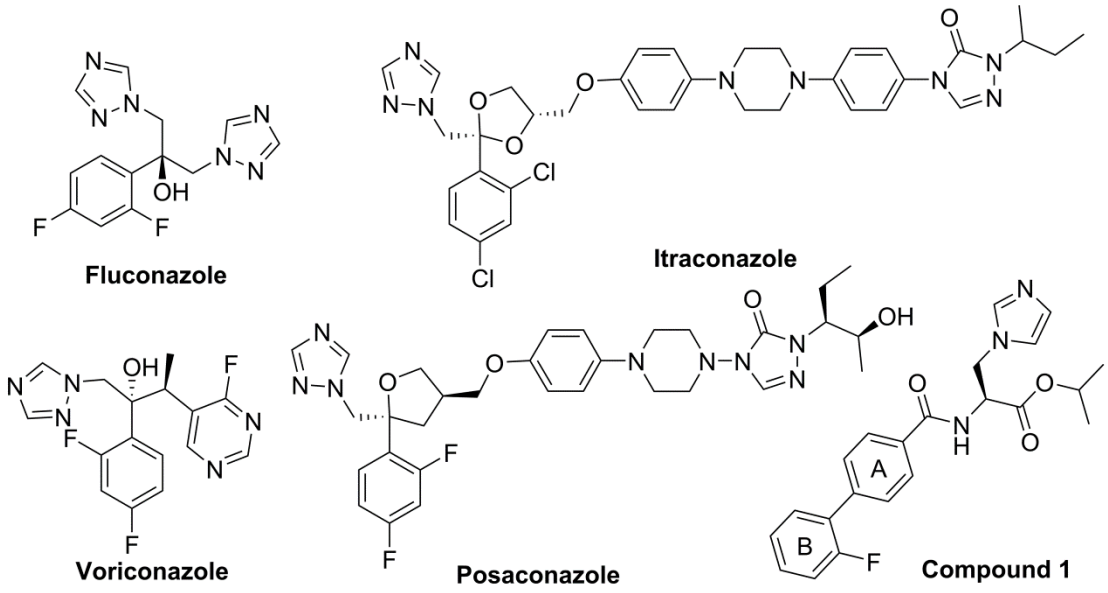



**Figure 1**. Chemical structures of azole antifungal agents and lead compound**.**

We have previously reported the synthesis and *in vitro* evaluation of antifungal activity of a new series of biphenyl imidazole derivatives[14, 15]. Most compounds displayed strong antifungal activities with MIC values in the range of 0.03125 μg/mL to 2 μg/mL against *Candida albicans* and *Cryptococcus neoformans.* However, almost all of the target compounds were

inactive against *Aspergillus fumigatus*, which prompted us to continue with studying the structural modification of potent original compounds in search of novel compounds with potent activity and broad spectrum.

A published crystal structure of voriconazole bound within the active site cavity of CYP51B of *Aspergillus fumigatus* (PDB ID:4UYM) served as a useful template for the rational structure-based design of novel drugs[16]. Voriconazole has much higher antifungal potency against *Aspergillus fumigatus* than fluconazole and itraconazole. The crystal structure suggests that the reason may be the formation of the hydrogen bonds between the 5-fluoropyrimidine ring of voriconazole and *A. fumigatus* CYP51 Tyr122 (Figure 2A).

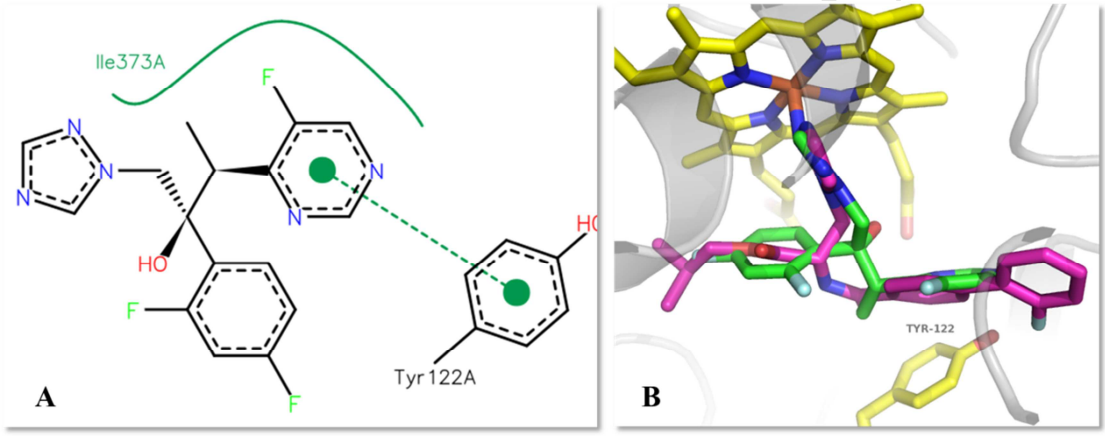



**Figure 2**. Crystal structure of sterol 14-alpha demethylase (CYP51B) from a pathogenic filamentous fungus *Aspergillus fumigatus* in complex with voriconazole (A and B) and the binding mode of compound **1** (B) in the active site.

To further explore the potential binding mode of biphenyl imidazole derivatives and guide the design of new compounds, compound **1** was docked into the active site of *A. fumigatus* CYP51B (PDB ID:4UYM, Figure 2B). Based on the interactions between compound **1** and *A. fumigatus* CYP51, along with the binding mode of voriconazole, we aimed to develop new heterocycles-linked compounds from biphenyl imidazole derivatives to formation hydrogen bonds interactions with Tyr122 of CYP51. The benzene ring (A ring of compound **1**) would be replaced by a five or six- membered heterocyclic ring, which might result in the development of more potent compounds with higher antifungal activities, especially the anti-*Aspergillus* efficacy.

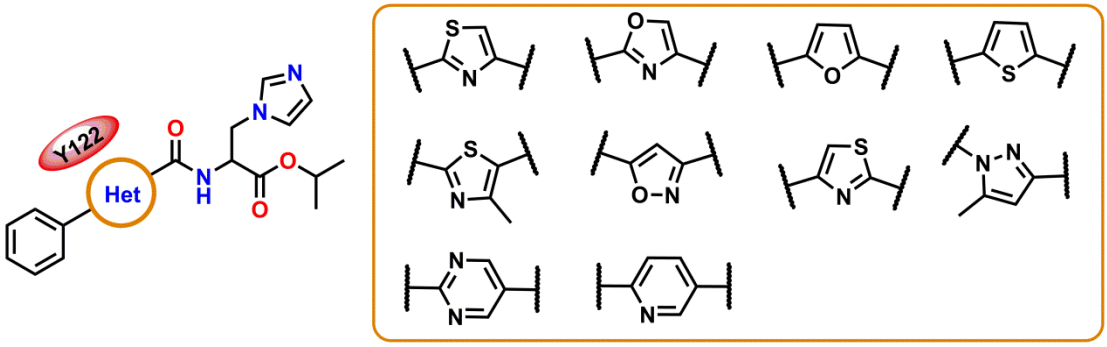


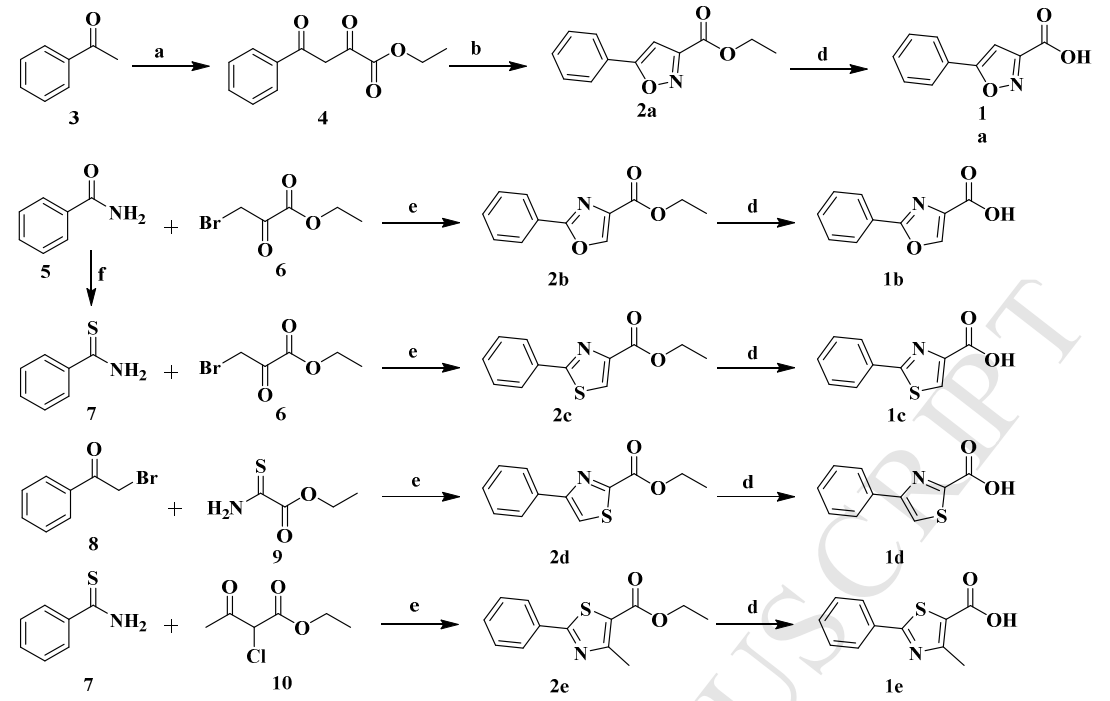

**Figure 3**. Our strategy for the formation hydrogen bonds interactions with CYP51B Tyr122 of *A. fumigatus* by replacing the middle benzene ring with 10 heteroaromatics.

