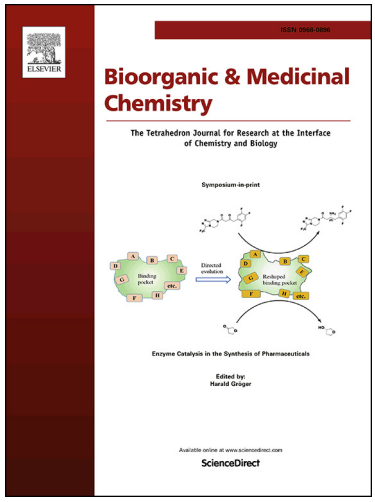


Design, synthesis and evaluation of benzoheterocycleanalogues as potent anti-fungal agents targeting CYP51

Shizhen Zhao, Peng Wei, Mengya Wu, Xiangqian Zhang, Liyu Zhao, Xiaolin Jiang, Chenzhou Hao, Xin Su, Dongmei Zhao, Maosheng Cheng

PII: S0968-0896(18)30132-9
DOI: https://doi.org/10.1016/j.bmc.2018.04.054
Reference: BMC 14337

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 22 January 2018
Revised Date: 25 April 2018
Accepted Date: 26 April 2018

Please cite this article as: Zhao, S., Wei, P., Wu, M., Zhang, X., Zhao, L., Jiang, X., Hao, C., Su, X., Zhao, D., Cheng, M., Design, synthesis and evaluation of benzoheterocycleanalogues as potent antifungal agents targeting CYP51, *Bioorganic & Medicinal Chemistry* (2018), doi: https://doi.org/10.1016/j.bmc.2018.04.054

Design, synthesis and evaluation of benzoheterocycle analogues as potent antifungal agents targeting CYP51

Shizhen Zhao[a], Peng Wei[a], Mengya Wu[a], Xiangqian Zhang[a], Liyu Zhao[a], Xiaolin Jiang[a], Chenzhou Hao[a], Xin Su[b], Dongmei Zhao[a,*], Maosheng Cheng[a]

[a]*Key Laboratory of Structure-Based Drug Design and Discovery, Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe District, Shenyang 110016, PR China*

[b]*The School of life Science and Biopharmaceutical, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe District, Shenyang, 110016, PR China*

[*]Corresponding author: Dongmei Zhao, E-mail: medchemzhao@163.com

ABSTRACT:

To further enhance the anti-*Aspergillus* efficacy of our previously discovered antifungal lead compound **1**, a series of benzoheterocycle analogues were designed, synthesized and evaluated for their *in vitro* antifungal activity. The most promising compounds **13s** and **14a** exhibited excellent antifungal activity against *C.albicans*, *C.neoformans*, *A.fumigatus* and fluconazole-resistant *C.albicans* strains, that was superior or comparable to those of the reference drugs fluconazole and voriconazole. GC-MS analyses suggested that the novel compound **13s** might have a similar mechanism to fluconazole by inhibiting fungal lanosterol 14α-demethylase (CYP51). Furthermore, compounds **13s** and **14a** exhibited low inhibition profiles for various human cytochrome P450 isoforms as well as excellent blood plasma stability.

Keywords:

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

1. Introduction

Fungal infections have been increasing dramatically and are currently estimated to directly affect approximately 1.2 billion people globally[1]. The incidence of invasive fungal infections (IFIs) and the emergence of resistant fungal pathogens have increased markedly, leading to high morbidity and mortality in immunocompromised patients, such as patients receiving organ

transplants, patients undergoing anticancer chemotherapy and patients with AIDS[2, 3]. Clinically, the three fungal genera *Aspergillus*, *Candida*, and *Cryptococcus* account for most fungal infections[4]. The common antifungal agents currently used in the clinic can be divided into four different classes based on their mode of action: polyenes (e.g., amphotericin B and nystatin)[5], echinocandins (e.g., caspofungin and micafungin)[6], azoles(e.g., fluconazole, voriconazole and itraconazole)[7], and antimetabolites (e.g., 5-fluorocytosine)[8]. Among these agents, azoles are most widely used as first-line antifungal therapy.

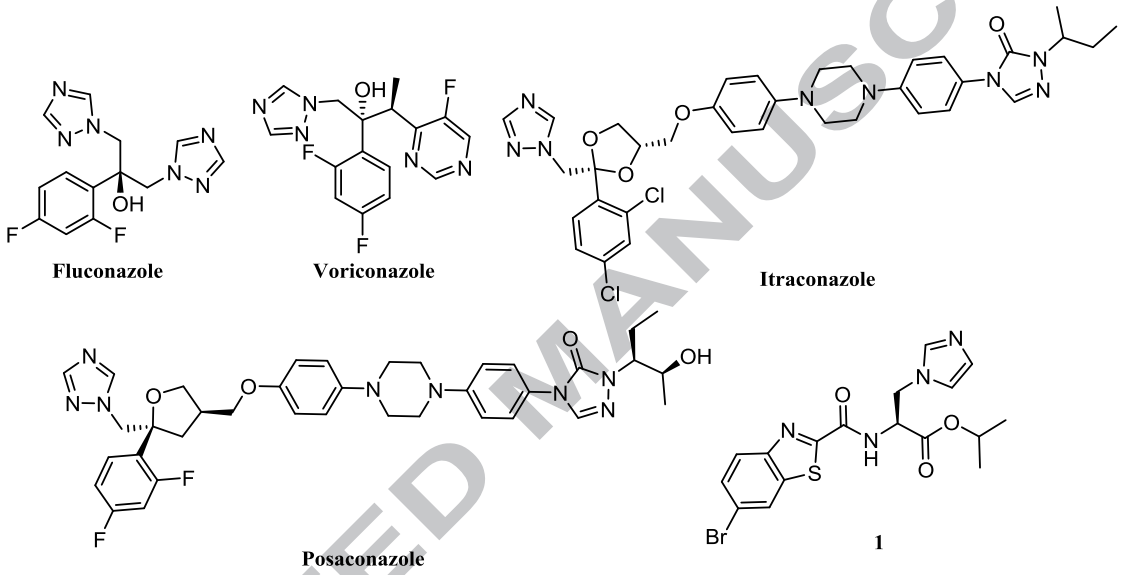


Figure 1. Chemical structures of azole antifungal agents and lead compound.

Azole antifungal agents inhibit fungal lanosterol 14α-demethylase (CYP51), which is involved in the biosynthesis of ergosterol, a significant cellular membrane component[9]. The inhibition of CYP51 would cause a reduction of the endogenous concentrations of ergosterol and an accumulation of lanosterol and other 14-methyl sterols, thus inhibiting the growth of fungal cells[10]. Azole antifungal drugs, such as fluconazole, itraconazole, voriconazole and posaconazole, have had a significant impact on the treatment of systemic fungal infections[11, 12]. However, many of the marketed azole drugs are limited in practical applications, due to their drug resistance, narrow antifungal spectrum, and low bioavailability[13, 14]. Therefore, there is still an urgent need for developing novel azole antifungal agents with potent activity, broad spectrum, low toxicity, and low resistance.

In our previous studies, a new series of benzothiazole derivatives were designed, synthesized and evaluated for their in vitro antifungal activity[15, 16]. Most of the compounds showed excellent

antifungal activity against *Candida albicans* and *Cryptococcus neoformans*, with MIC values in the range of 0.25 μg/mL to 2 μg/mL. Unfortunately, almost all of these target compounds were inactive against *Aspergillus fumigatus*. Therefore, this prompted us to continue studying the structural modification of our potent original compounds in search of novel compounds with improved anti-*Aspergillus fumigatus* efficacy.

The structure of the voriconazole-*A. fumigatus* CYP51B complex (PDB ID:4UYM) (Figure 2A) suggests that hydrogen bonds formed between the 5-fluoropyrimidine ring of voriconazole and *A. fumigatus* CYP51 Tyr122. This could explain why voriconazole has much higher antifungal potency against *Aspergillus fumigatus* than fluconazole does [17].

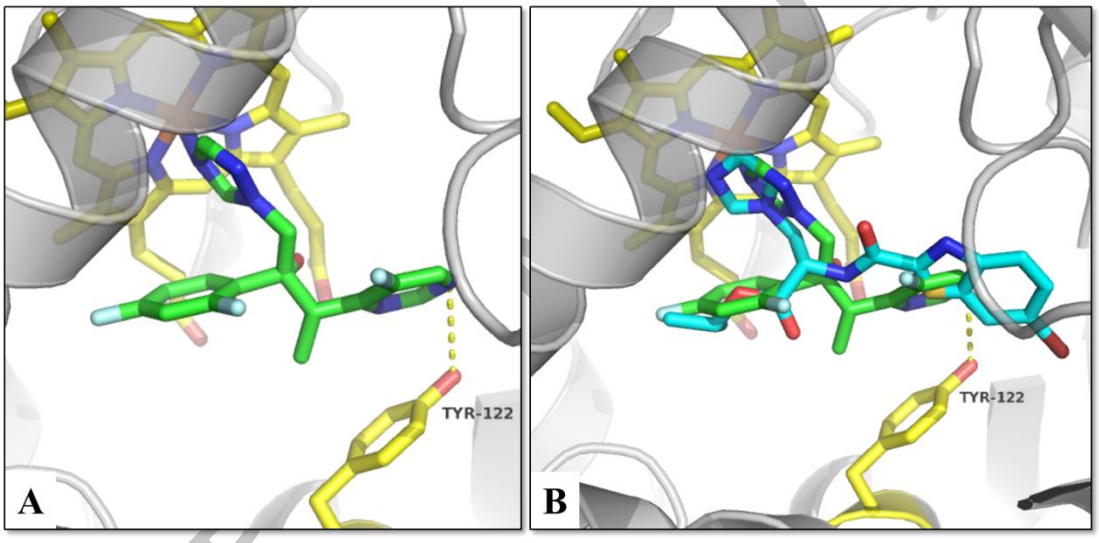


Figure 2. Crystal structure of sterol 14-alpha demethylase (CYP51B) from a pathogenic filamentous fungus *Aspergillus fumigatus* in complex with voriconazole (A and B) and the binding mode of compound **1** (B) in the active site.

To further explore the potent and broad antifungal spectrum of imidazole derivatives, 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, the benzothiazole ring was replaced by benzoheterocycles to facilitate the formation of hydrogen bond interactions with Tyr122 of CYP51. This might result in the development of more potent compounds with higher antifungal activities and a broader antifungal spectrum, especially with improved anti-*Aspergillus* efficacy.

