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Discovery of biphenyl imidazole derivatives as potent antifungal agents: Design, synthesis, and structure-activity relationship studies

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ABSTRACT:

Fungal infections have became a serious medical problem due to their high incidence and mortality. We describe the discovery and structure-activity relationships studies (SARs) of a series of novel biphenyl imidazole derivatives with excellent antifungal activities against *Candida albicans* and *Cryptococcus neoformans*. The most promising compounds **12f-g** and **19a-b** exhibited excellent activity with minimum inhibitory concentration (MIC) values in the range of 0.03125 to 2 μg/mL. Preliminary mechanism studies showed that the potent antifungal activity of compound **12g** stemed from inhibition of CYP51 in *Candida albicans*. Furthermore, compounds **12g** and **19b** exhibited low inhibition profiles for various human cytochrome P450 isoforms. The SARs and binding mode established in this study will be useful for further lead optimization.

KEYWORDS:

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

1. Introduction

Fungal infections have been increasing dramatically and present a serious threat to human health, especially in immunocompromised patients. The occurrence of these infections have

increased since the early 1980s and have resulted from many factors, such as patients undergoing organ transplants or anticancer chemotherapy and patients with AIDS as well as patients given potent pharmacologic immunomodulators or broad-spectrum antibiotics[1-4]. Clinically, the three major pathogens *Candida spp.*, *Cryptococcus neoformans* and *Aspergillus spp*. account for most fungal infections. Currently, the conventional antifungal agents to treat fungal infections can be divided into four categories based on their mode of action, including the 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 in front-line antifungal therapy [9].

Azole antifungal agents inhibit the activity of lanosterol 14-demethylase (CYP51), a member of the CYP51 class of cytochrome P450 enzymes[5]. The inhibition of CYP51 enzymes reduces endogenous concentrations of ergosterol, a significant cellular membrane component, and causes the accumulation of 14α-methylsterols (e.g., lanosterol and 14α-methyl-3-6-diol) [5, 10-12]. The active site of CYP51 contains a heme cofactor that can coordinate to an imidazole ring or the triazole ring of azole drugs[13]. Not only are these drugs essential for the clinical treatment of systemic fungal infections, but they are also a major focus in the search for new antifungals[14-16]. Several azole antifungal agents, such as itraconazole, voriconazole and bifonazole have been approved (Fig. 1) [17]. However, these antifungal agents exist many problems, such as drug resistance, narrow antifungal spectrum and low bioavailability, which negatively affect their clinical efficacy[12, 18]. Therefore, it is necessary to develop novel antifungal compounds with potent activity, broad spectrum and low resistance.

Based on results obtained from structure-activity relationships of azole antifungal agents and computational docking experiments, we performed a cell-based antifungal screening of an in-house library [19-21] using standard NCCLS (National Committee for Clinical Laboratory Standards) protocols. Compound **5**, which features biphenyl and imidazole scaffolds, showed modest antifungal activities, with an MIC value of 2 μg/mL against *Candida albicans*. The common phamacophore of classic azole antifungal drugs include a *N*-(phenethyl) azole skeleton and a tertiary alcohol group, both of which contribute to their antifungal activities[22]. Compound **5** contains an imidazole group but no tertiary alcohol group or *N*-(phenethyl) azole skeleton, rendering the chemical scaffold of compound **5** a novel structure compared to the classic azole

antifungal agents and necessitating the investigation of its structure-activity relationships (SARs) to design a novel antifungal lead compound.

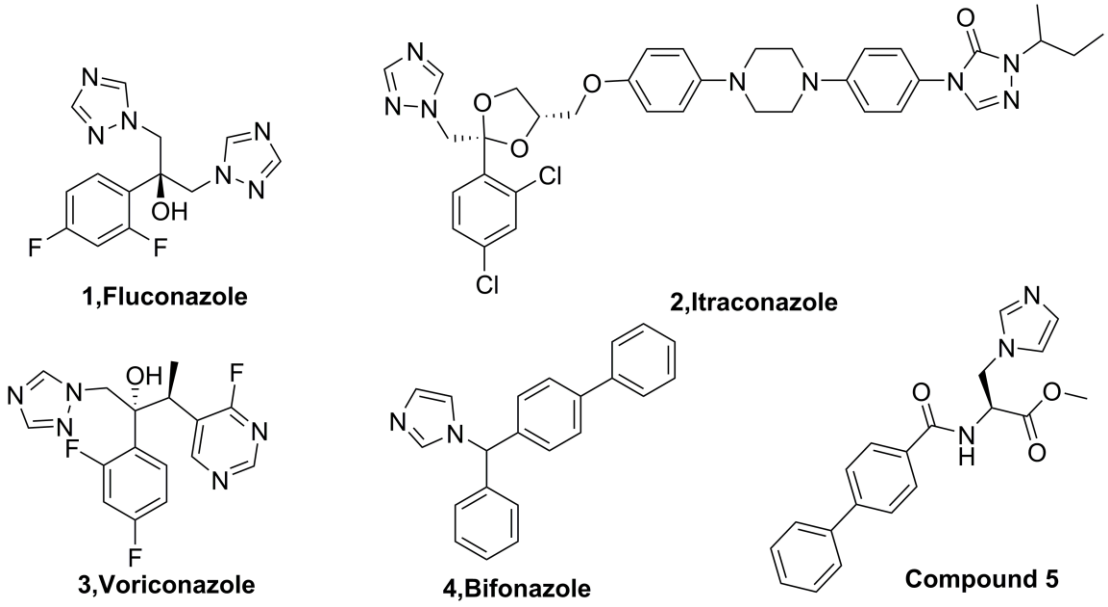
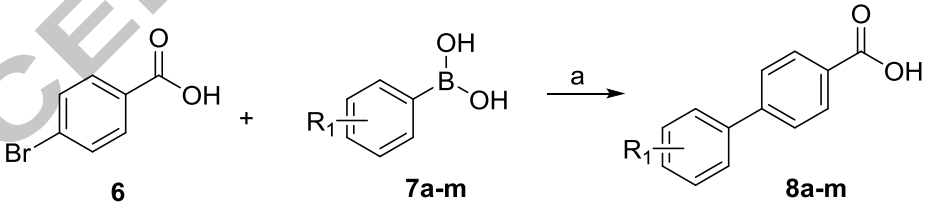


Figure 1. Chemical structures azole antifungal agents and lead compounds.

2. Results and discussion

2.1 Chemistry

The synthetic routes of the key intermediates **8a-m** are illustrated in Scheme 1. Commercially available 4-bromobenzoic acid **6** and the substituted phenylboronic acids **7a-m** were subjected to a Suzuki coupling in the presence of $Pd(PPh_3)_4$ to afford the key intermediates **8a-m**.