## 2. Results and discussion

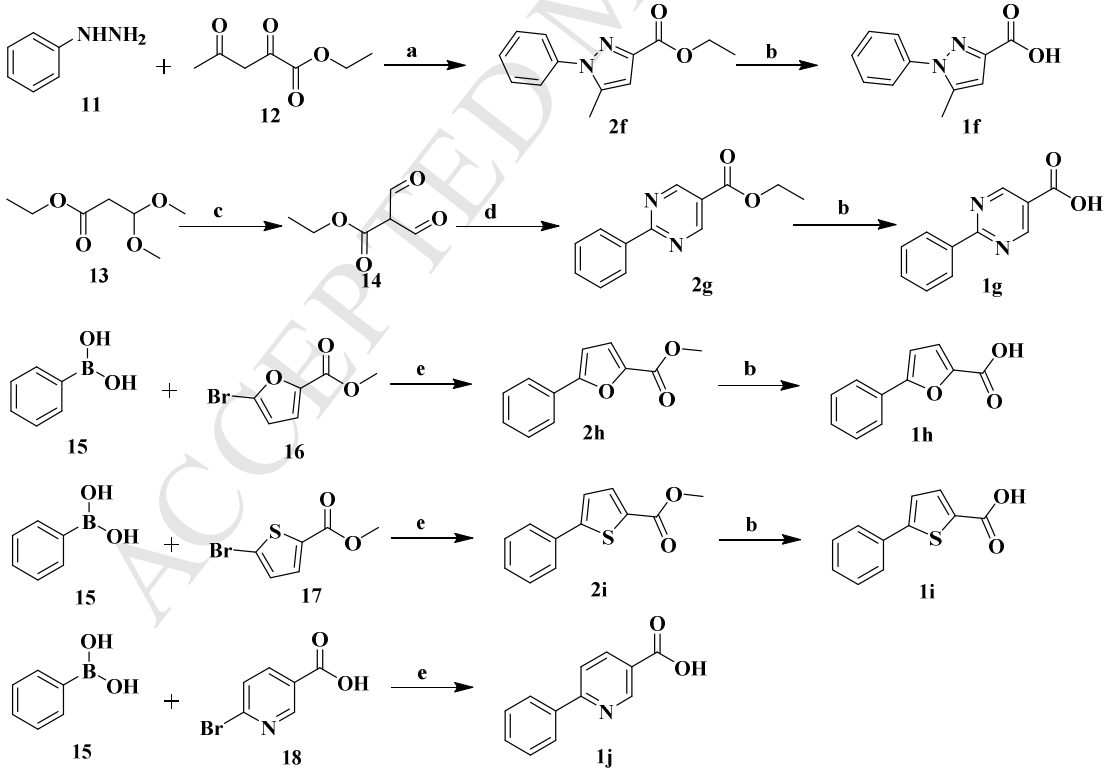
### *2.1 Chemistry*

The synthetic routes of the key intermediates **1a-j** are illustrated in Scheme 1 and 2. Acetophenone **3** was used as the starting material, and treated with diethyl oxalate in the presence of NaOEt by Claisen condensation to obtain intermediate **4**, which was used in a ring-closure reaction with hydroxylamine hydrochloride to form isoxazole **2a**. Replacing the oxygen atoms of benzamide **5** with sulphur via Lawesson's Reagent yielded thiobenzamide **7.** Then, benzamide **5** and thiobenzamide **7** were treated with ethyl bromopyruvate by intermolecular cyclization to obtain thiazole **2b** and oxazole **2c**, respectively. The thiazole **2d** was obtained from intermolecular cyclization between 2-Bromoacetophenone **8** and ethyl thiooxamate **9**.The ethyl 2-chloroacetoacetate **10** was condensed with thiobenzamide **6** to form thiazole **2e**.

The intermediate **2f** was obtained by ring-closure reaction between phenylhydrazine **11** and ethyl 2,4-dioxopentanoate **12**. Ethyl 3,3-diethoxy propionate **13** was treated with Ethyl formate in the presence of NaH by Claisen condensation to form intermediate **14**, which were combined with benzamidine hydrochloride hydrate in an intermolecular cyclization to yield intermediates pyrimidine **2g**. Commercially available **16,17** and **18** and phenylboronic acids **15** were subjected to Suzuki coupling in the presence of $Pd(PPh_3)_4$ to form the intermediates **2h**, **2i** and **1j**. Finally, intermediates **2a-i** were saponified with 2 N NaOH to obtain the key intermediates **1a-i**.

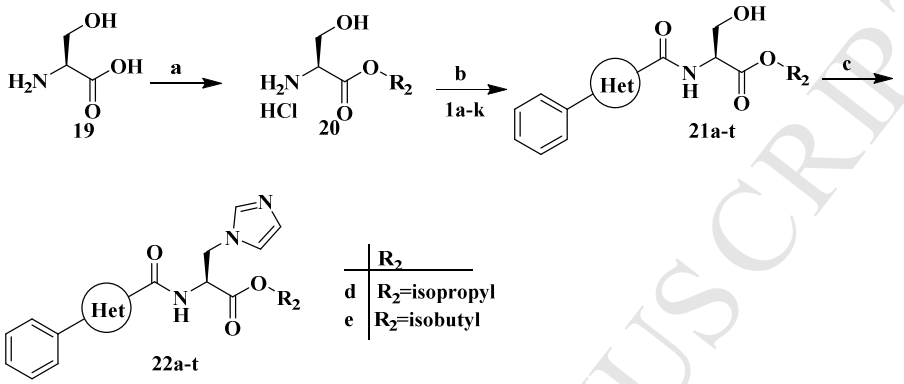


**Scheme 1**. Synthesis of intermediates **1a-e**. Reagents and conditions:(a) Diethyl oxalate, NaOEt, 18 h;(b) Hydroxylamine hydrochloride, EtOH, reflux, 2 h; (c) Hydrazine hydrate, EtOH, reflux, 2 h; (d) NaOH, MeOH/$H_2O$; (e) EtOH, reflux; (f) Lawesson's reagent, toluene, reflux, 7 h.



**Scheme 2**. Synthesis of intermediates **1f-j**. Reagents and conditions: (a) EtOH, reflux; (b) NaOH, MeOH/$H_2O$; (c) ethyl formate, NaH, THF; (d) benzamidine hydrochloride hydrate, $K_2CO_3$, DMF, 100$^oC$ ; (e) $Pd(PPh_3)_4$, $K_2CO_3$, reflux, 5h.

The target compounds **22a-v** were formed according to the reaction pathways illustrated in Scheme 3. L-serine **19** was treated with an alcohol (isopropanol or isobutanol) and refluxed with $SOCl_2$ to give the serine esters **20a-b**. The serine esters **20a-b** reacted with the key intermediates **1a-j** in the presence of EDCI and HOBt, to give compounds **21a-t**. Finally, the compounds **21a-t** were treated with imidazole/CDI, to obtain the target compounds **22a-t**.




**Scheme 3.** General synthesis of the target compounds **22a-t**. Reagents and conditions: (a) alcohol reagent, $SOCl_2$, reflux, 1–2 h; (b) EDCI, HOBt, DIEA, r.t., 7 h; (c) CDI, imidazole, $CH_3CN$, reflux, 7 h.

*2.2 In vitro antifungal activity*

To explore an optimal polar heteroaromatic to replace the middle phenyl of compound **1**, we evaluated the *in vitro* antifungal activities of the synthesized 19 heteroaromatic compounds according to the protocols from the NCCLS[17]. Broth microdilution methods were used to determine the minimum inhibitory concentrations (MICs) of the target compounds in 96-well microtest plates. Fluconazole (FLC) and Itraconazole (ITR) were used as reference drugs.

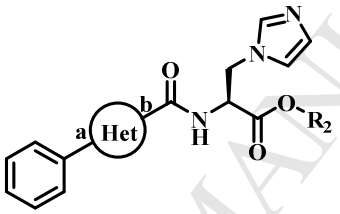
The *in vitro* antifungal activities of the target compounds are listed in Table 1. The MIC values revealed that most compounds showed a decrease in antifungal activity compared with the lead compound **1**. Interestingly, the heteroatom compounds such as thiophene (**22a-b**), 2-Methylthiazole (**22m-n**), isoxazole (**22e-f**), pyrimidine(**22q-s**) and pyridine(**22t**) exhibited moderate to good antifungal properties. Of these, isoxazole (**22e-f**) showed the most potent activity against *C. albicans*, *C. neoformans*, and *C. tropicalis* with MIC values in the range of 0.03125 to 1 μg/mL, which are superior or comparable to those of the reference drugs FLC and ITR. Interestingly, the isoxazole core (**22e-f**) showed excellent antifungal activities against *Aspergillus fumigatus* with an MIC value of 4 μg/mL, which was intended to increase the antifungal activities against *Aspergillus fumigatus*. Moreover, other combinations of heteroatoms

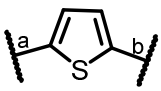
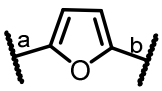
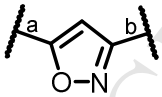
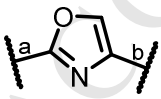
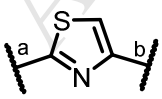
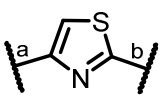
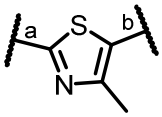
such as furans (**22c-d**), oxazole (**22g-h**) and thiazole(**22k-l**) did not show the desired potency, further confirming the importance of the electrical and hydrophobic effect in ligands.

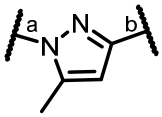
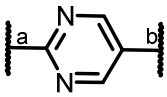
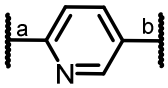
Based on the results above, we selected a scaffold of isoxazole core (**22e-f**) as our starting point for further modification. Our optimized efforts were directed toward replacing the terminal benzene ring with various substituents to expand the SAR studies (Table 2). Most of the compounds(**23a-h**) exhibited excellent antifungal properties with broad spectrums of activity. The results showed that the structure-activity relationships of these compounds were identical to our previously reported biphenyl imidazole derivatives[15]. Of these, compounds **23g-h** with 2-F substituents showed the best antifungal activity.

**Table 1**

In vitro antifungal activities of the target compounds (MIC, μg/mL) [a].



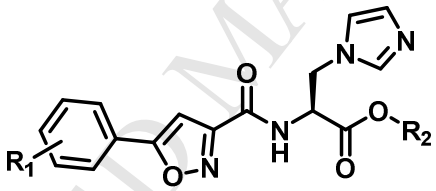

| Compd. | Het | $R_2$ | *C. alb.*(Ⅰ) | *C. alb*(Ⅱ). | *C. neo.* | *C.tro.* | *A. fum.* |
|---|---|---|---|---|---|---|---|
| **22a** |  | $-CH(CH_3)_2$ | 8 | 8 | 16 | 1 | >16 |
| **22b** | | $-CH_2CH(CH_3)_2$ | 2 | 4 | >16 | 0.125 | >16 |
| **22c** |  | $-CH(CH_3)_2$ | >16 | >16 | >16 | 8 | >16 |
| **22d** | | $-CH_2CH(CH_3)_2$ | >16 | >16 | >16 | 4 | >16 |
| **22e** |  | $-CH(CH_3)_2$ | 0.25 | 0.5 | 0.5 | 0.0625 | 4 |
| **22f** | | $-CH_2CH(CH_3)_2$ | 0.0625 | 1 | 0.25 | 0.03125 | 4 |
| **22g** |  | $-CH(CH_3)_2$ | >16 | >16 | >16 | 8 | >16 |
| **22h** | | $-CH_2CH(CH_3)_2$ | >16 | >16 | >16 | 4 | >16 |
| **22i** |  | $-CH(CH_3)_2$ | >16 | >16 | >16 | >16 | >16 |
| **22j** | | $-CH_2CH(CH_3)_2$ | >16 | >16 | >16 | >16 | >16 |
| **22k** |  | $-CH(CH_3)_2$ | >16 | >16 | 2 | 0.0625 | >16 |
| **22l** | | $-CH_2CH(CH_3)_2$ | >16 | >16 | 2 | 0.125 | >16 |
| **22m** |  | $-CH(CH_3)_2$ | 2 | 2 | 16 | 0.25 | >16 |
| **22n** | | $-CH_2CH(CH_3)_2$ | 0.25 | 1 | 8 | 0.125 | >16 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **22o** |  | $-CH(CH_3)_2$ | >16 | >16 | >16 | 4 | >16 |
| **22p** | | $-CH_2CH(CH_3)_2$ | >16 | >16 | >16 | 4 | >16 |
| **22q** |  | $-CH(CH_3)_2$ | 0.25 | 1 | 8 | 0.0625 | >16 |
| **22s** | | $-CH_2CH(CH_3)_2$ | 0.25 | 0.5 | 2 | 0.125 | >16 |
| **22t** |  | $-CH(CH_3)_2$ | 2 | 4 | 16 | 1 | >16 |
| **1** | | | 0.0625 | 0.25 | 1 | 0.0625 | >16 |
| **FCZ** | - | - | 1 | 2 | 4 | 0.25 | 16 |
| **ITZ** | - | - | 0.03125 | 0.125 | 1 | 0.03125 | 1 |

[a]Abbreviations: *C.alb.*(Ⅰ), *Candida albicans* (ATCC SC5314); *C.alb.*(II), *Candida albicans* (CPCC400523); *C. neo.*, *Cryptococcus neoformans* (cgmcc 2.3161); *A.fum.*, *Aspergillus fumigatus* (GIM 3.524); *C.tro.*, *Candida tropicalis* (cgmcc 2.3739); FCZ: Fluconazole; ITZ: Itraconazole.

