European Journal of Medicinal Chemistry 183 (2019) 111689

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Combating fluconazole-resistant fungi with novel β-azole-phenylacetone derivatives

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ARTICLE INFO

Article history:
Received 28 July 2019
Received in revised form
6 September 2019
Accepted 7 September 2019
Available online 13 September 2019

Keywords:
CYP51
Azole antifungals
Structure-activity relationship
Fluconazole-resistance

ABSTRACT

A series of β-azole-phenylacetone derivatives with novel structures were designed and synthesized to combat the increasing incidence of susceptible fungal infections and drug-resistant fungal infections. The antifungal activity of the synthesized compounds was assessed against five susceptible strains and five fluconazole-resistant strains. Antifungal activity tests showed that most of the compounds exhibited excellent antifungal activities against five pathogenic strains with MIC values in the range of 0.03−1 μg/mL. Compounds with R^1 = 3-F substituted and **15o** and **15ae** exhibited moderate antifungal activities against fluconazole-resistant *strains 17#* and *CaR* with MIC values in the range of 1−8 μg/mL. Compounds with R^1 = H or 2-F (such as **15a**, **15o**, **15p**) displayed moderate to good antifungal activity against fluconazole-resistant *strains 632*, *901* and *904* with MIC values in the range of 0.125−4 μg/mL. Notably, **15o** and **15ae** exhibited antifungal activity against five susceptible strains and five fluconazole-resistant strains. Preliminary mechanistic studies showed that the potent antifungal activity of compound **15ae** stemmed from inhibition of *C. albicans* CYP51. Compounds **15o**, **15z** and **15ae** were nearly nontoxic to mammalian A549 cells.

1. Introduction

During the past decades, the incidence of systemic fungal infections has increased dramatically, due to the growing number of immunocompromised patients, such as cancer patients undergoing chemotherapy or organ transplant and patients with AIDS [1–4]. *Candida* spp., *Cryptococcus neoformans* and *Aspergillus* spp. are the three major invasive fungal pathogens [5]. Among them, *Candida albicans* causes the most common fungal infections. Currently, antifungal agents in the clinic can be divided into four classes according to the mode of action: polyenes (e.g., amphotericin B and nystatin) [6], azoles (e.g., fluconazole and itraconazole) [7], echinocandins (e.g., caspofungin and micafungin) [8], and antimetabolites (e.g., 5-fluorocytosine) [9].

Among them, azole agents can block the biosynthesis of ergosterol (a very important component of cell membrane) by inhibiting fungal lanosterol 14α-demethylase (CYP51) [10–14]. Fluconazole, a typical azole antifungal agent, has been used in the clinic for almost thirty years. Given its high therapeutic index and more favorable safety profile, fluconazole has been used as a first-line antifungal agent and some derivatives have been developed, for example, itraconazole, voriconazole and albaconazole (Fig. 1). However, the widespread use of azole agents leads to severe drug resistance, which has significantly decreased the therapeutic efficacy. The mechanism of drug resistance mainly includes: (1) overexpression or gene mutation of CYP51 [15,16] (2) upregulation of drug excretion genes (e.g., CDR1, CDR2 and MDR1); [17]; (3) the formation of biofilm [18]. Severe drug resistance has promoted ongoing discoveries azole antifungal agents with novel structures, low toxicity and high efficiency.

Previously, a number of azole derivatives in our compound library were screened and evaluated for their *in vitro* antifungal activity according to the protocols of the Clinical Laboratory Standard Institute (CLSI). Compound **5** has a novel β-azole-phenylacetone scaffold, and it showed excellent antifungal activity against five

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https://doi.org/10.1016/j.ejmech.2019.111689
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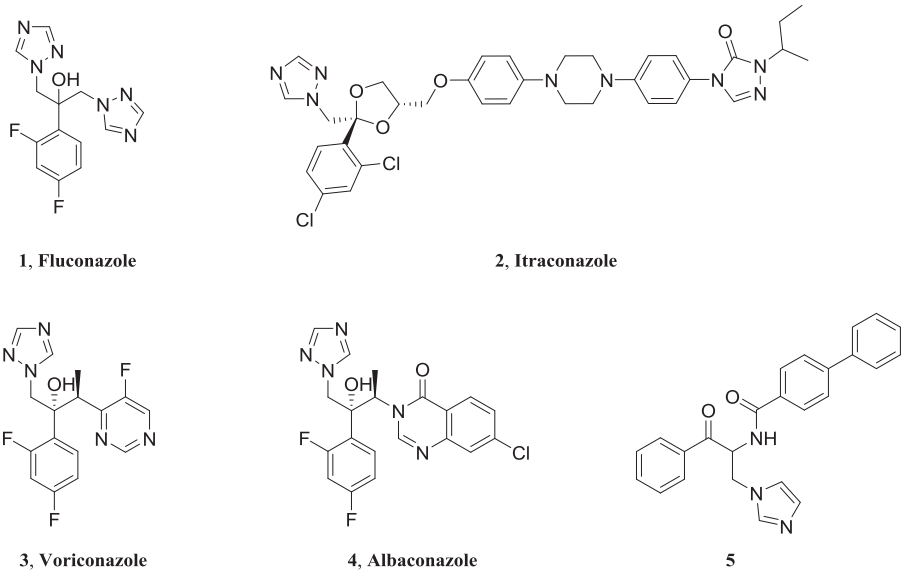
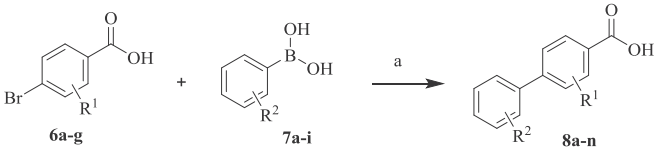


Fig. 1. Chemical structures of azole antifungal agents and lead compound **5**.

susceptible strains but was inactive against fluconazole-resistant *strains 17#* and *CaR*, which were isolated from AIDS patients. To discover compounds with activity against both five susceptible strains and five fluconazole-resistant strains and to explore the structure-activity relationship, a series of β-azole-phenylacetone derivatives have been designed and synthesized and their *in vitro* antifungal activity against susceptible strains and fluconazole-resistant strains was analyzed. The antifungal activity result showed that most compounds exhibited excellent antifungal activity against the five susceptible strains with MIC values in the range of 0.03−1 μg/mL. For the fluconazole-resistant strains, compounds **15b**, **15q**, **15t** and **15z** with R^1 = 3-F substitution and **15o** and **15ae** showed moderate antifungal activity against fluconazole-resistant *strains 17#* and *CaR* with MIC values in the range of 1−8 μg/mL. Compounds with R^1 = H or 2-F substitution, such as **15a**, **15o**, **15p**, displayed moderate to good antifungal activity against fluconazole-resistant *strains 632*, *901* and *904* with MIC values in the range of 0.125−4 μg/mL. Notably, **15o** and 15**ae** exhibited antifungal activity against five susceptible strains and five fluconazole-resistant strains.



	R^1
6a	R^1 = H
6b	R^1 = 2-F
6c	R^1 = 3-F
6d	R^1 = 2-Cl
6e	R^1 = 3-Cl
6f	R^1 = 2-CH_3
6g	R^1 = 3-CH_3

	R^2
7a	R^2 = H
7b	R^2 = 2-F
7c	R^2 = 3-F
7d	R^2 = 4-F
7e	R^2 = 2-Cl
7f	R^2 = 4-Cl
7g	R^2 = 2-CH_3
7h	R^2 = 3-CH_3
7i	R^2 = 4-CH_3

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

2. Results and discussion

2.1. Chemistry

The synthetic route of key intermediates **8a-n** was illustrated in Scheme 1. Commercially available substituted 4-bromobenzoic acid **6a-g** and substituted phenylboronic **7a-i** were reacted to afford **8a-n** via a palladium-catalyzed Suzuki coupling reaction [19].

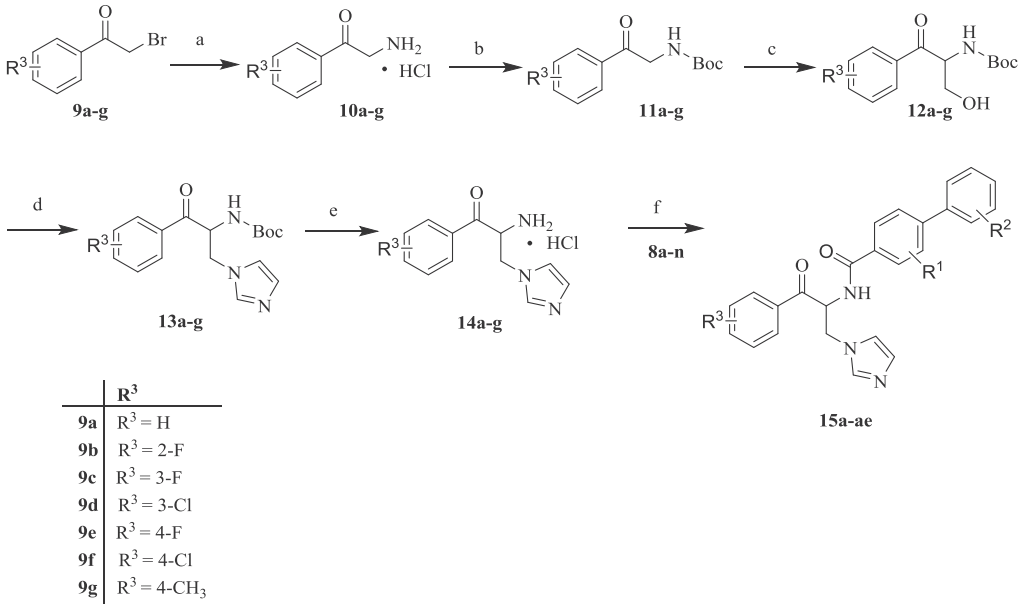
Target compounds **15a-ae** were prepared according the procedures shown in Scheme 2. The bromoacetophenones **9a-g** were reacted with hexamethylenetetramine and then refluxed in the presence of 37% HCl to afford the amino derivatives **10a-g**. The amino derivatives **10a-g** were protected with $(Boc)_2O$, and compounds **11a-g** were obtained. Treatment of compounds **11a-g** with formaldehyde and $NaHCO_3$ in $MeOH/H_2O$ afforded hydroxy compounds **12a-g** [20]. Compounds **12a-g** were reacted with CDI and imidazole to form imidazole derivatives **13a-g**, and then cleavage of the Boc protecting group of compounds **13a-g** with hydrochloric acid gave amino derivatives **14a-g**. The target compounds **15a-ae** were synthesized via amide reaction of amino **14a-g** and acid **8a-n** in DMF at room temperature.