	R_1
a	R_1=H
b	R_1=2-F
c	R_1=2-Cl
d	R_1=2-CH_3

	R_1
e	R_1=4-F
f	R_1=4-Cl
g	R_1=4-CH_3
h	R_1=4-OCF_3
i	R_1=4-CF_3

	R_1
j	R_1=3-F
k	R_1=2,4-F
l	R_1=3,4-F
m	R_1=2,5-F

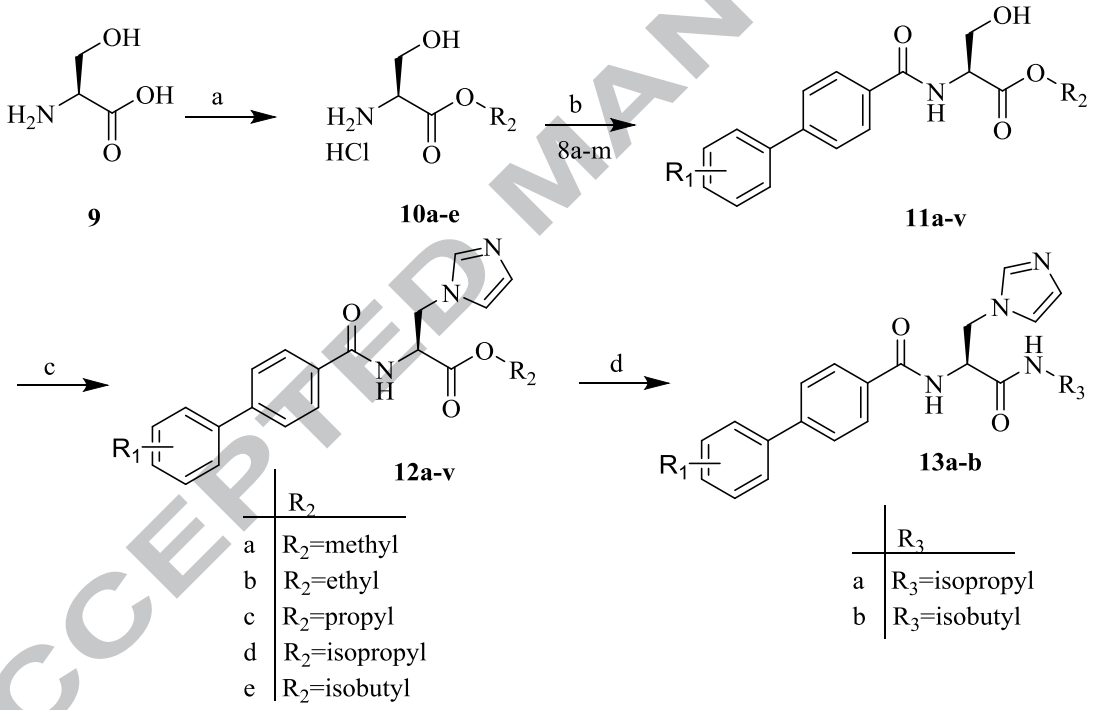
Scheme 1. Synthesis of the intermediates **8a-m.**Reagents and conditions: (a) $Pd(PPh_3)_4$, K_2CO_3, reflux, 5h.

The target compounds **12a-v** were synthesized according to our previously reported procedure (Scheme 2) [13, 21]. L-serine **9** was treated with an alcohol (methanol, ethanol, propanol, isopropyl

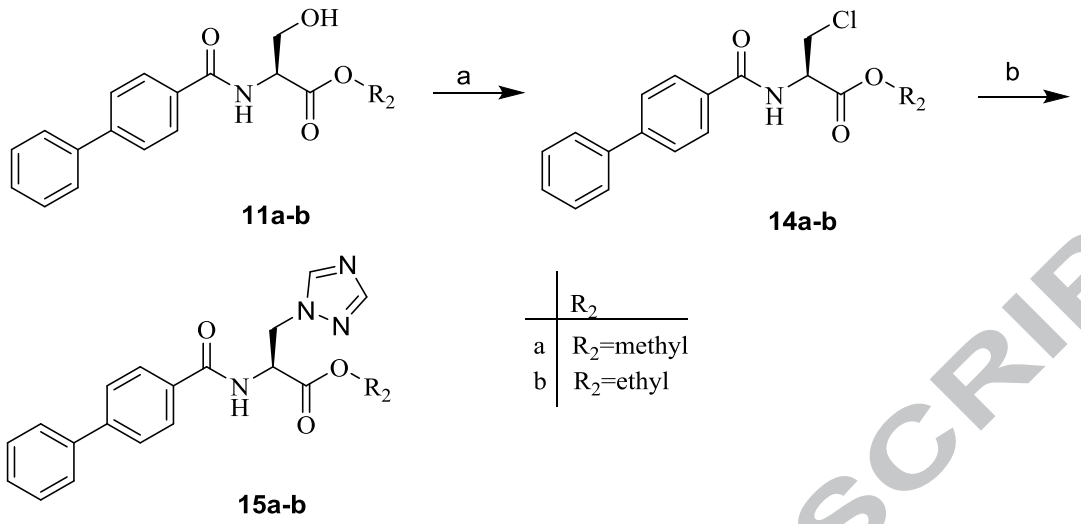
alcohol, or isobutyl alcohol) in the presence of $SOCl_2$ to afford serine ester **10a-e**. The serine esters **10a-e** were then treated with **8a-m** in the presence of a condensing agent to give intermediates **11a-v**. These compounds were treated with imidazole in the presence of CDI, to afford the target compounds **12a-v**. Subsequently, compounds **13a-b** were synthesized from compounds **12a-v** via hydrolyzation and amidation.

Compounds **15a-b** were prepared according to the procedure in Scheme 3. Alcohols **11a-b** were chlorinated with $SOCl_2$ to afford intermediate **14a-b**, which were then subjected to nucleophilic substitution with triazole to yield the target compounds.

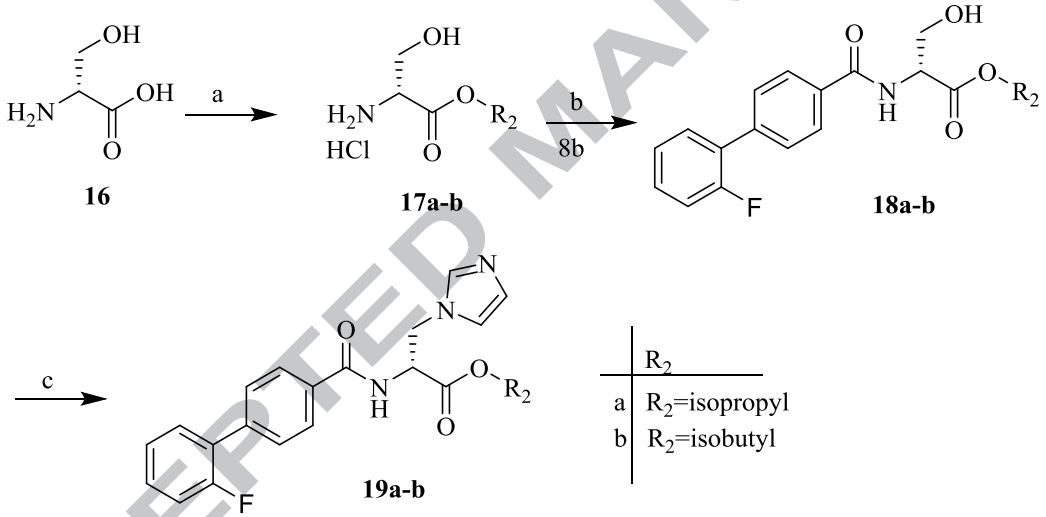
The synthetic procedure for compounds **19a-b** was similar to the synthesis of compounds **12a-v**, except that D-serine was used instead of L-serine(Scheme 4).



Scheme 2. General synthesis of the target compounds **12a-v** and **13a-b**.Reagents and conditions: (a) alcohol reagent, $SOCl_2$, reflux, 1–2 h; (b) EDCI, HOBt, DIEA, r.t., 7 h; (c) CDI, imidazole, CH_3CN, reflux, 7 h; (d) 1)NaOH, $MeOH/H_2O$; 2)R_3NH_2, EDCI, HOBt, DIEA, r.t., 7 h.



Scheme 3. General synthesis of the target compounds **15a-b**. Reagents and conditions: (a) $SOCl_2$, DMF, r.t., 2 h; (b) 1H-1,2,4-triazole, Triethylamine, DMF, r.t. 12 h.



Scheme 4. General synthesis of the target compounds **19a-b**. Reagents and conditions: (a) alcohol reagent, $SOCl_2$, reflux, 1-2 h; (b) EDCI, HOBt, DIEA, r.t., 7 h; (c) CDI, imidazole, CH_3CN, reflux, 7 h

2.2 In vitro antifungal activity

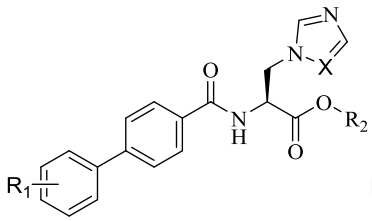
The target compounds were evaluated for their antifungal activity against five main fungal pathogens according to the protocols from the NCCLS. Broth microdilution methods was used to determine the minimum inhibitory concentrations (MICs) of the target compounds in 96-well microtest plates[16].

