Accepted Manuscript

Design, synthesis, and structure-activity relationship studies of l-amino alcohol derivatives as broad-spectrum antifungal agents

Liyu Zhao, Linfeng Tian, Nannan Sun, Yin Sun, Yixuan Chen, Xinran Wang, Shizhen Zhao, Xin Su, Dongmei Zhao, Maosheng Cheng

PII: S0223-5234(19)30457-X

DOI: https://doi.org/10.1016/j.ejmech.2019.05.047

Reference: EJMECH 11357

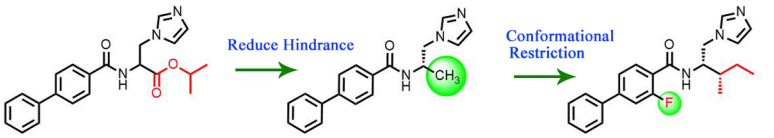
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 23 April 2019

Revised Date: 16 May 2019

Accepted Date: 16 May 2019

Please cite this article as: L. Zhao, L. Tian, N. Sun, Y. Sun, Y. Chen, X. Wang, S. Zhao, X. Su, D. Zhao, M. Cheng, Design, synthesis, and structure-activity relationship studies of l-amino alcohol derivatives as broad-spectrum antifungal agents, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.05.047.



5 *C.alb.* MIC = 0.25 μg/mL
A.fum. MIC > 64 μg/mL

14a *C.alb.* MIC = 4 μg/mL
A.fum. MIC = 1 μg/mL

14n *C.alb.* MIC < 0.03 μg/mL
A.fum. MIC = 2 μg/mL

Design, synthesis, and structure-activity relationship studies of L-amino alcohol derivatives as broad-spectrum antifungal agents

Liyu Zhao[a], Linfeng Tian[a], Nannan Sun[a], Yin Sun[a], Yixuan Chen[a], Xinran Wang[a], Shizhen Zhao[b], Xin Su[c], 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, China*

[b]*Key Laboratory of Receptors-Mediated Gene Regulation and Drug Discovery, School of Medicine, Henan University, Kaifeng 475004, China*

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

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

ABSTRACT:

To discover broad spectrum antifungal agents, two strategies were applied, and a novel class of L-amino alcohol derivatives were designed and synthesized. 3-F substituted compounds **14i**, **14n**, **14s** and **14v** exhibited excellent antifungal activities with broad antifungal spectra against *C. albicans* and *C. tropicalis*, with MIC values in the range of 0.03 - 0.06 μg/mL, and against *A. fumigatus* and *C. neoformans*, with MIC values in the range of 1 - 2 μg/mL. Notably, Compounds **14i**, **14n, 14s** and **14v** also displayed moderate activities against fluconazole-resistance strains *17#* and *CaR* that were isolated from AIDS patients. Moreover, only compounds in the *S*-configuration showed antifungal activity. Preliminary mechanistic studies showed that the potent antifungal activity of compound **14v** stemmed from inhibition of *C. albicans* CYP51. Compounds **14n** and **14v** were almost nontoxic to mammalian A549 cells, and their stability in human plasma was excellent.

Keywords:

CYP51; Azole antifungals; Structure-activity relationship; Fluconazole-resistance.

1. Introduction

Over the past few decades, the incidence of systemic fungal infections has increased

markedly, especially in immunocompromised hosts, such as patients undergoing organ transplants or anticancer chemotherapy and patients with AIDS [1-4]. *Candida spp.*, *Cryptococcus neoformans* and *Aspergillus spp.* are the three major pathogenic fungi that threaten human health [5]. Approximately 72.8 million people suffer from *Candida spp.* infections, 65.5 million people suffer from *Cryptococcus spp.* infections and 12.4 million people suffer from *Aspergillus spp.* infections each year [6]. Currently, clinical antifungal agents can be divided into four classes: polyenes (such as Amphotericin B and Nystatin) [7], azoles (such as Fluconazole and Itraconazole) [8], echinocandins (such as Caspofungin and Micafungin) [9] and antimetabolites (such as 5-fluorocytosine) [10].

Azoles, inhibitors of fungal lanosterol 14α-demethylase (CYP51), can prevent the biosynthesis of ergosterol, which is a very important component of biofilms [11, 12]. Fluconazole is a typical azole antifungal agent that has been used clinically for almost 30 years. Due to the high therapeutic index, azole drugs have been used as first-line antifungal agents, and some azole derivatives have been developed, such as itraconazole, voriconazole, ketoconazole (Fig. 1). However, some problems remain, such as severe drug resistance and cytotoxicity and a narrow antifungal spectrum [13, 14]. Therefore, an urgent clinical need exists to develop broad spectrum azole antifungal agents with novel structures, low toxicity and high efficiency.

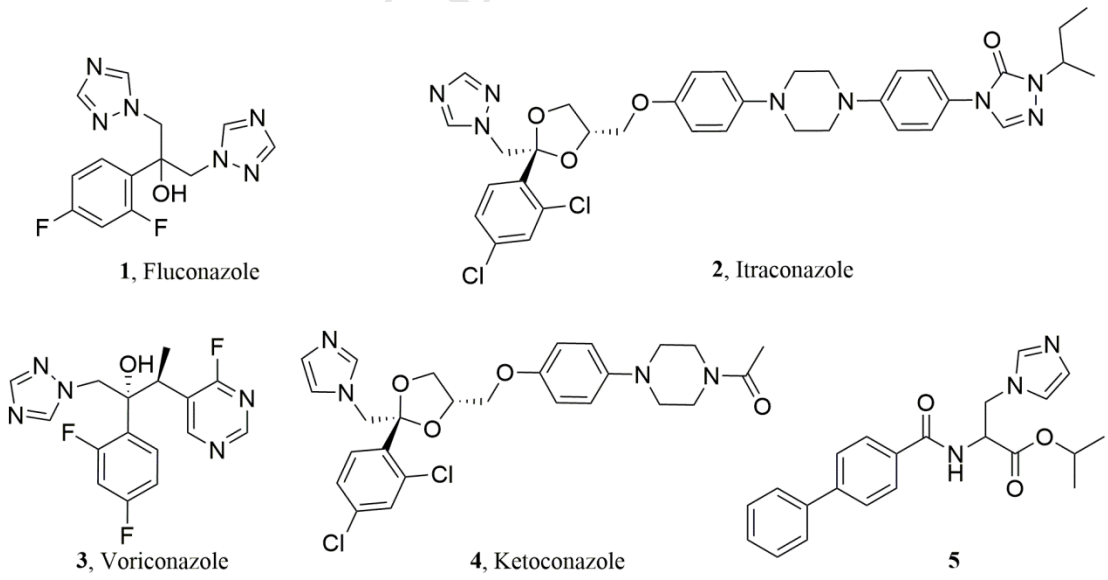


Figure 1 Chemical structures of azole antifungal agents and lead compound **5.**

In our previous studies [15-17], a series of azole derivatives with an ester group were designed and synthesized. Most of the compounds exhibited excellent *in vitro* antifungal activity

against *Candida albicans*. However, these compounds were inactive against *Aspergillus fumigatus*. The potent compound **5** displayed excellent antifungal activity against *Candida albicans (SC5314)* with an MIC value 0.03 μg/mL but was inactive against *Aspergillus fumigatus*. To discover antifungal agents with a broad spectrum, the *Candida albicans* CYP51 (PDB: 5TZ1) [18] and *Aspergillus fumigatus* CYP51 (PDB: 4UYM) [19] were overlaid, and we found that the amino acid residues Leu^{503} and Tyr^{122} made the cavity of *Aspergillus fumigatus* CYP51 more narrow than that of *Candida albicans* CYP51 (Fig. 2A). The steric clash between the phenyl group of ester compound **5** with Leu^{503} may be responsible for the inactivity against *Aspergillus fumigatus* (Fig. 2B). To avoid the clash and increase the compound's activity against *Aspergillus fumigatus*, two strategies were applied: (1) replacing the ester group with small alkyl side chains to reduce the steric hindrance, and (2) conformational restriction of the phenyl group of the compound by interacting with surrounding amino acid residues to prevent rotation (Fig. 3). A series of L-amino alcohol derivatives were designed and synthesized.

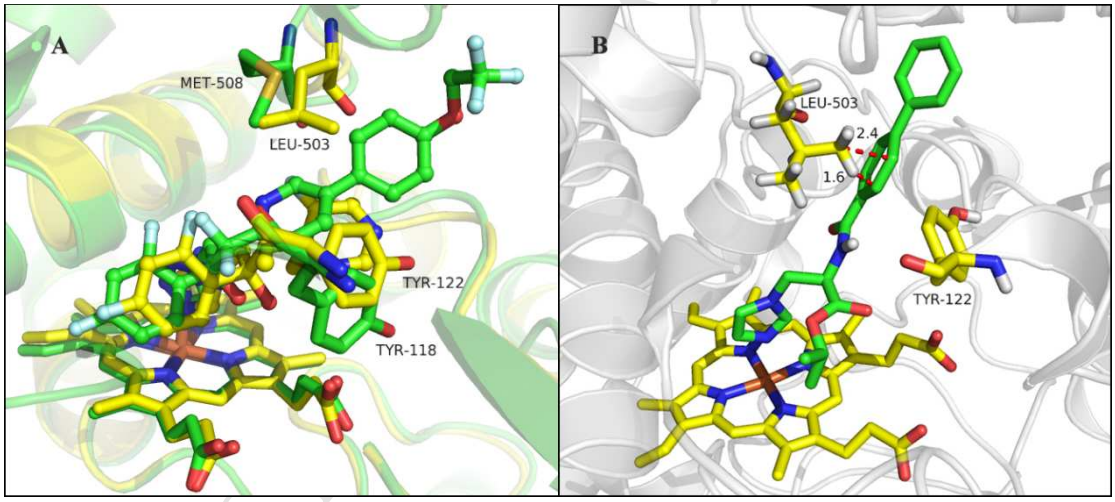


Figure 2 (A) Overlay of *Candida albicans* CYP51 (green, PDB: 5TZ1) and *Aspergillus fumigatus* CYP51 (yellow, PDB: 4UYM). (B) Predicted binding mode of **5** in *Aspergillus fumigatus* CYP51. The distances are shown as red dashed lines.

