New generation modified azole antifungals against multidrug-resistant *Candida auris*

Yiyuan Chen[1], Yunxiao Li[1], Charlotte K. Hind[2], Md. Mahbub Hasan[1, 4], Kazi S. Nahar[1,3], Melanie Clifford[2], J. Mark Sutton[1, 2] *, Khondaker Miraz Rahman[1] *

[1]Institute of Pharmaceutical Science, King's College London, 150 Stamford Street, London. SE1 9NH, United Kingdom.

[2]UK Health Security Agency, Vaccine Evaluation and Development Centre, Manor Farm Road, Salisbury. SP4 0JG, United Kingdom.

[3]Department of Natural Sciences, University of Middlesex, The Burroughs, Hendon, London NW4 4BT

[4]Department of Genetic Engineering and Biotechnology, Faculty of Biological Sciences, University of Chittagong, Chattogram, 4331, Bangladesh

Abstract

The increasing prevalence of antifungal resistance and the limited numbers of antifungal agents available to treat patients with invasive fungal disease underscores the urgent need for novel drug classes. *Candida auris* has emerged as a major public pathogen of global concern with reduced effective treatment options. A targeted modification of azole core-scaffold with a cyclic heteroaliphatic linker linked aromatic and heteroaromatic rings resulted in compounds with exceptional activity, with MICs ranging from 0.016 to 4 µg/mL against a panel of *Candida auris,* including azole-resistant isolates. The research employed a systematic approach, varying substitutions of the linkers and the terminal aromatic rings to develop a structure activity relationship (SAR). The compounds also showed excellent activity against public health Candida species including *C. albicans*, *N. glabrata*, *C. tropicalis* and *C. parasliosis,* with MICs less than 1 µg/mL for most compounds. The study identified compounds **7, 18** and **21** as promising lead candidates with superior potency than both fluconazole and voriconazole against both *C. auris* and diverse *Candida* strains. The compounds were found to be non-toxic up to 50 mg/Kg in a *Galleria mellonella* model while showing efficacy against *C. auris* at a dose of 5 mg/Kg. This study offers a new chemical scaffold that can be taken forward to develop new generation azole antifungal agents against *C. auris*.

Introduction

Antifungal resistance is the ability of a fungus to grow and survive in the presence of antifungal drugs. This can lead to severe infections that are hard to treat.[1, 2] Antifungal drugs are used to treat a variety of fungal infections, including candidiasis, aspergillosis, and cryptococcosis.[3-5] Resistance can occur naturally or develop over time due to exposure to antifungal drugs or fungicides. Improper use of antifungal drugs, such as low doses or short courses, can also contribute to resistance. [2, 6, 7] Some fungi, like *Aspergillus* and certain *Candida* species, are resistant to some or all types of antifungal drugs. [8-11] Among them, *Candida auris* is a new and highly resistant fungus that can spread quickly in healthcare settings.[12-14]

C. auris is an emerging fungal pathogen that is resistant to many antifungal drugs. It can cause serious infections in hospitals and other healthcare settings.[15-18] The US Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) both consider *C. auris* to be a major threat to public health.[19] It was first discovered in Japan in 2009 and has since been reported in over 47 countries worldwide, with 6 clades emerging.[20] A study of patients with echinocandin-susceptible *C. auris* bloodstream infection at three hospitals in Brooklyn, New York, found that 30.1% of patients died within 30 days and 44.6% died within 90 days.[21]

Resistant Candida strains are relatively prevalent in clinical settings. Collected data from the CDC showed that around 7% of the clinical Candida strains isolated from hospitalised patients suffering from bloodstream infections exhibited resistance to marketed antifungal drugs.[22, 23] In another study approximately 90% of the *C. auris* isolates were found to be resistant to at least one commercially available antifungal drug, and 30% of the clinical strains were non-susceptible to more than one antifungal on the market.[24] All the data from public health authorities makes it clear that innovation in the treatment of antifungal infections, particularly the drug resistant *C. auris* is urgently necessary.