As depicted in Scheme 3, the triazole derivative **18** was synthesized using procedures similar to those described above, except that 1,1′-Carbonyl-di(1,2,4-triazole) and triazole were used instead of CDI and imidazole.

2.2. In vitro antifungal activity

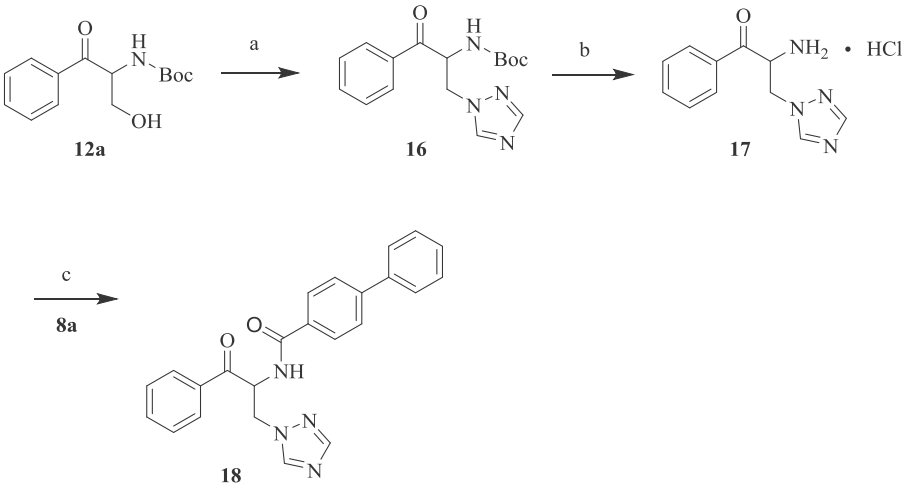
The *in vitro* antifungal activity of target compounds was evaluated against five pathogenic fungi according to the protocols of the Clinical Laboratory Standard Institute (CLSI) [21]. Fluconazole (FCZ) and itraconazole (ITZ) were used as reference drugs. The *in vitro* results of the antifungal activities are summarized in Table 1.

The results showed that most of the compounds exhibited moderate to good antifungal activities against *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, and *Candida parapsilosis*. Of these, compounds **15a**, **15b** and **15u-ae** show the excellent antifungal activities with the MIC values in the range of 0.03−1 μg/mL,



	R^3
9a	R^3 = H
9b	R^3 = 2-F
9c	R^3 = 3-F
9d	R^3 = 3-Cl
9e	R^3 = 4-F
9f	R^3 = 4-Cl
9g	R^3 = 4-CH_3

Scheme 2. General synthesis of the target compounds **15a-ae**. Reagents and conditions: (a) i) hexamethylenetetramine, $CHCl_3$, 50 °C; ii) 37% HCl, EtOH, reflux; (b) $(Boc)_2O$, $NaHCO_3$, MeOH, H_2O, rt.; (c) 37% CH_2O (aq), $NaHCO_3$, EtOH; (d) imidazole, CDI, CH_3CN, 70 °C; (e) HCl—EtOH, rt.; (f) HOBt, EDCI, DIEA, DMF, rt.



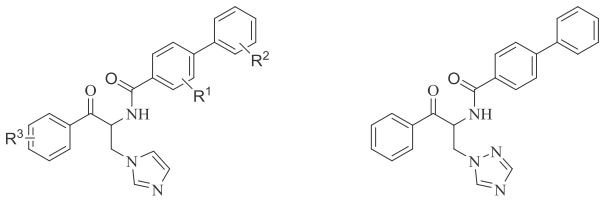
Scheme 3. Synthesis of the target compound **18**. Reagents and conditions: (a) Triazole, 1,1′-Carbonyl-di(1,2,4-triazole), CH_3CN, 70 °C; (b) HCl—EtOH, rt.; (c) HOBt, EDCI, DIEA, DMF, rt.

which are superior to the reference drug fluconazole. The antifungal activity of compound **15e** with 4′-F substituted on the biphenyl group almost disappeared. Compounds **15f-n** with -Cl or $-CH_3$ substituted on the biphenyl group was inactive against the fungi. Compound **18** with a triazole group exhibited a slightly decrease in antifungal activities against *Candida albicans* and *Candida tropicalis*.

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

In recent years, given the widespread use of antifungal agents, fungi have developed severe resistance to marketed drugs, especially fluconazole. Therefore, it is necessary to evaluate its antifungal activity against fluconazole-resistant strains in the development of antifungal drugs. *Strains 17#* and *CaR* are fluconazole-resistant strains of *Candida albicans*, which were isolated from AIDS patients. *Strains 632*, *901* and *904* are also fluconazole-resistant strains of *Candida albicans*, which were isolated from clinic. The resistance mechanism of *strain 17#* is correlated with the increase in mRNA levels of *ERG11*, *CDR1* (encodes a protein which belongs to the superfamily of ATP-binding cassette transporters) and *MDR1* (encodes a protein from the major facilitator superfamily). For the resistance *strain CaR*, increased *MDR1* mRNA levels and the mutation of *ERG11* (E266D, G464S, V488I) correlated with decreased drug susceptibility. The potent compounds were selected for evaluation of *in vitro* antifungal activity against the five fluconazole-resistant strains. The results are summarized in Table 2. The lead compound **5** was inactive against the fluconazole-resistant *strains 17#* and *CaR* with the MIC values > 64 μg/mL. Compounds **15b**, **15q**, **15t** and **15z** with R^1 = 3-F substituted and **15o** and **15ae** exhibited moderate antifungal activities against *strains 17#* and *CaR* with MIC values in the range of 1—8 μg/mL. Among them, compounds **15z** and **15ae** exhibited the

Table 1
In vitro antifungal activities of the target compounds (MIC, μg/mL)[a].



Compd.	R^1	R^2	R^3	*C. alb.*(I)	*C. alb.*(II)	*C. tro.*	*C. gla.*	*C. par.*
15a	2-F	H	H	0.06	0.125	0.06	0.25	0.125
15b	3-F	H	H	0.125	0.25	0.06	0.5	0.125
15c	H	2′-F	H	0.5	1	0.25	1	2
15d	H	3′-F	H	0.25	0.5	0.125	>64	1
15e	H	4′-F	H	4	>16	8	>64	>64
15f	2-Cl	H	H	>16	>16	>16	>64	>64
15g	3-Cl	H	H	8	>16	>16	>64	>64
15h	H	2′-Cl	H	2	>16	>16	4	>64
15i	H	4′-Cl	H	0.5	>16	>16	>64	>64
15j	2-CH_3	H	H	>16	>16	>16	>64	>64
15k	3-CH_3	H	H	>16	>16	>16	>64	>64
15l	H	2′-CH_3	H	>16	>16	>16	>64	>64
15m	H	3′-CH_3	H	>16	>16	>16	>64	>64
15n	H	4′-CH_3	H	2	>16	>16	>64	>64
15o	H	H	2-F	0.125	0.125	1	2	0.125
15p	2-F	H	2-F	0.5	0.5	4	>64	2
15q	3-F	H	2-F	0.5	1	1	>64	1
15r	H	H	3-F	0.06	0.125	0.25	1	0.5
15s	2-F	H	3-F	0.125	0.25	1	2	1
15t	3-F	H	3-F	0.125	0.5	0.5	2	0.5
15u	H	H	3-Cl	0.03	0.06	0.03	0.125	0.125
15v	2-F	H	3-Cl	0.06	0.125	0.125	0.25	0.5
15w	3-F	H	3-Cl	0.06	0.125	0.03	0.25	0.125
15x	H	H	4-F	0.06	0.25	0.25	0.5	0.25
15y	2-F	H	4-F	0.06	0.25	0.25	0.5	0.25
15z	3-F	H	4-F	0.06	0.25	0.125	0.125	0.125
15aa	H	H	4-Cl	0.06	0.125	0.06	0.5	0.25
15 ab	2-F	H	4-Cl	0.06	0.5	0.06	0.5	1
15ac	3-F	H	4-Cl	0.06	0.5	0.06	0.25	0.125
15ad	H	H	4-CH_3	0.06	0.125	0.03	0.25	0.5
15ae	2-F	H	4-CH_3	0.03	0.06	0.03	0.125	0.125
18				0.25	2	1	ND[b]	ND[b]
5				0.06	0.125	0.06	0.25	0.125
FCZ				0.5	2	0.5	4	2
ITZ				0.03	0.03	0.03	0.125	0.125

[a] Abbreviations: *C. alb.*(I), *Candida albicans* (SC5314); *C. alb.*(II), *Candida albicans* (GIM 2.194); *C. tro.*, *Candida tropicalis* (GIM 2.183); *C. gla., Candida glabrata; C. par.*, *Candida parapsilosis* (GIM 2.190); FCZ: Fluconazole; ITZ: Itraconazole.
[b] ND = not determined.

best antifungal activity against fluconazole-resistant strains with MIC values in the range of 1−2 μg/mL. Compounds with R^1 = H or 2-F substitution displayed moderate to good antifungal activity against *strains 632*, *901* and *904*, such as **15a**, **15o**, **15p**. Of these, compounds **15o**, **15p**, **15r** and **15s** with R^3 = 2-F or 3-F substitution showed excellent antifungal activity against *strains 632*, *901* and *904* with MIC values in the range of 0.125−1 μg/mL. Compounds **15u**, **15v**, **15x**, **15y** and **15aa** with R^3 = 3-Cl, 4-F or 4-Cl substitution showed moderate antifungal activity. In addition, compound **15o** exhibited good antifungal activity against five fluconazole-resistant strains.