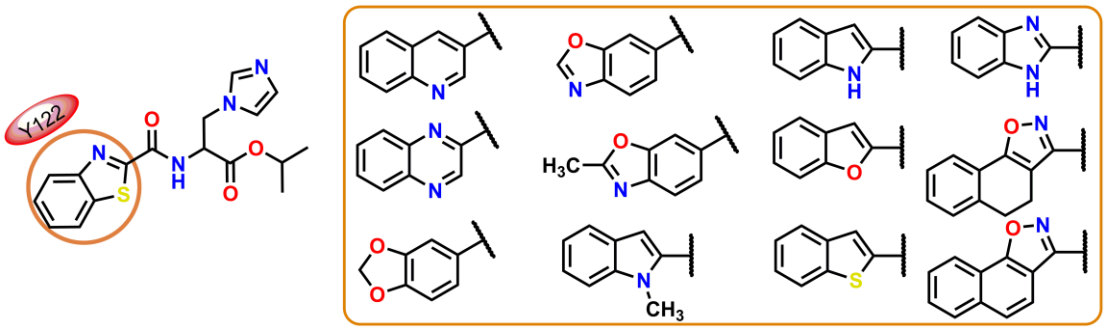
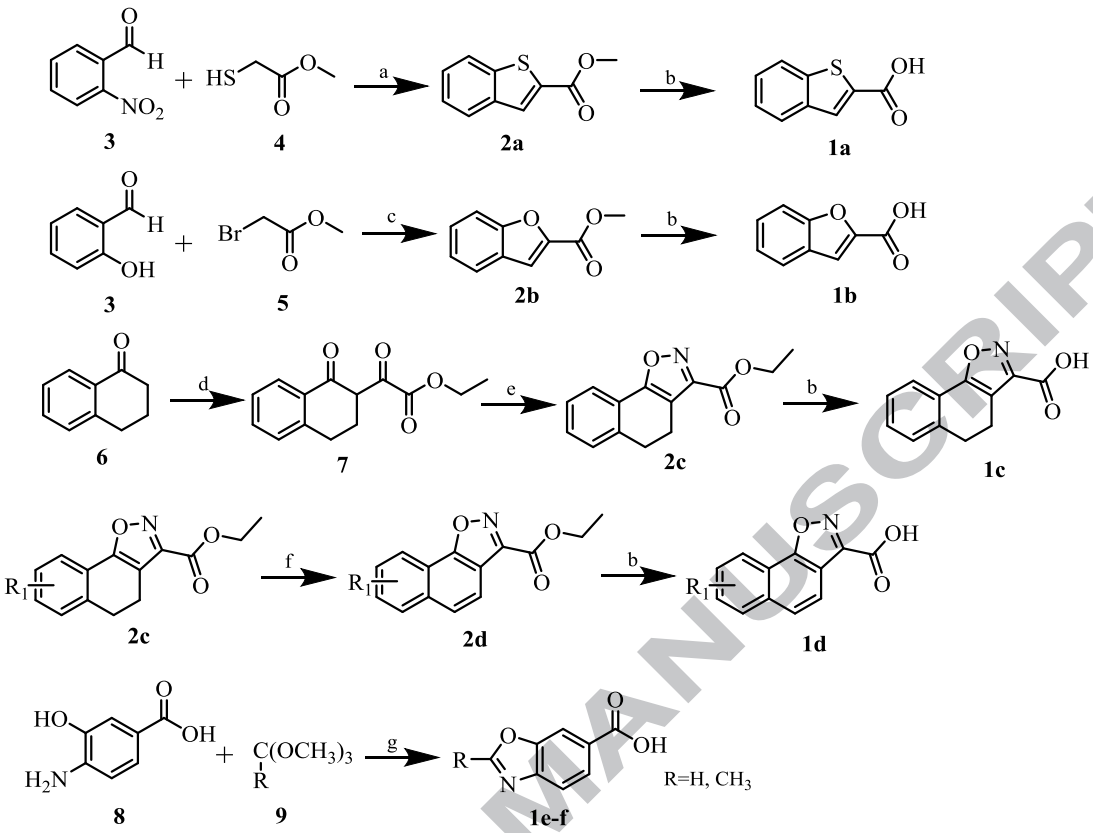


Figure 3. Our strategy to facilitate the formation of hydrogen bond interactions with CYP51B Tyr122 of *A. fumigatus* by replacing the benzothiazole ring with 12 benzoheterocycles.

2. Results and discussion

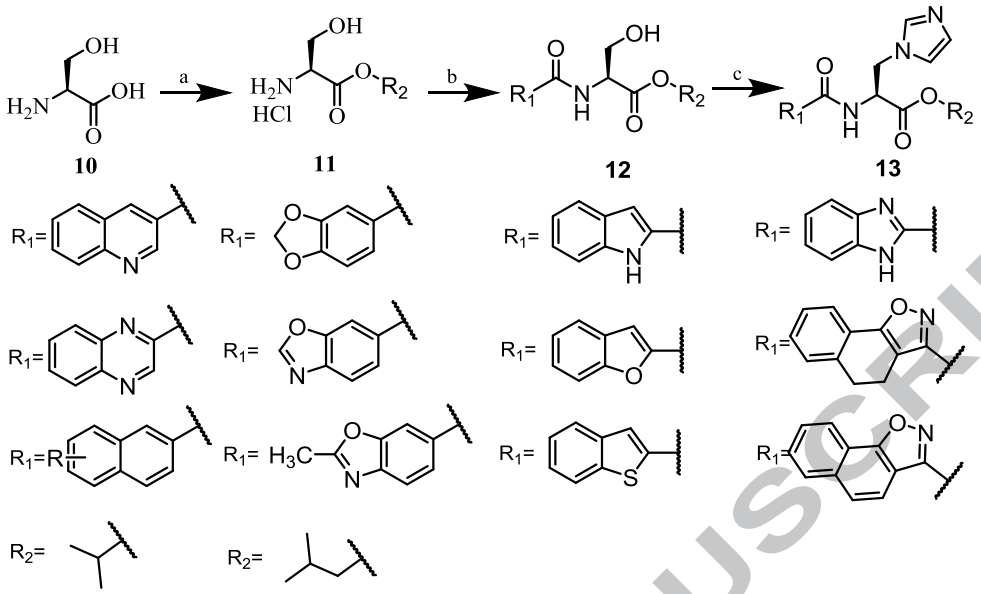
2.1 Chemistry

The synthetic routes of the key intermediates **1a-f** are shown in Scheme 1. The benzothiophene **2a** was obtained from the intermolecular cyclization between 2-nitrobenzaldehyde and methyl 2-mercaptoacetate. The salicylaldehyde was treated with methyl bromoacetate in the presence of K_2CO_3, and cyclization afforded the benzofuran **2b**. 1-tetralone **6** was treated with diethyl oxalate in the presence of LiHMDS by a Claisen condensation to afford intermediate **7**, which was then subjected to a ring-closure reaction with hydroxylamine hydrochloride to form isoxazole **2c**. The intermediate **2d** was obtained by DDQ dehydrogenation reaction from **2c**. The 4-amino-3-hydroxybenzoic acid was condensed with the substituted methyl orthoformate **9** to form the benzo[*d*]oxazole acids **1e-f**. Finally, intermediates **2a-d** were saponified with 2 N NaOH to obtain the key intermediates **1a-d**.



Scheme 1. Synthesis of intermediates **1a-f**. Reagents and conditions: (a) K_2CO_3, DMF; (b) NaOH, MeOH/H_2O, r.t., 7 h; (c) K_2CO_3, DMF, 80 °C, 7 h; (d) Diethyl oxalate, LiHMDS; (e) Hydroxylamine hydrochloride, EtOH, reflux, 2 h; (f) DDQ, 1,4-dioxane; (g) 80 °C, 2 h.

The syntheses of target compounds **13a-v** were carried out according to our previously reported procedure, as shown in Scheme 2. L-serine **10** was dissolved in alcohol (isopropanol or isobutanol) and refluxed with $SOCl_2$ to afford the serine esters **11a-b**. The serine esters **11a-b** were then treated with intermediates **1a-j** in the presence of a condensing reagent to give compounds **12a-u**. Finally, the target compounds **13a-u** were successfully obtained by introducing the imidazole group using CDI/imidazole.



Scheme 2. Reagents and conditions: (a) isopropanol or isobutanol, $SOCl_2$, reflux, 1–2 h; (b) EDCI, HOBt, DIEA, r.t., 7 h; (c) CDI, imidazole, CH_3CN, reflux, 7 h.

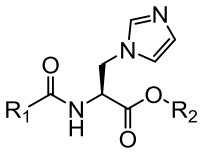
2.2 In vitro antifungal activity

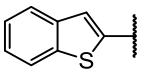
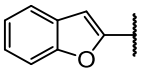
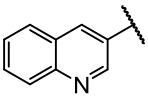
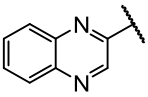
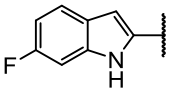
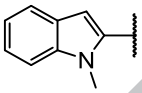
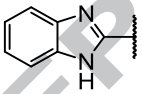
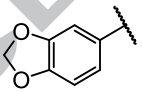
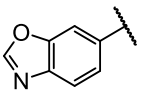
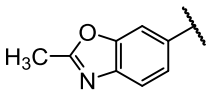
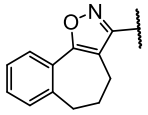
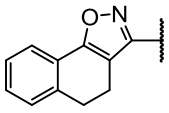
The *in vitro* minimum inhibitory concentration (MIC) of all target compounds was determined using the method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) and the serial dilution method in 96-well microtest plates[18, 19]. All of the target compounds were evaluated for their antifungal activity against five important fungal pathogens. Fluconazole (FLC) and itraconazole (ITR) were used as reference drugs. The *in vitro* antifungal activity results are summarized in Table 1.

As shown in Table 1, the introduction of different benzoheterocycle rings had a notable influence on the antifungal activity. Many of these modifications showed a decrease in antifungal activity compared with the lead compound **1**. Interestingly, the benzoheterocycle compounds such as benzothiophene (**13a-b**), benzofuran (**13c-d**), *N*-methylindole (**13j-k**), and 4,5-dihydronaphtho[2,1-*d*]isoxazole (**13s-t**) exhibited moderate to good antifungal properties with a broad spectrum of activity. Among these, 4,5-dihydronaphtho[2,1-*d*] isoxazole (**13s-t**) showed the most potent activity against *all of the tested strains*, with the exception of *Aspergillus fumigatus*, and was superior or comparable to those of the reference drugs FLC and ITR. When the middle six-membered ring of **13s** was changed to the seven-membered ring (**13q**) showed decreased antifungal activity.