**Table 2**

In vitro antifungal activities of the target compounds (MIC, μg/mL)[a].



| Compd. | $R_1$ | $R_2$ | *C. alb.*(Ⅰ) | *C. alb*(Ⅱ). | *C. neo.* | *C.tro.* | *A. fum.* |
|---|---|---|---|---|---|---|---|
| **22e** | H | $-CH(CH_3)_2$ | 0.25 | 0.5 | 0.5 | 0.0625 | 4 |
| **22f** | | $-CH_2CH(CH_3)_2$ | 0.0625 | 1 | 0.25 | 0.03125 | 4 |
| **23a** | 4-CH3 | $-CH(CH_3)_2$ | 0.25 | 0.5 | 2 | 0.03125 | 8 |
| **23b** | | $-CH_2CH(CH_3)_2$ | 0.125 | 0.5 | 0.5 | 0.03125 | 8 |
| **23c** | 4-F | $-CH(CH_3)_2$ | 1 | 2 | 2 | 0.03125 | 8 |
| **23d** | | $-CH_2CH(CH_3)_2$ | 0.5 | 2 | 0.5 | 0.03125 | 8 |
| **23e** | 4-Br | $-CH(CH_3)_2$ | 0.0625 | 0.25 | 2 | 0.03125 | >16 |
| **23f** | | $-CH_2CH(CH_3)_2$ | 0.03125 | 0.125 | 2 | 0.03125 | >16 |
| **23g** | 2-F | $-CH(CH_3)_2$ | 0.0625 | 0.25 | 0.5 | 0.03125 | 4 |
| **23h** | | $-CH_2CH(CH_3)_2$ | 0.03125 | 0.125 | 0.25 | 0.03125 | 4 |
| **FCZ** | - | - | 1 | 2 | 4 | 0.25 | >16 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **ITZ** | - | - | 0.03125 | 0.125 | 1 | 0.03125 | 1 |

[a]Abbreviations: *C.alb.*(Ⅰ), *Candida albicans* (ATCC SC5314); *C.alb.*(II), (CPCC400523); *C. neo.*, *Cryptococcus neoformans* (cgmcc 2.3161); *A.fum.*, *Aspergillus fumigatus* (GIM 3.524); *C.tro.*, *Candida tropicalis* (cgmcc 2.3739); FCZ: Fluconazole; ITZ: Itraconazole.

*2.3 In vitro antifungal activity against fluconazole-resistant strains of C. alb.*

Currently, the widespread use of Fluconazole, especially for prolonged treatment periods, has led to a severe increase in drug-resistance, which has become a major clinical problem in fungal infection therapy. Therefore, there is an urgent need to find new types of inhibitors that could be effective against fluconazole-resistant strains of *C. albicans*. The most potent compounds **23g** and **23h** were further evaluated against fluconazole-resistant strains of *C.alb*. (*strains 100* and *103*). Compounds **23g** and **23h** displayed strong antifungal activities against *strains 100* and *103*, with MIC values in the range from 0.125 to 1 μg/mL (Table 3).

**Table 3**

In vitro antifungal activities of the target compounds (MIC, μg/mL)[a].

| **Compd.** | **$R_1$** | **$R_2$** | ***C. alb.*** | |
|---|---|---|---|---|
| | | | ***Strain100*** | ***Strain103*** |
| **23g** | 2-F | $-CH(CH_3)_2$ | 1 | 1 |
| **23h** | 2-F | $-CH_2CH(CH_3)_2$ | 0.125 | 0.5 |
| **FCZ** | - | - | >64 | >64 |

[a]Abbreviations: *C.alb.*, *Candida albicans*; *strain 100*, fluconazole-resistant strains of *Candida albicans*; *strain 103*, fluconazole-resistant strains of *Candida albicans*; FCZ: Fluconazole.*Strain 100* and *strain 103* were provided by The Second Military Medical University.

*2.4 Dose-dependent effect on sterol composition in Candida albicans (ATCC SC5314)*

The antifungal mechanism of compound **23g** was confirmed by analysing the change in sterol composition in **23g**-treated *C. albicans SC5314* cells by GC-MS, which was compared to that of untreated or fluconazole-treated *C. albicans SC5314*. The assay has been successfully used in studying the mechanism of action of antifungal agents on the sterol biosynthesis pathways[18-20]. FLC was used as a reference drug and cholesterol was added as an internal standard. As shown in Fig. 4, **23g** and FLC reduced the content of ergosterol and increased the content of lanosterol and eburicol in a dose-dependent manner. The GC-MS analysis results are shown in Table 4. In the untreated control, the sterol fraction contained 98.7% ergosterol, while lanosterol was not

observed and other 14-methylated sterols (obtusifoliol and eburicol) contained only 1.0 %. When *C. albicans* was treated with FLC at 0.03125-8 μg/mL for 16 h, the content of ergosterol was reduced to 1.7% from 98.7% of the total amount of sterol fraction, whereas the lanosterol and eburicol contents were increased to 34.8 % and 37.2%, respectively. These changes were caused by the competitive inhibition of CYP51 in *C. albicans* by FLC and are consistent with previously published studies[15, 21-23]. Interestingly, treatment with **23g** also resulted in noticeable accumulation of eburicol and lanosterol and a significant reduction in the ergosterol content (reduced to 0.7%). These results suggested that the novel compound **23g** caused the potent disruption of sterol biosynthesis by inhibiting CYP51 activity in *C. albicans*, similar to the accepted mechanism of FLC.

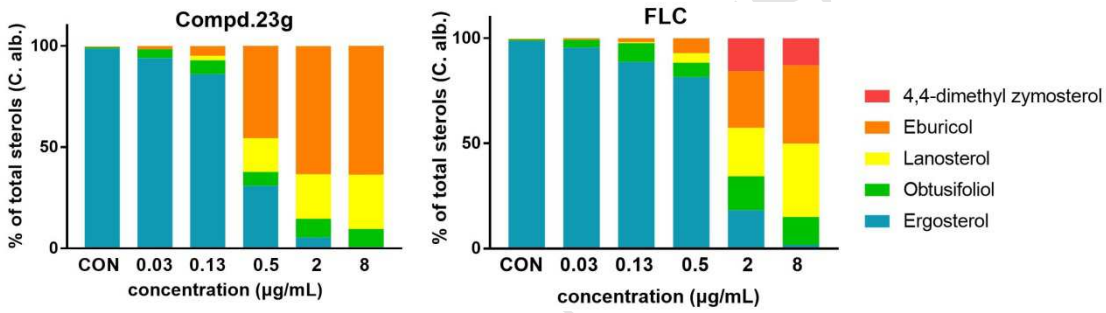



**Figure 4**. Sterol composition in **23g** or fluconazole-treated SC5314 *C. albicans* cells by GC-MS.

**Table 4.**

Analysis of sterol composition in *C.albicans* by GC-MS.[a]

| Compd. | concentration (μg/mL) | % of total sterols (*C. alb.*[a]) | | | | |
|---|---|---|---|---|---|---|
| | | Ergosterol | Obtusifoliol | Lanosterol | Eburicol | 4,4-dimethyl zymosterol |
| **23g**[b] | 0.03125 | 93.9 | 4.3 | - | 1.7 | - |
| | 0.125 | 85.9 | 6.9 | 2.2 | 4.9 | - |
| | 0.5 | 30.8 | 6.9 | 16.7 | 45.6 | - |
| | 2 | 5.4 | 9.2 | 22.0 | 63.3 | - |
| | 8 | 0.7 | 8.9 | 26.7 | 63.7 | - |
| **FLC**[c] | 0.03125 | 95.6 | 3.6 | - | 0.8 | - |
| | 0.125 | 88.7 | 8.9 | 0.4 | 2.0 | - |
| | 0.5 | 81.4 | 6.9 | 4.5 | 7.2 | - |
| | 2 | 18.2 | 16.1 | 23.0 | 27.0 | 15.7 |
| | 8 | 1.7 | 13.3 | 34.8 | 37.2 | 13.0 |
| **Control**[d] | - | 98.7 | 0.6 | - | 0.4 | - |

[a]Abbreviations: *C.alb.*, *Candida albicans* (ATCC SC5314); [b]Treated with compound **23g**; [c]Treated with FLC; [d]Control (no drug).

*2.5 Cytochrome P450 Inhibition Assay*

Cytochrome P450s (CYP) comprise a superfamily of enzymes that catalyse the oxidation of a wide variety of drugs. Drug-drug interactions (DDI) caused by inhibiting cytochrome P450s enzymes can result in dangerous side effects. However, many azole antifungals, such as ketoconazole and itraconazole, had greater inhibitory effects on Cytochrome P450 (CYP) enzymes[24]. For example, the $IC_{50}$ of ketoconazole and itraconazole for CYP3A4 inhibition were 25 and 32.6 nM, respectively[25]. The DDI potential for compounds **23g** and **23h** were tested against the five major human CYP isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4-M). As shown in Table 5, the compounds **23g** and **23h** showed weak inhibitory activity against CYP1A2, CYP2C9, CYP2C19, and CYP2D6 with $IC_{50}$ values of ≥50μM, while compounds **23g** and **23h** exhibited moderate activity against CYP3A4 with $IC_{50}$ values of 5.81 μM and 8.38 μM, respectively. The results showed that compounds **23g** and **23h** had a low potential for causing DDI.

**Table 5.**

In Vitro CYP Inhibition assessment of compounds.

| Compd. | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|
| | CYP1A2 | CYP2C9 | CYP2C19 | CYP2D6 | CYP3A4-M |
| **23g** | **>50** | **>50** | **>50** | **>50** | **5.81** |
| **23h** | **>50** | **>50** | **44.1** | **>50** | **8.38** |

*2.6 In vitro human Plasma Stability Assay*

The stability of compounds in human plasma is an important consideration in drug discovery. Based on their in vitro antifungal activities, compounds **23g** and **23h** were incubated with human Plasma. As shown in Table 6, compounds **23g** and **23h** exhibited excellent metabolic profiles in human plasma at 120 min (remaining 91.8% and 61.4%, respectively).