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

β-Azole-phenylacetone derivatives are a novel class of antifungal agents. To investigate the antifungal mechanism of compound **15ae**, gas chromatography-mass spectrometry (GC-MS) was used to analyze the sterol composition in cells membrane using fluconazole as the reference drug [22]. The sterol profile results were summarized in Table 3. The sterol profile in the absence of drug indicated that ergosterol was the only sterol (100%) detected. When *Candida albicans* were cultured with fluconazole at 0.25 μg/mL, the content of ergosterol in the cell membrane decreased sharply from 100% to 63%, and the contents of lanosterol, eburicol and 14α-methyl ergosta-8, 24(28)-dien-3β,6α-diol were accumulated. These changes in sterol levels were caused by inhibiting the sterol 14α-demethylase enzyme (CYP51) of the ergosterol biosynthetic pathway. When treated with compound **15ae** at sub-MIC levels of 0.015 μg/mL, the same changing trend of sterol contents in the cell membrane was detected compared with the fluconazole group. Moreover, we detected a relatively low amount of ergosterol and a high quantity of lanosterol, eburicol and 14α-methyl ergosta-8,24(28)-dien-3β,6α-diol. The results indicated that compound **15ae** acts by inhibiting the sterol 14α-demethylase enzyme (CYP51), thereby affecting the biosynthesis of ergosterol.

2.5. In vitro cytotoxicity assay

β-Azole-phenylacetone derivatives exhibited excellent antifungal activity against pathogenic fungi. Therefore, it is necessary to consider the potential toxicity to mammalian cells. Potent compounds **15o**, **15u**, **15z** and **15ae** were selected to evaluate the toxicity to human lung carcinoma A549 cells. The results are shown in Table 4. In general, concentration-dependent toxicity against the A549 cell line was observed for compounds **15u**, **15z** and **15ae**. When tested at the concentration of 10 μM/L, compounds **15o**, **15u**, **15z** and **15ae** were nontoxic, and cell survival was 100%, 96.35%, 86.78%, and 92.47%, respectively. Therefore, the compounds showed no toxicity to A549 cells.

2.6. Structure-activity relationships

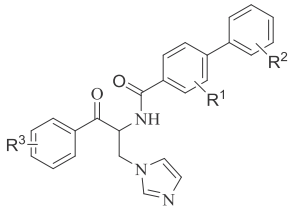
Compounds **15a** and **15b** with 2-F or 3-F substituted on the biphenyl group maintain antifungal activity against the fungi with the MIC values in the range of 0.06−0.5 μg/mL. The antifungal activity decreased when the compounds with 2′-F (**15c**) or 3′-F (**15d**) substituted on the biphenyl group. The antifungal activity of compound **15e** with 4′-F on the biphenyl group disappeared. In addition, compounds **15f-n** with -Cl or $-CH_3$ substituted on the biphenyl group were inactive against pathogenic fungi. This finding may be due to the fact that the hydrophobic cavity of CYP51 occupied by biphenyl group is relatively narrow, and the -Cl and $-CH_3$ of large groups easily cause the collision with the CYP51 protein.

Compounds **15o-t** with R^3 = 2-F or 3-F exhibited slightly weaker antifungal activity than compounds **15u-ae** with R^3 = 3-Cl, 4-F, 4-Cl or 4-CH_3. When R^3 is 3-Cl, 4-F, 4-Cl or 4-CH_3 group, compounds **15u-ae** exhibited similar activity, and the substitution on the biphenyl group has minimal effect on the antifungal activity. Replacing the imidazole group with a triazole, compound **18** showed slightly decreased activity.

For the resistant *strains 17#* and *CaR*, when R^3 is an H or F atom, only the compounds (**15b**, **15q**, **15t**, **15z**) with R^1 = 3-F and **15o** exhibit the moderate antifungal activity. The compounds are inactive when R^3 is 3-Cl and 4-Cl. Moreover, when R^3 is 4-CH_3, compound **15ae** with R^1 = 2-F shows best antifungal activity against the two resistant strains.

For the resistant *strains 632*, *901* and *904*, compounds (such as **15o**, **15p**) with R^1 = H or 2-F substituted displayed moderate to good antifungal activity. Of these, compounds with R^3 = 2-F or 3-F substituted showed excellent antifungal activity against *strains 632*, *901* and *904* with MIC values in the range of 0.125−1 μg/mL.

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



Compd.	R^1	R^2	R^3	*Candida albicans*				
				Strain 17#	Strain CaR	Strain 632	Strain 901	Strain 904
15a	2-F	H	H	>64	>64	2	0.25	2
15b	3-F	H	H	2	4	32	>64	8
15o	H	H	2-F	8	1	0.5	0.25	<0.125
15p	2-F	H	2-F	>64	>64	1	0.25	0.25
15q	3-F	H	2-F	8	4	8	>64	2
15r	H	H	3-F	>64	>64	1	0.25	0.5
15s	2-F	H	3-F	>64	>64	1	0.25	0.5
15t	3-F	H	3-F	4	2	>64	2	32
15u	H	H	3-Cl	>64	>64	4	1	4
15v	2-F	H	3-Cl	>64	>64	4	4	4
15w	3-F	H	3-Cl	>64	>64	>64	>64	>64
15x	H	H	4-F	>64	>64	2	1	2
15y	2-F	H	4-F	>64	>64	4	>64	4
15z	3-F	H	4-F	2	2	>64	>64	8
15aa	H	H	4-Cl	>64	>64	4	2	2
15 ab	2-F	H	4-Cl	>64	>64	>64	>64	>64
15ac	3-F	H	4-Cl	>64	>64	>64	>64	32
15ad	H	H	4-CH_3	>64	>64	>64	16	32
15ae	2-F	H	4-CH_3	2	1	16	16	16
5				>64	>64	2	0.5	1
FCZ				>64	>64	>64	>64	>64
ITZ				0.25	0.25	>64	>64	>64

[a]Abbreviations: *strain 17#*, fluconazole-resistant strain of *Candida albicans*; *strain CaR*, fluconazole-resistant strain of *Candida albicans*; *strain 632*, fluconazole-resistant strain of *Candida albicans*; *strain 901*, fluconazole-resistant strain of *Candida albicans*; *strain 904*, fluconazole-resistant strain of *Candida albicans*; FCZ: Fluconazole; ITZ: Itraconazole. *Strains 17#* and *CaR* were provided by Institute of Microbiology, Chinese Academy of Sciences. *Strains 632*, *901* and *904* were provided by the Second Military Medical University.

Table 3
Analysis of sterol composition in *C. albicans* by GC-MS[a].

Sterol	% of total sterols (*C. alb.*)		
	No drug	FCZ	15ae
Lanosterol	ND	16.74	23.17
Ergosterol	100.00	63.00	41.99
Eburicol	ND	4.27	9.56
14α-methyl ergosta-8,24(28)-dien-3β,6α-diol	ND	5.15	13.26
Unknown sterol	ND	4.23	3.04
Unknown sterol	ND	5.07	5.85
Unknown sterol	ND	1.54	3.13

[a] Abbreviations: The fungal strain was treated with DMSO (no drug), Fluconazole at 0.25 μg/mL, Compound **15ae** at 0.015 μg/mL, ND = Not detected.

Table 4
In vitro cytotoxicity of compounds in A549 cells.

Compd.	Cell survival (μM/L)			
	0.08	0.40	2	10
15o	100%	100%	100%	100%
15u	100%	97.42%	97.23%	96.35%
15z	100%	100%	100%	86.78%
15ae	100%	100%	96.96%	92.47%
FCZ	100%	99.07%	95.69%	95.34%

Compounds with R^3 = 3-Cl, 4-F or 4-Cl substituted showed moderate antifungal activity.

2.7. Molecular docking model analysis of compounds **15o** and **15z** in the active site of Candida albicans CYP51

To better understand the binding mode of compounds with *Candida albicans* CYP51 and provide information for further optimization, compounds **15o** and **15z** were docked into the active site of *Candida albicans* CYP51 using the CDOCKER program in the Discovery Studio 3.0 software. The published crystal structure of *Candida albicans* CYP51 (PDB ID: 5V5Z) served as a useful template for generating the binding mode [23]. As shown in Fig. 2A, the imidazole group of compound **15z** binds to the heme group through formation of a coordination bond with the iron atom. The benzoyl group forms hydrophobic interactions with Thr^{126}, Ile^{131} and Tyr^{132}. The biphenyl group extends into the CYP51 channel and mainly forms hydrophobic and Van der Waals interactions with the surrounding residues, such as Leu^{121}, Met^{508}, Ser^{507}, Pro^{230}, Tyr^{64}, and Phe^{380}. In addition, the 2-F on the biphenyl group can form halogen (fluorine) bonds with Met^{508}. The binding mode of compound **15o** with *Candida albicans* CYP51 is similar to that of **15z** (Fig. 2B).

Amino acid mutations (Y132H, Y132F, K143R, G307S, and S405F) in CYP51 from fluconazole-resistant *Candida albicans* that have not been found in fluconazole-susceptible strains might potentially influence fluconazole binding to CYP51. Of these, Y132 is the most common resistance mutation site seen in fluconazole resistant *Candida albicans*. The tertiary alcohol group of fluconazole interacts with the residue Y132 through a water molecule by forming

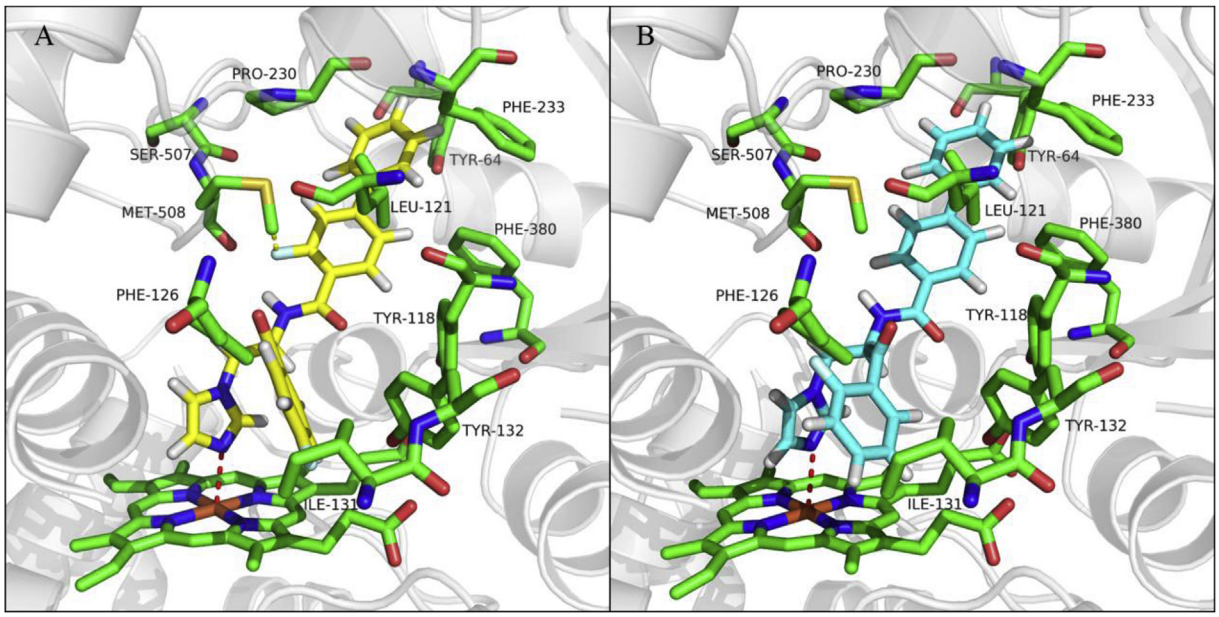


Fig. 2. (A) Predicted binding mode of **15z** in the active site of *Candida albicans* CYP51. (B) Predicted binding mode of **15o** in the active site of *Candida albicans* CYP51. The halogen bonds are shown as yellow dashed lines. Figures were generated using PyMOL. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

hydrogen bonds. However, when Y132 is mutated, the tertiary alcohol group of fluconazole can be not interacted with the CYP51, which reduces the binding force. β-Azole-phenylacetone derivatives without the tertiary alcohol group might be advantage for their antifungal activity against the fluconazole-resistance strains.