Table 1

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.*
13a		$-CH(CH_3)_2$	1	2	4	0.25	8
13b		$-CH_2CH(CH_3)_2$	0.5	1	1	0.25	8
13c		$-CH(CH_3)_2$	16	32	4	0.5	32
13d		$-CH_2CH(CH_3)_2$	8	8	4	0.25	32
13e		$-CH(CH_3)_2$	64	64	64	32	64
13f		$-CH_2CH(CH_3)_2$	64	64	64	32	64
13g		$-CH(CH_3)_2$	64	64	64	16	64
13h		$-CH_2CH(CH_3)_2$	64	64	64	16	64
13i		$-CH(CH_3)_2$	32	32	8	0.5	64
13j		$-CH(CH_3)_2$	8	8	64	0.5	64
13k		$-CH_2CH(CH_3)_2$	8	8	4	1	64
13l		$-CH(CH_3)_2$	64	64	64	64	64
13m		$-CH(CH_3)_2$	64	64	64	32	64
13n		$-CH_2CH(CH_3)_2$	64	64	64	16	64
13o		$-CH(CH_3)_2$	64	64	64	32	64
13p		$-CH(CH_3)_2$	64	64	64	64	64
13q		$-CH(CH_3)_2$	16	2	64	1	64
13s		$-CH(CH_3)_2$	0.25	0.25	8	0.125	8
13t		$-CH_2CH(CH_3)_2$	0.125	0.5	4	0.125	8

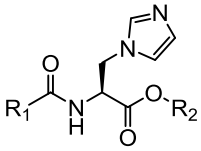
1			0.125	0.5	2	1	>64
FCZ	-	-	0.5	1	4	1	>64
ITZ			0.125	0.5	1	0.5	4
VRC			0.125	0.25	0.5	0.125	2

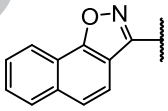
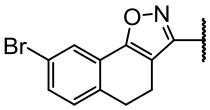
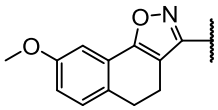
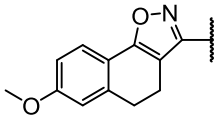
[a]Abbreviations: *C.alb.*(Ⅰ), *Candida albicans* (ATCC SC5314); *C.alb.*(II), *Candida albicans* (CPCC400523); *C. neo.*, *Cryptococcus neoformans* (cgmcc 2.3161); *A.fum.*, *Aspergillus fumigatus* (cgmcc 3.7795); *C.tro.*, *Candida tropicalis* (cgmcc 2.3739); FCZ: Fluconazole; ITZ: Itraconazole, VRC: Voriconazole.

Based on the results above, we selected a scaffold of 4,5-dihydronaphtho[2,1-*d*]isoxazole core (**13s-t**) as our starting point for further modification. When substituents were introduced at the 7 or 8 position of the 4,5-dihydronaphtho[2,1-*d*] isoxazole scaffold , the resulting compounds (**14c-g**) showed decreased *in vitro* antifungal activity. Especially, Compound **14c** with -Br groups at the 8-position showed no antifungal activity. Notably, the 4,5-dihydronaphtho[2,1-*d*] isoxazole nucleus, when replaced with a naphtho[2,1-*d*] isoxazole nucleus, led to improved antifungal activity in compounds **14a-b**. Of these, compound **14a** showed the best antifungal activity.

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.***
14a		-$CH(CH_3)_2$	0.125	0.25	0.25	0.125	4
14b		-$CH_2CH(CH_3)_2$	0.125	0.25	0.125	0.125	16
14c		-$CH(CH_3)_2$	64	64	64	64	64
14d		-$CH(CH_3)_2$	0.25	16	64	2	64
14e		-$CH_2CH(CH_3)_2$	0.25	32	64	4	64
14f		-$CH(CH_3)_2$	0.25	4	64	8	64
14g		-$CH_2CH(CH_3)_2$	0.25	64	64	32	64
FCZ	-	-	0.5	1	4	1	>64

ITZ	-	-	0.125	0.5	1	0.5	4
VRC			0.125	0.25	0.5	0.125	2

[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* (cgmcc 3.7795); *C.tro.*, *Candida tropicalis* (cgmcc 2.3739); FCZ: Fluconazole; ITZ: Itraconazole, VRC: Voriconazole.

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

The wide use of fluconazole has greatly increased the fluconazole resistance of *C. alb.* strains, which has become a major clinical problem for treating fungal infections. Therefore, there is an urgent need to find a new type of inhibitor that is effective against fluconazole-resistant strains of *C. alb*. Based on the results of the *in vitro* antifungal activity assays, the most potent compounds **13s-t** and **14a-b** were further evaluated against fluconazole-resistant strains of *C. alb*. (*strain 100* and *strain 103*). As shown in Table 3, compounds **13s-t** and **14a-b** displayed strong antifungal activity against *strain 100* and *strain 103*, with MIC values in the range from 0.5 to 2 μg/mL.

Table 3

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

Compd.	***C. alb.***	
	Strain100	***Strain103***
13s	2	0.5
13t	1	0.5
14a	2	1
14b	2	2
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 GC-MS analysis of sterol composition

To confirm the antifungal mechanism of compound **13s**, we analysed the sterol composition of *C. albicans* (ATCC SC5314) cells by using GC-MS methods. GC-MS methods have been successfully used to reflect the effect on *C. albicans* membrane and elucidate the mechanism of antifungal agent[16, 20-22]. FLC was used as a reference drug and cholesterol was added as an internal

standard. The GC-MS analysis results are shown in Table 4 and Figure 4. The untreated cell sterol fraction contained 98.7% ergosterol, whereas lanosterol was not observed, and other 14-methylated sterols (obtusifoliol and eburicol) were found at only 1.0%. When *C. albicans* was treated with **13s** and FLC at 0.03125-8 μg/mL for 16 h, they acted in a dose-dependent fashion to decrease the ergosterol content and increase the lanosterol and eburicol contents. The decrease of ergosterol and the concomitant accumulation of lanosterol and eburicol provide indirect evidence that compound **13s** might have a similar mechanism as FLC by inhibiting fungal lanosterol 14α-demethylase (CYP51), which catalyses the oxidative removal of the 14-methyl group of lanosterol or eburicol.

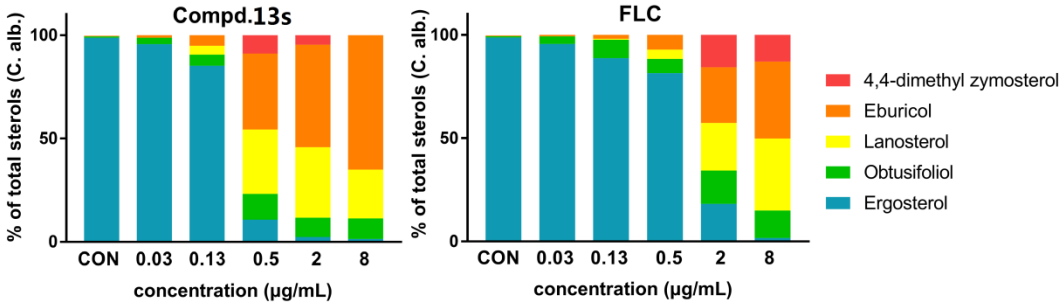


Figure 4. Sterol composition in **13s** or fluconazole-treated SC5314 *C. albicans* cells by GC-MS**.**

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**
13s[b]	0.03125	95.6	3.1	-	1.3	-
	0.125	85.2	5.3	4.2	5.3	-
	0.5	10.7	12.4	31.2	36.8	8.9
	2	2.3	9.4	34.1	49.5	4.7
	8	1.3	10.0	23.6	65.1	-
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 **13s**; [c]Treated with FLC; [d]Control (no drug).

2.5 Cytochrome P450 inhibition assay

Cytochrome P450 (CYP) enzymes are heme-thiolate proteins that are responsible for the oxidative metabolism of a wide variety of drugs. Inhibiting cytochrome P450 enzymes is one of the most common mechanisms leading to drug-drug interactions (DDI)[23]. Many azole antifungal agents have great inhibitory effects of CYP enzymes. For example, ketoconazole and itraconazole inhibit CYP3A4, a major drug-metabolizing P450 isoform in the human liver[24]. The compounds **13s** and **14a** were tested against the five major human CYP isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4-M). As shown in Table 4, compounds **13s** and **14a** showed no inhibitory activity against CYP1A2, CYP2C9, CYP2C19, and CYP2D6 with IC_{50} values of ≥50 μM, whereas compounds **13s** and **14a** exhibited weak activity against CYP3A4 with IC_{50} values of 7.27 μM and 14.6 μM, respectively. The results showed that compounds **13s** and **14a** had a low potential for causing DDI (for more information, see Supporting Information).

Table 5.

In vitro CYP inhibition assessment of compounds.

Compd.	IC50 (μM)				
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4-M
13s	>50	>50	>50	>50	7.27
14a	>50	>50	>50	>50	14.6

2.6 In vitro human Plasma Stability Assay

Plasma stability plays an important role in drug discovery and development. Based on their in vitro antifungal activities, compounds **13s** and **14a** were selected for an in vitro plasma stability study. As shown in **Table 6**, compounds **13s** and **14a** exhibited excellent metabolic profiles in human plasma.

Table 6.

In vitro Stability in Human Blood Plasma.

Compd.	Stability in Human Blood Plasma			
	Time Point(min)	%Remaining	Time Point(min)	%Remaining
13s	60	95.0	120	90.5
14a	60	95.8	120	91.7

*2.7 Molecular docking model analysis of compound **13s** in the active site of CYP51*

To investigate the binding mode of compound 13s and 14a, two highly active compounds, they were docked into the active site of CYP51 by using the CDOCKER program in the Discovery Studio 3.0 software. The published crystal structure of *C. albicans* CYP51(PDB ID:5FSA, Figure 5)[25] and *A. fumigatus* CYP51(PDB ID:4UYM, Figure 6) [17]served as a useful template for generating binding modes. As shown in Figure 5, the imidazole ring of compounds **13s** and **14a** coordinated the iron in the heme group, and the alkyl ester formed a hydrophobic interaction with Phe126 and Gly303. The 4,5-dihydronaphtho[2,1-*d*] isoxazole and naphtho[2,1-*d*] isoxazole side chains extended into the CYP51 channel to form van der Waals and hydrophobic interactions with the surrounding residues Ala62, Gly65, Leu88, Leu121, Pro230, Phe233, Phe380 and Met508.