The *in vitro* antifungal activities of the target compounds are listed in Table 1. Fluconazole (FLC) and Itraconazole (ITR) were used as reference drugs. Most of the synthesized compounds exhibited moderate to good antifungal properties with broad spectrums of activity. Of these,

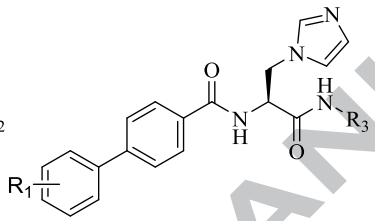
compounds **12a-b**, **12f-g**, **12q**, and **19a-b** showed the most potent activity against *C. albicans*, *C. neoformans*, and *C. tropicalis* with MIC values in the range of 0.03125 to 2 μg/mL, which are superior or comparable to those of the reference drugs FLC and ITR. Substitution in the alkyl ester side chain (**12a-b**) by an amide group (**15a-b)** led to obvious decrease of antifungal activity. Replacing the imidazole group (**16b**) with a triazole group (**16f**) resulted in a slight decrease in activity. The *R*-enantiomers(**19a-b)** and *S*-enantiomers(**14o-p)** show similar antifungal activities. However, most of the compounds were inactive against *Aspergillus fumigatus*.

Table 1

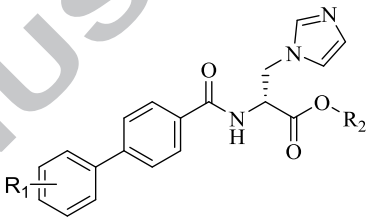
In vitro antifungal activities of the target compounds (MIC, μg/mL) [a].



12a-v X=C
15a-b X=N



13a-b



19a-b

Compd.	R_1	R_2/ R_3	*C. alb.*(Ⅰ)	*C. alb*(Ⅱ).	*C. neo.*	*C.tro.*	*A. fum.*
12a	H	$-CH(CH_3)_2$	0.03125	0.25	2	0.0625	>16
12b	H	$-CH_2CH(CH_3)_2$	0.03125	0.25	1	0.03125	>16
12c	2-F	$-CH_3$	16	16	2	4	>16
12d	2-F	$-CH_2CH_3$	0.25	1	1	0.125	>16
12e	2-F	$-CH_2CH_2CH_3$	0.125	0.25	0.5	0.0625	>16
12f	2-F	$-CH(CH_3)_2$	0.0625	0.25	1	0.0625	>16
12g	2-F	$-CH_2CH(CH_3)_2$	0.03125	0.125	0.5	0.03125	>16
12h	2-Cl	$-CH(CH_3)_2$	0.25	1	16	0.5	>16
12i	2-Cl	$-CH_2CH(CH_3)_2$	0.5	2	16	0.5	>16
12j	$2-CH_3$	$-CH(CH_3)_2$	0.5	2	16	2	>16
12k	$2-CH_3$	$-CH_2CH(CH_3)_2$	>16	>16	>16	4	>16
12l	4-F	$-CH(CH_3)_2$	1	8	>16	0.5	>16
12m	4-Cl	$-CH(CH_3)_2$	0.25	1	16	0.25	>16
12n	$4-CH_3$	$-CH(CH_3)_2$	0.0625	0.5	4	0.0625	>16
12o	$4-CF_3$	$-CH(CH_3)_2$	>16	>16	>16	2	>16

12p	4-OCF_3	-$CH(CH_3)_2$	0.125	0.125	16	0.5	>16
12q	3-F	-$CH(CH_3)_2$	0.0625	0.25	16	0.0625	>16
12s	3-CN	-$CH(CH_3)_2$	>16	>16	>16	8	>16
12t	2,4-F_2	-$CH(CH_3)_2$	1	2	8	0.5	>16
12u	3,4-F_2	-$CH(CH_3)_2$	>16	>16	>16	8	>16
12v	2,5-F_2	-$CH(CH_3)_2$	0.25	1	16	0.0625	>16
13a	H	-$CH(CH_3)_2$	>16	>16	>16	>16	>16
13b	H	-$CH_2CH(CH_3)_2$	>16	>16	>16	>16	>16
15a	H	-$CH(CH_3)_2$	0.25	4	16	2	>16
15b	H	-$CH_2CH(CH_3)_2$	1	8	16	4	>16
19a	2-F	-$CH(CH_3)_2$	0.125	0.5	>16	0.25	>16
19b	2-F	-$CH_2CH(CH_3)_2$	0.0625	0.5	>16	0.125	>16
5	-	-	2	2	>16	0.25	>16
FCZ	-	-	0.5	1	4	1	>16
ITZ	-	-	0.0625	0.25	1	0.5	4

[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.

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

Fluconazole is widely used today and can seriously increase the problem of drug-resistance[23]. Based on the results of *in vitro* antifungal activity assays, the most potent compounds **12f-g** and **19a-b** were further evaluated against fluconazole-resistant strains of *C. alb.* (*strain 100* and *strain 103*). As shown in Table 2, compounds **12f-g** and compounds **19a-b** showed moderate antifungal activities against *strain 100* and *strain 103*, with MIC values in the range of 2 to 8 μg/mL.

Table 2
In vitro antifungal activities of the target compounds (MIC, μg/mL)[a].

Compd.	**R_1**	**R_2**	***C. alb.***	
			Strain100	***Strain103***
12f	2-F	-$CH(CH_3)_2$	8	4
12g	2-F	-$CH_2CH(CH_3)_2$	2	4

19a	2-F	$-CH(CH_3)_2$	4	8
19b	2-F	$-CH_2CH(CH_3)_2$	4	4
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.

2.3 Analysis of sterol composition

To confirm the antifungal mechanism of compound **12g**, we analysed sterol composition in *C. albicans* by GC-MS. GC-MS methods have been successfully used to analyse the differences in sterol composition in *C. albicans* cells, to elucidate the mechanism of action of antifungal agents on sterol biosynthesis pathways[24, 25]. FLC was used as a reference drug and cholesterol was added as an internal standard. The GC-MS analysis results are shown in Table 3. In the untreated control, ergosterol was the major sterol, accounting for 89.4% of the total sterol content. While lanosterol comprised only 3.5% and other 14-methylated sterols (obtusifoliol and eburicol) were not observed. When the *C. albicans* was treated with FLC at 8 μg/mL for 16 h, the content of ergosterol was reduced to 2.0% of the total amount of sterol. In contrast, the content of lanosterol and obtusifoliol were increased to 18.2% and 6.8%, respectively. Especially, eburicol content was increased to 68.1% of the total sterol content. These changes in sterol composition were caused by the inhibition of CYP51 in *C. albicans* by FLC and are consistent with the earlier reports [21, 26, 27]. Treatment with **12g** also resulted in a marked increase in the lanosterol and eburicol contents and a reduction of the ergosterol content (reduced to 2.5%). The GC-MS results suggest that the novel compound **12g** might have a similar mechanism with FLC by inhibiting fungal lanosterol 14-demethylase (CYP51) in *C. albicans.*

Table 3

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

sterol	**% of total sterols(Candida albicans)**		
	control[a]	**FLC[b]**	**12g[c]**
Ergosterol	89.4	2.0	2.5
Obtusifoliol	--	6.8	6.6
Lanosterol	3.5	18.2	19.6

Eburicol	--	68.1	65.1
unidentified	7.1	4.9	6.2

[a]Abbreviations: *C.alb.*, *Candida albicans* (ATCC SC5314); [b]Control (no drug). [c]Treated with FLC at 8 μg/ mL. [d]Treated with compound **12g** at 8 μg/ mL.

2.4 Cytochrome P450 Inhibition Assay

Cytochrome P450 (CYP) enzymes play an important role in drug metabolism. However, many azole antifungals interfere with cytochrome P450 enzymes, such as CYP3A4 and CYP2D6, while maintaining a high activity against for fungal CYP51. For example, the IC_{50} of ketoconazole and itraconazole for inhibition of CYP3A4 are 31.7 and 32.6 nM[28], respectively. The compounds **12g** and **19b** were tested against the five major human CYP isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4-M). As shown in Table 4, compounds **12g** and **19b** showed weak-to-moderate inhibition against CYP1A2, CYP2C9, CYP2C19, and CYP2D6, while compounds **12g** and **19b** exhibited strong inhibition against CYP3A4 with IC_{50} values 0.155 μM and 0.224 μM, respectively. The *R*-enantiomer **19b** showed higher selectivity than the *S*-enantiomer **12g**.