3. Conclusions

To combat the increasing incidence of susceptible fungal infections and drug-resistant fungal infections, a number of azole derivatives in our compound library were screened, and their *in vitro* antifungal activity was evaluated. Compound **5** with a β-azole-phenylacetone scaffold showed excellent antifungal activity against the susceptible strains but was inactive against fluconazole-resistant *strains 17#* and *CaR*. To discover compounds active against both susceptible strains and fluconazole-resistant strains and to explore the structure-activity relationship, a series of β-azole-phenylacetone derivatives with novel structure were designed and synthesized. The synthesized compounds were evaluated for their antifungal activity against five susceptible strains and five fluconazole-resistant strains. Antifungal activity tests showed that compounds **15a**, **15b** and **15u-ae** exhibited excellent antifungal activity against pathogenic strains with MIC values in the range of 0.03−1 μg/mL. In addition, compounds **15b**, **15q**, **15t** and **15z** with R^1 = 3-F substituted and **15o** and **15ae** exhibited moderate antifungal activity against two fluconazole-resistant *strains 17#* and *CaR* with MIC values in the range of 1−8 μg/mL. For the resistant *strains 632*, *901* and *904*, compounds with R^1 = H or 2-F substituted displayed moderate to good antifungal activity. Of these, compounds **15o**, **15p**, **15r** and **15s** showed excellent antifungal activity against *strains 632*, *901* and *904* with MIC values in the range of 0.125−1 μg/mL. Compounds **15o** and **15ae** exhibited antifungal activity against the five susceptible strains and five fluconazole-resistant strains. Preliminary mechanistic studies showed that the potent antifungal activity of compound **15ae** stemmed from inhibition of *C. albicans* CYP51. Compounds **15o**, **15z** and **15ae** were almost nontoxic to mammalian A549 cells. Further investigations of the potent compounds **15o**, **15z** and **15ae** are still in progress.

4. Experimental section

4.1. Chemistry

All reagents used in the experiment were procured from Aladdin, Bide and Sinopharm Company (except special instruction) without further purification. The key intermediates **8a-n** and **12a-g** were synthesized according to the literature [19,24,25]. The solvents were purified according to the standard procedures. The reactions were monitored by TLC (Jiangyou, Yantai). TLC was performed on silica gel plates with fluorescence F-254 and visualized with UV light. Silica gel of 200−300 mesh (Jiangyou, Yantai) 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. 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 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 (1.1 equiv.), benzoic acid (1.0 equiv.), K_2CO_3 (2.0 equiv.), and Pd $[P(Ph)_3]_4$ (0.1 equiv.) were added to dioxane/H_2O (10:1). The mixture was heated at 105 °C for approximately 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*

Hexamethylenetetramine (1.0 equiv.) was added to a solution of

bromoacetophenone (1.0 equiv.) in $CHCl_3$. The mixture was heated at 50 °C for approximately 2 h. After filtering, the residue was washed with $CHCl_3$ and EtOH sequentially. Then, the residue was dissolved in EtOH, and 37% HCl (6.0 equiv.) was added to the mixture. The mixture was refluxed for 4 h and monitored with TLC. After filtering, the filtrate was evaporated in vacuum to afford compounds **10a-g**.

*4.3.1. 2-Amino-1-phenylethan-1-one hydrochloride (**10a**)*

Light white solid; yield: 89.5%. ^{1}H NMR (600 MHz, DMSO) δ 8.40 (s, 3H), 8.06–8.00 (m, 2H), 7.74 (t, J = 7.4 Hz, 1H), 7.62–7.58 (m, 2H), 4.63–4.59 (m, 2H).

*4.4. The procedure for the synthesis of the intermediates **11a-g***

Briefly, $(Boc)_2O$ (1.5 equiv.) and $NaHCO_3$ (2.5 equiv.) were added to a solution of the amino **10a-g** (1.0 equiv.) in MeOH/H_2O (1:1). The mixture was stirred at room temperature overnight, and then poured into ice-water, and the product precipitated. The mixture was filtered, and the residue was dried to give compounds **11a-g** as a white solid.

*4.5. The procedure for the synthesis of the intermediates **12a-g***

To a solution of the amino protected compounds **11a-g** (1.0 equiv.) in ethanol, $NaHCO_3$ (0.5 M, 0.5 equiv.), 37% CH_2O (1.5 equiv.) were added. The mixture was stirred at 50 °C for 48 h, and then the organic solvent was removed under vacuum. The residue was dissolved in DCM and washed with water, brine sequentially. The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated and purified by silica column to give the compounds **12a-g** as a white solid.

*4.5.1. ·Tert-butyl (3-hydroxy-1-oxo-1-phenylpropan-2-yl) carbamate (**12a**)*

Light white solid; yield: 70.6%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 (d, J = 7.5 Hz, 2H), 7.62 (t, J = 7.4 Hz, 1H), 7.50 (t, J = 7.8 Hz, 2H), 7.26 (s, 1H), 5.87 (s, 1H), 5.34 (s, 1H), 4.02 (dd, J = 11.6, 3.4 Hz, 1H), 3.86 (dd, J = 11.6, 4.6 Hz, 1H), 1.47 (s, 9H).

*4.6. The procedure for the synthesis of the intermediates **13a-g***

The intermediates **12a-g** (1.0 equiv.), CDI (1.2 equiv.), and imidazole (1.0 equiv.) were added to acetonitrile. The mixture was heated at 70 °C for 2 h and then cooled to room temperature. The solvent was removed under vacuum. The residue was dissolved in ethylacetate, washed with water thrice, dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford compounds **13a-g** as a white solid.

*4.6.1. Tert-butyl (3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)carbamate (**13a**)*

Light white solid; yield: 78.3%. ^{1}H NMR (600 MHz, DMSO) δ 7.96 (d, J = 7.4 Hz, 2H), 7.65 (t, J = 7.4 Hz, 1H), 7.59 (d, J = 8.8 Hz, 1H), 7.57 (s, 1H), 7.52 (t, J = 7.7 Hz, 2H), 7.17 (s, 1H), 6.85 (s, 1H), 5.30–5.26 (m, 1H), 4.43–4.39 (m, 1H), 4.18–4.14 (m, 1H), 1.26 (s, 9H).

*4.7. The procedure for the synthesis of the intermediates **14a-g***

The Boc protected compounds **13a-g** (1.0 equiv.) was added to HCl–EtOH (4 N, 7.0 equiv.); then the mixture was stirred at room temperature for 3 h. The mixture was filtered, and the residue was dried to afford **14a-g** as a white solid.

*4.8. The procedure for the synthesis of the compounds **15a-ae***

To a solution of acid **8a-n** (1.0 equiv.) in DMF, HOBt (1.1 equiv.) and EDCI (1.1 equiv.) were added and the mixture was stirred for 1 h at room temperature. Then the amino salt **14a-g** (1.1 equiv.) and DIEA (2.2 equiv.) were added. The reaction was stirred and monitored with TLC. The mixture was poured into water and extracted with ethylacetate 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) to afford target compounds **15a-ae**.

*4.8.1. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-2-fluoro-[1,1′-biphenyl]-4-carboxamide (**15a**)*

Light white solid; yield: 48.5%; mp: 136.7–137.0 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.28 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 7.5 Hz, 2H), 7.65 (ddd, J = 21.9, 11.7, 7.1 Hz, 6H), 7.58–7.54 (m, 3H), 7.53 (t, J = 7.7 Hz, 2H), 7.50 (t, J = 7.6 Hz, 2H), 7.44 (t, J = 7.3 Hz, 1H), 7.25 (s, 1H), 6.86 (s, 1H), 5.81–5.77 (m, 1H), 4.61 (dd, J = 14.1, 4.4 Hz, 1H), 4.43 (dd, J = 14.1, 9.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 197.28, 164.88, 159.95, 158.32, 138.41, 135.36, 134.76, 134.71, 134.62, 134.08, 131.93, 131.85, 131.44, 129.31, 129.18, 128.92, 128.78, 124.25, 120.53, 115.51, 115.35, 55.44, 46.27. HRMS (EI): m/z (M^+) for $C_{25}H_{20}FN_3O_2$: calcd. 413.154; found 413.1548.

*4.8.2. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-3-fluoro-[1,1′-biphenyl]-4-carboxamide (**15b**)*

Light white solid; yield: 43.1%; mp: 140.0–141.0 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.04 (dd, J = 8.3, 1.4 Hz, 1H), 8.05–8.01 (m, 2H), 7.74–7.71 (m, 2H), 7.66 (t, J = 6.8 Hz, 1H), 7.63 (s, 1H), 7.61–7.52 (m, 2H), 7.51–7.40 (m, 2H), 7.22 (s, 1H), 6.88 (s, 1H), 5.77–5.74 (m, 1H), 4.60 (dd, J = 14.2, 4.4 Hz, 1H), 4.40 (dd, J = 14.2, 9.4 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 197.16, 163.76, 160.85, 159.19, 145.29, 145.23, 138.40, 138.26, 135.31, 134.06, 130.86, 130.83, 129.56, 129.14, 128.84, 128.75, 127.41, 123.00, 121.99, 121.90, 120.51, 114.70, 114.54, 55.57, 46.31. HRMS (EI): m/z (M^+) for $C_{25}H_{20}FN_3O_2$: calcd. 413.154; found 413.1548.