There are only four major classes of antifungal drugs available on the market for treating systemic fungal infections, namely azoles (e.g., fluconazole, voriconazole, itraconazole, and posaconazole), polyenes (typically amphotericin B), echinocandins (e.g. micafungin and caspofungin), and pyrimidine analogues (mainly flucytosine or its salt form).[25, 26] Azoles (imidazoles and triazoles, containing two and three nitrogens on the azole ring, respectively) are a class of antifungal drugs widely used to treat fungal infections in humans.[27] They work by inhibiting the synthesis of ergosterol, an essential component of fungal cell membranes. This disruption weakens the cell membrane and leads to the death of the fungal pathogen.[28, 29]

Despite the potential of azoles, the increasing levels of resistance against them is becoming a concern in tackling the healthcare burden caused by fungal infections. The treatment of fungal pathogens using azoles triggers the upregulation of efflux pumps in *Candida spp.*, including CDR1, MDR1, RDC3, SNQ2, and YHD3 in *C. auris*.[30-32] Moreover, reports show that *Candida spp.* can also overexpress azole targets such as the ERG11 gene (that encodes lanosterol 14-alpha-demethylase),[33, 34] sequestrations of azoles within vacuole and biofilms (in fluconazole-resistant *C. albicans*),[35, 36] and by modification of the targets by mutation over time (ERG11 mutation triggered by the application of azoles in *Candida spp.*).[37, 38] To develop azoles that

can overcome resistance observed in *C. auris* and other drug-resistant *Candida* species, we made a targeted modification in the azole core scaffold with various types of hetero-aliphatic linkers with different lengths, sizes, and ring strains (Figure 1A), and modified the terminal aromatic and heteroaromatic ring. This medicinal chemistry led approach has led to compounds with activity against multidrug-resistant *C. auris* and other clinically important *Candida* species that can be taken forward towards pre-clinical development.