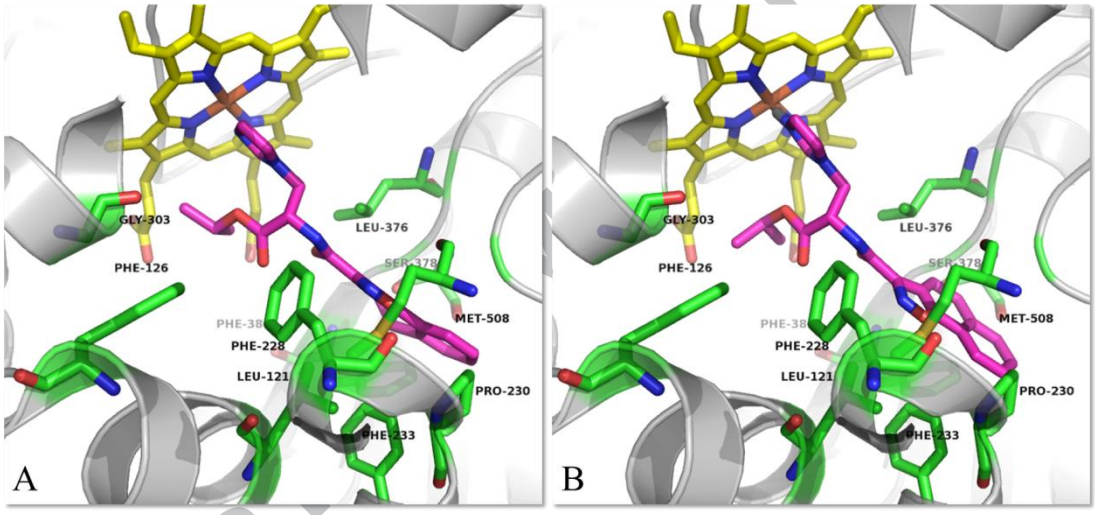


Figure 5. The binding mode of compounds **13s** (A) and **14a** (B) in the active site of CYP51of *C. albicans*(PDB ID: 5FSA).

As shown in Figure 6, the imidazole ring of compounds **13s** and **14a** coordinated the iron in the heme group, and the alkyl ester formed a hydrophobic interaction with Phe130, Val135 and Ala303. The 4,5-dihydronaphtho[2,1-*d*] isoxazole side chain extended into the CYP51 channel to form van der Waals and hydrophobic interactions with the surrounding residues Leu125, Phe229, Phe234, Ile373 and Leu503. In addition, hydrogen bonds formed between the isoxazole ring of compound **13s** and *A. fumigatus* CYP51 Tyr122.These results may provide a good explanation for the excellent in vitro antifungal activity of compounds **13s** and **14a**.

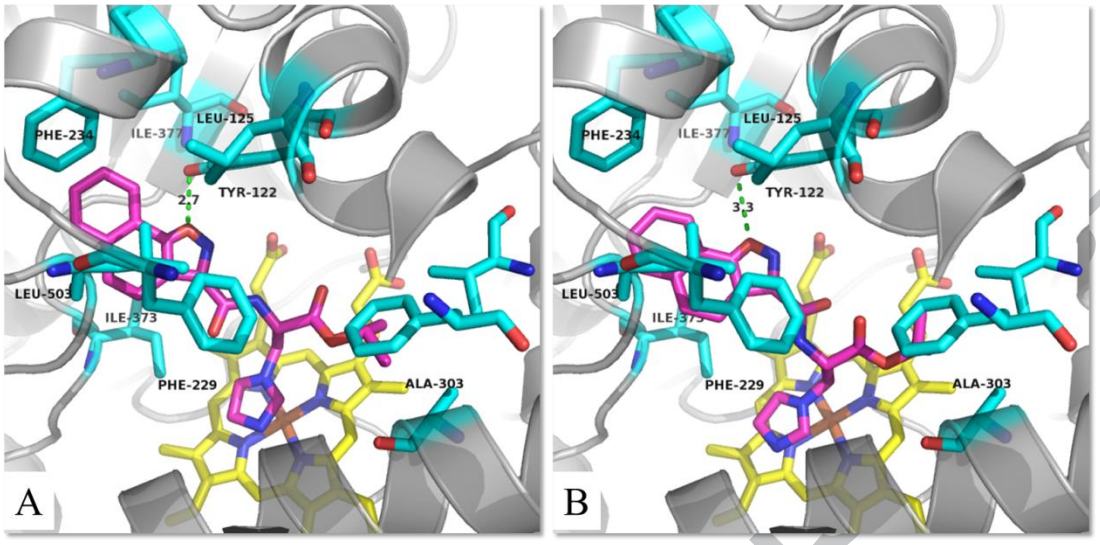


Figure 6. The binding mode of compounds **13s** (A) and **14a** (B) in the active site of *A. fumigatus* CYP51 (PDB ID: 4UYM).

3. Conclusions

To further enhance the anti-*Aspergillus* efficacy of our previous compounds, a novel class of benzoheterocycles ring derivatives were designed, synthesized and evaluated for their *in vitro* antifungal activity. Among these compounds, the 4,5-dihydronaphtho[2,1-*d*] isoxazole nucleus **13s** and naphtho[2,1-d] isoxazole nucleus **14a** exhibited the most remarkable *in vitro* activity against a variety of fungal pathogens, including *Candida spp.*, *C. neoformans* and *A. fumigatus* and fluconazole-resistant strains of *C. alb.*, that was superior or comparable to those of the reference drugs fluconazole and voriconazole. Further mechanistic investigations showed that the potent antifungal activity of novel compound **13s** might act by inhibiting the CYP51 of *Candida albicans*. Notably, a CYP enzyme inhibition assay showed that compounds **13s** and **14a** had weak inhibition for various human cytochrome P450 isoforms, which indicated they had a low potential to cause DDI. In addition, compounds **13s** and **14a** exhibited excellent blood plasma stability. Further studies on the antifungal mechanism and structural optimization are in progress.

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 performed 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 are 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 are reported in parts per million (ppm), and the coupling constants (*J*) are expressed in hertz (Hz). Peak multiplicities are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br).

*4.2 methyl benzo[b]thiophene-2-carboxylate (**2a**)*

A solution of 2-nitrobenzaldehyde (2.0 g, 13.1 mmol) and anhydrous potassium carbonate (7.32 g, 52.9 mmol) in DMF (60 mL) was cooled to 0°C. Next, methyl 2-mercaptoacetate (1.40 g, 13.23 mmol) was added dropwise to the reaction mixture. The resulting mixture was stirred for 30 min at 0°C, and then stirred for 4 h at ambient temperature. The reaction mixture was poured into ice water, and the resulting solid was filtered and dried to give the desired compound.

*4.3 methyl benzofuran-2-carboxylate (**2b**)*

A solution of salicylaldehyde (2.0 g, 16.2 mmol) and anhydrous potassium carbonate (9.05 g, 65.5 mmol) in DMF (60 mL) was cooled to 0°C. Then ethyl bromoacetate (5.01 g, 32.8 mmol) was added dropwise to the reaction mixture. The resulting mixture was stirred at 0°C for 30 min, and then at 80 °C for 5 h. After confirming that the reaction was complete by TLC analysis, the solution was cooled to room temperature and water (15 mL) was 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 **2b**.

*4.4 ethyl 2-oxo-2-(1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)acetate (**8**)*

A solution of 1-tetralone (3.0 g, 20.5 mmol) and diethyl oxalate (3.6 g, 24.6 mmol) in dry THF (100 mL) was cooled to 0°C under an argon atmosphere. Then LiHMDS (1 M in THF, 24.6 mL, 24.5 mmol) was added dropwise to the reaction mixture, and the resulting mixture was stirred for 4 h at ambient temperature. The solvent was removed under reduced pressure, and the residue

was used for the next step without further purification.

*4.5 ethyl 4,5-dihydronaphtho[2,1-d]isoxazole-3-carboxylate(**2c**)*

To a solution of the residue **8** in AcOH (40 mL) was added hydroxylamine hydrochloride (1.9 g, 27.3 mmol) at ambient temperature. The reaction was stirred at 80°C for 8 h. The reaction mixture was extracted with EtOAc and then 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 **2c**.

*4.6 ethyl naphtho[2,1-d]isoxazole-3-carboxylate(**2d**)*

2,3-Dicyano-5,6-dichlorobenzoquinone (6.10 g, 27.3 mmol) was added to a solution of ethyl 4,5-dihydronaphtho[2,1-*d*]isoxazole-3-carboxylate **2c** (2.20 g, 9.1 mmol) in ethanol at ambient temperature. The solution was refluxed for 12 h. After confirming that the reaction was complete by TLC analysis, the reaction was quenched with aqueous $NaHCO_3$. The reaction mixture was extracted with EtOAc and then brine. The organic phase was dried over Na_2SO_4 overnight and the solvent was removed *in* vacuum. The crude product was purified by silica gel column chromatography to yield the target product **2d**.

*4.6 General procedure for the synthesis of compounds (**1a-c**)*

To a solution of intermediate **2a-c** (1 equiv.) in methanol was added 2N sodium hydroxide 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. The precipitated white solid was collected by filtration and dried to give the carboxylic acid intermediate ***(1a-c)***.

*4.6.1 benzo[b]thiophene-2-carboxylic acid (**1a**)*

^{1}H-NMR (600 MHz, DMSO-d_6) δ 13.48 (s, 1H), 8.12 (s, 1H), 8.05 (d, *J* = 8.1 Hz, 1H), 8.01 (d, *J* = 7.9 Hz, 1H), 7.53 – 7.49 (m, 1H), 7.48 – 7.44 (m, 1H).

*4.6.2 benzofuran-2-carboxylic acid (**1b**)*

^{1}H-NMR (600 MHz, DMSO-d_6) δ 13.58 (s, 1H), 7.79 (d, *J* = 7.6 Hz, 1H), 7.70 (dd, *J* = 8.4, 0.7 Hz, 1H), 7.67 (d, *J* = 0.9 Hz, 1H), 7.52-4.49 (m, 1H), 7.38 – 7.33 (m, 1H).