**Table 6.**

*In Vitro* human Plasma Stability of compounds **23g** and **23h**.

| Compd. | Stablity in Human Blood Plasma | |
|---|---|---|
| | % Remaining at 60 min | % Remaining at 120 min |
| **23g** | 95.3 | 91.8 |
| **23h** | 74.6 | 61.4 |

## 3. Summary

A major focus of our optimization effort was to increase the anti-*Aspergillus* efficacy of our previous series of biphenyl imidazole derivatives to formation hydrogen bond interactions with Tyr122 of CYP51. A series of aromatic heterocyclic derivatives was designed, synthesized and evaluated for in vitro antifungal activity. Among these compounds, the pyrimidine and isoxazole nuclei was more suited for improving activity against *Candida spp.* and *Cryptococcus ssp*. Specifically, isoxazole nuclei were identified for anti-*Aspergillus* activity. Among these, 2-F substituted analogues **23g** and **23h** displayed the most remarkable in vitro activity against *Candida spp*., *C. neoformans*, *A. fumigatus* and fluconazole-resistant *C.alb.* strains, which was superior or comparable to those of the reference drugs fluconazole and voriconazole. Notably, the compounds **23g** and **23h** exhibited low inhibition profiles for various human cytochrome P450 isoforms and excellent blood plasma stability. Further developments of compounds **23g** and **23h** are ongoing in our laboratory.

## 4. Experimental section

*4.1 General procedure for the synthesis of compounds*

Unless otherwise noted, all reagents and solvents were obtained from commercially available sources and were used without purification. TLC analysis was performed on GF254 silica gel plates (Jiangyou, Yantai). Column chromatography was carried out with silica gel (200-300 mesh) from Qingdao Haiyang Chemicals (Qingdao, Shandong, China). Mass spectrometry was performed using ESI mode on an Agilent 1200 LC-MS (Agilent, Palo Alto, CA, USA). High-resolution accurate mass determinations (HRMS) were recorded on a Bruker Micromass Time of Flight mass spectrometer equipped with electrospray ionisation (ESI). Melting points (mp.) were determined using glass capillary tubes on a BüCHI Melting Point B-540 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. Nuclear magnetic resonance ($^{1}$H-NMR and $^{13}$C-NMR )spectra were recorded on a Bruker 400 MHz NMR spectrometer with TMS as an internal standard. The chemical shifts were reported in parts per million (ppm), the coupling constants (*J*) were expressed in hertz (Hz). Peak multiplicities were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br).

4.1 *ethyl 2,4-dioxo-4-phenylbutanoate (**4**)*

Na (5.2 g, 224.7 mmol) was was dissolved in 80 mL ethanol, cooled to < 0 °C using a salted ice bath. Diethyl oxalate (18.2 g, 124.8 mmol) and acetophenone (6.0 g, 49.9 mmol) were added to a stirred solution of NaOEt, and the resulting mixture was stirred for 8 h at ambient temperature. After confirming that the reaction was complete by using TLC analysis, the reaction was quenched with aqueous 3N HCl and the ethanol was removed under reduced pressure. The mixture was extracted with EtOAc, and then with brine. The organic phase was dried over $Na_2SO_4$ overnight and the solvent was removed *in vacuo* to give the target product **4**.

4.2 *ethyl 5-phenylisoxazole-3-carboxylate (**2a**)*

Hydroxylamine hydrochloride (1.26 g, 18.2 mmol)was added to a solution of ethyl 2, 4-dioxo-4-phenylbutanoate (2.00 g, 9.1 mmol) in EtOH (40 mL) at ambient temperature. The reaction was refluxed for 6 h and concentrated under reduced pressure. The reaction mixture was extracted with EtOAc followed by extraction with brine. The organic phase was dried over $Na_2SO_4$ overnight and the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography to yield the target product **2a**.$^1$H NMR (600 MHz, DMSO-$d_6$) δ 7.98 (dd, $J$ = 7.5, 1.9 Hz, 2H), 7.61 – 7.55 (m, 3H), 7.53 (s, 1H), 4.41 (q, $J$ = 7.1 Hz, 2H), 1.35 (t, $J$ = 7.1 Hz, 3H).

4.3 *ethyl 2-phenyloxazole-4-carboxylate(**2b**)*

Ethyl bromopyruvate (3.86 g, 19.8 mmol) was added to a solution of benzamide **5** (2.00 g, 16.5 mmol) in ethanol at ambient temperature. The solution was refluxed for 6 h. After confirming the reaction was complete by using TLC analysis, the solution was cooled to room temperature. The solvent was removed under reduced pressure, and the residue was extracted with ethyl acetate and washed with brine. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated. The crude product was purified by silica gel column chromatography to yield the target product **2b.**

4.4 *benzothioamide(7)*

A solution of benzamide **5** (5.00 g, 41.3 mmol) and Lawesson's reagent (8.35 g, 20.6 mmol) in toluene (60 mL) was stirred at 70 °C for 3 h. After confirming that the reaction was complete by using TLC analysis, the crude mixture was concentrated under reduced pressure. The resulting residue was subjected to flash chromatography purification to give the titled compound **7** as a red solid.

4.5 *ethyl 2-phenylthiazole-4-carboxylate(**2c**)*

Ethyl bromopyruvate (3.41 g, 17.5 mmol) was added to a solution of benzothioamide **7** (2.00 g, 14.6 mmol) in ethanol at ambient temperature. The solution was refluxed for 6 h. After confirming that the reaction was complete by using TLC analysis, the solution was cooled to room temperature. The solvent was removed under reduced pressure, and the residue was extracted with ethyl acetate and washed with brine. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated. The crude product was purified using silica gel column chromatography to yield the target product **2c**.

4.6 *ethyl 4-phenylthiazole-2-carboxylate（**2d**）*

A solution of ethyl thiooxamate (2.00 g, 10.1 mmol) and 2-Bromoacetophenone (1.29 g, 11.1 mmol) in 60 mL ethanol was heated to reflux for 8 h. After confirming that the reaction was complete by using TLC analysis, the solution was cooled to room temperature. The solvent was removed under reduced pressure, and the residue was extracted with ethyl acetate and washed with brine. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated. The crude product was purified by silica gel column chromatography to yield the target product **2d** as a white solid**.**

4.7 *ethyl 4-methyl-2-phenylthiazole-5-carboxylate (**2e**)*

A solution of benzothioamide **7** (2.0 g, 14.6 mmol) and ethyl 2-chloroacetoacetate (2.6 g, 16.1 mmol) in 50 mL ethanol was heated to reflux for 10 h. After confirming that the reaction was complete by using TLC analysis, the solvent was removed under reduced pressure, and the residue was extracted with ethyl acetate and washed with brine. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated. The crude product was purified by silica gel column chromatography to yield the target product **2e** as a white solid**.**

4.8 *ethyl 5-methyl-1-phenyl-1H-pyrazole-3-carboxylate (**2f**)*

A solution of phenylhydrazine hydrochloride (1.64 g, 15.18 mmol) and ethyl 2, 4-dioxopentanoate (2.19 g, 12.65 mmol) in 50 mL ethanol was heated to reflux for 4 h. At the end of the reaction, the solution was cooled to room temperature. The solvent was removed under reduced pressure, and the residue was extracted with ethyl acetate and washed with brine. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated. The crude product was purified by silica gel column chromatography to yield the target product **2f** as a white solid**.**

4.9 *ethyl 2-formyl-3-oxopropanoate(**14**)*

Sodium hydride (2.00 g, 50.6 mmol, 60%) was added to a solution of ethyl formate (10.13 g, 168.7 mmol) in tetrahydrofuran while maintaining the internal temperature below 0˚C. Then, ethyl 3,3-diethoxy propionate (5.00 g, 33.75 mmol) was added dropwise to the reaction mixture and stirred for 24 h at ambient temperature. After confirming that the reaction was complete by using TLC analysis, the reaction was quenched with 20 mL water and the tetrahydrofuran was removed under reduced pressure. The mixture was extracted with ethyl acetate and then brine. The organic phase was dried over $Na_2SO_4$ overnight and the solvent was removed in vacuo to give the title compound **14**.

4.10 *ethyl 2-phenylpyrimidine-5-carboxylate(**2g**)*

$K_2CO_3$ (2.1 g, 15.4 mmol) was added to a mixture of benzamidine hydrochloride hydrate (1.55 g, 10.0 mmol) and **14** (1.0 g, 7.7 mmol) in DMF at ambient temperature. The the solution was heated at 100°C for 4 h. After confirming that the reaction was complete by using TLC analysis, the solution was cooled to room temperature and water (15 mL) is added. The reaction mixture was extracted with EtOAc and brine. The organic phase was dried over $Na_2SO_4$ overnight and the solvent was removed under vacuum. The crude product was purified by silica gel column chromatography to give the target product **2g**.$^1$H NMR (600 MHz, DMSO-$d_6$) δ 9.31 (s, 2H), 8.49 – 8.44 (m, 2H), 7.59 (ddd, *J* = 15.9, 7.7, 3.6 Hz, 3H), 4.40 (q, *J* = 7.1 Hz, 2H), 1.37 (t, *J* = 7.1 Hz, 3H).

4.11 *General procedure for the synthesis of compounds (**2h,2i** and **1j**)*

Under an argon atmosphere, compound (**16, 17, 18**) (1 equiv.), boronic acid (1.2 equiv.) and $Pd[P(C_6H_5)_3]_4$(1 mol%) were dissolved in a mixed solution of dioxane/H2O (10:1). $K_2CO_3$ (2 equiv.) was added and the mixture was heated under reflux for 6 h. The reaction mixture was cooled to room temperature and the dioxane was removed by rotary evaporation. $H_2O$ was added and the solution was adjusted to pH=1-3 with 2 N HCl. The white solid precipitate was collected by filtration and dried to give the desired compound.

*4.11.1 methyl 5-phenylfuran-2-carboxylate (**2h**)*

Light white solid; yield: 71.7%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 7.85 – 7.80 (m, 2H), 7.50 (dd, *J* = 10.6, 4.8 Hz, 2H), 7.42 (dd, *J* = 10.6, 5.5 Hz, 2H), 7.19 (d, *J* = 3.6 Hz, 1H), 3.85 (s, 3H).

*4.11.2 6-phenylnicotinic acid(**1j**)*

Light white solid; yield: 67.1%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 13.37 (s, 1H), 9.22 – 9.04 (m, 1H), 8.33 (dd, *J* = 8.3, 2.2 Hz, 1H), 8.17 (dd, *J* = 8.2, 1.3 Hz, 2H), 8.14 – 8.09 (m, 1H), 7.53 (tdd, *J* = 6.9, 4.5, 2.1 Hz, 3H).

4.12 *General procedure for the synthesis of compounds (**1a-i**)*

Sodium hydroxide (2N) was added to a solution of intermediate **2a-i** (1 equiv.) in methanol at ambient temperature. The reaction mixture was stirred for 4 h and the methanol was removed by rotary evaporation. The resultant mixture was adjusted to pH=5-6 with 1 N HCl solution. The precipitated white solid was collected by filtration and dried to give the carboxylic acid intermediate ***(1a-i)***.

*4.12.1 5-phenylisoxazole-3-carboxylic acid(**1a**)*

Light white solid; yield: 91.5%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 7.95 (dd, *J* = 7.8, 1.7 Hz, 2H), 7.58 – 7.53 (m, 3H), 7.41 (s, 1H).

*4.12.2 2-phenyloxazole-4-carboxylic acid(**1b**)*

Light white solid; yield: 95.3%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 13.20 (s, 1H), 8.84 (s, 1H), 8.03 – 8.01 (m, 2H), 7.58 (dd, *J* = 5.1, 1.9 Hz, 3H).

*4.12.3 2-phenylthiazole-4-carboxylic acid(**1c**)*

Light white solid; yield: 94.9%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 13.12 (s, 1H), 8.51 (s, 1H), 7.98 (dd, *J* = 6.4, 3.2 Hz, 2H), 7.54 (dd, *J* = 5.0, 1.9 Hz, 3H).