Table 4.

In Vitro CYP Inhibition assessment of compounds.

Compd.	**IC50 (μM)**				
	CYP1A2	**CYP2C9**	**CYP2C19**	**CYP2D6**	**CYP3A4-M**
12g	21.0	2.36	1.16	7.59	0.155
19b	41.4	3.22	1.89	10.2	0.227

2.5 Structure-activity relationships

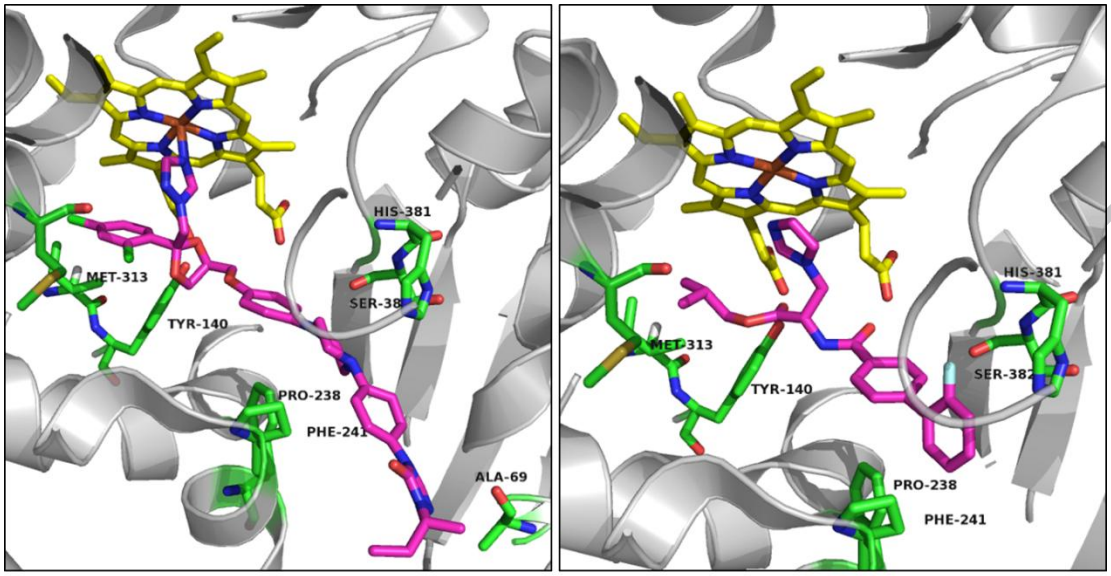
The relationship between various substituents on the aromatic ring was investigated (compounds **12a-v**). Compounds with electron-donating groups at the 4-position, such as $-CH_3$ and $-OCF_3$ moieties, exhibited higher antifungal activity than those with electron-withdrawing groups such as $-CF_3$ and halogens such as -F and -Cl. On the contrary, electron-donating groups such as methyl at the 2-position resulted in weaker activity compared to single halogens such as -F and -Cl. Among these electron-poor aryls, compounds **12f-g** with 2-F substituents showed the best antifungal activity. Moreover, Compounds **12t-v** with multi-halogens substituents showed no advantage.

The antifungal activities of compounds **12c-g,** which have differing alkyl ester side chains (methyl ester, ethyl ester, propyl ester, isopropyl ester and isobutyl ester) were investigated. The *in vitro* antifungal activity data showed that bulky groups such as isopropyl ester and isobutyl ester have the potential for higher antifungal activity. Replacement of the alkyl ester side chain by an amide group (**13a-b**) resulted in a significant decrease in antifungal activity. In addition, replacing the imidazole group (**12a-b**) with a triazole group (**15a-b**) resulted in a slight decrease in activity.

The influence of the stereochemical configuration was examined by synthesizing novel *R*-configured compounds (**19a-b**) and studying their antifungal activities. The results show that *R*- or *S*-configured compounds have similar antifungal activity.

*2.6 Molecular docking model analysis of compound **12g** in the active site of CYP51*

A molecular docking study was performed to investigate interactions between CYP51 and **12g** and to provide a basis for further structure-based drug design. The first full-length structure of a fungal CYP51 was from Saccharomyces cerevisiae and was determined by X-ray crystallography [29]. A published crystal structure of ITZ bound within the active site cavity of CYP51(PDB ID:5EQB, Figure 2A) served as a useful template for generating binding modes. Compound **12g** was docked into the active site using the CDOCKER program in Discovery Studio 3.0. Images depicting the proposed binding modes were generated using PyMOL. As shown in Figure 2B, the imidazole ring of compound **12g** coordinated the iron in the heme group, and the alkyl ester formed a hydrophobic interaction with Met313. Like the side chain of ITZ, the biphenyl side chain extended into CYP51 channel to form van der Waals and hydrophobic interactions with the surrounding residues Ala69, Leu95, Pro238, Phe241 and His381.



(A) **(B)**

Figure 2. The binding mode of ITZ (A) and compound **12g** (B) in the active site of CYP51.

3. Conclusion

In summary, we discovered a novel class of compounds with excellent antifungal activities. Further examination of their antifungal activity and structure-activity relationship(SAR) led to a series of novel biphenyl imidazole derivatives that were designed, synthesized and evaluated for *in vitro* antifungal activity. Most compounds displayed moderate or strong antifungal activities with MIC values in the range of 0.03125 μg/mL to 2 μg/mL against *Candida albicans* and *Cryptococcus neoformans*. Compounds **12f-g** and **14a-b** displayed moderate antifungal activities against fluconazole-resistant strains of *C.alb*. Notably, compounds **12g** and **19b** were selective for fungal targets compared to human CYP1A2, CYP2C9, CYP2C19, and CYP2D6 (IC50>1 μM).Preliminary mechanism studies showed that the potent antifungal activity of novel compound **12g** stems from inhibition of CYP51 in *Candida albicans*. Based on the SAR and a molecular docking study, compound **12g** was identified as a promising antifungal agent. Further optimization on these compounds is underway.

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), 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 General procedure for the synthesis of intermediates (**8a-m**)*

Under an argon atmosphere, 4-bromobenzoic acid (1 equiv.), substituted boronic acid (1.2 equiv.) and $Pd[P(C_6H_5)_3]_4$(1 mol%) were dissolved in a mixed solution of dioxane/H2O (10:1). K_2CO_3 (2 equiv.) was added and the mixture was heated under reflux for 6 h. The reaction mixture was cooled to room temperature and the dioxane was removed by rotary evaporation. H_2O was added and the solution was adjusted to pH=1-3 with 2 N HCl. The white solid precipitate was collected by filtration and dried to give the desired compound.

*4.3 General procedure for the synthesis of L-serine ester (**10a–e**)*

To a solution of L-serine (1 equiv.) in alcohol reagent (methanol, ethanol, propanol, isopropanol or isobutanol) was added dropwise thionyl chloride (3 equiv.) at less then 0 °C using a salted ice bath. The mixture was heated under reflux for 2-10 h. The reaction mixture was then concentrated under reduced pressure to yield a white solid.

*4.4 General procedure for the synthesis of compounds (**11a-v**)*

To a solution of the intermediate acid compound (1 equiv.) in anhydrous DMF was added EDCI(1.1 equiv) and HOBt(1.1 equiv), respectively.The reaction mixture was stirred for 2 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, the resulting solid was filtered and dried to give the desired compound.

*4.5 General procedure for the synthesis of compounds (**12a-v**)*

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 at 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 (CH_3OH : CH_2Cl_2 = 1:50, v/v) to give the target product **12a-v**.