*4.6.3 4,5-dihydronaphtho[2,1-d]isoxazole-3-carboxylic acid (**1c**)*

^{1}H-NMR (600 MHz, DMSO-d_6) δ 7.63 (d, *J* = 6.5 Hz, 1H), 7.14 – 7.08 (m, 2H), 7.03 (d, *J* = 6.8 Hz, 1H), 2.64 (t, *J* = 7.0 Hz, 2H), 2.32 – 2.27 (m, 2H).

*4.6.4 naphtho[2,1-d]isoxazole-3-carboxylic acid(**1d**)*

^{1}H-NMR (600 MHz, DMSO-d_6) δ 8.11 (d, *J* = 8.1 Hz, 1H), 7.42 (d, *J* = 7.7 Hz, 1H), 7.32 – 7.25 (m, 1H), 7.11 (t, *J* = 7.5 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.25 (d, *J* = 8.4 Hz, 1H).

4.7 *General procedure for the synthesis of compounds (**1e-f**)*

A solution of 4-amino-3-hydroxybenzoic acid (1 equiv.) and substituted trimethyl orthoformate (3 equiv.) was heated to 100°C for 3 h. At the end of the reaction, the solution was cooled to room temperature and diluted with MeOH. The solvent was removed under reduced pressure to give the target product **1e-f**.

*4.8 General procedure for the synthesis of L-serine ester (**11a–b**)*

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

*4.9 General procedure for the synthesis of compounds (**12a-t**)*

To a solution of the intermediate acid compound ***1a-f*** (1 equiv.) in anhydrous DMF was added EDCI(1.1 equiv) and HOBt(1.1 equiv), respectively. The reaction mixture was stirred for 1 h 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.

*4.10 General procedure for the synthesis of compounds (**13a-t** and **14a-g**)*

To a solution of the intermediate **11a-v** (1 equiv.) in CH_3CN was added CDI (2 equiv.) and imidazole (1.2 equiv.). 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 **12a-v**.

*4.10.1 isopropyl (S)-2-(benzo[b]thiophene-2-carboxamido)-3-(1H-imidazol-1-yl)propanoate (**13a**)*

Light white solid; yield:71.3 %. mp: 151.5-158.4 °C.^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.24 (d, *J* = 7.9 Hz, 1H), 8.12 (s, 1H), 8.03 (d, *J* = 7.8 Hz, 1H), 7.99 (dd, *J* = 7.0, 1.3 Hz, 1H), 7.65 (s, 1H), 7.53 – 7.39 (m, 2H), 7.23 (d, *J* = 1.0 Hz, 1H), 6.86 (s, 1H), 4.99 – 4,94 (m, 1H), 4.80 – 4.76 (m, 1H), 4.52 (dd, *J* = 14.1, 5.1 Hz, 1H), 4.39 (dd, *J* = 14.1, 9.6 Hz, 1H), 1.19 (dd, *J* = 9.5, 6.2 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.89, 161.82, 140.30, 138.98, 138.55, 137.78, 127.96,

126.50, 125.66, 125.39, 125.05, 122.85, 120.03, 68.88, 53.88, 45.91, 21.48, 21.43. HRMS calcd for $C_{18}H_{20}N_3O_3S$, $[M + H]^+$, 358.1225; found 358.1254.

*4.10.2 isobutyl (S)-2-(benzo[b]thiophene-2-carboxamido)-3-(1H-imidazol-1-yl)propanoate (**13b**)*

Light white solid; yield:69.5%. mp: 140.1-144.7 °C.^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.27 (d, *J* = 8.0 Hz, 1H), 8.10 (s, 1H), 8.03 (d, *J* = 7.9 Hz, 1H), 7.99 (d, *J* = 7.2 Hz, 1H), 7.66 (s, 1H), 7.51 – 7.43 (m, 2H), 7.23 (s, 1H), 6.86 (s, 1H), 4.85 – 4.81 (m, 1H), 4.55 (dd, *J* = 14.1, 4.8 Hz, 1H), 4.42 (dd, *J* = 14.1, 10.0 Hz, 1H), 3.94 – 4.87 (m, 2H), 1.90 – 1.84 (m, 1H), 0.86 (d, *J* = 6.7 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.37, 161.86, 140.31, 138.96, 138.55, 137.83, 128.36, 126.50, 125.59, 125.39, 125.04, 122.86, 119.87, 70.77, 53.86, 45.69, 27.23, 18.73(2C). HRMS calcd for $C_{19}H_{22}N_3O_3S$, $[M + H]^+$, 372.1382; found 372.1413.

*4.10.3 isopropyl (S)-2-(benzofuran-2-carboxamido)-3-(1H-imidazol-1-yl)propanoate (**13c**)*

Light white solid; yield:72.9%. mp: 54.1-59.4 °C.^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.18 (d, *J* = 8.1 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.64 (s, 1H), 7.58 (d, *J* = 0.7 Hz, 1H), 7.52 – 7.46 (m, 1H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.22 (s, 1H), 6.85 (s, 1H), 4.99 – 4.92 (m, 1H), 4.84 – 4.80 (m, 1H), 4.53 (dd, *J* = 14.1, 5.1 Hz, 1H), 4.42 (dd, *J* = 14.1, 9.7 Hz, 1H), 1.19 (t, *J* = 6.1 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.21, 158.71, 154.75, 148.58, 138.21, 128.43, 127.65, 127.45, 124.32, 123.41, 120.44, 112.34, 110.84, 69.37, 53.78, 46.27, 21.92, 21.89. HRMS calcd for $C_{18}H_{20}N_3O_4$, $[M + H]^+$, 342.1454; found 342.1474.

*4.10.4 isobutyl (S)-2-(benzofuran-2-carboxamido)-3-(1H-imidazol-1-yl)propanoate (**13d**)*

Light white solid; yield:68.1%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.23 (d, *J* = 8.2 Hz, 1H), 7.79 (d, *J* = 7.7 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.64 (s, 1H), 7.57 (d, *J* = 0.6 Hz, 1H), 7.52 – 7.46 (m, 1H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.22 (s, 1H), 6.84 (s, 1H), 4.91 – 4.87 (m, 1H), 4.56 (dd, *J* = 14.1, 4.8 Hz, 1H), 4.45 (dd, *J* = 14.1, 10.0 Hz, 1H), 3.95 – 3.85 (m, 2H), 1.90 – 1.84 (m, 1H), 0.86 (dd, *J* = 6.7, 1.4 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.24, 158.31, 154.31, 148.15, 137.80, 128.35, 127.19, 126.98, 123.86, 122.96, 119.84, 111.87, 110.36, 70.78, 53.25, 45.62, 27.23, 18.72(2C). HRMS calcd for $C_{19}H_{22}N_3O_4$, $[M + H]^+$, 356.1610; found 356.1639.

*4.10.5 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(quinoline-3-carboxamido)propanoate (**13e**)*

Light white solid; yield:69.7%. mp: 79.0-104.0 °C. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.32 (d, *J* = 7.8 Hz, 1H), 9.21 (d, *J* = 2.2 Hz, 1H), 8.79 (d, *J* = 2.0 Hz, 1H), 8.11 (dd, *J* = 14.4, 8.1 Hz, 2H), 7.89 (ddd, *J* = 8.4, 6.9, 1.3 Hz, 1H), 7.76 – 7.70 (m, 1H), 7.67 (s, 1H), 7.25 (s, 1H), 6.87 (s, 1H),

5.00 – 4.94 (m, 1H), 4.87 – 4.83 (m, 1H), 4.55 (dd, *J* = 14.1, 5.2 Hz, 1H), 4.43 (dd, *J* = 14.1, 9.5 Hz, 1H), 1.20 (dd, *J* = 10.2, 6.3 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.92, 165.26, 148.67, 148.64, 137.81, 135.84, 131.50, 129.20, 128.81, 127.82, 127.59, 126.42, 126.16, 120.16, 68.85, 53.91, 46.04, 21.49, 21.44. HRMS calcd for $C_{19}H_{21}N_4O_3$, $[M + H]^+$, 353.1614; found 353.1630.

*4.10.6 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(quinoxaline-2-carboxamido)propanoate (**13f**)*

Light white solid; yield:70.3%. mp: 115.2-128.0 °C.^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.36 (d, *J* = 7.9 Hz, 1H), 9.20 (d, *J* = 2.2 Hz, 1H), 8.78 (d, *J* = 1.9 Hz, 1H), 8.11 (dd, *J* = 13.8, 8.4 Hz, 2H), 7.91 – 7.88 (m, 1H), 7.73 – 7.69 (m, 1H), 7.67 (s, 1H), 7.24 (d, *J* = 9.6 Hz, 1H), 6.86 (s, 1H), 4.93 – 4.89 (m, 1H), 4.58 (dd, *J* = 14.1, 4.9 Hz, 1H), 4.46 (dd, *J* = 14.1, 9.8 Hz, 1H), 3.95 – 3.88 (m, 2H), 1.91 – 1.85 (m, 1H), 0.87 (d, *J* = 6.7 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.43, 165.27, 148.63(2C), 137.90, 135.82, 131.51, 129.20, 128.81, 128.42, 127.61, 126.41, 126.14, 119.93, 70.76, 53.90, 45.78, 27.23, 18.74(2C). HRMS calcd for $C_{20}H_{23}N_4O_3$, $[M + H]^+$, 367.1770; found 367.1788.

*4.10.7 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(quinoxaline-2-carboxamido)propanoate (**13g**)*

Light white solid; yield:65.3%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.43 (d, *J* = 7.8 Hz, 2H), 8.26 – 8.20 (m, 2H), 8.05 – 7.99 (m, 2H), 7.65 (s, 1H), 7.21 (s, 1H), 6.84 (s, 1H), 4.99 (dd, *J* = 12.5, 6.3 Hz, 1H), 4.96 – 4.93 (m, 1H), 4.62 – 4.54 (m, 2H), 1.22 (dd, *J* = 6.2, 3.4 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.50, 163.43, 143.59, 143.40, 143.16, 139.75, 137.54, 132.27, 131.55, 129.47, 129.18, 126.52, 120.54, 69.15, 53.37, 46.35, 21.46(2C). HRMS calcd for $C_{18}H_{19}N_5O_3$, $[M + Na]^+$, 376.1386; found 376.1404.