*4.8.3. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-2′-fluoro-[1,1′-biphenyl]-4-carboxamide (**15c**)*

Light white solid; yield: 47.8%. ^{1}H NMR (600 MHz, DMSO) δ 9.41 (d, J = 8.4 Hz, 1H), 8.05–8.02 (m, 2H), 7.86 (d, J = 8.4 Hz, 2H), 7.68 (s, 1H), 7.64–7.59 (m, 3H), 7.56–7.50 (m, 3H), 7.47–7.44 (m, 1H), 7.35–7.30 (m, 2H), 7.26 (s, 1H), 6.84 (s, 1H), 5.78–5.72 (m, 1H), 4.60 (dd, J = 14.1, 4.5 Hz, 1H), 4.49 (dd, J = 14.1, 9.6 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 197.45, 166.08, 160.32, 158.68, 138.67, 138.39, 135.46, 133.95, 132.98, 131.26, 130.71, 130.66, 129.24, 129.22, 129.12 (2C), 128.78 (2C), 128.58, 128.13 (2C), 127.81, 125.54, 125.51, 120.58, 116.73, 116.59, 55.53, 46.23. HRMS (EI): m/z (M^+) for $C_{25}H_{20}FN_3O_2$: calcd. 413.154; found 413.1549.

*4.8.4. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-3′-fluoro-[1,1′-biphenyl]-4-carboxamide (**15d**)*

Light white solid; yield: 45.5%; mp: 164.1–165.2 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.20 (t, J = 7.5 Hz, 1H), 8.03 (d, J = 6.9 Hz, 2H), 7.83–7.81 (m, 2H), 7.80–7.77 (m, 2H), 7.65–7.62 (m, 2H), 7.59–7.55 (m, 2H), 7.54–7.49 (m, 3H), 7.26–7.22 (m, 2H), 6.87–6.81 (m, 1H), 5.78–5.74 (m, 1H), 4.60 (dd, J = 14.0, 3.9 Hz, 1H), 4.46–4.41 (m, 1H). ^{13}C NMR (150 MHz, DMSO) δ 197.47, 166.06, 163.96, 162.34, 142.20, 141.97, 141.92, 138.39, 135.43, 134.01, 133.10, 131.47, 131.42, 129.15 (2C), 128.78 (2C), 128.74, 128.44 (2C), 127.28 (2C), 123.46, 120.53, 115.39, 115.25, 114.19, 114.05, 55.43, 46.28. HRMS (EI): m/z (M^+) for $C_{25}H_{20}FN_3O_2$: calcd. 413.154; found 413.1548.

*4.8.5. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-4′-fluoro-[1,1′-biphenyl]-4-carboxamide (**15e**)*

Light white solid; yield: 47.1%; mp: 110.0–110.9 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.18 (d, J = 8.3 Hz, 1H), 8.03 (d, J = 7.9 Hz, 2H), 7.80 (d, J = 8.3 Hz, 2H), 7.76–7.73 (m, 2H), 7.72 (d, J = 8.4 Hz, 2H), 7.65–7.61 (m, 2H), 7.52 (t, J = 7.8 Hz, 2H), 7.31 (t, J = 8.9 Hz, 2H), 7.23 (s, 1H), 6.85 (s, 1H), 5.76 (td, J = 9.0, 4.6 Hz, 1H), 4.60 (dd, J = 14.1, 4.4 Hz, 1H), 4.43 (dd, J = 14.1, 9.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 197.48, 166.12, 163.54, 161.91, 142.63, 138.38, 136.01, 135.99, 135.44, 134.00, 132.51, 129.47, 129.42, 129.14 (2C), 128.78 (2C), 128.73, 128.43 (2C), 127.04 (2C), 120.52, 116.39, 116.25, 55.41, 46.28. HRMS (EI): m/z (M^+) for $C_{25}H_{20}FN_3O_2$: calcd. 413.154; found 413.1539.

*4.8.6. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-2-chloro-[1,1′-biphenyl]-4-carboxamide (**15f**)*

Light white solid; yield: 48.5%; mp: 151.1–153.0 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.31 (d, J = 8.4 Hz, 1H), 8.03–8.00 (m, 2H), 7.89 (d, J = 1.1 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.65–7.62 (m, 2H), 7.53 (t, J = 7.7 Hz, 2H), 7.50–7.46 (m, 3H), 7.45–7.42 (m, 3H), 7.23 (s, 1H), 6.85 (s, 1H), 5.79–5.76 (m, 1H), 4.59 (dd, J = 14.2, 4.4 Hz, 1H), 4.42 (dd, J = 14.1, 9.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 197.26, 164.80, 143.28, 138.41, 138.32, 135.36, 134.25, 134.08, 132.14, 131.93, 129.54, 129.19, 128.95, 128.78, 128.69, 126.82, 120.54, 55.41, 46.27. HRMS (EI): m/z (M^+) for $C_{25}H_{20}ClN_3O_2$: calcd. 429.1244; found 429.1238.

*4.8.7. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-3-chloro-[1,1′-biphenyl]-4-carboxamide (**15g**)*

Light white solid; yield: 49.0%; mp: 141.5–142.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.21 (d, J = 8.6 Hz, 1H), 8.03 (d, J = 7.9 Hz, 2H), 7.71–7.64 (m, 6H), 7.56 (t, J = 7.7 Hz, 2H), 7.47 (t, J = 7.6 Hz, 2H), 7.41 (t, J = 7.2 Hz, 1H), 7.27 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 6.91 (s, 1H), 5.77 (td, J = 9.1, 4.4 Hz, 1H), 4.57 (dd, J = 14.1, 4.4 Hz, 1H), 4.37 (dd, J = 14.1, 9.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 197.16, 166.29, 143.43, 138.54, 138.29, 135.36, 134.75, 134.06, 131.12, 129.56 (3C), 129.11 (2C), 128.95 (2C), 128.82, 128.03, 127.38 (3C), 125.69, 120.58, 55.02, 46.26. HRMS (EI): m/z (M^+) for $C_{25}H_{20}ClN_3O_2$: calcd. 429.1244; found 429.1249.

*4.8.8. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-2′-chloro-[1,1′-biphenyl]-4-carboxamide (**15h**)*

Light white solid; yield: 54.8%; mp: 115.4–117.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.21 (d, J = 8.4 Hz, 1H), 8.05–8.00 (m, 2H), 7.79 (d, J = 8.4 Hz, 2H), 7.66–7.61 (m, 2H), 7.59–7.56 (m, 1H), 7.53 (t, J = 7.8 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.45–7.39 (m, 3H), 7.24 (s, 1H), 6.85 (s, 1H), 5.78–5.75 (m, 1H), 4.60 (dd, J = 14.1, 4.5 Hz, 1H), 4.43 (dd, J = 14.1, 9.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 197.42, 166.19, 142.33, 139.38, 138.40, 135.42, 134.03, 133.06, 131.87, 131.63, 130.36, 130.14, 129.78, 129.16, 128.78, 128.74, 128.07, 127.68, 120.55, 55.40, 46.29. HRMS (EI): m/z (M^+) for $C_{25}H_{20}ClN_3O_2$: calcd. 429.1244; found 429.1254.

*4.8.9. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-4′-chloro-[1,1′-biphenyl]-4-carboxamide (**15i**)*

Light white solid; yield: 52.6%; mp: 176.5–177.7 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.19 (d, J = 8.4 Hz, 1H), 8.02 (d, J = 7.3 Hz, 2H), 7.81 (d, J = 8.4 Hz, 2H), 7.76–7.71 (m, 4H), 7.66 (s, 1H), 7.63 (t, J = 7.4 Hz, 1H), 7.55–7.49 (m, 4H), 7.23 (s, 1H), 6.85 (s, 1H), 5.76 (td, J = 9.2, 4.6 Hz, 1H), 4.60 (dd, J = 14.1, 4.5 Hz, 1H), 4.42 (dd, J = 14.1, 9.6 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 197.47, 166.07, 142.31, 138.38, 138.33, 135.43, 134.01, 133.53, 132.85, 129.45 (2C), 129.16 (4C), 128.78 (2C), 128.73, 128.48 (2C), 127.08 (2C), 120.52, 55.41, 46.28. HRMS (EI): m/z (M^+) for $C_{25}H_{20}ClN_3O_2$: calcd. 429.1244; found 429.1250.

*4.8.10. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-2-methyl-[1,1′-biphenyl]-4-carboxamide (**15j**)*

Light white solid; yield: 49.5%; mp: 83.4–85.2 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.18 (s, 1H), 8.02 (d, 2H), 7.68–7.60 (m, 4H), 7.52 (t, J = 7.8 Hz, 2H), 7.45 (t, J = 7.5 Hz, 2H), 7.38 (t, J = 6.8 Hz, 1H), 7.34–7.32 (m, 2H), 7.27–7.22 (m, 2H), 6.86 (s, 1H), 5.76 (td, J = 9.1, 4.5 Hz, 1H), 4.60 (dd, J = 14.1, 4.5 Hz, 1H), 4.46–4.41 (m, 1H), 2.23 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 197.47, 166.19, 144.97, 140.82, 138.36, 135.44, 133.98, 132.53, 130.05, 129.74, 129.23 (2C), 129.13 (2C), 128.79 (4C), 128.59, 127.84, 125.34, 120.58, 55.28, 46.36, 20.64. HRMS (EI): m/z (M^+) for $C_{26}H_{23}N_3O_2$: calcd. 409.179; found 409.1791.