*4.12.4 4-phenylthiazole-2-carboxylic acid(**1d**)*

Light white solid; yield: 91.6%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 13.12 (s, 1H), 8.51 (s, 1H), 7.99 – 7.97 (m, 2H), 7.54 (dd, *J* = 5.0, 1.9 Hz, 3H).

*4.12.5 4-methyl-2-phenylthiazole-5-carboxylic acid(**1e**)*

Light white solid; yield: 89.3%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 13.39 (s, 1H), 7.98 (d, *J* = 6.7 Hz, 2H), 7.58 – 7.50 (m, 3H), 2.68 (s, 3H).

*4.12.6 5-methyl-1-phenyl-1H-pyrazole-3-carboxylic acid(**1f**)*

Light white solid; yield: 90.3%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 12.75 (s, 1H), 7.58 – 7.55 (m, 4H), 7.52 – 7.48 (m, 1H), 6.71 (d, *J* = 0.6 Hz, 1H), 2.33 (s, 3H).

*4.12.7 5-phenylthiophene-2-carboxylic acid(**1i**)*

Light white solid; yield: 91.7%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 13.24 (s, 1H), 8.20 (d, *J* = 1.5 Hz, 1H), 8.13 (d, *J* = 1.6 Hz, 1H), 7.78 – 7.73 (m, 2H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 1H).

*4.13 General procedure for the synthesis of L-serine ester (**20a,20b**)*

Thionyl chloride (3 equiv.) was added dropwise to a solution of L-serine (1 equiv.) in alcohol reagent (isopropanol or isobutanol) cooled to < 0 °C. The mixture was heated under reflux for 6 h. The reaction mixture was then concentrated under reduced pressure to yield a white solid.

*4.14General procedure for the synthesis of compounds (**21a-t**)*

EDCI (1.1 equiv.) and HOBt (1.1 equiv) were added to a solution of the intermediate acid compound ***1a-j*** (1 equiv.) in anhydrous DMF. The reaction mixture was stirred for 1h at ambient temperature, and the L-serine ester (1.1 equiv.) and DIEA (3 equiv.) were added. The solution was heated to 70 °C for 6 h and then cooled to room temperature. The reaction mixture was poured into ice water, and the resulting solid was filtered and dried to give the desired compound.

*4.15 General procedure for the synthesis of compounds (**22a-t**)*

CDI (2 equiv.) and imidazole (1.2 equiv.) were added to a solution of the intermediate **21a-t** (1 equiv.) in $CH_3CN$. The solution was heated to reflux for 6 h. The reaction mixture was extracted with EtOAc and brine. The organic phase was dried over $Na_2SO_4$ overnight and the solvent was removed under vacuum. The crude product was purified by silica gel column chromatography to give the target product **22a-t**.

*4.15.1 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(5-phenylthiophene-2-carboxamido)propanoate(**22a**)*

Light white solid; yield: 69.1%; mp: 126.1-128.9 °C.$^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.01 (d, *J* = 7.9 Hz, 1H), 8.25 (d, *J* = 1.2 Hz, 1H), 8.13 (d, *J* = 1.2 Hz, 1H), 7.70 (d, *J* = 7.3 Hz, 2H), 7.65 (s, 1H), 7.47 (t, *J* = 7.7 Hz, 2H), 7.35 (t, *J* = 7.4 Hz, 1H), 7.23 (s, 1H), 6.86 (s, 1H), 4.95 (dt, *J* = 12.5, 6.2 Hz, 1H), 4.77 (td, *J* = 9.1, 5.3 Hz, 1H), 4.50 (dd, *J* = 14.1, 5.2 Hz, 1H), 4.36 (dd, *J* = 14.0, 9.5 Hz, 1H), 1.24 – 1.14 (m, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.02, 161.21, 141.78, 139.22, 137.83, 134.47, 129.07(2C), 128.24, 127.63, 127.49, 126.18, 125.88(2C), 119.95, 68.85, 53.76, 45.98, 21.48, 21.41. HRMS calcd for $C_{20}H_{22}N_3O_3S$, $[M + H]^+$, 384.1382; found 384.1385.

*4.15.2 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(5-phenylthiophene-2-carboxamido)propanoate(**22b**)*

Light white solid; yield: 65.7%; mp: 102.4-104.5 °C.$^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.05 (d, *J* = 8.0 Hz, 1H), 8.25 (d, *J* = 15.0 Hz, 1H), 8.13 (d, *J* = 1.1 Hz, 1H), 7.69 (d, *J* = 7.4 Hz, 2H),

7.66 (s, 1H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.35 (t, *J* = 7.3 Hz, 1H), 7.23 (s, 1H), 6.86 (s, 1H), 4.83 (td, *J* = 9.4, 5.0 Hz, 1H), 4.54 (dd, *J* = 14.1, 4.9 Hz, 1H), 4.39 (dd, *J* = 14.0, 9.8 Hz, 1H), 3.97 – 3.81 (m, 2H), 1.95 – 1.77 (m, 1H), 0.87 (d, *J* = 6.5 Hz, 6H).$^{13}$C NMR (150 MHz, DMSO-$d_6$) δ 169.41, 161.30, 141.78, 139.17, 137.66, 134.46, 129.11(2C), 127.68, 127.48, 127.30, 126.28, 125.90(2C), 120.26, 70.81, 53.57, 46.16, 27.24, 18.76(2C). HRMS calcd for $C_{21}H_{24}N_3O_3S$, $[M + H]^+$, 398.1538; found 398.1538.

*4.15.3 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(5-phenylfuran-2-carboxamido)propanoate(**22c**)*

Light white solid; yield: 70.4%; mp: 131.3-133.7 °C.$^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.93 (d, *J* = 8.1 Hz, 1H), 7.91 (d, *J* = 7.4 Hz, 2H), 7.69 (s, 1H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 1H), 7.24 (s, 1H), 7.21 (d, *J* = 3.6 Hz, 1H), 7.13 (d, *J* = 3.6 Hz, 1H), 6.87 (s, 1H), 4.96 (dt, *J* = 12.5, 6.2 Hz, 1H), 4.79 (td, *J* = 9.5, 5.2 Hz, 1H), 4.54 (dd, *J* = 14.0, 5.2 Hz, 1H), 4.41 (dd, *J* = 14.0, 9.7 Hz, 1H), 1.20 (dd, *J* = 6.2, 4.2 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.06, 157.70, 154.90, 146.11, 137.76, 129.25, 128.96(2C), 128.77, 128.03, 124.39(2C), 119.97, 116.58, 107.74, 68.88, 53.16, 45.91, 21.48, 21.44. HRMS calcd for $C_{20}H_{22}N_3O_4$, $[M + H]^+$, 368.1610; found 368.1608.

*4.15.4 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(5-phenylfuran-2-carboxamido)propanoate(**22d**)*

Yellow oil; yield: 62.5%;$^1$H NMR (600 MHz, DMSO-$d_6$) δ 9.10 (d, *J* = 8.2 Hz, 1H), 7.92 (d, *J* = 7.3 Hz, 2H), 7.67 (s, 1H), 7.49 (s, 2H), 7.40 (d, *J* = 7.3 Hz, 1H), 7.25 (dd, *J* = 16.0, 5.5 Hz, 2H), 7.13 (dd, *J* = 3.5, 2.2 Hz, 1H), 6.83 (s, 1H), 4.86 (dd, *J* = 8.5, 4.9 Hz, 1H), 4.57 – 4.54 (m, 1H), 4.50 – 4.49 (m, 1H), 3.90 (dd, *J* = 6.5, 1.8 Hz, 2H), 1.91 – 1.84 (m, 1H), 0.87 (dd, *J* = 6.7, 2.2 Hz, 6H).$^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 169.96, 158.22, 155.36, 146.56, 138.17, 129.70, 129.42(2C), 129.24, 128.36, 124.84(2C), 120.46, 117.03, 108.21, 68.20, 53.47, 46.34, 27.69, 19.59, 19.19. HRMS calcd for $C_{21}H_{24}N_3O_4$, $[M + H]^+$, 382.1767; found 382.1768.

*4.15.5 isopropyl(S)-3-(1H-imidazol-1-yl)-2-(5-phenylisoxazole-3-carboxamido)propanoate(**22e**)*

Light white solid; yield: 63.8%; mp: 171.4-173.3 °C.$^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.31 (d, *J* = 8.1 Hz, 1H), 7.99 – 7.90 (m, 2H), 7.62 (s, 1H), 7.56 (dd, *J* = 5.2, 1.7 Hz, 3H), 7.36 (s, 1H), 7.21 (s, 1H), 6.85 (s, 1H), 4.96 (dt, *J* = 12.5, 6.2 Hz, 1H), 4.89 – 4.78 (m, 1H), 4.52 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.41 (dd, *J* = 14.0, 9.7 Hz, 1H), 1.20 (dd, *J* = 6.0, 4.8 Hz, 6H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 170.69, 168.45, 158.86, 158.69, 137.80, 130.95, 129.34(2C), 128.34, 126.18, 125.84(2C), 119.84, 99.88, 68.94, 53.42, 45.57, 21.45, 21.42. HRMS calcd for $C_{20}H_{22}N_5O_3$, [M +

H]$^+$, 380.1723; found 380.1720.

*4.15.6 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(5-phenylisoxazole-3-carboxamido)propanoate(**22f**)*

Light white solid; yield: 67.1%; mp: 107.1-109.5 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.37 (d, *J* = 8.3 Hz, 1H), 7.96 – 7.90 (m, 2H), 7.64 (s, 1H), 7.58 – 7.54 (m, 3H), 7.36 (s, 1H), 7.22 (s, 1H), 6.86 (s, 1H), 4.93 (td, *J* = 9.8, 4.8 Hz, 1H), 4.56 (dd, *J* = 14.0, 4.7 Hz, 1H), 4.44 (dd, *J* = 14.0, 10.1 Hz, 1H), 3.92 (dd, *J* = 6.5, 2.8 Hz, 2H), 1.88 (dp, *J* = 13.2, 6.6 Hz, 1H), 0.88 (d, *J* = 6.7 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.19, 169.35, 159.31, 159.21, 138.21, 131.43, 129.81(2C), 128.49, 126.62, 126.30(2C), 120.39, 100.32, 71.28, 53.70, 46.05, 27.69, 19.24, 19.19. HRMS calcd for $C_{21}H_{24}N_5O_3$, [M + H]$^+$, 394.1879; found 394.1875.

*4.15.7 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(2-phenyloxazole-4-carboxamido)propanoate(**22g**)*

Yellow oil; yield: 67.1%.$^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.77 (s, 1H), 8.75 (s, 1H), 8.04 (dd, *J* = 6.6, 3.0 Hz, 2H), 7.66 (s, 1H), 7.64 – 7.57 (m, 3H), 7.21 (s, 1H), 6.87 (s, 1H), 4.96 (dt, *J* = 12.5, 6.3 Hz, 1H), 4.83 (td, *J* = 8.7, 5.3 Hz, 1H), 4.56 – 4.44 (m, 2H), 1.20 (d, *J* = 6.2 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 168.82, 160.73, 160.12, 142.71, 137.73, 136.43, 131.33, 129.30(2C), 128.09, 126.33(2C), 126.12, 119.92, 68.92, 53.04, 45.87, 21.47, 21.44. HRMS calcd for $C_{19}H_{21}N_4O_4$, [M + H]$^+$, 369.1563; found 369.1560.