*4.10.8 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(quinoxaline-2-carboxamido)propanoate (**13h**)*

Light white solid; yield:68.7%. mp: 97.2-98.0 °C.^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.51 (d, *J* = 8.3 Hz, 1H), 9.43 (s, 1H), 8.26 – 8.19 (m, 2H), 8.06 – 7.98 (m, 2H), 7.68 (s, 1H), 7.23 (s, 1H), 6.85 (s, 1H), 5.05 – 5.01 (m, 1H), 4.66 – 4.57 (m, 2H), 3.93 (dd, *J* = 6.5, 1.8 Hz, 2H), 0.87 (dd, *J* = 6.7, 2.5 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.09, 163.45, 143.56, 143.43, 143.14, 139.75, 137.81, 132.25, 131.55, 129.44, 129.18, 128.10, 119.98, 70.88, 53.44, 45.83, 27.21, 18.72(2C). HRMS calcd for $C_{19}H_{22}N_5O_3$, $[M + H]^+$, 368.1723; found 368.1741.

*4.10.9 isopropyl (S)-2-(6-fluoro-1H-indole-2-carboxamido)-3-(1H-imidazol-1-yl)propanoate (**13i**)*

Light white solid; yield:62.6%. mp: 88.0-91.1°C.^{1}H-NMR (600 MHz, DMSO-d_6) δ 11.74 (s,

1H), 9.01 (d, *J* = 8.0 Hz, 1H), 7.63 (s, 1H), 7.44 (dd, *J* = 9.8, 2.5 Hz, 1H), 7.40 (dd, *J* = 8.9, 4.6 Hz, 1H), 7.21 (s, 1H), 7.15 (d, *J* = 1.5 Hz, 1H), 7.06 (td, *J* = 9.2, 2.6 Hz, 1H), 6.83 (s, 1H), 4.99 – 4.90 (m, 1H), 4.81 – 4.78 (m, 1H), 4.51 (dd, *J* = 14.1, 5.2 Hz, 1H), 4.38 (dd, *J* = 14.1, 9.7 Hz, 1H), 1.19 (dd, *J* = 11.5, 6.2 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.62, 161.43, 158.79(0.5C), 156.48(0.5C), 138.25, 133.78, 132.76, 128.64, 127.41, 120.40, 114.05, 113.00, 106.41, 103.86, 69.24, 53.99, 46.38, 21.93, 21.88. MS (ESI) *m/z* 359.3 $[M+H]^+$.

4.10.10 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(1-methyl-1H-indole-2-carboxamido)propanoate ***(13j)***

Light white solid; yield:72.9%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 8.94 (d, *J* = 8.0 Hz, 1H), 7.69 – 7.63 (m, 2H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.29 (t, *J* = 7.6 Hz, 1H), 7.23 (s, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 7.07 (s, 1H), 6.86 (s, 1H), 4.99 – 4.95 (m, 1H), 4.77 – 4.73 (m, 1H), 4.51 (dd, *J* = 14.1, 5.0 Hz, 1H), 4.38 (dd, *J* = 14.0, 9.9 Hz, 1H), 3.90 (s, 3H), 1.21 (dd, *J* = 9.4, 6.3 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.47, 162.41, 138.95, 138.27, 131.57, 128.25, 125.91, 124.33, 122.16, 120.73, 120.59, 111.01, 105.49, 69.18, 53.84, 46.41, 31.72, 21.96, 21.93. HRMS calcd for $C_{19}H_{23}N_4O_3$, $[M + H]^+$, 355.1770; found 355.1799.

*4.10.11 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(1-methyl-1H-indole-2-carboxamido)propanoate(****13k****)*

Light white solid; yield:71.4%. mp: 58.1-61.6 °C.^{1}H-NMR (600 MHz, DMSO-d_6) δ 8.98 (d, *J* = 8.1 Hz, 1H), 7.69 – 7.62 (m, 2H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.31 – 7.27 (m, 1H), 7.23 (s, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 7.07 (s, 1H), 6.86 (s, 1H), 4.85 – 4.81 (m, 1H), 4.55 (dd, *J* = 14.1, 4.7 Hz, 1H), 4.40 (dd, *J* = 14.0, 10.2 Hz, 1H), 3.90 (s, 3H), 3.79 – 3.69 (m, 2H), 1.88 (d, *J* = 6.6 Hz, 1H), 0.88 (d, *J* = 6.7 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.96, 162.43, 138.97, 138.21, 131.49, 128.25, 125.89, 124.35, 122.16, 120.73, 120.54, 111.02, 105.49, 71.15, 53.72, 46.34, 31.72, 27.73, 19.21, 19.16. HRMS calcd for $C_{20}H_{25}N_4O_3$, $[M + H]^+$, 369.1927; found 369.1955.

4.10.12 isopropyl (S)-2-(1H-benzo[d]imidazole-2-carboxamido)-3-(1H-imidazol-1-yl)propanoate ***(13l)***

Light white solid; yield:67.4%. mp: 100.6-103.2 °C.^{1}H-NMR (600 MHz, DMSO-d_6) δ 13.30 (s, 1H), 9.36 (d, *J* = 8.4 Hz, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.63 (s, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.33 (dd, *J* = 11.2, 4.0 Hz, 1H), 7.29 (dd, *J* = 11.2, 4.0 Hz, 1H), 7.20 (s, 1H), 6.82 (s, 1H), 4.99 – 4.95 (m, 1H), 4.92 – 4.89 (m, 1H), 4.55 (dd, *J* = 14.1, 5.0 Hz, 1H), 4.49 (dd, *J* = 14.1, 9.5 Hz, 1H), 1.20 (dd, *J* = 6.2, 4.0 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.54, 159.00, 144.67, 142.43,

137.70, 134.49, 127.55, 124.45, 122.76, 120.12, 120.02, 112.66, 68.97, 53.23, 45.98, 21.47, 21.45. HRMS calcd for $C_{17}H_{20}N_5O_3$, $[M + H]^+$, 342.1566; found 342.1589.

*4.10.13 isopropyl (S)-2-(benzo[d][1,3]dioxole-5-carboxamido)-3-(1H-imidazol-1-yl)propanoate (**13m**)*

Light white solid; yield:74.1%. mp: 60.9-63.3 °C.^{1}H-NMR (600 MHz, DMSO-d_6) δ 8.74 (d, *J* = 7.9 Hz, 1H), 7.61 (s, 1H), 7.39 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.31 (d, *J* = 1.7 Hz, 1H), 7.19 (s, 1H), 7.00 (d, *J* = 8.1 Hz, 1H), 6.84 (s, 1H), 6.10 (s, 2H), 4.96 – 4.90 (mz, 1H), 4.72 – 4.68 (m, 1H), 4.47 (dd, *J* = 14.0, 5.1 Hz, 1H), 4.35 (dd, *J* = 14.0, 9.8 Hz, 1H), 1.17 (dd, *J* = 8.2, 6.3 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.22, 165.70, 150.08, 147.38, 137.78, 128.11, 127.40, 122.48, 119.95, 107.96, 107.30, 101.79, 68.62, 53.92, 45.83, 21.50, 21.44. HRMS calcd for $C_{17}H_{19}N_3O_5$, $[M + Na]^+$, 368.1222; found 368.1243.

*4.10.14 isobutyl (S)-2-(benzo[d][1,3]dioxole-5-carboxamido)-3-(1H-imidazol-1-yl)propanoate (**13n**)*

Light white solid; yield:73.9%. mp: 109.4-113.0 °C. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 8.78 (d, *J* = 7.9 Hz, 1H), 7.61 (s, 1H), 7.38 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.30 (d, *J* = 1.7 Hz, 1H), 7.19 (s, 1H), 7.00 (d, *J* = 8.1 Hz, 1H), 6.84 (s, 1H), 6.10 (d, *J* = 1.0 Hz, 2H), 4.77 – 4.74 (m, 1H), 4.51 (dd, *J* = 14.0, 4.9 Hz, 1H), 4.38 (dd, *J* = 14.0, 10.0 Hz, 1H), 3.91 – 3,84 (m, 2H), 1.88 – 1.82 (m, 1H), 0.85 (dd, *J* = 6.7, 1.1 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 170.14, 166.19, 150.54, 147.83, 138.26, 128.79, 127.84, 122.90, 120.30, 108.41, 107.71, 102.25, 71.07, 54.32, 46.14, 27.69, 19.20. HRMS calcd for $C_{18}H_{21}N_3O_5$, $[M + Na]^+$, 382.1379; found 382.1402.

*4.10.15 isopropyl (S)-2-(benzo[d]oxazole-6-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**13o**)*

Light white solid; yield:71.5%. mp: 71.9-75.4 °C. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.30 (d, *J* = 7.8 Hz, 1H), 8.92 (s, 1H), 8.28 (d, *J* = 0.7 Hz, 1H), 7.91 (dt, *J* = 17.6, 4.9 Hz, 2H), 7.69 (s, 1H), 7.26 (s, 1H), 6.83 (s, 1H), 4.97 – 4.93 (m, 1H), 4.83 – 4.76 (m, 1H), 4.55 – 4.48 (m, 2H), 1.19 (dd, *J* = 8.9, 6.3 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.08, 165.90, 156.41, 149.08, 142.30, 137.91, 131.05, 128.29, 124.33, 119.92, 119.86, 110.55, 68.66, 54.14, 45.68, 21.50, 21.45. HRMS calcd for $C_{17}H_{19}N_4O_4$, $[M + H]^+$, 343.1406; found 343.1427.

*4.10.16 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(2-methylbenzo[d]oxazole-6-carboxamido) propanoate(**13p**)*

Light white solid; yield:70.7%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.01 (d, *J* = 7.8 Hz, 1H), 8.04 (d, *J* = 1.1 Hz, 1H), 7.80 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.74 (d, *J* = 8.3 Hz, 1H), 7.69 (s, 1H), 7.24 (s, 1H), 6.86 (s, 1H), 4.98 – 4.92 (m, 1H), 4.80 – 4.76 (m, 1H), 4.52 (dd, *J* = 14.1, 5.1 Hz, 1H), 4.40 (dd, *J* = 14.0, 9.7 Hz, 1H), 2.65 (s, 3H), 1.18 (dd, *J* = 8.3, 6.3 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.10, 166.43, 166.06, 150.08, 143.89, 138.00, 130.00, 128.11, 123.83, 120.09, 118.74, 109.57, 68.70, 54.00, 45.89, 21.49, 21.44, 14.32. HRMS calcd for $C_{18}H_{20}N_4O_4$, $[M + Na]^+$, 379.1382; found 379.1416.