*4.8.11. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-3-methyl-[1,1′-biphenyl]-4-carboxamide (**15k**)*

Light white solid; yield: 48.8%; mp: 147.9–149.6 °C. ^{1}H NMR (600 MHz, DMSO) δ 8.99 (d, J = 8.6 Hz, 1H), 8.02 (d, J = 7.4 Hz, 2H), 7.67 (t, J = 7.4 Hz, 2H), 7.63 (d, J = 7.4 Hz, 2H), 7.55 (t, J = 7.8 Hz, 2H), 7.47–7.43 (m, 4H), 7.37 (t, J = 7.3 Hz, 1H), 7.27 (s, 1H), 7.08–7.05 (m, 1H), 6.91 (s, 1H), 5.77–5.73 (m, 1H), 4.57 (dd, J = 14.1, 4.3 Hz, 1H), 4.38 (dd, J = 14.1, 10.0 Hz, 1H), 2.06 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 197.62, 168.95, 141.83, 139.80, 138.49, 136.75, 135.56, 135.06, 133.95, 129.41 (2C), 129.26, 129.08 (2C), 128.79 (3C), 128.29, 127.98, 127.22 (2C), 124.17, 120.59, 55.03, 46.28, 19.50. HRMS (EI): m/z (M^+) for $C_{26}H_{23}N_3O_2$: calcd. 409.179; found 409.1803.

*4.8.12. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-2′-methyl-[1,1′-biphenyl]-4-carboxamide (**15l**)*

Light white solid; yield: 46.5%; mp: 143.4–144.2 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.18 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 7.8 Hz, 2H), 7.77 (d, J = 8.1 Hz, 2H), 7.67–7.62 (m, 2H), 7.53 (t, J = 7.7 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.30–7.24 (m, 4H), 7.18 (d, J = 7.2 Hz, 1H), 6.86 (s, 1H), 5.79–5.75 (m, 1H), 4.59 (dd, J = 14.1, 4.3 Hz, 1H), 4.43 (dd, J = 14.1, 9.7 Hz, 1H), 2.20 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 197.45, 166.33, 145.05, 140.79, 138.40, 135.44, 135.13, 134.02, 132.31, 130.91, 129.87, 129.48, 129.17, 128.80, 128.76, 128.23, 127.72, 126.50, 120.53, 55.37, 46.29, 20.58. HRMS (EI): m/z (M^+) for $C_{26}H_{23}N_3O_2$: calcd. 409.179; found 409.1805.

*4.8.13. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-3′-methyl-[1,1′-biphenyl]-4-carboxamide (**15m**)*

Light white solid; yield: 53.7%; mp: 150.6–152.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.17 (d, J = 8.4 Hz, 1H), 8.05–8.01 (m, 2H), 7.80 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 8.4 Hz, 2H), 7.67 (s, 1H), 7.63 (t, J = 7.4 Hz, 1H), 7.54–7.50 (m, 3H), 7.48 (d, J = 7.8 Hz, 1H), 7.36 (t, J = 7.6 Hz, 1H), 7.24 (s, 1H), 7.21 (d, J = 7.3 Hz, 1H), 6.86 (s, 1H), 5.78–5.74 (m, 1H), 4.60 (dd, J = 14.1, 4.5 Hz, 1H), 4.43 (dd, J = 14.1, 9.6 Hz, 1H), 2.37 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 197.47, 166.18, 143.81, 139.48, 138.68, 138.34, 135.44, 134.01, 132.45, 129.38, 129.23, 129.15 (2C), 128.78 (2C), 128.55, 128.38 (2C), 127.96, 127.04 (2C), 124.48, 120.59, 55.37, 46.35, 21.54. HRMS (EI): m/z (M^+) for $C_{26}H_{23}N_3O_2$: calcd. 409.179; found 409.1789.

*4.8.14. N-(3-(1H-imidazol-1-yl)-1-oxo-1-phenylpropan-2-yl)-4′-methyl-[1,1′-biphenyl]-4-carboxamide (**15n**)*

Light white solid; yield: 46.3%; mp: 129.9–132.5 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.17 (d, J = 8.4 Hz, 1H), 8.04–8.00 (m, 2H), 7.79 (d, J = 8.4 Hz, 2H), 7.74–7.68 (m, 3H), 7.63 (t, J = 7.4 Hz, 1H), 7.59 (d, J = 8.1 Hz, 2H), 7.52 (t, J = 7.8 Hz, 2H), 7.28 (d, J = 8.0 Hz, 2H), 7.26 (s, 1H), 6.89 (s, 1H), 5.76 (td, J = 9.3, 4.5 Hz, 1H), 4.61 (dd, J = 14.1, 4.5 Hz, 1H), 4.43 (dd, J = 14.1, 9.6 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 197.49, 166.18, 143.61, 138.37, 138.07, 136.61, 135.44, 134.00, 132.23, 130.09 (2C), 129.14 (2C), 128.78 (2C), 128.67, 128.40 (2C), 127.18 (2C), 126.75 (2C), 120.55, 55.39, 46.31, 21.17. HRMS (EI): m/z (M^+) for $C_{26}H_{23}N_3O_2$: calcd. 409.179; found 409.1797.

*4.8.15. N-(1-(2-fluorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15o**)*

Light white solid; yield: 44.8%; mp: 176.7–178.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.25 (d, J = 7.7 Hz, 1H), 7.79 (td, J = 7.6, 1.6 Hz, 1H), 7.76 (d, J = 8.5 Hz, 2H), 7.72 (d, J = 8.5 Hz, 2H), 7.70–7.68 (m, 2H), 7.60 (s, 2H), 7.48 (t, J = 7.7 Hz, 2H), 7.40 (t, J = 7.4 Hz, 1H), 7.34–7.29 (m, 2H), 7.18 (s, 1H), 6.86 (s, 1H), 5.35 (td, J = 9.2, 4.2 Hz, 1H), 4.67 (dd, J = 14.2, 4.2 Hz, 1H), 4.42 (dd, J = 14.2, 9.5 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 195.95, 166.10, 161.05, 159.37, 143.26, 139.07, 137.84, 134.82, 134.76, 131.97, 130.62, 129.03 (2C), 128.37, 128.14, 127.91 (2C), 126.91 (2C), 126.63 (2C), 124.56, 124.46, 124.38, 119.96, 116.60, 116.45, 58.88, 58.86, 45.19. HRMS (EI): *m/z* (M^+) for $C_{25}H_{20}FN_3O_2$: calcd. 413.154; found 413.1552.

*4.8.16. 2-Fluoro-N-(1-(2-fluorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15p**)*

Light white solid; yield: 49.5%; mp: 163.0–163.9 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.34 (d, J = 7.7 Hz, 1H), 7.81–7.78 (m, 1H), 7.64–7.58 (m, 5H), 7.56 (d, J = 8.5 Hz, 2H), 7.49 (t, J = 7.5 Hz, 2H), 7.44 (t, J = 7.3 Hz, 1H), 7.34–7.30 (m, 2H), 7.18 (s, 1H), 6.87 (s, 1H), 5.39–5.35 (m, 1H), 4.67 (dd, J = 14.2, 4.2 Hz, 1H), 4.41 (dd, J = 14.2, 9.5 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 196.15, 165.27, 161.53, 159.89 (d, J = 10.7 Hz), 158.28, 138.32, 135.39, 135.34, 134.64, 134.61, 131.96, 131.87, 131.47, 131.44, 131.09, 129.31, 129.30, 129.22, 129.17 (2C), 128.90, 125.08, 124.81, 124.73, 124.20, 124.18, 120.41, 117.10, 116.95, 115.45, 115.29, 59.39, 59.37–59.35 (m), 45.62. HRMS (EI): *m/z* (M^+) for $C_{25}H_{19}F_2N_3O_2$: calcd. 431.1445; found 431.1457.

*4.8.17. 3-Fluoro-N-(1-(2-fluorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15q**)*

Light white solid; yield: 45.6%; mp: 126.3–128.2 °C. ^{1}H NMR (400 MHz, DMSO) δ 10.13 (d, J = 5.6 Hz, 1H), 9.14–9.06 (m, 1H), 7.83–7.76 (m, 2H), 7.73–7.70 (m, 2H), 7.65–7.63 (m, 1H), 7.60 (s, 1H), 7.59–7.54 (m, 2H), 7.50–7.46 (m, 2H), 7.44–7.39 (m, 2H), 7.35–7.31 (m, 2H), 7.18 (s, 1H), 6.89 (s, 1H), 5.37 (td, J = 8.6, 4.3 Hz, 1H), 4.67 (dd, J = 14.2, 4.3 Hz, 1H), 4.41 (dd, J = 14.2, 9.1 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 195.63, 163.64, 161.11, 160.34, 159.43, 158.68, 144.80, 144.75, 140.90, 137.79, 134.90, 134.84, 130.63, 130.29, 130.27, 129.09 (2C), 128.66, 128.38, 126.95 (2C), 124.58, 124.34, 124.26, 122.53, 122.51, 121.44, 121.34, 119.96, 116.61, 116.46, 114.22, 114.06, 58.93, 45.18. HRMS (EI): *m/z* (M^+) for $C_{25}H_{19}F_2N_3O_2$: calcd. 431.1445; found 431.1453.

*4.8.18. N-(1-(3-fluorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15r**)*

Light white solid; yield: 50.7%; mp: 145.2–146.7 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.21 (d, J = 8.3 Hz, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 8.5 Hz, 2H), 7.78–7.75 (m, 1H), 7.74 (d, J = 8.5 Hz, 2H), 7.71–7.68 (m, 2H), 7.62 (s, 1H), 7.58 (td, J = 8.0, 5.8 Hz, 1H), 7.49 (dt, J = 11.1, 6.1 Hz, 3H), 7.40 (t, J = 7.4 Hz, 1H), 7.22 (s, 1H), 6.84 (s, 1H), 5.72–5.69 (m, 1H), 4.60 (dd, J = 14.1, 4.5 Hz, 1H), 4.44 (dd, J = 14.1, 9.6 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 196.54, 166.26, 163.24, 161.62, 143.79, 139.51, 138.38, 137.73, 137.69, 132.40, 131.37, 131.32, 129.50 (2C), 128.73, 128.62, 128.41 (2C), 127.37 (2C), 127.14 (2C), 124.92, 120.92, 120.78, 120.54, 115.31, 115.16, 55.61, 46.13. HRMS (EI): *m/z* (M^+) for $C_{25}H_{20}FN_3O_2$: calcd. 413.154; found 413.155.