*4.5.1 isopropyl(S)-2-([1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12a**)*

Light white solid; yield: 53.3%; mp: 92.7-94.9 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, *J* = 7.9 Hz, 1H), 7.90 (d, *J* = 8.3 Hz, 2H), 7.80 (d, *J* = 8.3 Hz, 2H), 7.74 (d, *J* = 7.3 Hz, 2H), 7.65 (s, 1H), 7.50 (t, *J* = 7.5 Hz, 2H), 7.42 (t, *J* = 7.3 Hz, 1H), 7.24 (s, 1H), 6.87 (s, 1H), 4.96 (m, *J* = 18.7 Hz, 1H), 4.85 – 4.71 (m, 1H), 4.76 - 4.38 (m, 2H), 1.20 (t, *J* = 5.9 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.14, 166.25, 143.19, 139.09, 137.81, 132.29, 129.01(2C), 128.29, 128.10, 127.98(2C), 126.89(2C), 126.61(2C), 119.87, 68.63, 53.89, 45.77, 21.47, 21.42. HRMS calcd for $C_{22}H_{24}N_3O_3$, $[M + H]^+$, 378.1818; found 378.1812.

*4.5.2 isobutyl (S)-2-([1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate (**12b**)*

Yellow oil; yield: 59.1%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (d, *J* = 8.0 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.65 (s, 1H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.42 (t, *J* = 7.3 Hz, 1H), 7.23 (s, 1H), 6.86 (s, 1H), 4.84 (m, 1H), 4.58 – 4.40 (m, 2H), 3.91 (d, *J* = 6.1 Hz, 2H), 1.87 (m, 1H), 0.87 (d, *J* = 6.7 Hz, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.60, 166.29, 143.19, 139.07, 137.78, 132.25, 129.02(2C), 128.19, 128.12, 127.96(2C), 126.90(2C), 126.62(2C), 119.88, 70.64, 53.79, 45.72, 27.23, 18.74(2C). HRMS calcd for $C_{23}H_{26}N_3O_3$, $[M + H]^+$, 392.1974; found 392.1967.

*4.5.3 methyl(S)-2-(2'-fluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12c**)*

Light white solid; yield: 58.1%; mp: 199.8-201.3 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.06 (d, *J* = 8.0 Hz, 1H), 7.91 (d, *J* = 8.4 Hz, 2H), 7.69 – 7.66 (m, 2H), 7.65 (s, 1H), 7.59 (td, *J* = 7.9, 1.5 Hz, 1H), 7.50 – 7.44 (m, 1H), 7.37 – 7.32 (m, 2H), 7.22 (s, 1H), 6.86 (s, 1H), 4.87 (ddd, *J* = 9.9, 8.1, 4.7 Hz, 1H), 4.56 (dd, *J* = 14.1, 4.7 Hz, 1H), 4.42 (dd, *J* = 14.1, 10.0 Hz, 1H), 3.70 (s, 3H).^{13}C NMR (150 MHz, DMSO-d_6) δ 170.10, 166.14, 159.90, 158.26, 138.31, 137.85, 132.59, 130.84, 130.21, 129.43, 128.86, 128.38, 127.65(2C), 127.37, 125.08, 119.89, 116.14, 53.68, 52.33, 45.76.HRMS calcd for $C_{20}H_{19}FN_3O_3$, $[M + H]^+$, 368.1410; found 368.1414.

*4.5.4 ethyl(S)-2-(2'-fluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12d**)*

Light white solid; yield: 61.7%; mp: 109.5-110.8 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (d, J = 8.0 Hz, 1H), 7.92-7.90 (d, J = 8.0Hz, 2H), 7.69 – 7.60 (m, 3H), 7.58 – 7.48 (m, 1H), 7.48 (t, J = 12 Hz, 1H), 7.44-7.32 (m, 2H), 7.23 (s, 1H), 6.87 (s, 1H), 4.85 (m, 1H), 4.58 – 4.39 (m, 2H), 4.19 (q, J = 7.1 Hz, 2H), 1.21 (t, J = 14.2 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.57, 166.16, 160.29, 157.83, 138.25, 137.81, 132.67, 130.82, 130.24, 128.83, 128.80, 128.28, 127.61(2C), 127.26, 125.05, 125.02, 119.88, 116.30, 61.05, 53.75, 45.78, 13.99.HRMS calcd for $C_{21}H_{21}FN_3O_3$, $[M + H]^+$, 382.1567; found 382.1570.

*4.5.5 propyl(S)-2-(2'-fluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12e**)*

Light white solid; yield: 49.5%; mp: 67.9-71.3 °C.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.68 – 7.60 (m, 3H), 7.60 – 7.58 (m, 1H), 7.57-7.56 (m, 1H), 7.47-7.31 (m, 2H), 7.24 (s, 1H), 6.88 (s, 1H), 4.87 (m, 1H), 4.59 – 4.42 (m, 2H), 4.07 (t, J = 10.8 Hz, 2H), 1.61-1.56 (m, 2H), 0.87 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.61, 166.20, 160.29, 157.84, 138.24, 137.78, 132.69, 130.81, 130.23, 128.83, 128.80, 128.15, 127.59(2C), 127.25, 125.05, 119.91, 116.29, 66.41, 53.77, 45.77, 21.44, 10.16. HRMS calcd for $C_{22}H_{23}FN_3O_3$, $[M + H]^+$, 396.1723; found 396.1720.

*4.5.6 isopropyl(S)-2-(2'-fluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12f**)*

Light white solid; yield: 58.8%; mp: 75.4-78.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, J = 7.9 Hz, 1H), 7.91 (d, J = 8.3 Hz, 2H), 7.69 (d, J = 7.2 Hz, 2H), 7.66 (s, 1H), 7.61 (s, 1H), 7.49-7.34 (m, 3H), 7.23 (s, 1H), 6.86 (s, 1H), 4.99-4.95 (m, 1H), 4.78 - 4.76 (m, 1H), 4.55- 4.37 (m, 2H), 1.20 (t, J = 5.9 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.09, 166.21, 160.29, 157.84, 138.22, 137.82, 132.76, 130.79, 130.24, 128.83, 128.81, 128.31, 127.59(2C), 127.27, 125.02, 119.87, 116.30, 68.65, 53.89, 45.76, 21.48, 21.43. HRMS calcd for $C_{22}H_{23}FN_3O_3$, $[M + H]^+$, 396.1723; found 396.1716.

*4.5.7 isobutyl(S)-2-(2'-fluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12g**)*

Light white solid; yield: 63.1%; mp: 117.2-119.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.68 (t, J = 9.1 Hz, 3H), 7.59 - 7.55 (m, 1H), 7.46 -7.45 (m, 1H), 7.35 -7.30 (m, 2H), 7.27 (s, 1H), 6.90 (s, 1H), 4.92 - 4.87 (m, 1H), 4.62 - 4.45 (m, 2H), 3.92 (t, J = 6.1 Hz, 2H), 1.89 – 1.84 (m, 1H), 0.87 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ

169.56, 166.25, 160.29, 157.84, 138.24, 137.81, 132.74, 130.82, 130.16, 128.84, 128.31, 127.58(2C), 127.25, 125.05, 125.02, 119.86, 116.30, 116.07, 70.66, 53.81, 45.69, 27.23, 18.73. HRMS calcd for $C_{23}H_{25}FN_3O_3$, $[M + H]^+$, 410.1880; found 410.1876.

*4.5.8 isopropyl(S)-2-(2'-chloro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12h**)*

Light white solid; yield: 57.3%; mp: 122.8-124.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, *J* = 7.9 Hz, 1H), 7.87 (d, *J* = 8.3 Hz, 2H), 7.64 (s, 1H), 7.62 – 7.57 (m, 1H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.49 – 7.41 (m, 3H), 7.23 (s, 1H), 6.86 (s, 1H), 5.02 – 4.90 (m, 1H), 4.79 – 4.75 (m, 1H), 4.52 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.40 (dd, *J* = 14.0, 9.9 Hz, 1H), 1.20 (t, *J* = 5.8 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.11, 166.32, 141.86, 138.98, 137.86, 132.81, 131.44, 131.20, 129.92, 129.68, 129.35(2C), 128.37, 127.63, 127.26(2C), 119.90, 68.68, 53.91, 45.77, 21.51, 21.46. HRMS calcd for $C_{22}H_{23}ClN_3O_3$, $[M + H]^+$, 412.1428; found 412.1419.