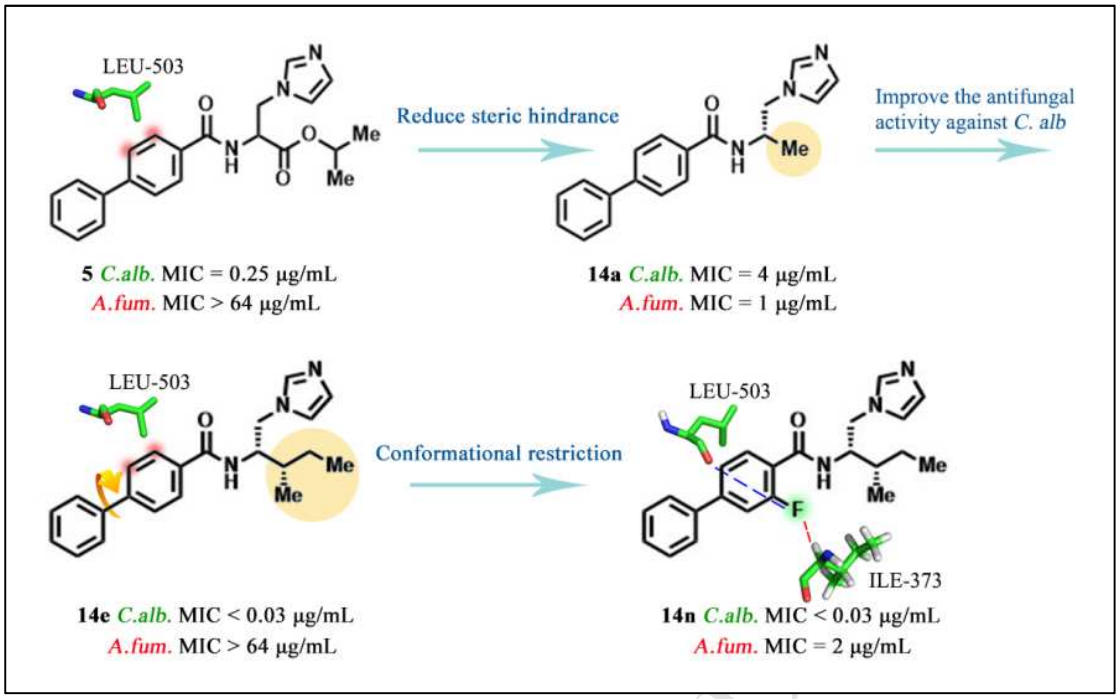
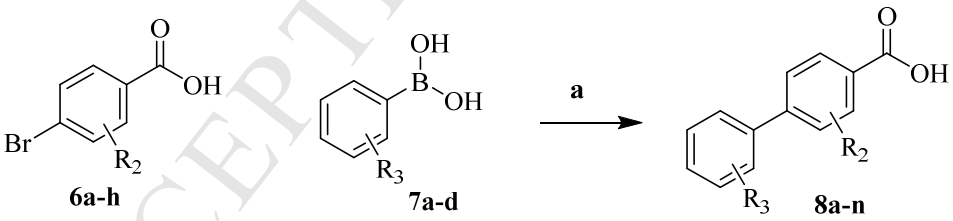


Figure 3 Design strategies of novel L-amino alcohol derivatives. *C. alb.*, *Candida albicans* (CPCC400523); *A. fum.*, *Aspergillus fumigatus* (cgmcc 3.7795).

2. Results and discussion

2.1. *Chemistry*

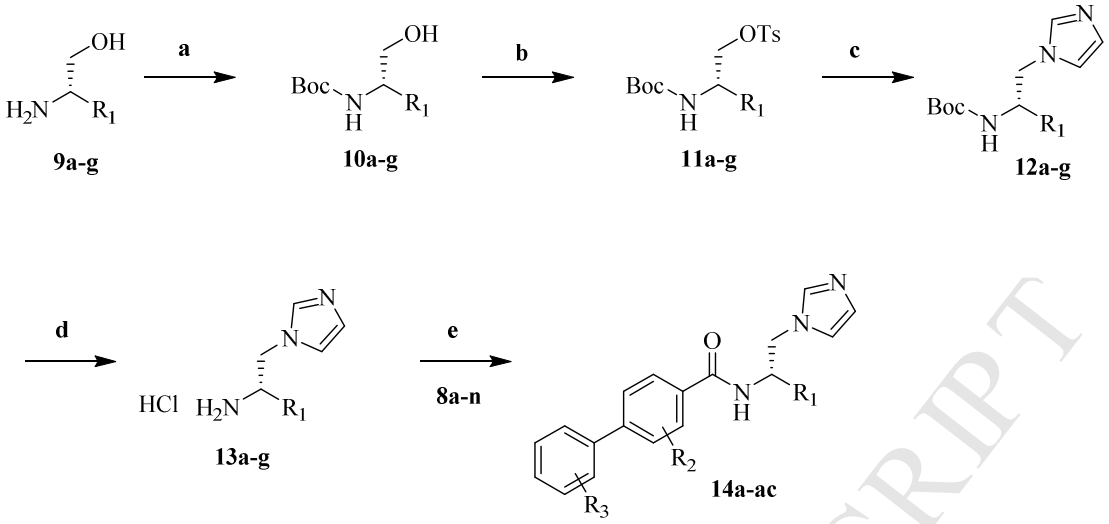
The key intermediates **8a-n** were prepared conveniently via palladium-catalyzed Suzuki coupling of commercially available substituted 4-bromobenzoic acid **6a-h** and substituted phenylboronic **7a-d** (Scheme 1).



	R_2
6a	R_2 = H
6b	R_2 = 2-F
6c	R_2 = 3-F
6d	R_2 = 2-Cl
6e	R_2 = 3-Cl
6f	R_2 = 2-CH_3
6g	R_2 = 3-CH_3
6h	R_2 = 2-OCH_3

	R_3
7a	R_3 = H
7b	R_3 = 2-F
7c	R_3 = 3-F
7d	R_3 = 4-F

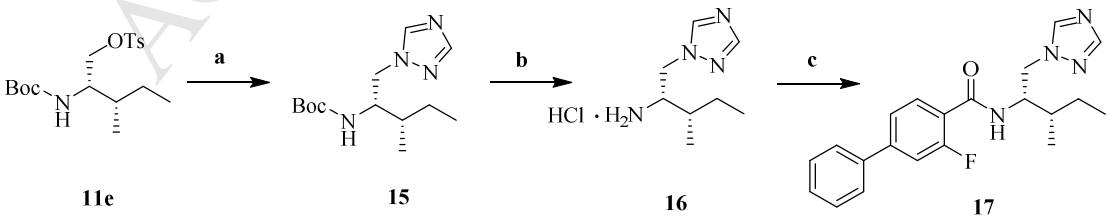
Scheme 1 Synthesis of intermediates **8a-n**. Reagents and conditions: (a) $Pd(PPh_3)_4$, K_2CO_3, dioxane, reflux, 5 h.



	R_1
a	R_1 = -CH_3
b	R_1 = -CH_2CH_3
c	R_1 = -$(CH_2)_2CH_3$
d	R_1 = -$CH(CH_3)_2$
e	R_1 = -$CH(CH_3)CH_2CH_3$
f	R_1 = -$CH_2CH(CH_3)_2$
g	R_1 = -CH_2Ph

Scheme 2 General synthesis of the target compounds **14a-ac**. Reagents and conditions: (a) $(Boc)_2O$, TEA, DCM, rt.; (b) TsCl, TEA, DMAP, DCM, rt.; (c) imidazole, NaH, DMF; (d) HCl-EtOH, rt.; (e) EDCI, HOBt, DIEA, r.t., 4 h.

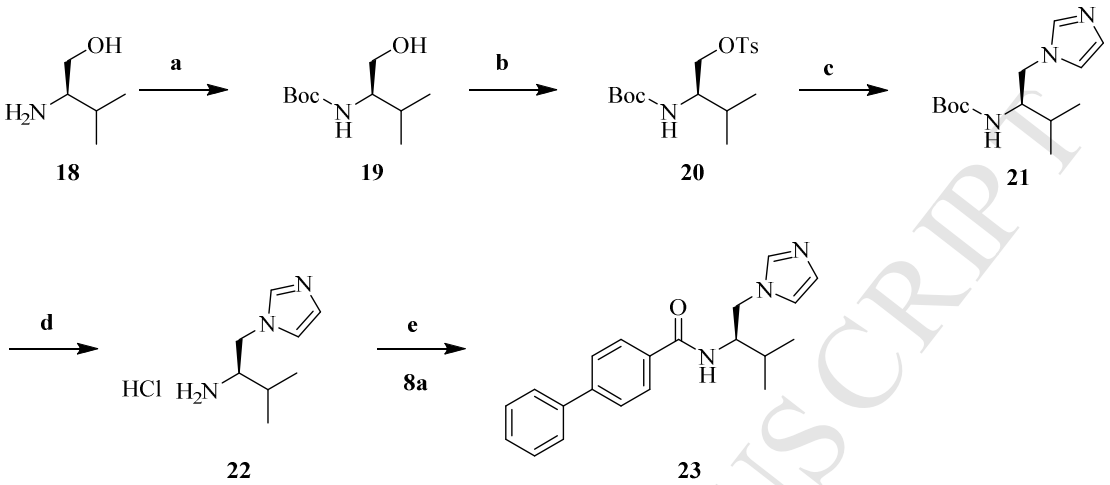
The alkyl compounds **14a-ac** were prepared according the procedures shown in Scheme 2. Various L-amino alcohol derivatives **9a-g** were protected with $(Boc)_2O$ to afford compounds **10a-g**, which were converted to **11a-g** through substitution reactions. Treatment of compounds **11a-g** with imidazole and NaH in dry DMF afforded **12a-g**, and then cleavage of the Boc protecting group with hydrochloric acid gave the key intermediates **13a-g**. The target compounds **14a-ac** were synthesized via amide reaction of amino **13a-g** and acid **8a-n** in DMF at room temperature.



Scheme 3 Synthesis of the target compound **17**. Reagents and conditions: (a) triazole, NaH, DMF; (b) EtOH-HCl, rt.; (c) EDCI, HOBt, DIEA, 4 h, rt.

As depicted in Scheme 3, the triazole derivative **17** was prepared by using procedures similar

to those described above, except that triazole was used instead of imidazole. The synthetic procedures for compound **23** of *R*-configuration were similar to the synthesis of compounds **14a-ac** (Scheme 4).



Scheme 4 Synthesis of the target compound **23**. Reagents and conditions: (a) $(Boc)_2O$, TEA, DCM, rt.; (b) TsCl, TEA, DMAP, DCM, rt.; (c) imidazole, NaH, DMF; (d) EtOH-HCl, rt.; (e) EDCI, HOBt, DIEA, r.t., 4 h.

2.2. *In vitro antifungal activity*

The target compounds were evaluated for their *in vitro* antifungal activity against five pathogenic fungi according to the protocols of the National Committee for Clinical Laboratory Standards (NCCLS). Fluconazole (FCZ) and itraconazole (ICZ) were used as the reference drugs. The results of *in vitro* antifungal activities are summarized in Table 1.

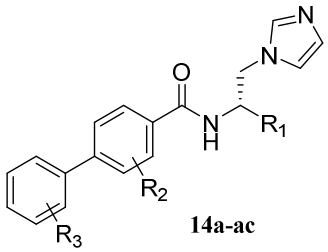
Compounds **14a-f** with different alkyl side chain lengths exhibited moderate to good antifungal activities. Compound **14a** with a methyl substitution increased the activity against *Aspergillus fumigatus* with an MIC value of 1 μg/mL. However, the antifungal activities of **14a** against *Candida albicans*, *Cryptococcus neoformans* and *Candida tropicalis* were moderate or even disappeared. Compounds **14e** and **14f** with bulk substitutions displayed excellent activities against *Candida albicans* and *Candida tropicalis* with MIC values < 0.03 μg/mL but were inactive against *Aspergillus fumigatus*.

Most of the compounds **14h-x** with fluorine substitution maintained excellent antifungal activities against *Candida albicans, Cryptococcus neoformans* and *Candida tropicalis.* Moreover, the 3-F substitution compounds **14i**, **14n**, **14s** and **14v** increased the antifungal activities against *Aspergillus fumigatus* with MIC values in the range of 1 - 2 μg/mL. Substitution of -Cl, $-CH_3$ and $-OCH_3$ (**14y-14ac**) led to disappearance of the antifungal activity against *Aspergillus fumigatus*.