*4.15.8 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(2-phenyloxazole-4-carboxamido)propanoate(**22h**)*

Yellow oil; yield: 59.4%.$^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.82 (d, *J* = 8.3 Hz, 1H), 8.75 (s, 1H), 8.03 (dd, *J* = 4.4, 2.8 Hz, 2H), 7.65 (s, 1H), 7.60 – 7.58 (m, 3H), 7.21 (s, 1H), 6.86 (s, 1H), 4.91 (td, *J* = 9.0, 5.1 Hz, 1H), 4.59 – 4.47 (m, 2H), 3.91 (d, *J* = 6.5 Hz, 2H), 1.91 – 1.83(m, 1H), 0.88 (dd, *J* = 6.7, 1.0 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.28, 160.75, 160.20, 142.74, 137.71, 136.45, 131.36, 129.32(2C), 128.00, 126.33(2C), 126.12, 119.94, 70.79, 52.91, 45.82, 27.24, 18.75(2C). HRMS calcd for $C_{20}H_{23}N_4O_4$, [M + H]$^+$, 383.1719; found 383.1716.

*4.15.9 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(2-phenylthiazole-4-carboxamido)propanoate(**22i**)*

Light white solid; yield: 58.9%; mp: 128.8-131.6 °C.$^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.88 (d, *J* = 8.2 Hz, 1H), 8.34 (s, 1H), 8.06 (dd, *J* = 6.5, 3.0 Hz, 2H), 7.63 (s, 1H), 7.60 – 7.53 (m, 3H), 7.20 (s, 1H), 6.84 (s, 1H), 4.97 (dt, *J* = 12.5, 6.2 Hz, 1H), 4.85 (td, *J* = 8.6, 5.3 Hz, 1H), 4.59 – 4.47 (m, 2H), 1.21 (d, *J* = 6.3 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 168.95, 167.40, 160.52, 149.58, 137.79, 132.34, 130.89, 129.30(2C), 128.32, 126.49(2C), 125.03, 119.86, 68.96, 53.34, 45.87, 21.48, 21.45. HRMS calcd for $C_{19}H_{21}N_4O_3S$, [M + H]$^+$, 385.1334; found 385.1332.

4.15.10 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(2-phenylthiazole-4-carboxamido)propanoate(**22j**)

Yellow oil; yield: 63.7%.$^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 9.39 (d, *J* = 8.6 Hz, 1H), 8.49 (s, 1H), 8.09 (d, *J* = 7.3 Hz, 2H), 7.63 (s, 1H), 7.51 (t, *J* = 7.7 Hz, 2H), 7.42 (t, *J* = 7.3 Hz, 1H), 7.22 (d, *J* = 11.0 Hz, 1H), 6.83 (d, *J* = 4.0 Hz, 1H), 5.00 – 4.85 (m, 1H), 4.63 – 4.49 (m, 2H), 3.92 (d, *J* = 6.3 Hz, 2H), 1.88 (m, 1H), 0.82 (d, *J* = 6.7 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.94, 167.45, 160.56, 149.52, 137.78, 132.35, 130.93, 129.32(2C), 128.21, 126.53(2C), 125.16, 119.93, 67.76, 52.45, 45.92, 27.25, 19.15, 18.76. HRMS calcd for $C_{20}H_{23}N_4O_3S$, $[M + H]^+$, 399.1491; found 399.1491.

*4.15.11 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(4-phenylthiazole-2-carboxamido)propanoate(**22k**)*

Light white solid; yield: 65.2%; mp: 130.7-133.7 °C.$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 9.29 (d, *J* = 8.3 Hz, 1H), 8.46 (s, 1H), 8.08 (d, *J* = 7.4 Hz, 2H), 7.66 (s, 1H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.42 (t, *J* = 7.4 Hz, 1H), 7.22 (s, 1H), 6.85 (s, 1H), 5.01 – 4.95 (m, 1H), 4.86 (td, *J* = 8.9, 5.2 Hz, 1H), 4.58 (dd, *J* = 14.0, 5.1 Hz, 1H), 4.50 (dd, *J* = 14.0, 9.5 Hz, 1H), 1.21 (d, *J* = 6.2 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 168.55, 162.19, 159.32, 155.42, 137.80, 133.33, 128.87(2C), 128.70, 128.25, 126.31(2C), 120.08, 119.89, 69.08, 53.57, 45.69, 21.44(2C). HRMS calcd for $C_{19}H_{21}N_4O_3S$, $[M + H]^+$, 385.1334; found 385.1334.

*4.15.12 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(4-phenylthiazole-2-carboxamido)propanoate(**22l**)*

Yellow oil; yield: 58.7%.$^{1}$H NMR (400 MHz, DMSO) δ 8.95 (t, *J* = 9.0 Hz, 1H), 8.33 (d, *J* = 4.9 Hz, 1H), 8.10 – 8.03 (m, 2H), 7.63 (s, 1H), 7.58 – 7.50 (m, 3H), 7.20 (d, *J* = 6.0 Hz, 1H), 6.84 (s, 1H), 4.97 – 4.94 (m, 1H), 4.61 – 4.50 (m, 2H), 3.92 (d, *J* = 6.5 Hz, 2H), 1.97 – 1.80 (m, 1H), 0.82 (d, *J* = 6.7 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.44, 162.63, 159.87, 155.89, 138.19, 133.79, 129.34(2C), 129.18, 128.35, 126.77(2C), 120.57, 120.47, 71.35, 53.88, 46.18, 27.68, 19.18(2C). HRMS calcd for $C_{20}H_{23}N_4O_3S$, $[M + H]^+$, 399.1491; found 399.1489.

*4.15.13 isopropyl(S)-3-(1H-imidazol-1-yl)-2-(4-methyl-2-phenylthiazole-5-carboxamido)propanoate(**22m**)*

Light white solid; yield: 66.9%; mp: 149.4-157.4 °C.$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 8.81 (d, *J* = 7.7 Hz, 1H), 7.95 (dd, *J* = 6.5, 3.0 Hz, 2H), 7.64 (s, 1H), 7.57 – 7.46 (m, 3H), 7.22 (s, 1H), 6.89 (s, 1H), 5.00 - 4.92 (m, 1H), 4.79 – 4.66 (m, 1H), 4.50 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.37 (dd, *J* = 14.0, 9.8 Hz, 1H), 2.52 (s, 3H), 1.21 (t, *J* = 5.7 Hz, 6H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 168.75, 166.41, 161.34, 155.54, 137.84, 132.30, 131.02, 129.39(2C), 128.36, 126.31(2C), 125.25,

119.86, 68.79, 54.00, 45.59, 21.49, 21.46, 16.90. HRMS calcd for $C_{20}H_{23}N_4O_3S$, $[M + H]^+$, 399.1491; found 399.1490.

*4.15.14 isobutyl(S)-3-(1H-imidazol-1-yl)-2-(4-methyl-2-phenylthiazole-5-carboxamido)propanoate(**22n**)*

Light white solid; yield: 63.9%; mp: 83.7-85.9 °C.$^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.86 (d, *J* = 7.8 Hz, 1H), 7.94 (dd, *J* = 6.4, 3.0 Hz, 2H), 7.64 (s, 1H), 7.57 – 7.49 (m, 3H), 7.23 (s, 1H), 6.88 (s, 1H), 4.80 (td, *J* = 9.9, 4.9 Hz, 1H), 4.53 (dd, *J* = 14.0, 4.8 Hz, 1H), 4.41 (dd, *J* = 14.0, 10.1 Hz, 1H), 3.98 – 3.85 (m, 2H), 2.52 (s, 3H), 1.94 – 1.84 (m, 1H), 0.89 (d, *J* = 6.7 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.25, 166.44, 161.38, 155.73, 137.83, 132.29, 131.05, 129.41(2C), 128.23, 126.32(2C), 125.14, 119.90, 70.77, 53.88, 45.56, 27.27, 18.78(2C), 16.96. HRMS calcd for $C_{21}H_{25}N_4O_3S$, $[M + H]^+$, 413.1647; found 413.1644.

*4.15.15 isopropyl(S)-3-(1H-imidazol-1-yl)-2-(5-methyl-1-phenyl-1H-pyrazole-3-carboxamido)propanoate(**22o**)*

Yellow oil; yield: 51.5%.$^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.58 (d, *J* = 8.2 Hz, 1H), 7.60 – 7.55 (m, 5H), 7.53 – 7.46 (m, 1H), 7.15 (s, 1H), 6.83 (s, 1H), 6.64 (s, 1H), 4.94 (dt, *J* = 12.5, 6.2 Hz, 1H), 4.78 (td, *J* = 8.7, 5.2 Hz, 1H), 4.51 – 4.39 (m, 2H), 2.32 (s, 3H), 1.19 (dd, *J* = 6.2, 3.3 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.09, 161.48, 145.70, 140.95, 138.87, 137.76, 129.30(2C), 128.47, 128.27, 125.07(2C), 119.83, 107.16, 68.77, 53.05, 45.88, 21.49, 21.45, 12.00. HRMS calcd for $C_{20}H_{23}N_5O_3$, $[M + Na]^+$,404.1699; found 404.1688.

*4.15.16 isobutyl(S)-3-(1H-imidazol-1-yl)-2-(5-methyl-1-phenyl-1H-pyrazole-3-carboxamido)propanoate(**22p**)*

Yellow oil; yield: 54.9%.$^1$H NMR (600 MHz, DMSO-$d_6$) δ 8.70 (d, *J* = 8.4 Hz, 1H), 7.62 – 7.55 (m, 5H), 7.54 – 7.45 (m, 1H), 7.17 (s, 1H), 6.82 (s, 1H), 6.65 (d, *J* = 0.7 Hz, 1H), 4.86 (td, *J* = 9.1, 5.0 Hz, 1H), 4.52 – 4.46 (m, 2H), 3.93 – 3.84 (m, 2H), 2.32 (s, 3H), 1.86 (dt, *J* = 13.3, 6.7 Hz, 1H), 0.87 (dd, *J* = 6.7, 2.1 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.56, 161.56, 145.72, 140.93, 138.87, 137.74, 129.29(2C), 128.46, 128.22, 125.06(2C), 119.84, 107.15, 67.75, 52.93, 45.80, 27.25, 19.14, 18.77, 12.00. HRMS calcd for $C_{21}H_{26}N_5O_3$, $[M + H]^+$, 396.2036; found 396.2027.

*4.15.17 isopropyl(S)-3-(1H-imidazol-1-yl)-2-(2-phenylpyrimidine-5-carboxamido)propanoate(**22q**)*

Light white solid; yield: 71.2%; mp: 148.2-150.9 °C. $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 9.34 (d, *J* = 7.8 Hz, 1H), 9.19 (s, 2H), 8.45 (dd, *J* = 8.1, 1.5 Hz, 2H), 7.68 (s, 1H), 7.58 (ddd, *J* = 16.1, 7.7, 3.6 Hz, 3H), 7.25 (s, 1H), 6.88 (s, 1H), 5.02 – 4.91 (m, 1H), 4.84 (ddd, *J* = 9.2, 8.0, 5.2 Hz, 1H), 4.53 (dd, *J* = 14.2, 5.2 Hz, 1H), 4.40 (dd, *J* = 14.2, 9.3 Hz, 1H), 1.21 (dd, *J* = 8.6, 6.3 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 168.62, 165.11, 163.51, 156.74(2C), 137.73, 136.27, 131.76, 128.94(2C), 128.29(2C), 127.18, 124.75, 120.43, 69.02, 53.68, 46.23, 21.48, 21.44. HRMS calcd for $C_{19}H_{21}N_4O_4$, $[M + H]^+$, 369.1563; found 369.1565.