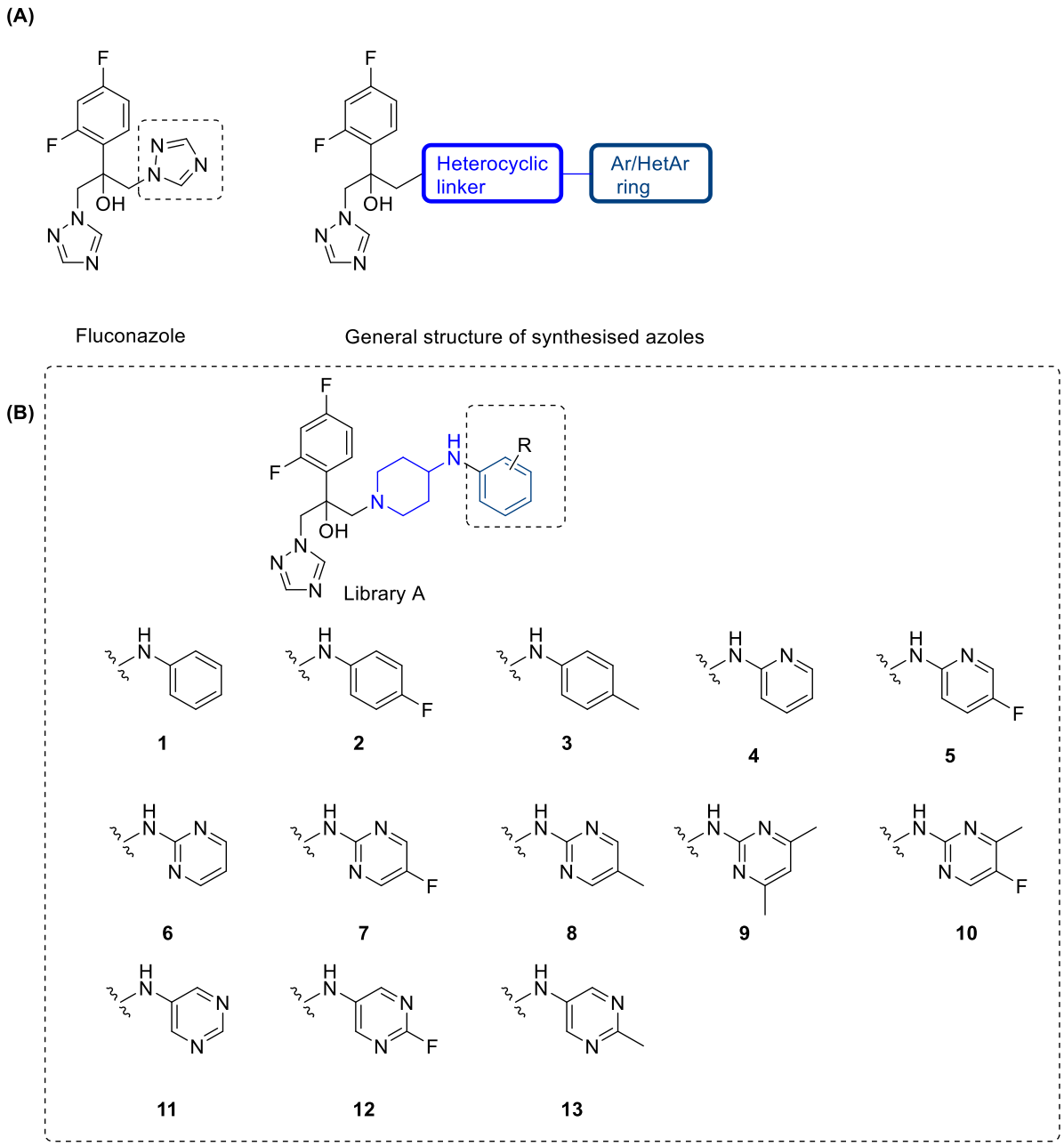


Figure 1. (A) general structure of the modified azole compounds. (B) compounds of library A containing a piperidine linker and different aromatic or heteroaromatic rings.

Results and Discussion

Design of modified azole compounds

We aimed to design fluconazole analogues with improved interaction with the fungal lanosterol 14α-demethylase (LDM) which is the target for azole antifungals and is involved in the biosynthesis of ergosterol.[39] For all compounds, one of the triazole rings of fluconazole was replaced by a heteroaliphatic linker connected with an aromatic or heteroaromatic ring (Figure 1A). For library A compounds, we designed compounds with a six-membered heterocyclic piperidine ring as the linker as it has been previously been used to generate azole modifications and compounds with this linker have shown MICs comparable to fluoconazole[40, 41]. The piperidine ring was substituted at the 4-position with an amino group which was considered as a point of diversity, and was substituted with a number of six-membered aromatic and heteroaromatic rings to establish the structure activity relationship (Figure 1B).

Table 1. The comparative binding affinity of novel azole analogues and commercially available azoles with fungal target lanosterol 14α-demethylase (LDM).

Compound	*C. albicans* calLDM (PDB ID 5TZ1)		*C. auris* carLDM (A0A2H4QC40)	
	Chem Score	ΔG (kcal/mol)	Chem Score	ΔG (kcal/mol)
Fluconazole	18.16	-18.94	16.07	-18.8
Voriconazole	24.6	-26.72	21.03	-26.48
1	40.46	-42.6	36.16	-39.65
2	41.22	-42.69	37.32	-38.34
3	36.3	-37.52	32.25	-33.7
4	34.39	-37.4	33.68	-34.13
5	31.77	-33.34	30.77	-33.33
6	32.88	-35.63	30.96	-32.1
7	41.22	-42.69	37.32	-38.34
8	39.45	-41.6	35.6	-34.54
9	40.8	-37.6	37.6	-37.4
10	38.6	-36.54	38.45	-38.33
11	34.31	-35.65	30.44	-33.61
12	33.45	-33.66	31.32	-34.7
13	35.74	-37.4	33.22	-35.6