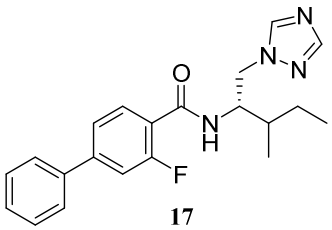
Replacing the imidazole group (**14n**) with a triazole group (**17**) resulted in a slight decrease in antifungal activities against *Candida albicans*, *Candida tropicalis* and *Aspergillus fumigatus*. Notably, the compound of *R*-configuration (**23**) displayed no antifungal activity compared to the *S*-configuration (**14d**). It turns out that the *S*-configuration is the pharmacodynamic configuration.

Table 1

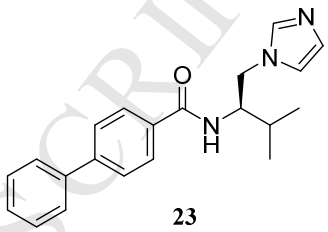
In vitro antifungal activities of target compounds (MIC, μg/mL) [a].



14a-ac



17



23

Compd.	R_1	R_2	R_3	*C. alb.*(I)	*C. alb.*(II)	*C. neo.*	*C. tro.*	*A. fum.*
14a	$-CH_3$	H	H	4	0.5	>64	0.25	1
14b	$-CH_2CH_3$	H	H	0.25	<0.06	16	0.125	4
14c	$-(CH_2)_2CH_3$	H	H	0.06	<0.03	2	<0.03	4
14d	$-CH(CH_3)_2$	H	H	0.06	<0.03	1	<0.03	8
14e	$-CH(CH_3)CH_2CH_3$	H	H	<0.03	<0.03	0.5	<0.03	>64
14f	$-CH_2CH(CH_3)_2$	H	H	<0.03	<0.03	1	<0.03	>64
14g	$-CH_2Ph$	H	H	0.125	<0.03	0.25	<0.03	>64
14h	$-CH(CH_3)_2$	2-F	H	0.06	<0.03	2	0.06	>64
14i	$-CH(CH_3)_2$	3-F	H	0.06	<0.03	2	<0.03	1
14j	$-CH(CH_3)_2$	2'-F	H	0.06	<0.03	1	0.06	>64
14k	$-CH(CH_3)_2$	3'-F	H	0.06	<0.03	2	0.06	>64
14l	$-CH(CH_3)_2$	4'-F	H	0.5	0.25	1	0.25	32
14m	$-CH(CH_3)CH_2CH_3$	2-F	H	0.06	<0.03	2	0.06	>64
14n	$-CH(CH_3)CH_2CH_3$	3-F	H	<0.03	<0.03	1	<0.03	2
14o	$-CH(CH_3)CH_2CH_3$	2'-F	H	0.06	<0.03	1	0.06	>64
14p	$-CH(CH_3)CH_2CH_3$	3'-F	H	0.125	<0.03	2	0.06	>64
14q	$-CH(CH_3)CH_2CH_3$	4'-F	H	0.25	0.125	1	0.125	>64
14r	$-CH_2CH(CH_3)_2$	2-F	H	0.06	<0.03	4	<0.03	>64
14s	$-CH_2CH(CH_3)_2$	3-F	H	0.06	<0.03	2	<0.03	2
14t	$-CH_2Ph$	2-F	H	0.06	<0.03	0.5	<0.03	>64
14u	$-CH_2Ph$	3-F	H	0.06	<0.03	>64	<0.03	>64
14v	$-CH(CH_3)CH_2CH_3$	3-F	2'-F	0.06	<0.03	1	<0.03	2
14w	$-CH(CH_3)CH_2CH_3$	3-F	3'-F	0.06	<0.03	4	<0.03	4
14x	$-CH(CH_3)CH_2CH_3$	3-F	4'-F	0.25	0.125	4	0.125	8
14y	$-CH(CH_3)CH_2CH_3$	2-Cl	H	4	0.5	4	0.25	>64
14z	$-CH(CH_3)CH_2CH_3$	3-Cl	H	0.25	0.06	>64	0.125	>64

14aa	-$CH(CH_3)CH_2CH_3$	2-CH_3	H	>16	>16	>64	2	>64
14ab	-$CH(CH_3)CH_2CH_3$	3-CH_3	H	1	0.25	>64	0.5	>64
14ac	-$CH(CH_3)CH_2CH_3$	3-OCH_3	H	1	0.25	>64	0.125	>64
17	-	-	-	0.25	0.125	>64	0.25	2
23	-	-	-	>16	>16	>64	8	>64
5	-	-	-	0.25	<0.03	2	<0.06	>64
FCZ	-	-	-	1	0.5	4	1	>64
ITZ	-	-	-	<0.03	<0.03	<0.125	<0.03	<0.125

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

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

Azole antifungal agents have been used clinically for many years, especially fluconazole, which has been applied in the clinic for approximately 30 years. The widespread use of azole antifungal agents has led to severe drug-resistance, which has been a major clinical problem for treating systemic fungal infection. Therefore, it is necessary to further evaluate the antifungal activity of the compounds against fluconazole-resistant strains when developing innovative drugs. The potent compounds **14i**, **14n**, **14s** and **14v** were selected to evaluate their antifungal activity against fluconazole-resistant strains of *C. albicans* (*strain 17#* and *strain CaR)*, which were isolated from AIDS patients. The results are summarized in Table 2. The compounds showed moderate antifungal activities against strain 17# with MIC values in the range of 1 - 4 μg/mL and against strain *CaR* with MIC values in the range of 4 - 32 μg/mL.

Table 2

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

Compd.	*Candida albicans*	
	Strain 17#	*Strain CaR*
14i	4	32
14n	2	4
14s	1	4
14v	2	8
FCZ	>64	>64

[a]Abbreviations: *strain 17#*, fluconazole-resistant strain of *Candida albicans*; *strain CaR*, fluconazole-resistant strain of *Candida albicans*; FCZ: Fluconazole. *Strain 17#* and *strain CaR* were provided by Institute of Microbiology, Chinese Academy of Sciences.

2.4. *GC-MS analysis of sterol composition in Candida albicans (SC5314)*

L-amino alcohol derivatives are a novel class of antifungal agents, and only compounds in the *S*-configuration showed antifungal activity. To investigate the antifungal mechanism of compound **14v**, gas chromatography-mass spectrometry (GC-MS) was used to analyze the sterol composition in cells using fluconazole as the reference drug. The sterol profile results are summarized in Table 3. Ergosterol was the major sterol (100%) in cells, and other sterols were not detected in the absence of drug. When *Candida albicans* were cultured for 16 h with different concentrations of fluconazole, the sterol components of the cells changed in a concentration-dependent manner. The content of ergosterol decreased sharply from 97.7% to 0% with increasing fluconazole concentration, and the obtusifoliol, lanosterol and eburicol content increased dramatically. These changes in sterol contents were caused by inhibiting the sterol 14α-demethylase enzyme (CYP51) of the ergosterol biosynthetic pathway. The same changes in sterol content were shown when *Candida albicans* was cultured with different concentrations of compound **14v**. The ergosterol content decreased and the obtusifoliol, lanosterol, eburicol contents increased with increasing concentration of **14v**. The results indicate that the antifungal mechanism of compound **14v** is similar to fluconazole, and the target is fungal lanosterol 14α-demethylase (CYP51).

Table 3

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
14v[b]	0.13	98.0	0	2.0	0
	0.5	36.0	12.2	21.0	30.8
	2	6.5	23.8	20.0	49.7
	8	0	31.3	23.8	44.9
FLC[c]	0.13	97.7	0	2.3	0
	0.5	90.1	0	9.9	0
	2	17.2	17.7	28.0	37.1
	8	0	37.2	22. 9	39.9
Control[d]	-	100	0	0	0

[a]Abbreviations: *C. alb.*, *Candida albicans* (ATCC SC5314); [b]Treated with compound **14v**; [c]Treated with FLC; [d]Control (no drug).

2.5. *In vitro* cytotoxicity assay

Fungus is a type of eukaryote that is similar to mammalian cells. The compounds designed as

antifungal agents may cause side-effects on mammalian cells. Therefore, it is necessary to consider the toxicity of the compounds to mammalian cells. The potent compounds **14n** and **14v** were selected to evaluate their toxicity against A549 cells (human lung carcinoma) according to the protocol described previously [20], and voriconazole was selected as a control. The results are shown in Table 4. Compounds **14n** and **14v** displayed 14% and 13.9% inhibition, respectively, against A549 cells at a concentration of 50 μM/L, and these results were superior to voriconazole, which displayed 37% inhibition. Therefore, compounds **14n** and **14v** were almost nontoxic to A549 cells.

Table 4

In vitro cytotoxicity of compounds in A549 cells.

Compd.	Inhibition (μM/L)				
	0.08	0.40	2	10	50
14n	1.2%	1.0%	1.2%	3.1%	14.0%
14v	5.4%	6.6%	7.3%	8.3%	13.9%
voriconazole	17.1%	20.3%	25.5%	32%	36.7%

2.6. *In vitro human plasma stability assay*

The stability of compounds in human plasma is an important property that must be considered in the development of new drugs. In general, ester groups are unstable in human plasma and can be easily hydrolyzed. Replacing ester groups with alkyl side chains, the potent alkyl derivatives **14n** and **14v** with broad antifungal spectra were selected to evaluate their human plasma stability. The results are summarized in Table 5 and show that **14n** and **14v** both exhibited excellent stability in human plasma, and 113.1% and 113.6% of each compound remained, respectively, when incubated in plasma for 60 min.

Table 5

In vitro human plasma stability of compounds **14n** and **14v**.

Compd.	Stability in human blood plasma	
	% Remaining at 60 min	% Remaining at 120 min
14n	113.1	86.3
14v	113.6	92.6

2.7. *Structure-activity relationships*

Compounds **14a-f** with different lengths of alkyl side chains were investigated. The results showed that the activities of **14a-f** against *Candida albicans*, *Cryptococcus neoformans* and *Candida tropicalis* increased with extension of the alkyl side chains. However, the activities against *Aspergillus fumigatus* decreased with extension of the alkyl side chains.

Fluorine substituted compounds **14h-x** with bulky alkyl side chains maintained excellent activities against *Candida albicans*, *Cryptococcus neoformans* and *Candida tropicalis*. Moreover, compounds of 3-F substitution on the biphenyl group displayed activity against *Aspergillus fumigatus*, such as the compounds **14i**, **14n**, **14s** and **14v**. Compounds with -Cl, -Me or -OCH_3 substitution on the biphenyl group showed moderate or even disappearing antifungal activity.

In addition, the influence of the stereochemical configuration was also investigated. Compared to the *S*-configured compound **14d**, compound **23** in its *R*-configuration lost all activity against the fungi. It turns out that the *S*-configuration is the pharmacodynamic configuration.

2.8. *Molecular docking model analysis of compounds **14n** and **14v** in the active site of CYP51*

To better understand the binding mode of compounds with *Aspergillus fumigatus* CYP51 and provide information for further optimization, compounds **14n** and **14v** were docked into the active site of *Aspergillus fumigatus* CYP51 by using the CDOCKER program in the Discovery Studio 3.0 software. The published crystal structure of *Aspergillus fumigatus* CYP51 (PDB ID: 4UYM) served as a useful template for generating the binding mode [18]. As shown in Fig. 4A, the imidazole of compound **14n** binds to the heme group through formation of a coordination bond with the iron atom. The alkyl side chain forms hydrophobic interactions with Thr^{126}, Phe^{130} and Tyr^{136}. The biphenyl group extends into the CYP51 channel and mainly forms hydrophobic and Van der Waals interactions with the surrounding residues such as Leu^{91}, Phe^{234}, Ile^{377} and Leu^{503}. Moreover, the 3-F atom on the biphenyl group can form a halogen (fluorine) bond with Leu^{503} and a hydrogen bond with Ile^{373} (Ile^{373} as the H-donor and fluorine as the H-acceptor). Therefore, the conformation of the phenyl of compound **14n** can be restricted to avoid the steric clash with Leu^{503}. The binding mode of compound **14v** with *Aspergillus fumigatus* CYP51 is similar to that of **14n** (Fig. 4B).