*4.15.18 isobutyl(S)-3-(1H-imidazol-1-yl)-2-(2-phenylpyrimidine-5-carboxamido)propanoate(**22s**)*

Yellow oil; yield: 61.1%. $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 9.92 (s, 1H), 9.33 (s, 2H), 8.46 (d, *J* = 6.9 Hz, 2H), 7.77 (s, 1H), 7.61 – 7.56 (m, 3H), 7.32 (s, 1H), 6.85 (s, 1H), 4.91 (d, *J* = 6.7 Hz, 1H), 4.57 (t, *J* = 6.6 Hz, 2H), 3.91 (qd, *J* = 10.5, 6.6 Hz, 2H), 1.87 (td, *J* = 13.3, 6.6 Hz, 1H), 0.87 (d, *J* = 6.7 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.13, 165.13, 163.49, 156.68(2C), 137.82, 136.24, 131.74, 128.90(2C), 128.28(2C), 127.88, 124.71, 120.11, 70.84, 53.69, 45.93, 27.21, 18.73. HRMS calcd for $C_{20}H_{22}N_4O_4$, $[M + Na]^+$, 405.1539; found 405.1536.

*4.15.19 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(6-phenylnicotinamido)propanoate(**22t**)*

Light white solid; yield: 64.9%; mp: 60.9-62.8 °C. $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 9.32 (d, *J* = 7.8 Hz, 1H), 9.04 (d, *J* = 1.8 Hz, 1H), 8.26 (dd, *J* = 8.3, 2.2 Hz, 1H), 8.20 – 8.14 (m, 2H), 8.11 (d, *J* = 8.3 Hz, 1H), 7.73 (s, 1H), 7.53 (t, *J* = 7.3 Hz, 2H), 7.50 (d, *J* = 7.1 Hz, 1H), 7.28 (s, 1H), 6.88 (s, 1H), 4.96 (dt, *J* = 12.5, 6.2 Hz, 1H), 4.84 – 4.76 (m, 1H), 4.54 (dd, *J* = 14.1, 4.6 Hz, 1H), 4.45 (dd, *J* = 14.0, 9.8 Hz, 1H), 1.20 (dd, *J* = 8.0, 6.4 Hz, 6H).$^{13}$C NMR (150 MHz, DMSO-$d_6$) δ 168.93, 165.03, 158.45, 148.67, 137.71, 136.26, 134.09, 129.86, 128.91(2C), 127.43, 127.34, 126.96(3C), 119.78, 68.77, 53.87, 48.58, 21.50, 21.46. HRMS calcd for $C_{21}H_{23}N_4O_3$, $[M + H]^+$, 379.1770; found 379.1767.

*4.15.20 isopropyl(S)-3-(1H-imidazol-1-yl)-2-(5-(p-tolyl)isoxazole-3-carboxamido)propanoate(**23a**)*

Light white solid; yield: 72.7%; mp: 168.4-170.5 °C. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 9.28 (d, *J* = 8.1 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.61 (s, 1H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.28 (s, 1H), 7.20 (s, 1H), 6.84 (s, 1H), 4.96 (dt, *J* = 12.5, 6.2 Hz, 1H), 4.83 (td, *J* = 9.4, 5.0 Hz, 1H), 4.52 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.40 (dd, *J* = 14.0, 9.7 Hz, 1H), 2.38 (s, 3H), 1.20 (d, *J* = 1.4 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 170.85, 168.46, 158.81, 158.74, 140.94, 137.80, 129.87(2C),

128.37, 125.79(2C), 123.53, 119.83, 99.23, 68.93, 53.41, 45.57, 21.45, 21.42, 21.04. HRMS calcd for $C_{20}H_{22}N_4O_4$, $[M + Na]^+$, 405.1539; found 409.1537.

*4.15.21 isobutyl(S)-3-(1H-imidazol-1-yl)-2-(5-(p-tolyl)isoxazole-3-carboxamido)propanoate(**23b**)*

Light white solid; yield: 69.5%; mp: 112.7-114.9 °C. $^1$H NMR (400 MHz, DMSO) δ 9.34 (d, *J* = 8.3 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.63 (s, 1H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.28 (s, 1H), 7.21 (s, 1H), 6.85 (s, 1H), 4.92 (td, *J* = 9.8, 4.8 Hz, 1H), 4.55 (dd, *J* = 14.0, 4.7 Hz, 1H), 4.43 (dd, *J* = 14.0, 10.0 Hz, 1H), 3.97 – 3.86 (m, 2H), 2.38 (s, 3H), 1.88 (dp, *J* = 13.3, 6.6 Hz, 1H), 0.88 (d, *J* = 6.7 Hz, 6H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.34, 169.36, 159.25, 141.42, 138.21, 130.34(2C), 128.57, 126.25(2C), 123.98, 120.36, 99.66, 71.27, 53.70, 46.02, 27.70, 21.50, 19.24, 19.19. HRMS calcd for $C_{21}H_{25}N_4O_4$, $[M + H]^+$, 397.1876; found 397.1875.

*4.15.22 isopropyl(S)-2-(5-(4-fluorophenyl)isoxazole-3-carboxamido)-3-(1H-imidazol-1-yl)propan oate(**23c**)*

Light white solid; yield: 64.9%; mp:196.2-199.3 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.31 (d, *J* = 8.1 Hz, 1H), 8.01 (dd, *J* = 8.7, 5.4 Hz, 2H), 7.61 (s, 1H), 7.42 (t, *J* = 8.8 Hz, 2H), 7.36 (s, 1H), 7.20 (s, 1H), 6.85 (s, 1H), 4.96 (dt, *J* = 12.5, 6.2 Hz, 1H), 4.83 (td, *J* = 9.1, 5.1 Hz, 1H), 4.52 (dd, *J* = 14.1, 5.0 Hz, 1H), 4.40 (dd, *J* = 14.0, 9.7 Hz, 1H), 1.20 (dd, *J* = 6.0, 4.6 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.78, 168.44, 164.67(0.5C), 162.19(0.5C), 158.91, 158.64, 137.81, 128.50, 128.41, 128.38, 122.93(0.5C), 122.91(0.5C), 119.82, 116.61, 116.39, 99.85, 68.94, 53.42, 45.56, 21.44, 21.41. HRMS calcd for $C_{19}H_{19}FN_4O_4$, $[M + H]^+$, 409.1288; found 409.1287.

*4.15.23 isobutyl(S)-2-(5-(4-fluorophenyl)isoxazole-3-carboxamido)-3-(1H-imidazol-1-yl)propano ate(**23d**)*

Light white solid; yield: 72.4%; mp: 136.2-138.0 °C. $^1$H NMR (400 MHz, DMSO) δ 9.36 (d, *J* = 8.3 Hz, 1H), 8.02 (dd, *J* = 8.8, 5.4 Hz, 2H), 7.63 (s, 1H), 7.43 (d, *J* = 8.9 Hz, 2H), 7.36 (s, 1H), 7.21 (s, 1H), 6.85 (s, 1H), 4.93 (td, *J* = 9.9, 4.8 Hz, 1H), 4.56 (dd, *J* = 14.0, 4.7 Hz, 1H), 4.44 (dd, *J* = 14.0, 10.1 Hz, 1H), 3.92 (dd, *J* = 6.5, 2.8 Hz, 2H), 1.87 (td, *J* = 13.3, 6.6 Hz, 1H), 0.88 (d, *J* = 6.7 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.83, 168.91, 164.69(0.5C), 162.22(0.5C), 158.92, 158.71, 137.78, 128.53, 128.44, 128.20, 122.93(0.5C), 122.90(0.5C), 119.89, 116.64, 116.42, 99.84, 70.83, 53.27, 45.55, 27.25, 18.78, 18.74. HRMS calcd for $C_{20}H_{22}FN_4O_4$, $[M + H]^+$, 401.1625; found 401.1619.

*4.15.24 isopropyl(S)-2-(5-(4-bromophenyl)isoxazole-3-carboxamido)-3-(1H-imidazol-1-yl)propan*

*oate(**23e**)*

Light white solid; yield: 67.3%; mp: 135.0-136.8 °C. $^{1}$H NMR (400 MHz, DMSO) δ 9.33 (d, *J* = 8.1 Hz, 1H), 7.90 (d, *J* = 8.6 Hz, 2H), 7.78 (d, *J* = 8.6 Hz, 2H), 7.63 (s, 1H), 7.43 (s, 1H), 7.21 (s, 1H), 6.85 (s, 1H), 4.96 (dt, *J* = 12.5, 6.2 Hz, 1H), 4.88 – 4.79 (m, 1H), 4.52 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.41 (dd, *J* = 14.0, 9.7 Hz, 1H), 1.20 (dd, *J* = 6.1, 4.3 Hz, 6H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.66, 168.41, 158.94, 158.56, 137.79, 132.36(2C), 128.30, 127.81(2C), 125.35, 124.45, 119.85, 100.51, 68.95, 53.42, 45.58, 21.44, 21.41. HRMS calcd for $C_{19}H_{20}BrN_4O_4$, $[M + H]^+$, 447.0668; found 447.0679.

*4.15.25 isobutyl(S)-2-(5-(4-bromophenyl)isoxazole-3-carboxamido)-3-(1H-imidazol-1-yl)propano*

*ate(**23f**)*

Light white solid; yield: 64.5%; mp: 123.7-126.6 °C. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 9.39 (d, *J* = 8.3 Hz, 1H), 7.90 (d, *J* = 8.6 Hz, 2H), 7.77 (d, *J* = 8.5 Hz, 2H), 7.63 (s, 1H), 7.43 (s, 1H), 7.21 (s, 1H), 6.86 (s, 1H), 4.93 (td, *J* = 9.8, 4.7 Hz, 1H), 4.56 (dd, *J* = 14.0, 4.7 Hz, 1H), 4.44 (dd, *J* = 14.0, 10.1 Hz, 1H), 3.97 – 3.87 (m, 2H), 1.93 – 1.83 (m, 1H), 0.88 (d, *J* = 6.7 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.72, 168.87, 158.95, 158.63, 137.73, 132.40(2C), 127.94(2C), 125.34, 124.49, 120.01, 100.51, 70.85, 53.24, 45.65, 27.25, 18.75. HRMS calcd for $C_{20}H_{22}BrN_4O_4$, $[M + H]^+$, 461.0824; found 461.0831.