*4.5.9 isobutyl(S)-2-(2'-chloro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12i**)*

Yellow oil; yield: 59.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 8.3 Hz, 2H), 7.64 (s, 1H), 7.63 – 7.58 (m, 1H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.48 – 7.41 (m, 3H), 7.23 (s, 1H), 6.86 (s, 1H), 4.91 – 4.76 (m, 1H), 4.55 (dd, *J* = 14.0, 4.8 Hz, 1H), 4.43 (dd, *J* = 14.0, 10.1 Hz, 1H), 3.96 – 3.87 (m, 2H), 1.91 – 1.85 (m, 1H), 0.88 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.58, 166.36, 141.86, 138.96, 137.85, 132.79, 131.44, 131.20, 129.92, 129.68, 129.35(2C), 128.39, 127.63, 127.23(2C), 119.87, 70.69, 53.82, 45.69, 27.26, 18.77(2C). HRMS calcd for $C_{23}H_{25}ClN_3O_3$, $[M + H]^+$, 426.1584; found 426.1581.

*4.5.10 isopropyl(S)-3-(1H-imidazol-1-yl)-2-(2'-methyl-[1,1'-biphenyl]-4-carboxamido)propanoate (**12j**)*

Light white solid; yield: 56.9%; mp: 80.1-82.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98 (d, *J* = 7.9 Hz, 1H), 7.85 (d, *J* = 8.3 Hz, 2H), 7.72 (d, *J* = 1.0 Hz, 1H), 7.64 (s, 1H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.34 – 7.25 (m, 3H), 7.23 (s, 1H), 6.86 (s, 1H), 4.99 – 4.92 (m, 1H), 4.82 – 4.74 (m, 1H), 4.51 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.39 (dd, *J* = 14.1, 9.8 Hz, 1H), 2.24 (s, 3H), 1.20 (t, *J* = 6.1 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.16, 166.44, 144.57, 140.39, 137.85, 134.69, 132.04, 130.46, 129.42, 129.03(2C), 128.37, 127.77, 127.30(2C), 126.05, 119.88, 68.66, 53.91, 45.77, 21.51, 21.46, 20.13. HRMS calcd for $C_{23}H_{26}N_3O_3$, $[M + H]^+$, 392.1974; found 392.1975.

4.5.11 isobutyl(S)-3-(1H-imidazol-1-yl)-2-(2'-methyl-[1,1'-biphenyl]-4-carboxamido)propanoate(***12k****)*

Yellow oil; yield: 51.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 8.3 Hz, 2H), 7.72 (d, *J* = 1.2 Hz, 1H), 7.66 (s, 1H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.34 – 7.27 (m, 3H), 7.24 (s, 1H), 6.87 (s, 1H), 4.85 (ddd, *J* = 10.1, 8.1, 4.9 Hz, 1H), 4.56 (dd, *J* = 14.0, 4.8 Hz, 1H), 4.44 (dd, *J* = 14.1, 10.1 Hz, 1H), 3.92 – 3.89 (m, 2H), 2.24 (s, 3H), 1.94 – 1.81 (m, 1H), 0.88 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.64, 166.51, 144.59, 140.39, 137.86, 134.70, 132.03, 130.46, 129.42, 129.04(2C), 128.38, 127.77, 127.29(2C), 126.05, 119.87, 70.69, 53.84, 45.73, 27.27, 20.12, 18.77(2C). HRMS calcd for $C_{24}H_{28}N_3O_3$, $[M + H]^+$, 406.2131; found 406.2130.

4.5.12 isopropyl(S)-2-(4'-fluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(***12l****)*

Light white solid; yield: 62.6%; mp: 98.4-100.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (s, 1H), 7.91 (d, *J* = 8.4 Hz, 2H), 7.69 – 7.68(m, 2H), 7.66 (d, *J* = 7.7 Hz, 1H), 7.57 (s, 1H), 7.49 - 7.34 (m, 3H), 7.23 (s, 1H), 6.86 (s, 1H), 4.99 – 4.95 (m, 1H), 4.78 - 4.76(m, 1H), 4.55 – 4.37 (m, 2H), 1.21 (t, *J* = 5.9 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.14, 166.22, 163.49, 161.04, 142.13, 137.81, 135.08, 132.25, 129.02, 128.94, 128.18, 128.01, 127.94, 126.58, 121.50, 119.93, 115.96, 115.75, 68.66, 53.89, 45.81, 21.48, 21.44. HRMS calcd for $C_{22}H_{23}FN_3O_3$, $[M + H]^+$, 396.1723; found 396.1719.

4.5.13 isopropyl(S)-2-(4'-chloro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate ***(12m)***

Light white solid; yield: 54.8%; mp: 171.9-173.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (d, *J* = 7.9 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.79 (dd, *J* = 11.1, 8.5 Hz, 4H), 7.64 (s, 1H), 7.56 (d, *J* = 8.6 Hz, 2H), 7.22 (s, 1H), 6.86 (s, 1H), 4.98 – 4.92 (m, 1H), 4.81 – 4.71 (m, 1H), 4.51 (dd, *J* = 14.0, 5.1 Hz, 1H), 4.39 (dd, *J* = 14.0, 9.8 Hz, 1H), 1.21 – 1.18 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.11, 166.13, 141.80, 137.87, 137.81, 133.04, 132.58, 128.97(2C), 128.67(2C), 128.32, 128.03(2C), 126.59(2C), 119.85, 68.63, 53.89, 45.75, 21.47, 21.42. HRMS calcd for $C_{22}H_{23}ClN_3O_3$, $[M + H]^+$, 412.1428; found 412.1423.

4.5.14 isopropyl(S)-3-(1H-imidazol-1-yl)-2-(4'-methyl-[1,1'-biphenyl]-4-carboxamido)propanoate ***(12n)***

Light white solid; yield: 52.3%; mp: 121.0-123.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm) : 8.96 (d, *J* = 7.9 Hz, 1H),7.98 (d, *J* = 8.4 Hz, 2H), 7.88 (d, *J* = 8.4Hz, 2H), 7.81 (s, 1H), 7.76 (s, 1H), 7.66-7.63 (m, 3H), 7.22 (s, 1H), 6.86 (s, 1H), 4.99 – 4.94 (m, 1H), 4.79 - 4.74 (m, 1H), 4.54 – 4.37 (m, 2H), 2.51 (s, 3H), 1.21 (d, *J* = 5.8 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.15, 166.25, 143.09, 137.79, 137.56, 136.16, 131.96, 129.60(2C), 128.27, 127.95(2C), 126.70(2C), 126.27(2C), 119.86, 68.61, 53.88, 45.77, 21.47, 21.42, 20.68. HRMS calcd for $C_{23}H_{26}N_3O_3$, $[M + H]^+$, 392.1974; found 392.1971.

*4.5.15 isopropyl(S)-3-(1H-imidazol-1-yl)-2-(4'-(trifluoromethyl)-[1,1'-biphenyl]-4-carboxamido)propanoate(**12o**)*

Light white solid; yield: 58.0%; mp: 173.5-175.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (d, *J* = 7.9 Hz, 1H), 8.00 – 7.82 (m, 8H), 7.64 (s, 1H), 7.22 (s, 1H), 6.85 (s, 1H), 4.99 – 4.93 (m, 1H), 4.83 – 4.71 (m, 1H), 4.52 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.40 (dd, *J* = 14.0, 9.8 Hz, 1H), 1.21 – 1.18 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.09, 166.10, 143.11, 141.54, 137.83, 133.20, 128.56, 128.33, 128.10, 127.76, 127.10, 125.86, 119.87, 68.66, 53.90, 45.75, 21.48, 21.43. HRMS calcd for $C_{23}H_{23}F_3N_3O_3$, $[M + H]^+$, 446.1692; found 446.1688.

*4.5.16 isopropyl(S)-3-(1H-imidazol-1-yl)-2-(4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-carboxamido)propanoate(**12p**)*

Light white solid; yield: 57.1%; mp: 140.9-142.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (d, *J* = 7.9 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 2H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.64 (s, 1H), 7.49 (d, *J* = 8.1 Hz, 2H), 7.22 (s, 1H), 6.86 (s, 1H), 5.00 – 4.91 (m, 1H), 4.84 – 4.71 (m, 1H), 4.52 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.40 (dd, *J* = 14.0, 9.8 Hz, 1H), 1.21 – 1.18 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.12, 166.18, 148.31, 141.71, 138.41, 137.83, 132.70, 128.88, 128.26, 128.05, 126.82, 121.50, 119.91, 68.66, 53.90, 45.80, 21.47, 21.43. HRMS calcd for $C_{23}H_{23}F_3N_3O_4$, $[M + H]^+$, 462.1641; found 462.1642.