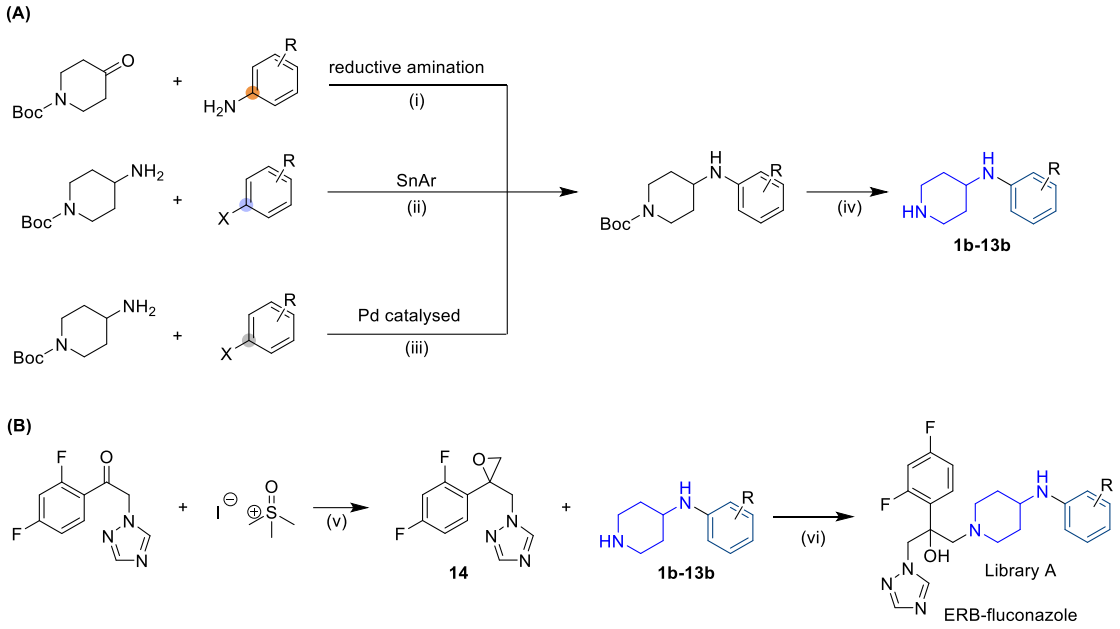
Designed compounds having phenyl, pyridine and pyrimidine as terminal aromatic or heteroaromatic fragments were then utilised for the computational analysis against the target LDM from different *Candida* species along with the clinically relevant drugs Fluconazole and

Voriconazole (Table 1). The data from the computational analysis showed that the newly designed modified fluconazole compounds showed better binding affinity to LDM enzyme from both *C. albicans* and *C. auris* compared to boith fluconazole and voriconazole. In terms of both ChemScore and Gibbs free energy ΔG most of the modified azole compounds showed almost twice the binding affinity compared to Fluconazole. The results of binding affinity remained similar across both species of *Candida* (Table 1).

Synthesis of the library A

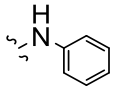
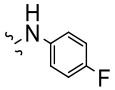
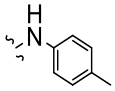
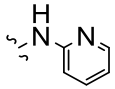
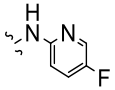
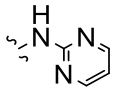
This library consisted of 13 compounds in a two-step process, the fragments were synthesised or purchased (compounds **2b**, **4b** – **7b, 11b**) and then connected to the azole core by an epoxide ring-opening reaction to form the final compounds. Fragments were synthesised by following three different approaches based on the electronic environment of the connecting carbon (Scheme 1).

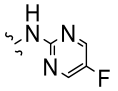
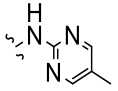
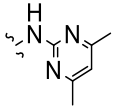
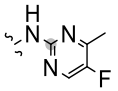
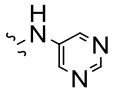
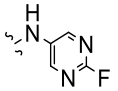
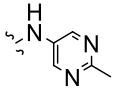
The fragments were synthesised either by reductive amination (**1a, 2a, 12a** and **13a**) or by simple SNAr reactions depending on the electronic environment of the terminal aromatic/heteroaromatic ring (**Scheme 1A**). In the case of fragment **10a**, a palladium-catalysed organometallic reaction was carried out to obtain the fragment. The epoxide core of the azole compounds was synthesised using the classic Corey-Chaykovsky reaction conditions (Scheme 1B). The final compounds were synthesised by reacting the amine fragments **1b-13b** with the epoxide core 14 with a nucleophilic epoxide ring opening reaction. This resulted in racemic final compounds **1** to **13** which were evaluated for their antifungal activity (Scheme 1).[42, 43]