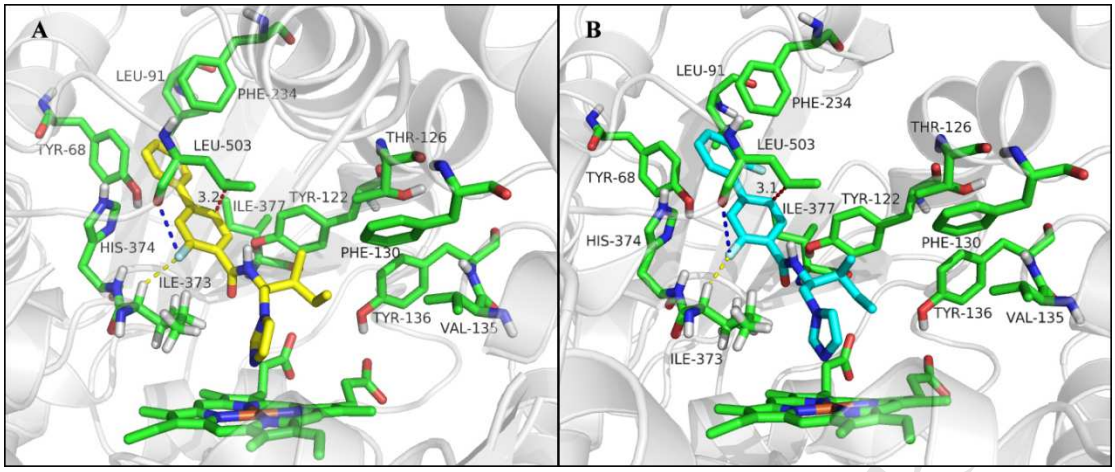


Figure 4 (A) Predicted binding mode of **14n** in the active site of *Aspergillus fumigatus* CYP51. (B) Predicted binding mode of **14v** in the active site of *Aspergillus fumigatus* CYP51. The distances are shown as red dashed lines. The halogen bonds are shown as blue dashed lines. The hydrogen bonds are shown as yellow dashed lines. Figures were generated using PyMOL.

3. Conclusions

The steric clash with Leu^{503} is the main reason that caused ester compound **5** to be inactive against *A. fumigatus*. To avoid this clash and increase the activity against *A. fumigatus*, the bulky ester group was replaced with small alkyl side chains. Compounds with different lengths of alkyl side chains were synthesized. The *in vitro* activities showed that the activities against *A. fumigatus* increased when the alkyl side chains were shortened, but the activities against *Candida albicans*, *Cryptococcus neoformans* and *Candida tropicalis* decreased when the alkyl side chains were shortened. Compound **14a** with methyl substitution increased the antifungal activity against *A. fumigatus* with an MIC value of 1 μg/mL. However, the activities against *Candida albicans*, *Cryptococcus neoformans* and *Candida tropicalis* were moderate or even lost.

To maintain excellent activities against *Candida albicans*, *Cryptococcus neoformans* and *Candida tropicalis*, the isopropyl, sec-butyl and isobutyl substitutions were selected. Moreover, in order to be effective against *A. fumigatus*, the strategy of conformational restriction of the compound was applied. A series of fluorine-substituted compounds were synthesized. Of these, 3-F substitution compounds **14i**, **14n**, **14s** and **14v** exhibited the excellent activities against *Candida albicans*, *Cryptococcus neoformans* and *Candida tropicalis* and displayed antifungal activity against *A. fumigatus* with MIC values in the range of 1 - 2 μg/mL.

Notably, compounds **14n**, **14s** and **14v** showed moderate activities against the fluconazole resistant strains of *C. albicans* (*strain 17#* and *strain CaR*) which were isolated from AIDS

patients. In addition, the configuration of the compounds had a great influence on the antifungal activity, and only compounds of the *S*-configuration showed antifungal activity. Preliminary mechanistic studies showed that the potent antifungal activity of compound **14v** stemmed from inhibition of *C. albicans* CYP51. Compounds **14n**, **14v** were almost nontoxic to A549 cells at 50 μM/L and exhibited excellent stabilities in human plasma. Molecular docking results showed that the 3-F can interact with Leu^{503} and Ile^{373}, which is very important for the conformational restriction of the compounds.

4. Experimental section

4.1. *Chemistry*

All reagents used were commercially available without further purification. Solvent were purified according to standard procedures. The reactions were monitored by TLC, which was performed on silica gel plates with fluorescence F-254 and visualized with UV light. Silica gel of 200-300 mesh was used for column chromatography. The melting points of the compounds were determined on a BüCHI Melting Point B-540 melting point apparatus and were uncorrected. Mass spectrometry was performed on an Agilent 1200 LC-MS (Agilent, Palo Alto, CA, USA). High-resolution mass spectra (HRMS) were performed on an Agilent 6530 accurate-mass Q-TOF LC-MS system. GC−MS analysis was performed on an Agilent 1200 LC−MS using ESI mode. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III-600 or Bruker Avance III-400 instruments (600 or 400 MHz for ^{1}H and 150 or 100 MHz for ^{13}C) with TMS as an internal standard. The coupling constants (*J*) are reported in hertz and the peak multiplicities were described as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad peak.

4.2. *General procedure for the synthesis of intermediates (**8a-n**)*

Under an argon atmosphere, substituted 4-bromobenzoic acid (1 equiv), benzoic acid (1.1 equiv), K_2CO_3 (2 equiv), $Pd[P(Ph)_3]_4$ (0.1 equiv) were added to dioxane/H_2O (10:1). The mixture was stirred in an 110 ^{o}C oil bath for 5 h. The reaction mixture was cooled to room temperature, and the solvent was removed by rotary evaporation. The residue was dissolved in water, and the solution was acidified with 3 N HCl until the product precipitated. The precipitate was filtered to afford compounds **8a-n**.

4.3. *General procedure for the synthesis of intermediates (**10a-g**)*

L-amino alcohol (1 equiv), $(Boc)_2O$ (1.2 equiv) and trimethylamine (2 equiv) were added to

dichloromethane. The mixture was stirred under ice bath conditions. The reaction was followed by TLC and diluted with dichloromethane. The organic phase was washed with citric acid (1 N) and saturated $NaHCO_3$ then dried over Na_2SO_4 and filtered. The filtrate was concentrated and purified by a silica column to afford compounds **10a-g**.

4.3.1. *Tert-butyl ((2S,3S)-1-hydroxy-3-methylpentan-2-yl)carbamate (**10e**)*

Light yellow oil; yield: 91%; ^{1}H NMR (400 MHz, DMSO) δ 6.36 (d, *J* = 8.6 Hz, 1H), 4.38 (t, *J* = 5.4 Hz, 1H), 3.43 – 3.28 (m, 3H), 1.55 – 1.48 (m, 1H), 1.41 – 1.36 (m, 10H), 1.09 – 1.00 (m, 1H), 0.85 – 0.78 (m, 6H).

4.4. *General procedure for the synthesis of intermediates (**11a-g**)*

To a solution of intermediates **10a-g** (1 equiv) in dichloromethane, TsCl (1.2 equiv), DMAP (0.2 equiv) and trimethylamine (3 equiv) were added. The solution was stirred at -20 ^{o}C. The reaction was followed by TLC and quenched with water. The organic phase was washed with citric acid (1 N) and saturated $NaHCO_3$ then dried over Na_2SO_4 and filtered. The filtrate was concentrated and purified by a silica column to afford compounds **11a-g** as a white solid.

4.4.1. *(S)-2-((tert-butoxycarbonyl)amino)propyl 4-methylbenzenesulfonate (**11a**)*

Light white solid; yield 85.1%; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 7.26 (s, 1H), 4.03 – 3.88 (m, 3H), 2.45 (s, 3H), 1.40 (s, 9H), 1.16 (d, J = 6.8 Hz, 3H).

4.5. *General procedure for the synthesis of intermediates (**12a-g**)*

Under an argon atmosphere, the imidazole (2 equiv) and NaH (3 equiv) were added to dry DMF at 0 ^{o}C. The mixture was stirred for 1 h. Then, intermediates **11a-g** (1 equiv) was added and stirred at room temperature for 3 - 4 h, controlled by TLC. Upon completion of the reaction, the reaction was quenched with water and extracted with ethyl acetate three times. The combined organic layers were dried over Na_2SO_4 and evaporated in a vacuum. The crude product was purified by silica gel column chromatography (CH_3OH:CH_2Cl_2 = 40:1).

4.5.1. *tert-butyl ((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)carbamate (**12e**)*

Light white solid; yield 35.3%; ^{1}H NMR (400 MHz, DMSO) δ 7.08 (s, 1H), 6.83 (m, 2H), 4.07 (dd, *J* = 13.9, 3.8 Hz, 1H), 3.82 (dd, *J* = 13.8, 10.3 Hz, 1H), 3.56 (m, 1H), 1.48 – 1.42 (m, 2H), 1.30 (s, 9H), 1.14 – 1.07 (m, 1H), 0.90 – 0.85 (m, 6H).

4.6. *General procedure for the synthesis of intermediates (**13a-g**)*

Imidazole derivatives **12a-g** were dissolved in a solution of HCl-EtOH (4 N). The mixture was stirred at room temperature. The reaction was monitored with TLC. The mixture was filtered to afford compounds **13a-g** as a white solid.

4.7. *General procedure for the synthesis of compounds (**14a-ac**)*

To a solution of acid **8a-n** (1 equiv) in DMF, HOBt (1.1 equiv), EDCI (1.1 equiv) was added and the mixture was stirred for 1 h at room temperature. Then, the amino salt **13a-g** (1.1 equiv) and DIEA (2.2 equiv) were added, and the reaction was stirred for 4 h and monitored with TLC. The reaction was poured into water and extracted with ethyl acetate three times. The combined organic layers were dried over Na_2SO_4 and evaporated in vacuum. The residue was purified by silica gel column chromatography (CH_3OH:CH_2Cl_2 = 40:1) to afford the target compounds **14a-ac**.

4.7.1. *(S)-N-(1-(1H-imidazol-1-yl)propan-2-yl)-[1,1'-biphenyl]-4-carboxamide (**14a**)*

Light white solid; yield: 59.0%; mp: 237.1 – 239.6 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.43 (d, *J* = 8.1 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 2H), 7.76 (d, *J* = 8.3 Hz, 2H), 7.73 (d, *J* = 7.4 Hz, 2H), 7.60 (s, 1H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.3 Hz, 1H), 7.15 (s, 1H), 6.85 (s, 1H), 4.34 – 4.30 (m, 1H), 4.15 – 4.08 (m, 2H), 1.16 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 166.04, 143.22, 139.66, 138.10, 133.67, 129.50 (2C), 128.61, 128.52, 128.37 (2C), 127.35 (2C), 126.94 (2C), 120.36, 50.62, 46.71, 18.46. HRMS (EI): *m/z* (M^+) for $C_{19}H_{19}N_3O$: calcd. 305.1528; found 305.1531.