*4.5.17 isopropyl(S)-2-(3'-fluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12q**)*

Light white solid; yield: 48.8%; mp: 122.7-124.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, *J* = 7.9 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 2H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.67 – 7.48 (m, 4H), 7.31 – 7.18 (m, 2H), 6.86 (s, 1H), 5.01 – 4.91 (m, 1H), 4.81 – 4.73 (m, 1H), 4.52 (dd, *J* = 14.0, 5.1 Hz, 1H), 4.40 (dd, *J* = 14.0, 9.8 Hz, 1H), 1.19 (t, *J* = 5.7 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ

169.14, 166.17, 163.93, 161.51, 141.73, 141.48, 137.85, 132.85, 131.03, 128.36, 128.03(2C), 126.83(2C), 123.01, 119.88, 114.74, 113.77, 68.67, 53.92, 45.77, 21.49, 21.44. HRMS calcd for $C_{22}H_{23}FN_3O_3$, $[M + H]^+$, 396.1723; found 396.1722.

*4.5.18 isopropyl(S)-2-(3'-cyano-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12s**)*

Yellow oil; yield: 57.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (d, *J* = 7.9 Hz, 1H), 8.26 (s, 1H), 8.10 (d, *J* = 8.4 Hz, 1H), 7.94 – 7.88 (m, 5H), 7.71 (t, *J* = 7.8 Hz, 1H), 7.65 (s, 1H), 7.23 (s, 1H), 6.85 (s, 1H), 5.00 - 4.91 (m, 1H), 4.82 – 4.73 (m, 1H), 4.52 (dd, *J* = 14.0, 5.1 Hz, 1H), 4.42 (dd, *J* = 14.0, 9.8 Hz, 1H), 1.19 (t, *J* = 5.7 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.10, 166.07, 140.92, 140.19, 137.86, 133.09, 131.70, 131.65, 130.50, 130.23, 128.30, 128.13(2C), 126.94(2C), 119.89, 118.69, 112.20, 68.65, 53.93, 45.73, 21.48, 21.44. HRMS calcd for $C_{23}H_{23}N_4O_3$, $[M + H]^+$, 403.1770; found 403.1764.

*4.5.19 isopropyl(S)-2-(2',4'-difluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12t**)*

Light white solid; yield: 50.7%; mp: 118.6-120.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (d, *J* = 7.9 Hz, 1H), 7.88 (d, *J* = 8.3 Hz, 2H), 7.74 – 7.54 (m, 4H), 7.48 – 7.35 (m, 1H), 7.28 – 7.18 (m, 2H), 6.85 (s, 1H), 4.99 – 4.92 (m, 1H), 4.80 – 4.74 (m, 1H), 4.51 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.39 (dd, *J* = 14.0, 9.9 Hz, 1H), 1.21 – 1.18 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.09, 166.18, 163.36, 163.24, 160.46, 160.33, 137.83, 137.39, 132.83, 132.00, 128.80, 128.34, 127.64(2C), 124.05, 119.87, 112.08, 104.62, 68.66, 53.90, 45.76, 21.47, 21.43. HRMS calcd for $C_{22}H_{22}F_2N_3O_3$, $[M + H]^+$, 414.1629; found 414.1626.

*4.5.20 isopropyl(S)-2-(3',4'-difluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propanoate(**12u**)*

Light white solid; yield: 63.5%; mp: 104.2-106.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (d, *J* = 7.9 Hz, 1H), 7.93 – 7.77 (m, 5H), 7.64 – 7.52 (m, 3H), 7.22 (s, 1H), 6.86 (s, 1H), 5.00 – 4.91 (m, 1H), 4.82 – 4.72 (m, 1H), 4.52 (dd, *J* = 14.0, 5.1 Hz, 1H), 4.40 (dd, *J* = 14.0, 9.9 Hz, 1H), 1.21 – 1.18 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.10, 166.07, 151.07, 150.63, 148.63, 148.29, 140.86, 137.81, 136.67, 132.73, 128.32, 127.99(2C), 126.72(2C), 123.79, 119.85, 118.09, 116.11, 68.64, 53.89, 45.75, 21.46, 21.42. HRMS calcd for $C_{22}H_{22}F_2N_3O_3$, $[M + H]^+$, 414.1629; found 414.1627.

*4.5.21 isopropyl(S)-2-(2',5'-difluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-imidazol-1-yl)propan oate(**12v**)*

Light white solid; yield: 55.7%; mp: 107.8-110.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (d, *J* = 7.9 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.64 (s, 1H), 7.52 – 7.47 (m, 1H), 7.45 – 7.41 (m, 1H), 7.35- 7.29 (m, 1H), 7.22 (s, 1H), 6.86 (s, 1H), 5.01 - 4.91 (m, 1H), 4.82 – 4.72 (m, 1H), 4.52 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.39 (dd, *J* = 14.0, 9.8 Hz, 1H), 1.21 – 1.18 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.06, 166.10, 159.56, 157.17, 156.47, 154.04, 137.82, 137.07, 133.20, 128.90, 128.87, 128.28, 127.63(2C), 119.89, 117.90, 117.06, 116.53, 68.67, 53.89, 45.77, 21.47, 21.43. HRMS calcd for $C_{22}H_{22}F_2N_3O_3$, $[M + H]^+$, 414.1629; found 414.1630.

*4.6 General procedure for the synthesis of compounds (**13a-b**)*

To a solution of intermediate **12a-v** (1 equiv.) in methanol was added 2N sodium hydroxide at ambient temperature. The reaction mixture was stirred for 3 h and the methanol was removed by rotary evaporation. The resultant mixture was adjusted to pH=5-7 with 1 N HCl solution. The precipitated white solid was collected by filtration and dried to give the carboxylic acid intermediate.

To a solution of the carboxylic acid intermediate (1 equiv.) in anhydrous DMF was added EDCI (1.1 equiv.) and HOBt (1.1 equiv.), respectively.The reaction mixture was stirred for 2 h at ambient temperature, and the aliphatic amine (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 titled compound.

*4.6.1 (S)-N-(3-(1H-imidazol-1-yl)-1-(isopropylamino)-1-oxopropan-2-yl)-[1,1'-biphenyl]-4-carbox amide(**13a**)*

Light white solid; yield: 71.1%; mp: 220.5-224.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (d, *J* = 8.1 Hz, 1H), 8.06 (d, *J* = 7.1 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 2H), 7.79 – 7.73 (m, 4H), 7.62 (s, 1H), 7.58 – 7.48 (m, 2H), 7.42 (d, *J* = 7.1 Hz, 1H), 7.17 (s, 1H), 6.83 (s, 1H), 4.83 (s, 1H), 4.39 – 4.36 (m, 1H), 4.33 – 4.21 (m, 1H), 3.87 (dd, *J* = 13.3, 6.6 Hz, 1H), 1.08 (dd, *J* = 13.7, 6.4 Hz, 6H).^{13}C NMR (150 MHz, DMSO-d_6) δ 167.97, 166.07, 143.07, 139.16, 137.72, 132.56, 129.09(2C), 128.20, 128.16(2C), 126.93(2C), 126.54(2C), 119.81, 54.45, 47.15, 40.80, 22.26(2C). HRMS calcd for $C_{22}H_{25}N_4O_2$, $[M + H]^+$, 377.1978; found 377.1981.