*4.15.26 isopropyl(S)-2-(5-(2-fluorophenyl)isoxazole-3-carboxamido)-3-(1H-imidazol-1-yl)propan*

*oate(**23g**)*

Light white solid; yield: 59.9%; mp: 151.3-154.0 °C. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 9.45 (d, *J* = 8.1 Hz, 1H), 8.00 (td, *J* = 7.7, 1.5 Hz, 1H), 7.69 – 7.60 (m, 2H), 7.46 (ddd, *J* = 16.1, 9.7, 4.7 Hz, 2H), 7.23 (s, 1H), 7.20 (d, *J* = 2.9 Hz, 1H), 6.86 (s, 1H), 4.96 (dt, *J* = 12.5, 6.2 Hz, 1H), 4.89 – 4.81 (m, 1H), 4.53 (dd, *J* = 14.1, 5.0 Hz, 1H), 4.43 (dd, *J* = 14.0, 9.7 Hz, 1H), 1.20 (dd, *J* = 6.1, 4.5 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 168.41, 165.07, 158.77, 158.39, 157.22, 137.83, 133.15, 128.27, 127.95, 125.47, 119.88, 116.63, 114.23, 102.74, 68.96, 53.48, 45.54, 21.45, 21.41. HRMS calcd for $C_{19}H_{20}FN_4O_4$, $[M + H]^+$, 387.1469; found 387.1463.

*4.15.27 isobutyl(S)-2-(5-(2-fluorophenyl)isoxazole-3-carboxamido)-3-(1H-imidazol-1-yl)propano*

*ate(**23h**)*

Light white solid; yield: 64.7%; mp: 85.5-87.9 °C. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 9.43 (d, *J* = 8.2 Hz, 1H), 8.00 (td, *J* = 7.8, 1.5 Hz, 1H), 7.64 (d, *J* = 11.0 Hz, 2H), 7.52 – 7.39 (m, 2H), 7.23

(s, 1H), 7.14 (d, *J* = 2.9 Hz, 1H), 6.87 (s, 1H), 4.93 (td, *J* = 9.8, 4.8 Hz, 1H), 4.56 (dd, *J* = 14.0, 4.7 Hz, 1H), 4.44 (dd, *J* = 14.0, 10.1 Hz, 1H), 4.03 – 3.83 (m, 2H), 1.92 – 1.85 (m, 1H), 0.88 (d, *J* = 6.7 Hz, 6H).$^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 168.85, 165.16, 158.75, 158.44, 157.23, 137.76, 133.27, 133.19, 128.11, 127.96, 125.44, 119.91, 116.84, 116.64, 114.21, 102.67, 70.84, 53.30, 45.55, 27.23, 18.72. HRMS calcd for $C_{20}H_{21}FN_4O_4$, $[M + Na]^+$, 423.1445; found 423.1447.

4.16 *In vitro antifungal testing*

The in vitro minimum inhibitory concentrations (MIC) were determined by serial dilution in 96-well microtiter plates based on the standard guidelines described by the National Committee for Clinical Laboratory Standards (NCCLS). The MIC values were defined as the lowest concentrations of an antimicrobial that would inhibit the visible growth of the fungi. FLC and ITR were purchased for use as positive control drugs. All of the compounds were dissolved in DMSO and serially diluted into the growth medium.

*4.17 GC-MS analysis of sterol composition*

GC-MS was performed with an Agilent Technologies (AT) 6890N Network GC system equipped with an AT 5975 quadrupole mass selector detector using He as the carrier gas. The sterols were extracted from *C. albicans* and analysed by GC-MS. The GC-MS data were analysed using Agilent software (Agilent MSD productivity ChemStation for GC and GC/MS systems data analysis application) and matched to known MS data using the NIST Spectrum Database (NIST MS search 2.0).

4.18 *Cytochrome P450 Inhibition Assay*

Cytochrome P450 inhibition was evaluated in human liver microsomes (0.25 mg/mL) using five specific probe substrates (CYP1A2, 10 μM phenacetin; CYP2C9, 5 μM diclofenac; CYP2C19, 30 μM S-mephenytoin; CYP2D6, 5 μM dextromethorphan; and CYP3A4, 2 μM midazolam) in the presence of multiple concentrations of the test compound (0.05-50 μM ). After pre-incubation at 37°C for 10 min, the reaction was initiated with by additing 20 μL NADPH to a final concentration of 10 mM. The mixture were incubated at 37°C for 10 min and the reaction was terminated by additing 400 μL cold stop solution (200 ng/mL tolbutamide and 200 ng/mL labetalol in acetonitrile). After the reactions were terminated, the plates were centrifuged, and the supernatants were analysed by LC/MS/MS.

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## References

[1] M. Victoria Castelli, E. Butassi, M. Candida Monteiro, L.A. Svetaz, F. Vicente, S.A. Zacchino, Novel antifungal agents: a patent review (2011-present), Expert Opin. Ther. Pat., 24 (2014) 323-338.

[2] G.D. Brown, D.W. Denning, N.A.R. Gow, S.M. Levitz, M.G. Netea, T.C. White, Hidden Killers: Human Fungal Infections, Sci. Transl. Med., 4 (2012) 1-9.

[3] D.W. Denning, W.W. Hope, Therapy for fungal diseases: opportunities and priorities, Trends Microbiol., 18 (2010) 195-204.

[4] M. Richardson, C. Lass-Flörl, Changing epidemiology of systemic fungal infections, Clin. Microbiol. Infect., 14 (2008) 5-24.

[5] D.W. Denning, Echinocandin antifungal drugs, Lancet, 362 (2003) 1142-1151.

[6] D. Allen, D. Wilson, R. Drew, J. Perfect, Azole antifungals: 35 years of invasive fungal infection management, Expert review of anti-infective therapy, 13 (2015) 787.

[7] V. Moudgal, J. Sobel, Antifungals to treat Candida albicans, Expert Opin. Pharmacother., 11 (2010) 2037-2048.

[8] C. Onyewu, J.R. Blankenship, P.M. Del, J. Heitman, Ergosterol biosynthesis inhibitors become fungicidal when combined with calcineurin inhibitors against Candida albicans, Candida glabrata, and Candida krusei, Antimicrob. Agents Chemother., 47 (2003) 956-964.

[9] H. Hof, A new, broad-spectrum azole antifungal: posaconazole--mechanisms of action and resistance, spectrum of activity, Mycoses, 49 (2006) 2-6.

[10] B.C. Monk, T.M. Tomasiak, M.V. Keniya, F.U. Huschmann, J.D.A. Tyndall, J.D. O'Connell, III, R.D. Cannon, J.G. McDonald, A. Rodriguez, J.S. Finer-Moore, R.M. Stroud, Architecture of a single membrane spanning cytochrome P450 suggests constraints that orient the catalytic domain relative to a bilayer, Proc. Natl. Acad. Sci. U. S. A., 111 (2014) 3865-3870.

[11] J. Heeres, L. Meerpoel, P. Lewi, Conazoles, Molecules, 15 (2010) 4129-4188.

[12] G. Rodríguez, Duque, Castellsague, Pérez-Gutthann, Stricker, A cohort study on the risk of acute liver injury among users of ketoconazole and other antifungal drugs, Br. J. Clin. Pharmacol., 48 (1999) 847.

[13] S. F, P. R, M. PA, M. CA, N. CC, H. R, L. D, B. TA, M. PM, In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts, Antimicrob. Agents Chemother., 50 (2006) 2009-2015.

[14] S. Zhao, L. Zhao, X. Zhang, C. Liu, C. Hao, H. Xie, B. Sun, D. Zhao, M. Cheng, Design, synthesis, and structure-activity relationship studies of benzothiazole derivatives as antifungal agents, Eur. J. Med. Chem., 123 (2016) 514-522.

[15] D. Zhao, S. Zhao, L. Zhao, X. Zhang, P. Wei, C. Liu, C. Hao, B. Sun, X. Su, M. Cheng, Discovery of biphenyl imidazole derivatives as potent antifungal agents: Design, synthesis, and structure-activity

relationship studies, Bioorg. Med. Chem., 25 (2016) 750-758.

[16] T.Y. Hargrove, Z. Wawrzak, D.C. Lamb, F.P. Guengerich, G.I. Lepesheva, Structure-Functional Characterization of Cytochrome P450 Sterol 14α-Demethylase (CYP51B) from Aspergillus fumigatus and Molecular Basis for the Development of Antifungal Drugs, J. Biol. Chem., 290 (2015) 23916.

[17] Z. Jiang, J. Gu, C. Wang, S. Wang, N. Liu, Y. Jiang, G. Dong, Y. Wang, Y. Liu, J. Yao, Design, synthesis and antifungal activity of novel triazole derivatives containing substituted 1,2,3-triazole-piperdine side chains, Eur. J. Med. Chem., 82 (2014) 490-497.

[18] R.-m. Liang, Y.-b. Cao, K.-h. Fan, Y. Xu, P.-h. Gao, Y.-j. Zhou, B.-d. Dai, Y.-h. Tan, S.-h. Wang, H. Tang, H.-t. Liu, Y.-y. Jiang, 2-Amino-nonyl-6-methoxyl-tetralin muriate inhibits sterol C-14 reductase in the ergosterol biosynthetic pathway, Acta Pharmacologica Sinica, 30 (2009) 1709-1716.

[19] A.G. Warrilow, C.M. Hull, J.E. Parker, E.P. Garvey, W.J. Hoekstra, W.R. Moore, R.J. Schotzinger, D.E. Kelly, S.L. Kelly, The clinical candidate VT-1161 is a highly potent inhibitor of Candida albicans CYP51 but fails to bind the human enzyme, Antimicrob. Agents Chemother., 58 (2014) 7121.

[20] I.J. Sud, D.S. Feingold, Mechanisms of action of the antimycotic imidazoles, The Journal of investigative dermatology, 76 (1981) 438-441.

[21] S.Z. Wang, Y. Wang, W. Liu, N. Liu, Y.Q. Zhang, G.Q. Dong, Y. Liu, Z.G. Li, X.M. He, Z.Y. Miao, J.Z. Yao, J. Li, W.N. Zhang, C.Q. Sheng, Novel Carboline Derivatives as Potent Antifungal Lead Compounds: Design, Synthesis, and Biological Evaluation, ACS Med. Chem. Lett., 5 (2014) 506-511.

[22] X. Jia, Y. Wang, Y. Jia, P. Gao, Y. Xu, L. Wang, Y. Cao, Y. Cao, L. Zhang, Y. Jiang, RTA2 is involved in calcineurin-mediated azole resistance and sphingoid long-chain base release in Candida albicans, Cell. Mol. Life Sci., 66 (2009) 122-134.

[23] K.S. Barker, S. Crisp, N. Wiederhold, R.E. Lewis, B. Bareither, J. Eckstein, R. Barbuch, M. Bard, P.D. Rogers, Genome-wide expression profiling reveals genes associated with amphotericin B and fluconazole resistance in experimentally induced antifungal resistant isolates of Candida albicans, J. Antimicrob. Chemother., 54 (2004) 376-385.

[24] D.W. Bates, D.T. Yu, Clinical impact of drug-drug interactions with systemic azole antifungals, Drugs of Today, 39 (2003) 801-813.

[25] T. Niwa, T. Shiraga, A. Takagi, Effect of antifungal drugs on cytochrome P450 (CYP) 2C9, CYP2C19, and CYP3A4 activities in human liver microsomes, Biol. Pharm. Bull., 28 (2005) 1805-1808.

# Highlights

- 27 new compounds with aromatic heterocyclic scaffolds were designed and synthesised.
- The isoxazole scaffolds were more suited for improving activity against *Aspergillus spp*.
- Compounds **23g** and **23h** showed better antifungal activity than fluconazole.
- Compound **23g** reduced the content of ergosterol in a dose-dependent manner.
- Compounds **23g** exhibited low inhibition profiles for human cytochrome P450 isoforms.