4.7.2. *(S)-N-(1-(1H-imidazol-1-yl)butan-2-yl)-[1,1'-biphenyl]-4-carboxamide (**14b**)*

Light white solid; yield: 57.2%; mp: 195.8 –197.9 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.31 (d, *J* = 8.2 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.74 – 7.70 (m, 2H), 7.57 (s, 1H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.13 (s, 1H), 6.83 (s, 1H), 4.17 (dd, *J* = 15.9, 5.2 Hz, 2H), 4.08 (d, *J* = 5.1 Hz, 1H), 1.60 – 1.56 (m, 1H), 1.53 – 1.49 (m, 1H), 0.92 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 166.43, 143.18, 139.71, 138.08, 133.71, 129.50 (2C), 128.53, 128.49, 128.40 (2C), 127.35 (2C), 126.93 (2C), 120.35, 52.30, 49.54, 25.38, 10.86. HRMS (EI): *m/z* (M^+) for $C_{20}H_{21}N_3O$: calcd. 319.1685; found 319.1687.

4.7.3. *(S)-N-(1-(1H-imidazol-1-yl)pentan-2-yl)-[1,1'-biphenyl]-4-carboxamide (**14c**)*

Light white solid; yield: 51.6%; mp: 185.0 – 186.6 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.32 (d, *J* = 8.5 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.74 – 7.70 (m, 2H), 7.58 (s,

1H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.13 (s, 1H), 6.84 (s, 1H), 4.29 – 4.24 (m, 1H), 4.16 (dd, *J* = 13.9, 4.7 Hz, 1H), 4.07 (dd, *J* = 13.9, 8.6 Hz, 1H), 1.54 – 1.47 (m, 2H), 1.43 – 1.37 (m, 1H), 1.35 – 1.29 (m, 1H), 0.89 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 166.36, 143.22, 139.70, 138.05, 133.71, 129.50 (2C), 128.54, 128.51, 128.34 (2C), 127.36 (2C), 126.96 (2C), 120.37, 50.44, 49.95, 34.31, 19.19, 14.32. HRMS (EI): *m/z* (M^+) for $C_{21}H_{23}N_3O$: calcd. 333.1841; found 333.1840.

4.7.4. *(S)-N-(1-(1H-imidazol-1-yl)-3-methylbutan-2-yl)-[1,1'-biphenyl]-4-carboxamide (**14d**)*

Light white solid; yield: 43.0%; mp: 177.4 – 179.5 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.26 (d, *J* = 8.8 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.73 – 7.70 (m, 2H), 7.59 (s, 1H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.15 (s, 1H), 6.80 (s, 1H), 4.23 (dd, *J* = 13.2, 3.2 Hz, 1H), 4.16 – 4.13 (m, 1H), 4.12 – 4.09 (m, 1H), 1.87 (td, *J* = 13.4, 6.7 Hz, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 166.55, 143.19, 139.73, 138.07, 133.82, 129.49 (2C), 128.49 (2C), 128.33 (2C), 127.36 (2C), 126.95 (2C), 120.27, 55.86, 47.80, 31.01, 19.93, 18.89. HRMS (EI): *m/z* (M^+) for $C_{21}H_{23}N_3O$: calcd. 333.1841; found 333.1840.

4.7.5. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-[1,1'-biphenyl]-4-carboxamide (**14e**)*

Light white solid; yield: 60.9%; mp: 203.2 – 205.7 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.31 (d, *J* = 8.8 Hz, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.73 – 7.70 (m, 2H), 7.62 (s, 1H), 7.49 (t, *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.16 (s, 1H), 6.80 (s, 1H), 4.25 (dd, *J* = 13.5, 3.3 Hz, 1H), 4.20 – 4.16 (m, 1H), 4.10 (dd, *J* = 13.5, 10.3 Hz, 1H), 1.67 – 1.62 (m, 1H), 1.58 – 1.53 (m, 1H), 1.22 – 1.17 (m, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 166.37, 143.19, 139.73, 138.18, 133.78, 129.49 (2C), 128.49, 128.37, 128.32 (2C), 127.36 (2C), 126.95 (2C), 120.39, 54.93, 47.41, 37.50, 25.54, 15.81, 11.77. HRMS (EI): *m/z* (M^+) for $C_{22}H_{25}N_3O$: calcd. 347.1998; found 347.2004.

4.7.6. *(S)-N-(1-(1H-imidazol-1-yl)-4-methylpentan-2-yl)-[1,1'-biphenyl]-4-carboxamide (**14f**)*

Light white solid; yield: 61.1%. ^{1}H NMR (600 MHz, DMSO) δ 8.30 (d, *J* = 8.7 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.74 – 7.71 (m, 2H), 7.56 (s, 1H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.12 (s, 1H), 6.83 (s, 1H), 4.35 – 4.31 (m, 1H), 4.14 (dd, *J* = 13.9, 4.9 Hz, 1H), 4.04 (dd, *J* = 13.9, 8.4 Hz, 1H), 1.66 – 1.62 (m, 1H), 1.55 – 1.51 (m, 1H), 1.27 – 1.24

(m, 1H), 0.92 – 0.87 (m, 6H). ^{13}C NMR (150 MHz, DMSO) δ 166.25, 143.22, 139.71, 138.06, 133.69, 129.50 (2C), 128.52, 128.51, 128.34 (2C), 127.36 (2C), 126.96 (2C), 120.38, 50.39, 48.85, 41.14, 24.81, 23.80, 22.20. HRMS (EI): *m/z* (M^+) for $C_{22}H_{25}N_3O$: calcd. 347.1998; found 347.2008.

4.7.7. *(S)-N-(1-(1H-imidazol-1-yl)-3-phenylpropan-2-yl)-[1,1'-biphenyl]-4-carboxamide (**14g**)*

Light white solid; yield: 55.7%; mp: 204.9 – 206.5 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.41 (d, *J* = 8.6 Hz, 1H), 7.78 (d, *J* = 8.3 Hz, 2H), 7.74 – 7.69 (m, 4H), 7.58 (s, 1H), 7.49 (t, *J* = 7.7 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 1H), 7.29 – 7.24 (m, 4H), 7.20 – 7.16 (m, 1H), 7.15 (s, 1H), 6.84 (s, 1H), 4.52 – 4.48 (m, 1H), 4.24 (dd, *J* = 13.9, 4.5 Hz, 1H), 4.15 (dd, *J* = 13.9, 8.7 Hz, 1H), 2.91 – 2.86 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 166.24, 143.22, 139.69, 138.72, 138.11, 133.67, 129.60 (2C), 129.48 (2C), 128.67 (2C), 128.49, 128.23 (2C), 127.34 (2C), 126.93 (2C), 126.68, 120.37, 52.23, 49.81, 37.99. HRMS (EI): *m/z* (M^+) for $C_{25}H_{23}N_3O$: calcd. 381.1841; found 381.1863.

4.7.8. *(S)-N-(1-(1H-imidazol-1-yl)-3-methylbutan-2-yl)-2-fluoro-[1,1'-biphenyl]-4-carboxamide (**14h**)*

Light white solid; yield: 52.3%; mp: 160.4 – 162.7 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.34 (d, *J* = 8.8 Hz, 1H), 7.74 – 7.67 (m, 2H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.61 – 7.55 (m, 3H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.45 (t, *J* = 7.3 Hz, 1H), 7.16 (s, 1H), 6.81 (s, 1H), 4.25 (dd, *J* = 13.5, 3.3 Hz, 1H), 4.18 – 4.13 (m, 1H), 4.09 (dd, *J* = 13.4, 10.3 Hz, 1H), 1.90 – 1.84 (m, 1H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 165.21, 159.97, 158.33, 138.09, 136.01, 135.96, 134.79, 131.37, 131.28, 131.26, 131.23, 129.32, 129.31, 129.18 (2C), 128.83, 128.52, 124.19, 124.17, 120.27, 115.39, 115.22, 56.04, 47.81, 30.95, 19.93, 18.85. HRMS (EI): *m/z* (M^+) for $C_{21}H_{22}FN_3O$: calcd. 351.1747; found 351.1746.

4.7.9. *(S)-N-(1-(1H-imidazol-1-yl)-3-methylbutan-2-yl)-3-fluoro-[1,1'-biphenyl]-4-carboxamide (**14i**)*

Light white solid; yield: 56.8%; mp: 161.2 – 163.0 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.25 (d, *J* = 9.1 Hz, 1H), 7.77 – 7.71 (m, 2H), 7.63 – 7.56 (m, 3H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.16 (s, 1H), 6.86 (s, 1H), 4.21 (dd, *J* = 13.8, 3.9 Hz, 1H), 4.16 – 4.11 (m, 1H), 4.02 (dd, *J* = 13.7, 10.1 Hz, 1H), 1.84 (dd, *J* = 13.1, 6.6 Hz, 1H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 163.77, 160.07, 158.43, 144.05, 143.99, 138.03, 137.66, 130.18, 130.15, 129.10 (2C), 128.52, 128.08, 126.92 (2C), 123.38, 123.28, 122.49, 122.48, 119.78,

114.08, 113.92, 55.32, 47.44, 30.25, 19.52, 18.01. HRMS (EI): *m/z* (M^+) for $C_{21}H_{22}FN_3O$: calcd. 351.1747; found 351.1755.

4.7.10. *(S)-N-(1-(1H-imidazol-1-yl)-3-methylbutan-2-yl)-2'-fluoro-[1,1'-biphenyl]-4-carboxamide* ***(14j)***

Light white solid; yield: 51.9%; mp: 159.8 – 161.4 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.29 (d, *J* = 8.8 Hz, 1H), 7.86 (d, *J* = 8.3 Hz, 2H), 7.66 – 7.59 (m, 3H), 7.57 (td, *J* = 7.8, 1.4 Hz, 1H), 7.46 (ddd, *J* = 9.2, 7.2, 1.6 Hz, 1H), 7.37 – 7.31 (m, 2H), 7.16 (s, 1H), 6.81 (s, 1H), 4.24 (dd, *J* = 13.3, 3.2 Hz, 1H), 4.18 – 4.13 (m, 1H), 4.10 (dd, *J* = 13.3, 10.3 Hz, 1H), 1.90 – 1.84 (m, 1H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 166.53, 160.36, 158.73, 138.17, 138.06, 134.31, 131.30, 131.28, 130.62, 130.56, 129.15, 129.13, 128.42, 127.99, 127.95 (2C), 127.90, 125.53, 125.51, 120.30, 116.74, 116.59, 55.88, 47.85, 30.99, 19.94, 18.87. HRMS (EI): *m/z* (M^+) for $C_{21}H_{22}FN_3O$: calcd. 351.1747; found 351.1753.

4.7.11. *(S)-N-(1-(1H-imidazol-1-yl)-3-methylbutan-2-yl)-3'-fluoro-[1,1'-biphenyl]-4-carboxamide* ***(14k)***

Light white solid; yield: 50.9%; mp: 161.2 – 163.5 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.29 (d, *J* = 8.8 Hz, 1H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.80 (d, *J* = 8.3 Hz, 2H), 7.62 – 7.56 (m, 3H), 7.56 – 7.51 (m, 1H), 7.25 (td, *J* = 8.3, 1.8 Hz, 1H), 7.15 (s, 1H), 6.81 (s, 1H), 4.24 (dd, *J* = 13.3, 3.2 Hz, 1H), 4.18 – 4.14 (m, 1H), 4.10 (dd, *J* = 13.2, 10.3 Hz, 1H), 1.87 (dt, *J* = 13.3, 6.7 Hz, 1H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 166.42, 163.98, 162.37, 142.17, 142.11, 141.70, 141.69, 138.07, 134.36, 131.47, 131.41, 128.50, 128.35 (2C), 127.13 (2C), 123.45, 123.43, 120.26, 115.28, 115.14, 114.17, 114.03, 55.89, 47.80, 31.00, 19.93, 18.90. HRMS (EI): *m/z* (M^+) for $C_{21}H_{22}FN_3O$: calcd. 351.1747; found 351.1747.