*4.8.19. 2-Fluoro-N-(1-(3-fluorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15s**)*

Light white solid; yield: 54.8%; mp: 128.2–130.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.30 (d, J = 8.3 Hz, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.78–7.75 (m, 1H), 7.67–7.61 (m, 4H), 7.59–7.54 (m, 3H), 7.50 (t, J = 7.6 Hz, 3H), 7.46–7.42 (m, 1H), 7.22 (t, J = 1.1 Hz, 1H), 6.85 (d, J = 0.9 Hz, 1H), 5.73 (td, J = 9.4, 4.6 Hz, 1H), 4.60 (dd, J = 14.2, 4.5 Hz, 1H), 4.43 (dd, J = 14.2, 9.6 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 196.32, 164.97, 163.26, 161.63, 159.96, 158.32, 138.40, 137.63, 137.59, 134.59, 134.54, 132.02, 131.93, 131.51, 131.49, 131.42, 131.37, 129.32, 129.30, 129.18 (2C), 128.94, 128.71, 124.93, 124.23, 124.21, 121.02, 120.87, 120.56, 115.50, 115.34, 115.31, 115.15, 55.62, 46.12. HRMS (EI): *m/z* (M^+) for $C_{25}H_{19}F_2N_3O_2$: calcd. 431.1445; found 431.1449.

*4.8.20. 3-Fluoro-N-(1-(3-fluorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15t**)*

Light white solid; yield: 38.9%; mp: 142.2–144.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.08 (d, J = 6.9 Hz, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.78–7.75 (m, 1H), 7.72 (d, J = 7.3 Hz, 2H), 7.65 (s, 1H), 7.61–7.56 (m, 3H), 7.54–7.50 (m, 1H), 7.49 (t, J = 7.6 Hz, 2H), 7.46–7.41 (m, 2H), 7.23 (s, 1H), 6.89 (s, 1H), 5.70 (td, J = 9.0, 4.5 Hz, 1H), 4.60 (dd, J = 14.2, 4.4 Hz, 1H), 4.42 (dd, J = 14.2, 9.4 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 196.23, 163.85, 163.24, 161.61, 160.84, 159.18, 145.37, 145.31, 138.40, 138.23, 137.60, 137.56, 131.35, 131.30, 130.80, 130.79, 129.56 (2C), 129.15, 128.63, 127.41 (2C), 124.96, 124.94, 123.07, 123.05, 121.82, 121.73, 120.94, 120.80, 120.56, 115.39, 115.24, 114.73, 114.57, 55.82, 46.13. HRMS (EI): *m/z* (M^+) for $C_{25}H_{19}F_2N_3O_2$: calcd. 431.1445; found 431.1456.

*4.8.21. N-(1-(3-chlorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15u**)*

Light white solid; yield: 52.5%; mp: 126.6–127.8 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.22 (d, J = 8.2 Hz, 1H), 7.99 (t, J = 1.8 Hz, 1H), 7.94 (d, J = 7.9 Hz, 1H), 7.79 (d, J = 8.5 Hz, 2H), 7.74 (d, J = 8.5 Hz, 2H), 7.71–7.68 (m, 3H), 7.62 (s, 1H), 7.55 (t, J = 7.9 Hz, 1H), 7.48 (t, J = 7.7 Hz, 2H), 7.40 (t, J = 7.4 Hz, 1H), 7.21 (s, 1H), 6.84 (s, 1H), 5.69 (td, J = 8.9, 4.6 Hz, 1H), 4.59 (dd, J = 14.1, 4.5 Hz, 1H), 4.43 (dd, J = 14.1, 9.5 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 196.62, 166.27, 143.79, 139.50, 138.38, 137.41, 133.93, 133.57, 132.40, 131.09, 129.49 (2C), 128.74, 128.62, 128.39 (3C), 127.37 (2C), 127.30, 127.14 (2C), 120.53, 55.61, 46.09. HRMS (EI): *m/z* (M^+) for $C_{25}H_{20}ClN_3O_2$: calcd. 429.1244; found 429.1256.

*4.8.22. N-(1-(3-chlorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-2-fluoro-[1,1′-biphenyl]-4-carboxamide (**15v**)*

Light white solid; yield: 47.9%; mp: 81.3–83.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.31 (d, J = 8.2 Hz, 1H), 7.98 (t, J = 1.8 Hz, 1H), 7.95–7.91 (m, 1H), 7.71–7.69 (m, 1H), 7.67–7.60 (m, 4H), 7.58–7.54 (m, 3H), 7.52–7.48 (m, 2H), 7.46–7.42 (m, 1H), 7.23 (s, 1H), 6.87 (s, 1H), 5.72 (td, J = 9.3, 4.6 Hz, 1H), 4.60 (dd, J = 14.1, 4.5 Hz, 1H), 4.43 (dd, J = 14.1, 9.5 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 195.95, 164.53, 159.50, 157.86, 137.95, 136.87, 134.14, 134.13, 134.10, 133.52, 133.17, 131.56, 131.47, 131.03, 131.01, 130.65, 128.84, 128.82, 128.69 (2C), 128.45, 128.35, 127.93, 126.84, 123.75, 123.73, 120.06, 115.03, 114.87, 55.17, 45.61. HRMS (EI): *m/z* (M^+) for $C_{25}H_{19}ClFN_3O_2$: calcd. 447.115; found 447.1171.

*4.8.23. N-(1-(3-chlorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-3-fluoro-[1,1′-biphenyl]-4-carboxamide (**15w**)*

Light white solid; yield: 47.2%; mp: 126.0–128.0 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.09 (dd, J = 8.2, 1.3 Hz, 1H), 7.99 (t, J = 1.8 Hz, 1H), 7.94 (d, J = 7.9 Hz, 1H), 7.73–7.71 (m, 3H), 7.64 (s, 1H), 7.60–7.55 (m, 3H), 7.49 (t, J = 7.6 Hz, 2H), 7.46–7.41 (m, 2H), 7.22 (s, 1H), 6.89 (s, 1H), 5.69 (td, J = 8.9, 4.5 Hz, 1H), 4.60 (dd, J = 14.2, 4.5 Hz, 1H), 4.41 (dd, J = 14.2, 9.3 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 196.32, 163.83, 160.83, 159.17, 145.38, 145.33, 138.39, 138.22, 137.30, 133.93, 133.59, 131.07, 130.79, 130.77, 129.56 (2C), 129.15, 128.69, 128.44, 127.42 (2C), 127.34, 123.07, 123.05, 121.80, 121.70, 120.54, 114.73, 114.57, 55.80, 46.08. HRMS (EI): *m/z* (M^+) for $C_{25}H_{19}ClFN_3O_2$: calcd. 447.115; found 447.1162.

*4.8.24. N-(1-(4-fluorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15x**)*

Light white solid; yield: 50.6%; mp: 81.7–83.5 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.18 (d, *J* = 8.3 Hz, 1H), 8.10 (dd, *J* = 8.8, 5.5 Hz, 2H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 8.4 Hz, 2H), 7.71–7.68 (m, 2H), 7.63 (s, 1H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 1H), 7.36 (t, *J* = 8.8 Hz, 2H), 7.22 (s, 1H), 6.84 (s, 1H), 5.76–5.72 (m, 1H), 4.58 (dd, *J* = 14.1, 4.6 Hz, 1H), 4.43 (dd, *J* = 14.1, 9.6 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 195.65, 165.96, 165.73, 164.28, 143.27, 139.05, 137.90, 132.00, 131.73, 131.35, 131.28, 129.96, 129.02 (2C), 128.21, 127.96 (2C), 126.90 (2C), 126.64 (2C), 120.07, 115.84, 115.70, 54.87, 45.78. HRMS (EI): *m/z* (M^+) for $C_{25}H_{20}FN_3O_2$: calcd. 413.154; found 413.1553.

*4.8.25. 2-Fluoro-N-(1-(4-fluorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15y**)*

Light white solid; yield: 49.0%; mp: 130.5–131.4 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.27 (d, *J* = 8.2 Hz, 1H), 8.10 (dd, *J* = 8.4, 5.6 Hz, 2H), 7.68–7.61 (m, 4H), 7.58–7.55 (m, 2H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.46–7.42 (m, 1H), 7.37 (t, *J* = 8.8 Hz, 2H), 7.22 (s, 1H), 6.84 (s, 1H), 5.78–5.74 (m, 1H), 4.58 (dd, *J* = 14.1, 4.4 Hz, 1H), 4.42 (dd, *J* = 14.0, 9.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 195.92, 166.47, 164.91, 164.80, 159.96, 158.32, 138.40, 134.67, 134.61, 132.10, 131.98, 131.89, 131.83, 131.77, 131.45, 129.31, 129.30, 129.18, 128.93, 128.77, 124.25, 120.53, 116.36, 116.22, 115.53, 115.36, 55.37, 46.22. HRMS (EI): *m/z* (M^+) for $C_{25}H_{19}F_2N_3O_2$: calcd. 431.1445; found 431.1447.

*4.8.26. 3-Fluoro-N-(1-(4-fluorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15z**)*

Light white solid; yield: 41.2%; mp: 120.3–122.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.05 (d, *J* = 8.2 Hz, 1H), 8.10 (dd, *J* = 8.6, 5.5 Hz, 2H), 7.73–7.71 (m, 2H), 7.64 (s, 1H), 7.61–7.56 (m, 2H), 7.50–7.45 (m, 3H), 7.44–7.41 (m, 1H), 7.38 (t, *J* = 8.8 Hz, 2H), 7.22 (s, 1H), 6.88 (s, 1H), 5.74 (td, *J* = 8.8, 4.6 Hz, 1H), 4.59 (dd, *J* = 14.2, 4.5 Hz, 1H), 4.40 (dd, *J* = 14.2, 9.3 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 195.80, 166.46, 164.79, 163.79, 160.84, 159.18, 145.33, 145.27, 138.38, 138.25, 132.09, 131.88, 131.82, 130.85, 130.83, 129.56 (2C), 129.13, 128.67, 127.41 (2C), 123.05, 123.03, 121.92, 121.83, 120.54, 116.30, 116.16, 114.71, 114.55, 55.55, 46.26. HRMS (EI): *m/z* (M^+) for $C_{25}H_{19}F_2N_3O_2$: calcd. 431.1445; found 431.1456.