4.6.2 (S)-N-(3-(1H-imidazol-1-yl)-1-(isobutylamino)-1-oxopropan-2-yl)-[1,1'-biphenyl]-4-carboxa

*mide(**13b**)*

Light white solid; yield: 69.5%; mp: 176.5-178.7 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.78 (d, *J* = 8.5 Hz, 1H), 8.18 (t, *J* = 5.7 Hz, 1H), 7.92 (d, *J* = 8.3 Hz, 2H), 7.79 – 7.73 (m, 5H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.24 (s, 1H), 6.91 (s, 1H), 4.90 – 4.86 (m, 1H), 4.45 (dd, *J* = 13.9, 4.4 Hz, 1H), 4.33 (dd, *J* = 13.8, 10.3 Hz, 1H), 2.94 (t, *J* = 6.4 Hz, 2H), 1.73 – 1.68 (m, 1H), 0.84 (dd, *J* = 6.6, 1.2 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.85, 166.12, 143.05, 139.13, 137.55, 132.51, 129.04(2C), 128.14(2C), 128.11, 126.90(2C), 126.48(2C), 120.01, 54.44, 47.19, 46.17, 28.01, 20.06(2C). HRMS calcd for $C_{23}H_{27}N_4O_2$, $[M + H]^+$, 391.2134; found 391.2129.

*4.7 General procedure for the synthesis of compounds (**15a-b**)*

To a solution of intermediate 11a-b (1 equiv.) in anhydrous DMF was added thionyl chloride (2 equiv) dropwise. The reaction mixture was stirred for 2 h at ambient temperature, and 1H-1,2,4-triazole (4 equiv.) and triethylamine (3 equiv.) were added.The mixture was stirred at room temperature overnight. When the reaction was complete, as determined by TLC, the reaction mixture was extracted with EtOAc and washed with brine. The organic phase was dried over Na_2SO_4 overnight and the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography (CH_3OH : CH_2Cl_2 = 1:40, v/v) to yield the target product **15a-b**.

*4.7.1 isopropyl(S)-2-([1,1'-biphenyl]-4-carboxamido)-3-(1H-1,2,4-triazol-1-yl)propanoate(**15a**)*

Light white solid; yield: 47.4%; mp: 132.5-135.2 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.97 (d, *J* = 7.8 Hz, 1H), 8.51 (s, 1H), 7.99 (s, 1H), 7.88 (d, *J* = 8.5 Hz, 2H), 7.79 (d, *J* = 8.5 Hz, 2H), 7.76 – 7.71 (m, 2H), 7.53 – 7.47 (m, 2H), 7.45 – 7.40 (m, 1H), 4.98 – 4.92 (m, 1H), 4.87 – 4.82 (m, 1H), 4.71 (dd, *J* = 14.0, 5.3 Hz, 1H), 4.65 (dd, *J* = 14.0, 8.9 Hz, 1H), 1.18 (dd, *J* = 6.2, 3.3 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.84, 166.19, 151.66, 144.91, 143.24, 139.08, 132.18, 129.04(2C), 128.14, 127.98(2C), 126.91(2C), 126.64(2C), 68.73, 52.83, 48.56, 21.46, 21.42. HRMS calcd for $C_{21}H_{22}N_4O_3$, $[M + Na]^+$, 401.1590; found 401.1595.

*4.7.2 isobutyl(S)-2-([1,1'-biphenyl]-4-carboxamido)-3-(1H-1,2,4-triazol-1-yl)propanoate(**15b**)*

Light white solid; yield: 50.5%; mp: 108.4-111.9 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.03 (d, *J* = 7.8 Hz, 1H), 8.52 (s, 1H), 8.00 (s, 1H), 7.88 (d, *J* = 8.3 Hz, 2H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.74 (d, *J* = 7.4 Hz, 2H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.42 (t, *J* = 7.4 Hz, 1H), 4.93 – 4.90 (m, 1H), 4.75 (dd, *J* = 14.0, 5.2 Hz, 1H), 4.70 (dd, *J* = 14.0, 9.1 Hz, 1H), 3.94 – 3.86 (m, 2H), 1.86 (dt, *J* =

13.3, 6.7 Hz, 1H), 0.86 (dd, *J* = 6.7, 1.1 Hz, 6H).^{13}C NMR (150 MHz, DMSO-d_6) δ 169.35, 166.21, 151.66, 144.88, 143.23, 139.06, 132.15, 129.02(2C), 128.13, 127.96(2C), 126.90(2C), 126.62(2C), 70.73, 52.73, 48.48, 27.19, 18.73. HRMS calcd for $C_{22}H_{24}N_4O_3$, $[M + Na]^+$, 415.1746; found 415.1748.

*4.8 General procedure for the synthesis of compounds (**19a-b**)*

The synthetic procedure used for compounds **19a-19b** was similar to that used for compounds **13a-14v**.

*4.8.1 isopropyl(R)-2-(2'-fluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-1,2,4-triazol-1-yl)propanoate(**19a**)*

Light white solid; yield: 58.7%; mp: 116.3-119.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (d, *J* = 7.9 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 2H), 7.67 (d, *J* = 7.2 Hz, 2H), 7.64 (s, 1H), 7.61 – 7.56 (m, 1H), 7.51 – 7.43 (m, 1H), 7.40 – 7.30 (m, 2H), 7.22 (s, 1H), 6.86 (s, 1H), 5.04 – 4.90 (m, 1H), 4.80 – 4.74 (td, *J* = 9.6, 5.2 Hz, 1H), 4.52 (dd, *J* = 14.0, 5.0 Hz, 1H), 4.40 (dd, *J* = 14.0, 9.8 Hz, 1H), 1.20 (t, *J* = 5.7 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.11, 166.21, 159.89, 158.26, 138.23, 137.84, 132.76, 130.82, 130.25, 128.83, 128.35, 127.61(2C), 127.30, 125.07, 125.05, 119.89, 116.28, 68.65, 53.91, 45.75, 21.49, 21.45. HRMS calcd for $C_{22}H_{23}FN_3O_3$, $[M + H]^+$, 396.1723; found 396.1723.

*4.8.2 isobutyl(R)-2-(2'-fluoro-[1,1'-biphenyl]-4-carboxamido)-3-(1H-1,2,4-triazol-1-yl)propanoate(**19b**)*

Light white solid; yield: 56.6%; mp: 118.6-121.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.67 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.64 (s, 1H), 7.61 – 7.56 (m, 1H), 7.50 -7.44 (m, 1H), 7.40 – 7.30 (m, 2H), 7.22 (s, 1H), 6.86 (s, 1H), 4.88 – 4.78 (m, 1H), 4.55 (dd, *J* = 14.0, 4.8 Hz, 1H), 4.42 (dd, *J* = 14.0, 10.1 Hz, 1H), 3.96 – 3.85 (m, 2H), 1.87 (dp, *J* = 13.3, 6.7 Hz, 1H), 0.87 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.58, 166.25, 159.89, 158.26, 138.24, 137.83, 132.73, 130.82, 130.25, 128.85, 128.83, 128.38, 127.59(2C), 127.28, 125.05, 119.86, 116.28, 70.66, 53.82, 45.67, 27.24, 18.75(2C). HRMS calcd for $C_{23}H_{25}FN_3O_3$, $[M + H]^+$, 410.1880; found 410.1880.

4.9 *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 will inhibit the visible growth of the fungi. FLC and ITR were purchased for use as positive control drugs. All of compounds were dissolved in DMSO and serially diluted into the growth medium.

4.10 GC-MS analysis of sterol composition

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

4.11 *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.

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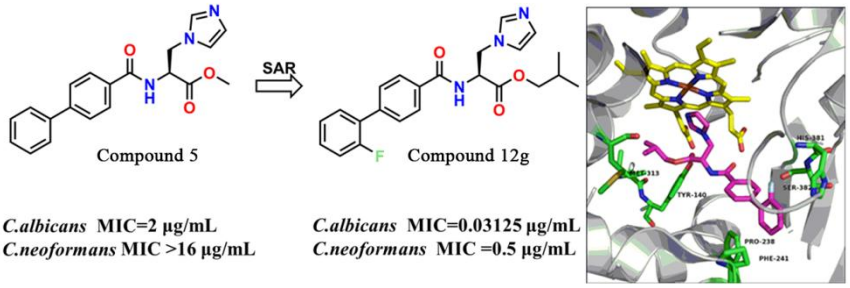
Graphical abstract



Highlights

- 27 new compounds with biphenyl imidazole scaffolds were designed and synthesized.
- Compounds **12f-g** and **19a-b** showed better antifungal activity than fluconazole.
- The structure-activity relationships of compounds were discussed.
- Mechanism studies revealed that the **12g** might act by inhibiting the CYP51.