4.7.12. *(S)-N-(1-(1H-imidazol-1-yl)-3-methylbutan-2-yl)-4'-fluoro-[1,1'-biphenyl]-4-carboxamide* ***(14l)***

Light white solid; yield: 55.7%; mp: 198.4 – 200.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.27 (d, *J* = 8.8 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 2H), 7.79 – 7.75 (m, 2H), 7.74 (d, *J* = 8.4 Hz, 2H), 7.59 (s, 1H), 7.33 (t, *J* = 8.9 Hz, 2H), 7.15 (s, 1H), 6.80 (s, 1H), 4.24 (dd, *J* = 13.2, 3.1 Hz, 1H), 4.18 – 4.13 (m, 1H), 4.10 (dd, *J* = 13.2, 10.3 Hz, 1H), 1.87 (dq, *J* = 13.3, 6.7 Hz, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 166.49, 163.49, 161.86, 142.12, 138.08, 136.20, 136.18, 133.77, 129.45, 129.39, 128.50, 128.34 (2C), 126.90 (2C), 120.26, 116.38,

116.24, 55.86, 47.80, 31.00, 19.93, 18.89. HRMS (EI): *m/z* (M^+) for $C_{21}H_{22}FN_3O$: calcd. 351.1747; found 351.1752.

4.7.13. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-2-fluoro-[1,1'-biphenyl]-4-carboxamide (**14m**)*

Light white solid; yield: 53.6%; mp: 153.8 – 155.7 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.38 (d, *J* = 8.8 Hz, 1H), 7.73 – 7.66 (m, 2H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.61 – 7.56 (m, 3H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.45 (t, *J* = 7.3 Hz, 1H), 7.15 (s, 1H), 6.81 (s, 1H), 4.25 (dd, *J* = 13.7, 3.4 Hz, 1H), 4.20 – 4.15 (m, 1H), 4.09 (dd, *J* = 13.6, 10.3 Hz, 1H), 1.67 – 1.62 (m, 1H), 1.58 – 1.53 (m, 1H), 1.22 – 1.18 (m, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 163.93, 158.89, 157.26, 137.06, 134.90, 134.85, 133.71, 130.30, 130.21, 130.18, 130.16, 128.25, 128.23, 128.10 (2C), 127.76, 127.45, 123.10, 123.08, 119.24, 114.30, 114.14, 54.03, 46.28, 36.34, 24.43, 14.74, 10.66. HRMS (EI): *m/z* (M^+) for $C_{22}H_{24}FN_3O$: calcd. 365.1903; found 365.1915.

4.7.14. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-3-fluoro-[1,1'-biphenyl]-4-carboxamide (14**n**)*

Light white solid; yield: 53.0%; mp: 169.8 – 171.7 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.28 (d, *J* = 9.0 Hz, 1H), 7.73 (d, *J* = 7.4 Hz, 2H), 7.62 – 7.56 (m, 3H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.43 (td, *J* = 7.6, 2.3 Hz, 2H), 7.16 (s, 1H), 6.85 (s, 1H), 4.22 (dd, *J* = 13.8, 3.6 Hz, 1H), 4.18 – 4.14 (m, 1H), 4.02 (dd, *J* = 13.8, 10.1 Hz, 1H), 1.62 – 1.55 (m, 2H), 1.23 – 1.18 (m, 1H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 164.05, 160.55, 158.90, 144.53, 144.47, 138.49, 138.16, 130.66, 130.63, 129.56, 128.99, 128.54, 127.38, 123.78, 123.68, 122.95, 120.30, 114.54, 114.39, 54.93, 47.45, 37.24, 25.19, 15.84, 11.71. HRMS (EI): *m/z* (M^+) for $C_{22}H_{24}FN_3O$: calcd. 365.1903; found 365.1917.

4.7.15. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-2'-fluoro-[1,1'-biphenyl]-4-carboxamide (**14o**)*

Light white solid; yield: 52.5%; mp: 172.8 – 174.5 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.34 (d, *J* = 8.8 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 7.0 Hz, 2H), 7.60 (s, 1H), 7.58 – 7.55 (m, 1H), 7.48 – 7.44 (m, 1H), 7.37 – 7.31 (m, 2H), 7.15 (s, 1H), 6.81 (s, 1H), 4.24 (dd, *J* = 13.6, 3.3 Hz, 1H), 4.21 – 4.16 (m, 1H), 4.10 (dd, *J* = 13.5, 10.3 Hz, 1H), 1.67 – 1.62 (m, 1H), 1.59 – 1.53 (m, 1H), 1.22 – 1.17 (m, 1H), 1.02 – 0.96 (m, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 166.32, 160.36, 158.72, 138.17, 138.12, 134.27, 131.30, 131.28, 130.61, 130.56, 129.15,

129.13, 128.51, 127.99, 127.94 (2C), 127.90, 125.53, 125.50, 120.31, 116.73, 116.59, 54.98, 47.37, 37.48, 25.52, 15.82, 11.77. HRMS (EI): *m/z* (M^+) for $C_{22}H_{24}FN_3O$: calcd. 365.1903; found 365.1907.

4.7.16. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-3'-fluoro-[1,1'-biphenyl]-4-carbox amide (**14p**)*

Light white solid; yield: 69.8%; mp: 170.0 – 172.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.33 (d, *J* = 8.8 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 2H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.62 (s, 1H), 7.60 – 7.57 (m, 2H), 7.55 – 7.51 (m, 1H), 7.25 (td, *J* = 8.0, 1.5 Hz, 1H), 7.16 (s, 1H), 6.81 (s, 1H), 4.25 (dd, *J* = 13.5, 3.3 Hz, 1H), 4.20 – 4.16 (m, 1H), 4.11 (dd, *J* = 13.5, 10.3 Hz, 1H), 1.67 – 1.62 (m, 1H), 1.58 – 1.53 (m, 1H), 1.22 – 1.18 (m, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 166.22, 163.98, 162.37, 142.16, 142.11, 141.71, 138.06, 134.30, 131.47, 131.41, 128.34 (3C), 127.13 (2C), 123.45, 123.43, 120.36, 115.28, 115.14, 114.17, 114.02, 54.96, 47.40, 37.47, 25.54, 15.82, 11.76. HRMS (EI): *m/z* (M^+) for $C_{22}H_{24}FN_3O$: calcd. 365.1903; found 365.1904.

4.7.17. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-4'-fluoro-[1,1'-biphenyl]-4-carbox amide (**14q**)*

Light white solid; yield: 48.6%; mp: 187.5 – 189.4 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.29 (d, *J* = 8.4 Hz, 1H), 7.85 (d, *J* = 8.5 Hz, 2H), 7.79 – 7.71 (m, 4H), 7.60 (s, 1H), 7.32 (t, *J* = 8.9 Hz, 2H), 7.14 (s, 1H), 6.80 (s, 1H), 4.24 (dd, *J* = 12.8, 2.9 Hz, 1H), 4.20 – 4.14 (m, 1H), 4.10 (dd, *J* = 12.6, 9.8 Hz, 1H), 1.68 – 1.51 (m, 2H), 1.24 – 1.17 (m, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 166.30, 163.89, 161.46, 142.12, 138.07, 136.21, 136.18, 133.76, 129.45, 129.37, 128.43, 128.32 (2C), 126.89 (2C), 120.31, 116.41, 116.19, 54.95, 47.39, 37.48, 25.54, 15.82, 11.75. HRMS (EI): *m/z* (M^+) for $C_{22}H_{24}FN_3O$: calcd. 365.1903; found 365.1906.

4.7.18. *(S)-N-(1-(1H-imidazol-1-yl)-4-methylpentan-2-yl)-2-fluoro-[1,1'-biphenyl]-4-carboxamid e (**14r**)*

Light white solid; yield: 46.8%; mp: 150.6 – 152.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.38 (d, *J* = 8.6 Hz, 1H), 7.75 – 7.69 (m, 2H), 7.64 (t, *J* = 8.0 Hz, 1H), 7.61 – 7.56 (m, 3H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.45 (t, *J* = 7.4 Hz, 1H), 7.13 (s, 1H), 6.84 (s, 1H), 4.36 – 4.31 (m, 1H), 4.15 (dd, *J* = 13.9, 4.8 Hz, 1H), 4.03 (dd, *J* = 13.9, 8.4 Hz, 1H), 1.67 – 1.62 (m, 1H), 1.55 – 1.50 (m, 1H), 1.30

– 1.26 (m, 1H), 0.90 (d, *J* = 6.7 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 164.91, 164.90, 159.99, 158.36, 138.10, 135.91, 135.87, 134.78, 131.40, 131.31, 131.27, 131.25, 129.32, 129.30, 129.18 (2C), 128.84, 128.60, 124.18, 124.16, 120.40, 115.41, 115.25, 50.34, 49.03, 41.02, 24.80, 23.78, 22.15. HRMS (EI): *m/z* (M^+) for $C_{22}H_{24}FN_3O$: calcd. 365.1903; found 365.1910.

4.7.19. *(S)-N-(1-(1H-imidazol-1-yl)-4-methylpentan-2-yl)-3-fluoro-[1,1'-biphenyl]-4-carboxamide* ***(14s)***

Light white solid; yield: 50.2%; mp: 137.3 – 139.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.25 (d, *J* = 8.6 Hz, 1H), 7.77 – 7.71 (m, 2H), 7.64 – 7.55 (m, 3H), 7.53 – 7.47 (m, 3H), 7.45 – 7.41 (m, 1H), 7.15 (s, 1H), 6.87 (s, 1H), 4.33 – 4.27 (m, 1H), 4.14 (dd, *J* = 13.9, 4.8 Hz, 1H), 4.01 (dd, *J* = 13.9, 8.3 Hz, 1H), 1.73 – 1.67 (m, 1H), 1.49 – 1.44 (m, 1H), 1.27 – 1.24 (m, 1H), 0.90 (t, *J* = 6.4 Hz, 6H). ^{13}C NMR (150 MHz, DMSO) δ 163.98, 160.61, 158.96, 144.60, 144.55, 138.45, 138.13, 130.77, 130.75, 129.57 (2C), 129.01, 128.61, 127.39 (2C), 123.62, 123.51, 122.95, 122.94, 120.38, 114.56, 114.41, 50.27, 48.91, 40.95, 24.73, 23.79, 22.11. HRMS (EI): *m/z* (M^+) for $C_{22}H_{24}FN_3O$: calcd. 365.1903; found 365.1908.