Scheme 1. General synthetic routes for Library A where different aromatic/heteroaromatic fragments (A) were connected to the fluconazole core via a piperidine linker (B). Conditions: (i) NaBH(OAc)3, AcOH, DCM / MeOH, overnight; (ii) DIPEA, MeCN, DMA, 160 – 180 °C, 2 – 3 h or TEA, ethylene glycol, 200 °C, 15 min; (iii) rac-BINAP, Pd2(dba)3, KOtBu, toluene, 100 °C, overnight; (iv) 4M HCl in 4-dioxane, r.t., 1 – 2 h; (v) NaOH (aq.), Toluene, 80 °C, Microwave 50 min; (vi) TEA, EtOH, 80 °C, overnight.

140 **Table 2**. Antifungal activity (μg/mL) of modified fluconazole compounds against *Candida,* and their corresponding LogP value.

	Log P	*C. albicans NCPF3281*	*C. glabrata NCPF8018*	*C. tropicalis NCPF8760*	*C. parapsilosis NCPF3209*	*C. auris*							
						TDG1912	*TDG2512*	*TDG1102*	*TDG2211*	*TDG2506*	*NCPF8984*	*NCPF8971*	*NCPF8977*
Fluconazole	0.87	0.06 – 0.125	4	16	0.25	> 128	8	64	64	128	128	32	16
 **1**	2.49	0.12	0.12	0.12 – 0.5	0.12	0.12 – 0.25	0.03	0.5	0.25	1	1	0.0625	0.0625
 **2**	2.65	0.12	0.12	1	0.12	0.5 – 1	0.06	0.5	0.01563	2	4	0.0625	0.0625
 **3**	2.98	0.002 – 0.004	0.125	0.0625	< 0.001	0.002 – 0.016	0.0156	0.0625	1	0.0625	0.125	< 0.002 – 0.5	< 0.001
 **4**	1.87	≤ 0.25	4	2	≤ 0.25	2	0.125	2	0.25	4	4	0.125	0.25
 **5**	2.03	≤ 0.25	0.5 – 2	0.25 – 0.5	≤ 0.25	≤ 0.25	0.06	0.5	0.5	2	2	0.0625	0.25
 **6**	1.06	0.12	0.12 – 0.25	0.5	0.12	1	0.125	2	1	4	4	0.25	0.5

	Log P	*C. albicans NCPF3281*	*C. glabrata NCPF8018*	*C. tropicalis NCPF8760*	*C. parapsilosis NCPF3209*	*C. auris*							
						TDG1912	*TDG2512*	*TDG1102*	*TDG2211*	*TDG2506*	*NCPF8984*	*NCPF8971*	*NCPF8977*
 **7**	1.22	≤ 0.125	0.25	0.25	≤ 0.125	0.25	0.06	4	0.5	≤ 0.03	≤ 0.03	0.25	≤ 0.03
 **8**	1.55	≤ 0.125	0.25	1	≤ 0.125	0.5	0.25	1	16	8	8	0.5	0.25
 **9**	2.47	≤ 0.06 – 0.125	1	1	≤ 0.06	0.25 – 1	0.0625	0.5	0.25	0.25	2	0.0625	0.25
 **10**	1.93	≤ 0.125	0.25	0.25	≤ 0.125	≤ 0.125	0.06	0.25	0.125	0.5	0.5	0.125	0.125
 **11**	0.55	0.12	0.12-8	4 – 16	0.12 – 0.25	8 – 32	2	8	0.5	32	32	2	2
 **12**	0.97	0.0313	8	4	0.06 – 0.12	0.5 – 1	0.5	2	8	16	16	0.5	0.5
 **13**	1.46	< 0.125 – 0.25	128	16	< 0.125 – 1	4	1 – 2	1 – > 16	1 – > 16	32 – 64	2 – 64	0.5 – > 16	2