*4.10.17 isopropyl (S)-2-(5,6-dihydro-4H-benzo[3,4]cyclohepta[1,2-d]isoxazole-3-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**13q**)*

Light white solid; yield:68.1%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.23 (d, *J* = 8.1 Hz, 1H), 7.94 – 7.87 (m, 1H), 7.61 (s, 1H), 7.41 – 7.38 (m, 2H), 7.32 (dd, *J* = 5.6, 3.3 Hz, 1H), 7.20 (s, 1H), 6.86 (s, 1H), 5.00 – 4.93 (m, 1H), 4.83 – 4.79 (m, 1H), 4.51 (dd, *J* = 14.1, 4.9 Hz, 1H), 4.39 (dd, *J* = 14.1, 9.7 Hz, 1H), 2.93 – 2.87 (m, 2H), 2.86 – 2.73 (m, 2H), 1.91 – 1.88 (m, 2H), 1.21 (t, *J* = 6.5 Hz, 6H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.42, 164.51, 159.72, 157.15, 141.23, 137.82, 130.09, 130.04, 128.32, 126.73, 126.20, 125.70, 119.83, 115.43, 68.90, 53.24, 45.59, 34.53, 24.18, 23.76, 21.44, 21.42. HRMS calcd for $C_{22}H_{25}N_4O_4$, $[M + H]^+$, 409.1876; found 409.1907.

*4.10.18 isopropyl (S)-2-(4,5-dihydronaphtho[2,1-d]isoxazole-3-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**13s**)*

Light white solid; yield:68.3%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.23 (d, *J* = 8.1 Hz, 1H), 7.67 (t, *J* = 8.4 Hz, 1H), 7.62 (s, 1H), 7.42 – 7.36 (m, 3H), 7.20 (s, 1H), 6.85 (s, 1H), 4.99 – 4.93 (m, 1H), 4.84 – 4.80 (m, 1H), 4.51 (dd, *J* = 14.1, 5.0 Hz, 1H), 4.41 (dd, *J* = 14.1, 9.7 Hz, 1H), 3.01 (t, *J* = 7.9 Hz, 2H), 2.89 – 2.79 (m, 2H), 1.20 (t, *J* = 6.5 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.43, 166.47, 159.34, 154.86, 137.78, 136.86, 130.52, 128.79, 128.00, 127.24, 123.80, 121.47, 119.99, 113.12, 68.97, 53.26, 45.68, 27.82, 21.44(2C), 17.75. HRMS calcd for $C_{21}H_{22}N_4O_4$, $[M + Na]^+$, 417.1539; found 417.1573.

*4.10.19 isobutyl (S)-2-(4,5-dihydronaphtho[2,1-d]isoxazole-3-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**13t**)*

Light white solid; yield:67.8%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.29 (d, *J* = 8.2 Hz, 1H), 7.66 (d, *J* = 7.3 Hz, 1H), 7.63 (s, 1H), 7.40 (ddd, *J* = 13.3, 6.9, 3.1 Hz, 3H), 7.21 (s, 1H), 6.86 (s, 1H), 4.92 – 4.88 (m, 1H), 4.55 (dd, *J* = 14.1, 4.7 Hz, 1H), 4.44 (dd, *J* = 14.1, 10.0 Hz, 1H), 3.94 –

3.88 (m, 2H), 3.01 (t, *J* = 7.9 Hz, 2H), 2.83 (dd, *J* = 16.7, 8.1 Hz, 2H), 1.90 – 1.86 (m, 1H), 0.88 (dd, *J* = 6.7, 1.6 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 169.39, 166.94, 159.84, 155.28, 138.28, 137.30, 130.98, 129.24, 128.81, 127.69, 124.25, 121.93, 120.31, 113.56, 71.27, 53.63, 45.95, 28.28, 27.70, 19.18(2C), 18.21. HRMS calcd for $C_{22}H_{24}N_4O_4$, $[M + Na]^+$, 431.1695; found 431.1727.

*4.10.20 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(naphtho[2,1-d]isoxazole-3-carboxamido) propanoate(**14a**)*

Light white solid; yield:74.1%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.60 (d, *J* = 8.0 Hz, 1H), 8.45 (dd, *J* = 6.0, 3.4 Hz, 1H), 8.24 – 8.16 (m, 1H), 7.94 (dd, *J* = 21.7, 8.7 Hz, 2H), 7.85 – 7.80 (m, 2H), 7.66 (s, 1H), 7.25 (s, 1H), 6.85 (s, 1H), 5.02 – 4.98 (m, 1H), 4.97 – 4.90 (m, 1H), 4.58 (dd, *J* = 14.1, 5.0 Hz, 1H), 4.47 (dd, *J* = 14.1, 9.7 Hz, 1H), 1.22 (t, *J* = 6.5 Hz, 6H).^{13}C-NMR (150 MHz, DMSO-d_6) δ 168.36, 161.68, 159.03, 152.16, 137.83, 133.59, 129.11, 128.74, 128.29, 128.05, 126.43, 121.14, 119.88, 118.55, 118.11, 115.39, 69.00, 53.45, 45.61, 21.44, 21.42. HRMS calcd for $C_{21}H_{21}N_4O_4$, $[M + H]^+$, 393.1563; found 393.1594.

*4.10.21 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(naphtho[2,1-d]isoxazole-3-carboxamido) propanoate(**14b**)*

Light white solid; yield:73.9%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.65 (d, *J* = 8.2 Hz, 1H), 8.45 (dd, *J* = 6.0, 3.5 Hz, 1H), 8.25 – 8.17 (m, 1H), 7.94 (dd, *J* = 21.7, 8.7 Hz, 2H), 7.83 (dd, *J* = 6.2, 3.2 Hz, 2H), 7.67 (s, 1H), 7.26 (s, 1H), 6.86 (s, 1H), 5.04 – 5.00 (m, 1H), 4.61 (dd, *J* = 14.1, 4.8 Hz, 1H), 4.50 (dd, *J* = 14.1, 10.0 Hz, 1H), 3.98 – 3.91 (m, 2H), 1.91 – 1.87 (m, 1H), 0.88 (dd, *J* = 6.7, 1.2 Hz, 6H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.83, 161.70, 159.09, 152.13, 137.82, 133.58, 129.12, 128.74, 128.30, 128.05, 126.44, 121.14, 119.85, 118.55, 118.10, 115.37, 70.85, 53.32, 45.53, 27.21, 18.69, 18.66. HRMS calcd for $C_{22}H_{23}N_4O_4$, $[M + H]^+$, 407.1719; found 407.1753.

*4.10.22 isopropyl (S)-2-(8-bromo-4,5-dihydronaphtho[2,1-d]isoxazole-3-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**14c**)*

Light white solid; yield:71.7%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.27 (d, *J* = 8.1 Hz, 1H), 7.80 (d, *J* = 2.0 Hz, 1H), 7.62 (s, 1H), 7.60 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.20 (s, 1H), 6.85 (s, 1H), 4.98 – 4.93 (m, 1H), 4.86 – 4.79 (m, 1H), 4.51 (dd, *J* = 14.1, 5.0 Hz, 1H), 4.40 (dd, *J* = 14.1, 9.7 Hz, 1H), 2.99 (t, *J* = 7.9 Hz, 2H), 2.87 – 2.78 (m, 2H), 1.20 (t, *J* = 6.3 Hz,

6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.38, 165.04, 159.11, 154.90, 137.81, 136.07, 133.03, 132.94, 130.92, 128.28, 125.72, 123.84, 119.86, 114.14, 68.94, 53.28, 45.58, 27.29, 21.44, 21.41, 17.52. HRMS calcd for $C_{21}H_{22}BrN_4O_4$, $[M + H]^+$, 475.0804; found 475.0843.

*4.10.23 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(8-methoxy-4,5-dihydronaphtho[2,1-d]isoxazole-3-carboxamido)propanoate(**14d**)*

Light white solid; yield: 69.3%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.22 (d, *J* = 8.1 Hz, 1H), 7.61 (s, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 2.7 Hz, 2H), 6.98 (dd, *J* = 8.4, 2.7 Hz, 1H), 6.85 (s, 1H), 4.99 – 4.93 (m, 1H), 4.83 – 4.79 (m, 1H), 4.51 (dd, *J* = 14.1, 5.0 Hz, 1H), 4.40 (dd, *J* = 14.1, 9.7 Hz, 1H), 3.82 (s, 3H), 2.93 (t, *J* = 7.9 Hz, 2H), 2.86 – 2.76 (m, 2H), 1.20 (t, *J* = 6.4 Hz, 6H).^{13}C-NMR (150 MHz, DMSO-d_6) δ 168.36, 161.68, 159.03, 152.16, 137.83, 133.59, 129.11, 128.74, 128.29, 128.05, 126.43, 121.14, 119.88, 118.55, 118.11, 115.39, 69.00, 53.45, 45.61, 21.44(2C), 21.42. HRMS calcd for $C_{22}H_{25}N_4O_5$, $[M + H]^+$, 425.1825; found 425.1859.

*4.10.24 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(8-methoxy-4,5-dihydronaphtho[2,1-d]isoxazole-3-carboxamido)propanoate(**14e**)*

Light white solid; yield: 71.6%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.29 (d, *J* = 8.2 Hz, 1H), 7.62 (s, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.21 (s, 1H), 7.19 (d, *J* = 2.7 Hz, 1H), 6.98 (dd, *J* = 8.4, 2.7 Hz, 1H), 6.85 (s, 1H), 4.98 – 4.88 (m, 1H), 4.55 (dd, *J* = 14.1, 4.8 Hz, 1H), 4.44 (dd, *J* = 14.1, 10.0 Hz, 1H), 3.94 – 3.88 (m, 2H), 3.82 (s, 3H), 2.93 (t, *J* = 7.9 Hz, 2H), 2.85 – 2.75 (m, 2H), 1.91 – 1.85 (m, 1H), 0.88 (dd, *J* = 6.7, 1.6 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.89, 166.45, 159.34, 158.29, 154.87, 137.78, 129.86, 128.66, 128.29, 124.54, 119.81, 116.29, 113.44, 106.56, 70.79, 55.40, 53.15, 45.48, 27.21, 26.98, 18.70, 18.00. HRMS calcd for $C_{23}H_{27}N_4O_5$, $[M + H]^+$, 439.1981; found 439.2019.