4.7.20. *(S)-N-(1-(1H-imidazol-1-yl)-3-phenylpropan-2-yl)-2-fluoro-[1,1'-biphenyl]-4-carboxamide* ***(14t)***

Light white solid; yield: 59.4%; mp: 173.5 – 175.4 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.48 (d, *J* = 8.5 Hz, 1H), 7.66 – 7.54 (m, 6H), 7.50 (t, *J* = 7.5 Hz, 2H), 7.44 (t, *J* = 7.3 Hz, 1H), 7.31 – 7.23 (m, 4H), 7.20 – 7.17 (m, 1H), 7.16 (s, 1H), 6.85 (s, 1H), 4.53 – 4.48 (m, 1H), 4.25 (dd, *J* = 13.9, 4.3 Hz, 1H), 4.14 (dd, *J* = 13.9, 8.8 Hz, 1H), 2.92 (dd, *J* = 13.8, 4.6 Hz, 1H), 2.86 (dd, *J* = 13.7, 9.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 164.89, 160.34, 157.89, 138.61, 138.12, 135.89, 135.82, 134.75, 131.43, 131.26, 131.23, 129.58, 129.31, 129.28, 129.16, 128.83, 128.69, 128.58, 126.72, 124.07, 124.04, 120.41, 115.33, 115.08, 52.36, 49.82, 37.92. HRMS (EI): *m/z* (M^+) for $C_{25}H_{22}FN_3O$: calcd. 399.1747; found 399.1756.

4.7.21. *(S)-N-(1-(1H-imidazol-1-yl)-3-phenylpropan-2-yl)-3-fluoro-[1,1'-biphenyl]-4-carboxamide* ***(14u)***

Light white solid; yield: 53.9%; mp: 188.9 – 190.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.35 (d, *J* = 8.6 Hz, 1H), 7.73 (d, *J* = 7.9 Hz, 2H), 7.61 (s, 1H), 7.59 (d, *J* = 11.8 Hz, 1H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.49 (t, *J* = 7.5 Hz, 2H), 7.43 (t, *J* = 7.3 Hz, 1H), 7.37 (t, *J* = 7.7 Hz, 1H), 7.32 – 7.23 (m,

4H), 7.21 (t, *J* = 6.9 Hz, 1H), 7.18 (s, 1H), 6.89 (s, 1H), 4.50 – 4.46 (m, 1H), 4.25 (dd, *J* = 13.9, 4.3 Hz, 1H), 4.15 – 4.11 (m, 1H), 2.90 (dd, *J* = 13.8, 4.5 Hz, 1H), 2.81 (dd, *J* = 13.7, 9.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 163.87, 161.02, 158.55, 144.68, 144.60, 138.52, 138.42, 138.11, 130.73, 130.69, 129.63, 129.55, 129.00, 128.65, 128.49, 127.37, 126.73, 123.34, 123.19, 122.87, 122.85, 120.41, 114.59, 114.36, 52.31, 49.77, 37.90. HRMS (EI): *m/z* (M^+) for $C_{25}H_{22}FN_3O$: calcd. 399.1747; found 399.1759.

4.7.22. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-2',3-difluoro-[1,1'-biphenyl]-4-car boxamide (**14v**)*

Light white solid; yield: 51.5%; mp: 123.0 – 125.0 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.32 (d, *J* = 8.8 Hz, 1H), 7.61 – 7.56 (m, 2H), 7.50 – 7.45 (m, 2H), 7.45 – 7.41 (m, 2H), 7.38 – 7.31 (m, 2H), 7.16 (s, 1H), 6.86 (s, 1H), 4.23 (dd, *J* = 13.4, 3.6 Hz, 1H), 4.19 – 4.13 (m, 1H), 4.02 (dd, *J* = 13.3, 9.8 Hz, 1H), 1.63 – 1.53 (m, 2H), 1.24 – 1.18 (m, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 162.90, 159.21, 158.84, 157.58, 157.19, 138.15, 138.09, 137.06, 130.17, 130.16, 130.00, 129.95, 129.15, 129.12, 127.39, 125.76, 125.68, 124.52, 124.50, 124.22, 123.37, 123.27, 119.25, 115.74, 115.59, 53.85, 46.41, 36.14, 24.08, 14.76, 10.62. HRMS (EI): *m/z* (M^+) for $C_{22}H_{23}F_2N_3O$: calcd. 383.1809; found 383.1812.

4.7.23. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-3',3-difluoro-[1,1'-biphenyl]-4-car boxamide (**14w**)*

Light white solid; yield: 62.0%; mp: 130.1 – 132.2 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.28 (d, *J* = 8.8 Hz, 1H), 7.69 – 7.52 (m, 6H), 7.42 (t, *J* = 7.8 Hz, 1H), 7.29 – 7.23 (m, 1H), 7.15 (s, 1H), 6.85 (s, 1H), 4.23 (dd, *J* = 13.4, 3.5 Hz, 1H), 4.19 – 4.12 (m, 1H), 4.02 (dd, *J* = 13.3, 9.8 Hz, 1H), 1.64 – 1.53 (m, 2H), 1.24 – 1.19 (m, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 164.37, 163.95, 161.95, 160.91, 158.44, 142.99, 142.91, 140.90, 138.15, 131.55, 131.47, 130.66, 130.63, 128.52, 124.42, 124.26, 123.49, 123.12, 123.09, 120.28, 115.81, 115.60, 114.86, 114.63, 114.32, 114.10, 54.94, 47.47, 37.23, 25.19, 15.84, 11.69. HRMS (EI): *m/z* (M^+) for $C_{22}H_{23}F_2N_3O$: calcd. 383.1809; found 383.1811.

4.7.24. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-4',3-difluoro-[1,1'-biphenyl]-4-car boxamide (**14x**)*

Light white solid; yield: 61.5%; mp: 132.4 – 134.8 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.25 (d, *J* = 8.7 Hz, 1H), 7.82 – 7.75 (m, 2H), 7.63 – 7.52 (m, 3H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.33 (t, *J* = 8.9

Hz, 2H), 7.17 (s, 1H), 6.87 (s, 1H), 4.23 (dd, *J* = 13.4, 3.5 Hz, 1H), 4.19 – 4.12 (m, 1H), 4.03 (dd, *J* = 13.3, 9.8 Hz, 1H), 1.64 – 1.52 (m, 2H), 1.24 – 1.18 (m, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 164.15, 164.01, 161.70, 160.95, 158.49, 143.46, 143.38, 138.05, 134.96, 130.69, 130.65, 129.56, 129.47, 128.30, 123.73, 123.58, 122.89, 122.86, 120.41, 116.49, 116.27, 114.56, 114.33, 54.90, 47.57, 37.25, 25.20, 15.84, 11.68. HRMS (EI): *m/z* (M^+) for $C_{22}H_{23}F_2N_3O$: calcd. 383.1809; found 383.1798.

4.7.25. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-2-chloro-[1,1'-biphenyl]-4-carbox amide (**14y**)*

Light white solid; yield: 73.7%; mp: 189.1 – 191.0 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.40 (d, *J* = 8.6 Hz, 1H), 7.93 (d, *J* = 1.7 Hz, 1H), 7.80 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.58 (s, 1H), 7.52 – 7.43 (m, 6H), 7.14 (s, 1H), 6.81 (s, 1H), 4.25 (dd, *J* = 13.1, 3.1 Hz, 1H), 4.20 – 4.14 (m, 1H), 4.09 (dd, *J* = 13.1, 10.0 Hz, 1H), 1.69 – 1.62 (m, 1H), 1.59 – 1.51 (m, 1H), 1.24 – 1.17 (m, 1H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 164.97, 142.76, 138.49, 138.11, 135.49, 131.95, 131.79, 129.57 (2C), 128.82, 128.77 (2C), 128.61, 128.54, 126.76, 120.29, 55.11, 47.40, 37.38, 25.49, 15.84, 11.70. HRMS (EI): *m/z* (M^+) for $C_{22}H_{24}ClN_3O$: calcd. 381.1608; found 381.1608.

4.7.26. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-3-chloro-[1,1'-biphenyl]-4-carbox amide (**14z**)*

Light white solid; yield: 65.0%; mp: 178.3 – 180.9 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.41 (d, *J* = 8.8 Hz, 1H), 7.75 (d, *J* = 1.5 Hz, 1H), 7.74 – 7.63 (m, 3H), 7.60 (s, 1H), 7.49 (t, *J* = 7.4 Hz, 2H), 7.42 (t, *J* = 7.3 Hz, 1H), 7.23 – 7.11 (m, 2H), 6.90 (s, 1H), 4.25 – 4.12 (m, 2H), 3.97 (dd, *J* = 13.3, 9.8 Hz, 1H), 1.66 – 1.55 (m, 2H), 1.27 – 1.22 (m, 1H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.91 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 166.51, 142.97, 138.53, 138.26, 136.46, 130.79, 129.57 (2C), 129.43, 128.84, 128.59, 127.83, 127.37 (2C), 125.75, 120.35, 54.64, 47.52, 37.33, 25.15, 15.90, 11.72. HRMS (EI): *m/z* (M^+) for $C_{22}H_{24}ClN_3O$: calcd. 381.1608; found 381.1616.

4.7.27. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-2-methyl-[1,1'-biphenyl]-4-carbox amide (**14aa**)*

Light white solid; yield: 55.7%; mp: 181.2 – 183.5 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.23 (d, *J* = 8.4 Hz, 1H), 7.69 (s, 1H), 7.67 – 7.63 (m, 1H), 7.58 (s, 1H), 7.49 – 7.44 (m, 2H), 7.41 – 7.33 (m, 3H), 7.27 (d, *J* = 7.9 Hz, 1H), 7.14 (s, 1H), 6.80 (s, 1H), 4.24 (dd, *J* = 12.8, 2.9 Hz, 1H), 4.20

– 4.14 (m, 1H), 4.09 (dd, *J* = 12.7, 9.8 Hz, 1H), 2.26 (s, 3H), 1.69 – 1.61 (m, 1H), 1.56 (ddd, *J* = 13.3, 7.4, 4.0 Hz, 1H), 1.24 – 1.17 (m, 1H), 1.02 – 0.97 (m, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 166.47, 144.42, 141.01, 138.09, 135.24, 133.84, 129.88, 129.62, 129.27 (2C), 128.78 (2C), 128.49, 127.76, 125.21, 120.29, 54.89, 47.44, 37.49, 25.51, 20.68, 15.85, 11.74. HRMS (EI): *m/z* (M^+) for $C_{23}H_{27}N_3O$: calcd. 361.2154; found 361.2166.

4.7.28. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-3-methyl-[1,1'-biphenyl]-4-carbox amide (**14ab**)*

Light white solid; yield: 55.5%; mp: 213.0 – 214.4 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.18 (d, *J* = 8.8 Hz, 1H), 7.65 (d, *J* = 7.4 Hz, 2H), 7.58 (s, 1H), 7.51 – 7.44 (m, 4H), 7.38 (t, *J* = 7.3 Hz, 1H), 7.21 – 7.13 (m, 2H), 6.88 (s, 1H), 4.25 – 4.15 (m, 2H), 3.97 (dd, *J* = 14.4, 11.6 Hz, 1H), 2.20 (s, 3H), 1.67 – 1.54 (m, 2H), 1.27 – 1.20 (m, 1H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.92 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 169.29, 141.33, 140.07, 138.20, 136.90, 136.16, 129.42 (2C), 129.06, 128.53, 128.17, 127.78, 127.20 (2C), 124.14, 120.29, 54.23, 47.59, 37.57, 25.31, 19.63, 15.93, 11.74. HRMS (EI): *m/z* (M^+) for $C_{23}H_{27}N_3O$: calcd. 361.2154; found 361.2159.