*4.8.27. N-(1-(4-chlorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15aa**)*

Light white solid; yield: 48.3%; mp: 149.7–150.4 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.20 (d, *J* = 8.3 Hz, 1H), 8.01 (d, *J* = 8.6 Hz, 2H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 7.5 Hz, 2H), 7.62–7.58 (m, 3H), 7.48 (t, *J* = 7.7 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 1H), 7.21 (s, 1H), 6.84 (s, 1H), 5.71 (td, *J* = 9.0, 4.6 Hz, 1H), 4.59 (dd, *J* = 14.1, 4.5 Hz, 1H), 4.42 (dd, *J* = 14.1, 9.5 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 196.63, 166.21, 143.77, 139.52, 138.83, 138.38, 134.23, 132.42, 130.62 (2C), 129.49 (2C), 129.27 (2C), 128.76, 128.61, 128.42 (2C), 127.37 (2C), 127.12 (2C), 120.52, 55.49, 46.16. HRMS (EI): *m/z* (M^+) for $C_{25}H_{20}ClN_3O2$: calcd. 429.1244; found 429.1251.

*4.8.28. N-(1-(4-chlorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-2-fluoro-[1,1′-biphenyl]-4-carboxamide (**15 ab**)*

Light white solid; yield: 39.0%; mp: 147.1–148.3 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.28 (d, *J* = 8.1 Hz, 1H), 8.01 (d, *J* = 8.1 Hz, 2H), 7.67–7.60 (m, 6H), 7.58–7.55 (m, 2H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.46–7.42 (m, 1H), 7.22 (s, 1H), 6.85 (s, 1H), 5.74 (td, *J* = 8.7, 4.7 Hz, 1H), 4.59 (dd, *J* = 14.2, 4.5 Hz, 1H), 4.42 (dd, *J* = 14.0, 9.6 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 196.42, 164.92, 159.96, 158.32, 138.91, 138.39, 134.60, 134.58, 134.15, 132.00, 131.91, 131.48, 131.46, 130.62, 129.31, 129.18, 128.93, 128.78, 124.24, 124.22, 120.53, 115.52, 115.36, 55.50, 46.15. HRMS (EI): *m/z* (M^+) for $C_{25}H_{19}ClFN_3O2$: calcd. 447.115; found 447.1158.

*4.8.29. N-(1-(4-chlorophenyl)-3-(1H-imidazol-1-yl)-1-oxopropan-2-yl)-3-fluoro-[1,1′-biphenyl]-4-carboxamide (**15ac**)*

Light white solid; yield: 40.5%; mp: 120.2–122.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.06 (dd, *J* = 8.2, 1.5 Hz, 1H), 8.02 (d, *J* = 8.6 Hz, 2H), 7.73 (d, *J* = 7.3 Hz, 2H), 7.63–7.61 (m, 4H), 7.59–7.56 (m, 1H), 7.51–7.45 (m, 4H), 7.43 (t, *J* = 7.3 Hz, 1H), 7.21 (s, 1H), 6.88 (s, 1H), 5.71 (td, *J* = 8.9, 4.5 Hz, 1H), 4.59 (dd, *J* = 14.2, 4.5 Hz, 1H), 4.41 (dd, *J* = 14.2, 9.3 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 196.30, 163.79, 160.84, 159.18, 145.36, 145.30, 138.86, 138.40, 138.24, 134.12, 130.86, 130.84, 130.68 (2C), 129.56 (2C), 129.26 (2C), 129.14, 128.77, 127.41 (2C), 123.04, 121.84, 121.75, 120.50, 114.72, 114.56, 55.67, 46.15. HRMS (EI): *m/z* (M^+) for $C_{25}H_{19}ClFN_3O2$: calcd. 447.115; found 447.117.

*4.8.30. N-(3-(1H-imidazol-1-yl)-1-oxo-1-(p-tolyl)propan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**15ad**)*

Light white solid; yield: 48.3%; mp: 76.6–78.0 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.14 (d, *J* = 8.4 Hz, 1H), 7.94 (d, *J* = 8.1 Hz, 2H), 7.82 (d, *J* = 8.2 Hz, 2H), 7.73 (d, *J* = 8.3 Hz, 2H), 7.70 (d, *J* = 7.6 Hz, 2H), 7.63 (s, 1H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.22 (s, 1H), 6.83 (s, 1H), 5.76–5.72 (m, 1H), 4.57 (dd, *J* = 14.1, 4.5 Hz, 1H), 4.41 (dd, *J* = 14.1, 9.6 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 196.92, 166.08, 144.54, 143.68, 139.54, 138.38, 132.87, 132.58, 129.73 (2C), 129.50 (2C), 128.93 (2C), 128.72, 128.60 (2C), 128.44, 127.37 (2C), 127.08 (2C), 120.52, 55.24, 46.35, 21.65. HRMS (EI): *m/z* (M^+) for $C_{26}H_{23}N_3O_2$: calcd. 409.179; found 409.1804.

*4.8.31. N-(3-(1H-imidazol-1-yl)-1-oxo-1-(p-tolyl)propan-2-yl)-2-fluoro-[1,1′-biphenyl]-4-carboxamide (**15ae**)*

Light white solid; yield: 42.3%; mp: 148.3–150.1 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.24 (d, *J* = 8.5 Hz, 1H), 7.93 (d, *J* = 8.2 Hz, 2H), 7.69–7.62 (m, 4H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.50 (t, *J* = 7.6 Hz, 2H), 7.45–7.42 (m, 1H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.23 (s, 1H), 6.84 (s, 1H), 5.78–5.74 (m, 1H), 4.57 (dd, *J* = 14.1, 4.5 Hz, 1H), 4.40 (dd, *J* = 14.1, 9.7 Hz, 1H), 2.36 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 196.71, 164.79, 159.95, 158.32, 144.64, 138.40, 134.78, 134.73, 134.62, 132.80, 131.91, 131.82, 131.45, 131.43, 129.77, 129.31, 129.30, 129.18, 128.93, 128.74, 124.26, 124.24, 120.53, 115.52, 115.36, 55.27, 46.34, 21.66. HRMS (EI): *m/z* (M^+) for $C_{26}H_{22}FN_3O_2$: calcd. 427.1696; found 427.1709.

*4.9. The procedure for the synthesis of compound **18***

The synthetic procedures of compound **18** were similar to those used for compounds **15a-ae.**

*4.9.1. N-(1-oxo-1-phenyl-3-(1H-1,2,4-triazol-1-yl)propan-2-yl)-[1,1′-biphenyl]-4-carboxamide (**18**)*

Light white solid; yield: 40.6%; mp: 117.1–119.0 °C. ^{1}H NMR (600 MHz, DMSO) δ 9.21 (d, *J* = 8.3 Hz, 1H), 8.52 (s, 1H), 7.98 (d, *J* = 7.3 Hz, 2H), 7.96 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.71–7.68 (m, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 2H), 7.48 (t, *J* = 7.7 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 1H), 5.83 (td, *J* = 8.5, 5.0 Hz, 1H), 4.83 (dd, *J* = 14.0, 4.9 Hz, 1H), 4.66 (dd, *J* = 14.0, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, DMSO) δ 196.90, 166.14, 151.98, 145.36, 143.73, 139.52, 135.34, 133.99, 132.47, 129.50, 129.15, 128.68, 128.61, 128.43, 127.37, 127.09, 54.51, 48.96. HRMS (EI): *m/z* (M^+) for $C_{24}H_{20}N_4O_2$: calcd. 396.1586; found 396.1589.

4.10. In vitro antifungal testing

Five pathogenic fungi (Guangdong culture collection center) and five fluconazole-resistant strains of *Candida albicans* were selected to determine the *in vitro* minimum inhibitory concentrations (MIC) according to the protocols of the Clinical Laboratory Standard Institute (CLSI, formerly NCCLS). Fluconazole and itraconazole were

used as positive control drugs. The RPMI 1640 medium (Gibco, USA) was adjusted to pH 7.0 with 3-[*N*-morpholino]- propanesulfonic acid (MOPS, Genyiew, USA). Fluconazole, itraconazole and the target compounds were dissolved in DMSO and serially diluted into the RPMI 1640 medium to achieve final concentrations ranged from 0.03 to 16 μg/mL or 0.125−64 μg/mL, and the initial concentration of the fungal suspension in RPMI 1640 medium was $1-5 \times 10^3$ CFU/mL. A drug-free RPMI 1640 medium with fungi and a fungus-free medium were used as the growth and blank controls, respectively. The 96-well plates were then incubated at 35 °C for 24 h. The MIC values were defined as the lowest concentrations of an antimicrobial that would inhibit the visible growth of the fungi.

4.11. GC-MS analysis of sterol composition

Candida albicans (SC5314) was treated with compound **15ae** (0.015 μg/mL) and fluconazole (0.25 μg/mL) at their sub-MIC values and incubated in RPMI 1640 medium (50 mL, Gibco, USA) for ~18 h at 30 °C with continuous agitation (200 rpm). Cells were harvested by centrifugation 3000*g* for 10 min. Then the cells were washed with PBS (HyClone, Utah) thrice, and saponified at 80 °C for 60 min with 3 mL of ethanol, 2 mL of pyrogallol (Bide, Shanghai) dissolved in ethanol, and 2 mL of KOH (60%, w/v). The nonsaponifiable sterols were then extracted three times with 5 mL of heptane. The combined extracts were evaporated in a vacuum, and the residue was dissolved in 500 μL of heptane and derivatized with 250 μL of *N*-methyl-*N*-(trimethylsilyl) trifluorooacetaminde (MSTFA, J&K, Hebei) at 70 °C for 20 min. 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.

4.12. In vitro cytotoxicity assays

A549 cells were plated in 96-well microtiter plates at a concentration of 3000 cells per well. The A549 cells were then incubated in RPMI 1640 medium (Gibco, USA) at 37 °C with 5% CO_2 for 16 h. Compounds **15o**, **15u**, **15z**, **15ae** and fluconazole were dissolved in DMSO and diluted with medium to achieve final concentrations of 0.08, 0.4, 2.0, 10 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 96-well microtiter plates and incubated for another 4 h. Each test well was measured at $\lambda_{490\ nm}$ [26].

Acknowledgements

The authors thank Hongwei Liu from Institute of Microbiology, Chinese Academy of Sciences for providing the fluconazole-resistant strains of *Candida albicans* (*strains 17#* and *CaR*). The authors thank Prof. Yongbing Cao from School of Pharmacy, the Second Military Medical University for providing the fluconazole-resistant *strains 632*, *901* and *904*. This work was supported by Program for Innovative Research Team of the Ministry of Education and Program for Liaoning Innovative Research Team in University.

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.ejmech.2019.111689. These data include MOL files and InChiKeys of the most important compounds described in this article.

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