*4.10.25 isopropyl (S)-3-(1H-imidazol-1-yl)-2-(7-methoxy-4,5-dihydronaphtho[2,1-d]isoxazole-3-carboxamido)propanoate(**14f**)*

Light white solid; yield: 73.2%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.17 (d, *J* = 8.1 Hz, 1H), 7.60 (d, *J* = 8.7 Hz, 2H), 7.20 (s, 1H), 7.01 (d, *J* = 2.2 Hz, 1H), 6.93 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.85 (s, 1H), 5.00 – 4.92 (m, 1H), 4.84 – 4.77 (m, 1H), 4.50 (dd, *J* = 14.1, 5.0 Hz, 1H), 4.40 (dd, *J* = 14.1, 9.7 Hz, 1H), 3.81 (s, 3H), 2.98 (t, *J* = 7.9 Hz, 2H), 2.87 – 2.75 (m, 2H), 1.20 (t, *J* = 6.4 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.44, 166.66, 160.94, 159.43, 154.67, 139.18, 137.78, 128.30, 123.07, 119.82, 116.83, 114.54, 112.45, 111.04, 68.90, 55.36, 53.24, 45.57, 28.24,

21.43(2C), 17.75. HRMS calcd for $C_{22}H_{25}N_4O_5$, $[M + H]^+$, 425.1825; found 425.1863.

*4.10.26 isobutyl (S)-3-(1H-imidazol-1-yl)-2-(7-methoxy-4,5-dihydronaphtho[2,1-d]isoxazole-3-carboxamido)propanoate(**14g**)*

Light white solid; yield: 76.6%. ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.23 (d, J = 8.3 Hz, 1H), 7.64 – 7.57 (m, 2H), 7.20 (s, 1H), 7.01 (d, J = 2.4 Hz, 1H), 6.92 (dd, J = 8.5, 2.5 Hz, 1H), 6.85 (s, 1H), 4.91 – 4.87 (m, 1H), 4.54 (dd, J = 14.1, 4.7 Hz, 1H), 4.43 (dd, J = 14.1, 10.0 Hz, 1H), 3.94 – 3.88 (m, 2H), 3.81 (s, 3H), 2.98 (t, J = 7.9 Hz, 2H), 2.86 – 2.74 (m, 2H), 1.91 – 1.84 (m, 1H), 0.88 (dd, J = 6.7, 1.6 Hz, 6H).^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.91, 166.69, 160.95, 159.49, 154.63, 139.18, 137.77, 128.32, 123.08, 119.80, 116.82, 114.55, 112.46, 111.03, 70.78, 55.36, 53.12, 45.48, 28.25, 27.21, 18.70(2C), 17.76. HRMS calcd for $C_{23}H_{26}N_4O_5$, $[M + Na]^+$, 461.1801; found 461.1834.

4.11 *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 in the National Committee for Clinical Laboratory Standards (NCCLS). The MIC values were defined as the lowest concentration of an antimicrobial that inhibited the visible growth of the fungi. FLC and ITR were purchased for serve as the positive control drugs. All test compounds were dissolved in DMSO and serially diluted into the growth medium.

4.12 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 the 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).

4.13 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 the addition of 20 μL NADPH to a final concentration of 10 mM. The mixture were incubated at 37°C for 10 min and the reaction terminated with the addition of a 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.

4.14 Plasma Stability Assay

Test compounds were added to human plasma to a final concentration of 2 μM and incubated at 37 °C. At each time point (0, 10, 30, 60 and 120 min), the reaction was terminated with the addition of a 200 μL cold stop solution (200 ng/mL tolbutamide plus 20 ng/mL buspirone in 50% MeOH/ACN) to precipitate protein. After the reactions were terminated, the plates were centrifuged, and the supernatants were analysed by LC/MS/MS.

Acknowledgements

The authors thank Prof. Yongbing Cao from School of Pharmacy, the Second Military Medical University, for providing the fluconazole-resistant strains of *Candida albicans*(*strain 100* and *strain 103*). This work was supported by Program for Innovative Research Team of the Ministry of Education and Program for Liaoning Innovative Research Team in University.

References

1. Denning, D. W.; Bromley, M. J. *Science* **2015,** *347*, 1414.
2. Brown, G. D.; Denning, D. W.; Gow, N. A. R.; Levitz, S. M.; Netea, M. G.; White, T. C. *Sci. Transl. Med.* **2012,** *4*, 9.
3. Campoy, S.; Adrio, J. L. *Biochem. Pharmacol.* **2017,** *133*, 86.
4. Denning, D. W.; Hope, W. W. *Trends Microbiol.* **2010,** *18*, 195.
5. Surarit, R.; Shepherd, M. G. *Journal of medical and veterinary mycology : bi-monthly publication of the International Society for Human and Animal Mycology* **1987,** *25*, 403.
6. Hospenthal, D. R. *Infect. Med.* **2004,** *21*, 476.
7. Allen, D.; Wilson, D.; Drew, R.; Perfect, J. *Expert Review Of Anti-Infective Therapy* **2015,** *13*, 787.
8. Vermes, A.; Guchelaar, H. J.; Dankert, J. *J. Antimicrob. Chemother.* **2000,** *46*, 171.
9. Onyewu, C.; Blankenship, J. R.; Del Poeta, M.; Heitman, J. *Antimicrob. Agents Chemother.* **2003,** *47*, 956.
10. Sheehan, D. J.; Hitchcock, C. A.; Sibley, C. M. *Clin. Microbiol. Rev.* **1999,** *12*, 40.
11. Heeres, J.; Meerpoel, L.; Lewi, P. *Molecules* **2010,** *15*, 4129.
12. Lass-Florl, C. *Drugs* **2011,** *71*, 2405.
13. Garcia Rodriguez, L. A.; Duque, A.; Castellsague, J.; Perez-Gutthann, S.; Stricker, B. H. *Br. J. Clin. Pharmacol.* **1999,** *48*, 847.

14. Bruggemann, R. J. M.; Alffenaar, J. W. C.; Blijlevens, N. M. A.; Billaud, E. M.; Kosterink, J. G. W.; Verweij, P. E.; Burger, D. M. *Clin. Infect. Dis.* **2009,** *48*, 1441.

15. Zhao, S.; Zhao, L.; Zhang, X.; Liu, C.; Hao, C.; Xie, H.; Sun, B.; Zhao, D.; Cheng, M. *Eur. J. Med. Chem.* **2016,** *123*, 514.

16. Zhao, S.; Zhang, X.; Wei, P.; Su, X.; Zhao, L.; Wu, M.; Hao, C.; Liu, C.; Zhao, D.; Cheng, M. *Eur. J. Med. Chem.* **2017,** *137*, 96.

17. Hargrove, T. Y.; Wawrzak, Z.; Lamb, D. C.; Guengerich, F. P.; Lepesheva, G. I. *J. Biol. Chem.* **2015,** *290*, 23916.

18. Jiang, Z. G.; Gu, J. L.; Wang, C.; Wang, S. Z.; Liu, N.; Jiang, Y.; Dong, G. Q.; Wang, Y.; Liu, Y.; Yao, J. Z.; Miao, Z. Y.; Zhang, W. N.; Sheng, C. Q. *Eur. J. Med. Chem.* **2014,** *82*, 490.

19. Cao, X. F.; Sun, Z. S.; Cao, Y. B.; Wang, R. L.; Cai, T. K.; Chu, W. J.; Hu, W. H.; Yang, Y. S. *J. Med. Chem.* **2014,** *57*, 3687.

20. Sud, I. J.; Feingold, D. S. *The Journal of investigative dermatology* **1981,** *76*, 438.

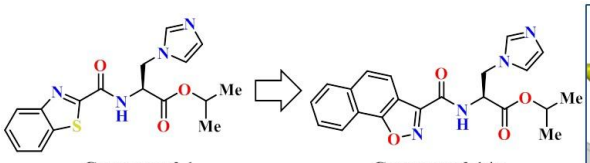
21. Liang, R. M.; Cao, Y. B.; Fan, K. H.; Xu, Y.; Gao, P. H.; Zhou, Y. J.; Dai, B. D.; Tan, Y. H.; Wang, S. H.; Tang, H.; Liu, H. T.; Jiang, Y. Y. *Acta Pharmacologica Sinica* **2009,** *30*, 1709.

22. Warrilow, A. G. S.; Hull, C. M.; Parker, J. E.; Garvey, E. P.; Hoekstra, W. J.; Moore, W. R.; Schotzinger, R. J.; Kelly, D. E.; Kelly, S. L. *Antimicrob. Agents Chemother.* **2014,** *58*, 7121.

23. Bates, D. W.; Yu, D. T. *Drugs Of Today* **2003,** *39*, 801.

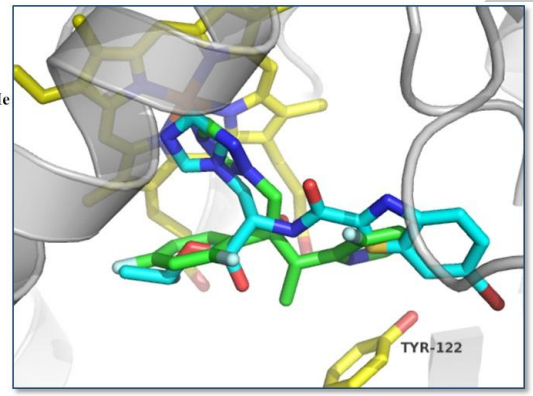
24. Niwa, T.; Shiraga, T.; Takagi, A. *Biol. Pharm. Bull.* **2005,** *28*, 1805.

25. Hargrove, T. Y.; Friggeri, L.; Wawrzak, Z.; Qi, A. D.; Hoekstra, W. J.; Schotzinger, R. J.; York, J. D.; Guengerich, F. P.; Lepesheva, G. I. *J. Biol. Chem.* **2017,** *292*, 6728.



Compound 1 Compound 14a

Compd.	MIC(μg/mL)			
	C. alb.	*C. neo.*	*C.tro.*	*A. fum*
14a	**0.125**	**0.25**	**0.125**	**4**



Highlights

- 26 new compounds with benzoheterocycle scaffolds were designed and synthesized.
- The naphthoisoxazole was selected to improve activity against *Aspergillus spp.*
- Compounds **13s** and **14a** showed better antifungal activity than fluconazole.
- Compound **13s** reduced the content of ergosterol in a dose-dependent manner.
- Compounds **13s** and **14a** exhibited low inhibition profiles for human cytochrome P450 isoforms.