4.7.29. *N-((2S,3S)-1-(1H-imidazol-1-yl)-3-methylpentan-2-yl)-3-methoxy-[1,1'-biphenyl]-4-carbo xamide (**14ac**)*

Light white solid; yield: 45.6%; mp: 126.3 – 128.2 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.03 (d, *J* = 8.5 Hz, 1H), 7.73 (d, *J* = 7.3 Hz, 2H), 7.62 – 7.56 (m, 2H), 7.49 (t, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.3 Hz, 1H), 7.34 (d, *J* = 1.1 Hz, 1H), 7.29 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.15 (s, 1H), 6.85 (s, 1H), 4.24 – 4.16 (m, 2H), 4.10 (dd, *J* = 14.5, 10.4 Hz, 1H), 3.98 (s, 3H), 1.64 – 1.53 (m, 2H), 1.23 – 1.15 (m, 1H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.91 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 165.43, 157.52, 144.32, 139.92, 138.17, 131.01, 129.41 (2C), 128.54 (2C), 127.48 (2C), 123.19, 120.26, 119.29, 110.81, 56.51, 54.58, 47.50, 37.03, 25.07, 15.90, 11.82. HRMS (EI): *m/z* (M^+) for $C_{23}H_{27}N_3O2$: calcd. 377.2103; found 377.2105.

4.8. *Procedures for the synthesis of compounds **17** and **23***

The synthetic procedures of compounds **17** and **23** were similar to those used for compounds **14a-ac.**

4.8.1. *3-fluoro-N-((2S,3S)-3-methyl-1-(1H-1,2,4-triazol-1-yl)pentan-2-yl)-[1,1'-biphenyl]-4-car boxamide (**17**)*

Light white solid; yield: 58.7%; mp: 173.4 – 175.6 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.41 (s, 1H), 8.28 (d, *J* = 8.3 Hz, 1H), 7.96 (s, 1H), 7.75 – 7.72 (m, 2H), 7.61 – 7.56 (m, 2H), 7.51 – 7.46 (m, 3H), 7.43 (t, *J* = 7.3 Hz, 1H), 4.41 (dd, *J* = 13.3, 3.2 Hz, 1H), 4.32 – 4.26 (m, 2H), 1.65 – 1.61 (m, 1H), 1.57 – 1.53 (m, 1H), 1.23 – 1.19 (m, 1H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO) δ 163.91, 160.59, 158.94, 151.81, 144.97, 144.61, 144.55, 138.46, 130.75, 130.72, 129.57 (2C), 129.01, 127.39 (2C), 123.57, 123.47, 122.95, 122.93, 114.55, 114.39, 53.85, 50.11, 36.73, 25.23, 15.80, 11.58. HRMS (EI): *m/z* (M^+) for $C_{21}H_{23}FN_4O$: calcd. 366.1856; found 366.1855.

4.8.2. *(R)-N-(1-(1H-imidazol-1-yl)-3-methylbutan-2-yl)-[1,1'-biphenyl]-4-carboxamide* ***(23)***

Light white solid; yield: 65.0%; mp: 201.3 – 203.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.26 (d, *J* = 8.7 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.73 – 7.70 (m, 2H), 7.59 (s, 1H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.15 (s, 1H), 6.80 (s, 1H), 4.23 (dd, *J* = 13.0, 2.9 Hz, 1H), 4.17 – 4.13 (m, 1H), 4.10 (dd, *J* = 13.1, 10.3 Hz, 1H), 1.89 – 1.85 (m, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 166.76, 143.22, 139.74, 138.42, 133.78, 129.49 (2C), 128.49, 128.33 (2C), 127.35 (2C), 126.97 (2C), 121.23, 55.62, 48.61, 31.04, 19.87, 18.90. HRMS (EI): *m/z* (M^+) for $C_{21}H_{23}N_3O$: calcd. 333.1841; found 333.1858.

4.9. *In vitro antifungal testing*

Five sensitive pathogenic fungi and two fluconazole-resistant strains of *Candida albicans* were selected to determine the *in vitro* minimum inhibitory concentrations (MIC) according to the protocols of 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. Fluconazole and itraconazole were used as positive control drugs. All of the compounds were dissolved in DMSO and serially diluted into the growth medium.

4.10. *GC-MS analysis of sterol composition*

Candida albicans (ATCC SC5314) was treated with different concentrations of compound **14v** and fluconazole and incubated at 30 °C for ~16 h with continuous agitation (200 rpm). Cells were harvested by centrifugation 3000 g for 10 min. Then, the cells were washed with PBS three times and saponified at 80 °C for 60 min with NaOH in ethanol. The nonsaponifiable sterols were then extracted three times with 6 mL petroleum ether. The combined extracts were evaporated under vacuum, and the residue was dissolved in hexamethylene. The sterols were analyzed by

GC-MS. The GC-MS data were analyzed 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).

4.11. *In vitro human plasma stability assay*

Frozen human plasma was thawed in a water bath at 37 °C prior to experiments. Then, the human plasma was centrifuged at 4000 rpm for 5 min. Compounds and positive control propantheline solutions (100 μM) were prepared by diluting. Blank plasma (98 μL) was mixed with 2 μL of dosing solution (100 μM) to achieve 2 μM of the final concentration in duplicate, and samples were incubated at 37 °C in a water bath. At each time point (0, 10, 30, 60 and 120 min), 400 μL of stop solution (200 ng/mL tolbutamide and 200 ng/mL labetalol in 50% ACN/MeOH) was added to terminate the reactions. The sample plates were centrifuged at 4000 rpm for approximately 10 min. The supernatant (50 μL) was mixed with 100 μL ultrapure water. The samples were shaken at 800 rpm for 10 min. Samples were analyzed by LC/MS/MS. Disappearance of test compounds are assessed base on the peak area ratio of analyte/IS (no standard curve).

4.12. *In vitro cytotoxicity assays*

A549 cells were plated in 96-well microtiter plates at a concentration of 3000 cells per well. The 96-well microtiter plates were allowed to incubate at 37 °C with 5% CO_2 for 16 h. Compounds **14n, 14v** and voriconazole were dissolved in DMSO and diluted with medium to achieve final concentrations of 0.08, 0.4, 2.0, 10 and 50 μM/L. Then, the old medium of the tested well was removed by vacuum suction, and 200 μL of the freshly prepared medium containing the different concentrations of compounds was added. Afterwards, the 96-well microtiter plates were incubated at 37 °C with 5% CO_2 for 24 h. Finally, MTT was added to the 96-well microtiter plates and incubated for another 4 h. Observation of each test well was performed at λ_{490nm}.

Acknowledgements

The authors thank Hongwei Liu from the Institute of Microbiology, Chinese Academy of Sciences, for providing the fluconazole-resistant strains of *Candida albicans*(*strain 17#* and *strain CaR*). The authors thank Prof. Yongbing Cao from School of Pharmacy, the Second Military Medical University, for providing the strains of *Candida albicans*(*SC5314*). This work was

supported by the Program for Innovative Research Team of the Ministry of Education and Program for Liaoning Innovative Research Team in University.

References

[1] E.M. Carmona, A.H. Limper, Overview of Treatment Approaches for Fungal Infections, Clinics in Chest Medicine, 38 (2017) 393-402.

[2] C. Pannuti, R. Gingrich, M.A. Pfaller, C. Kao, R.P. Wenzel, Nosocomial pneumonia in patients having bone marrow transplant. Attributable mortality and risk factors, Cancer, 69 (1992) 2653-2662.

[3] P. Fischer, S. Jungwirth, S. Weissgram, K.H. Tragl, Emerging fungal infections among children: A review on its clinical manifestations, diagnosis, and prevention, Journal of Pharmacy & Bioallied Sciences, 2 (2010) 314-320.

[4] A.H. Groll, J. Lumb, New developments in invasive fungal disease, Future Microbiology, 7 (2012) 179-184.

[5] S. Chunquan, Z. Wannian, J. Haitao, Z. Min, S. Yunlong, X. Hui, Z. Jie, M. Zhenyuan, J. Qingfen, Y. Jianzhong, Structure-based optimization of azole antifungal agents by CoMFA, CoMSIA, and molecular docking, Journal of medicinal chemistry, 49 (2006) 2512.

[6] M.A. Pfaller, D.J. Diekema, Epidemiology of invasive candidiasis: a persistent public health problem, Clin Microbiol Rev, 20 (2007) 133-163.

[7] S. Arikan, J.H. Rex, Nystatin LF (Aronex/Abbott), Curr Opin Investig Drugs, 2 (2001) 488-495.

[8] 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) 787-798.

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

[10] V. Moudgal, J. Sobel, Antifungals to treat Candida albicans, Expert Opinion on Pharmacotherapy, 11 (2010) 2037.

[11] B.H. Vanden, L. Koymans, H. Moereels, P450 inhibitors of use in medical treatment: focus on mechanisms of action, Pharmacology & Therapeutics, 67 (1995) 79-100.

[12] S. Emami, P. Tavangar, M. Keighobadi, An overview of azoles targeting sterol 14α-demethylase for antileishmanial therapy, European journal of medicinal chemistry, 135 (2017) 241.

[13] J.C.R. Corrêa, H.R.N. Salgado, Review of Fluconazole Properties and Analytical Methods for Its Determination, Critical Reviews in Analytical Chemistry, 41 (2011) 124-132.

[14] P. Kale, L.B. Johnson, Second-generation azole antifungal agents, Drugs Today, 41 (2005) 91-105.

[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, Bioorganic & medicinal chemistry, 25 (2017) 750-758.

[16] S. Zhao, P. Wei, M. Wu, X. Zhang, L. Zhao, X. Jiang, C. Hao, X. Su, D. Zhao, M. Cheng, Design, synthesis and evaluation of benzoheterocycle analogues as potent antifungal agents targeting CYP51, Bioorganic & medicinal chemistry, 26 (2018) 3242-3253.

[17] 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, European journal of medicinal chemistry, 123 (2016) 514-522.

[18] T.Y. Hargrove, L. Friggeri, Z. Wawrzak, A. Qi, W.J. Hoekstra, R.J. Schotzinger, J.D. York, F.P. Guengerich, G.I. Lepesheva, Structural analyses of Candida albicans sterol 14alpha-demethylase complexed with azole drugs address the molecular basis of azole-mediated inhibition of fungal sterol

biosynthesis, The Journal of biological chemistry, 292 (2017) 6728-6743.

[19] T.Y. Hargrove, Z. Wawrzak, D.C. Lamb, F.P. Guengerich, G.I. Lepesheva, Structure-Functional Characterization of Cytochrome P450 Sterol 14alpha-Demethylase (CYP51B) from Aspergillus fumigatus and Molecular Basis for the Development of Antifungal Drugs, The Journal of biological chemistry, 290 (2015) 23916-23934.

[20] S.K.S. Nishad Thamban Chandrika, † Huy X. Ngo,† Oleg V. Tsodikov,† Kaitlind C. Howard,†and Sylvie Garneau-Tsodikova, Alkylated Piperazines and Piperazine-Azole Hybrids as Antifungal Agents, Journal of Medical Chemistry, 61 (2018) 158-173.

Highlights

- Compounds **14i**, **14n, 14s** and **14v** with 3-F substitution on the biphenyl group exhibited excellent antifungal activities with broad antifungal spectra.
- Compounds **14i**, **14n, 14s** and **14v** showed moderate antifungal activities against fluconazole-resisitent *Candida albicans* (strains *17#* and *CaR*).
- Compound **14v** reduced the content of ergosterol in a dose-dependent manner.
- Compounds **14n** and **14v** were almost nontoxic to mammalian A549 cells.
- Compound **14v** showed excellent stability in human plasma.