141

Antifungal activity of Library A

All 13 compounds were tested against a panel of 4 strains from different clinically important *Candida* species and an extended panel of *C. auris* (8 strains) and their antifungal activity and lipophilicity was compared against the reference compound, Fluconazole (Table 2). The *C. auris* panel covers a range of azole susceptibility profiles and clades (Clade I; TDG2211, NCPF8971, Presumptive Clade I; TDG2512, Clade II; TDG2506, NCPF8984, Clade III; TDG1102, NCPF8977, TDG1912). Whilst there is no defined EUCAST or CLSI resistance breakpoint for fluconazole in *C. auris,* there is a presumed breakpoint of 32 µg/mL, therefore the panel contains both azole 'susceptible' and azole 'resistant' isolates.

The goals of the structural modification was to understand the role of Fluorine and methyl at the 4-position of the terminal aromatic or heteroaromatic ring, and the effect of introduction of heteroatoms to the phenyl ring on antifungal activity of these compounds. Compound **1** showed excellent activity across all Candida strains including the drug-resistant *C. auris* strains. The MICs against all Candida strans was between 0.03 to 1 µg/mL, compared to fluconazole which was inactive against the majority of the *C. auris* panel. Introduction of F at the 4-position of the phenyl ring maintained activity across all strains with the exception of *C. auris* NCPF 8984 where a 4-fold reduction in activity was observed.

On the other hand, introducing a methyl group at the 4-position of the phenyl ring resulted in significantly improved activity across all strains, including *C. auris* strains. In some cases, the activity increased by as much as 20-fold. Introducing a heteroatom at the 2 position in the form of nitrogen maintained activity across all strains, but a slight reduction was observed in a few strains, particularly *N. glabrata* NCPF8018, where a 32-fold reduction was noted. A 4-fold reduction was noted for *C. auris* TDG2506 and NCPF8984. Interestingly, the introduction of a fluorine atom as seen in compound **5** restored activity to the levels observed for compound **1** across all strains tested. This suggests that fluorine at the 4-position of the pyridine ring is preferred over an unsubstituted pyridine, a preference not observed in the case of the phenyl ring.

For the next set of compounds, the phenyl ring was replaced with a pyrimidine ring. Compounds **6, 7, 8, 9**, and **10** contained a pyrimidine ring with nitrogen atoms that are *ortho*- to 2º amine linkage. In the case of the unsubstituted pyrimidine (compound **6**), it maintained excellent activity across all strains tested, with MICs ranging from 0.12 to 4 µg/mL. However, for *C. auris* TDG2506 and NCPF8984, a 4-fold reduction was observed. Similar to what was observed for compound **5**, where the introduction of fluorine restored activity, compound **7**, which contains a 4-substituted fluorine, also showed a significant improvement in activity, with MIC values comparable or in some cases better than compound **1**. Interestingly, only for *C. auris* strain TDG1102 was an 8-fold reduction in activity observed. In contrast, for other strains like TDG2506 and NCPF8984, a 30-fold improvement in activity was noted.

When the fluorine was replaced by a methyl group, as in compound **8**, activity was reduced, particularly across all *C. auris* strains. This was surprising, given that in the case of compound **3**, which contains a phenyl ring, replacing fluorine with methyl resulted in improved activity. This suggests that the electronic environment of the terminal heteroaromatic ring, in this case, pyrimidine, played a key role in the interaction of these compounds with the target enzyme, affecting their activity.

In compound **9**, a dimethyl substitution was made at the 3 and 5 positions of the pyrmidine ring. Surprisingly, this compound showed a significant improvement in activity, making it one of the most active compounds, with activity comparable to that observed for compounds **1** and **5**. Introducing a methyl substitution at the **3**-position of compound **7,** as seen in compound **10**, maintained activity, suggesting that these hydrophobic substitutions are well tolerated in pyrimidine ring-containing compounds where nitrogen atoms are *ortho*- to 2º amine linkage.

Finally, for the Library A compounds, three further compounds were synthesized, where the positions of the nitrogens in the pyrimidine ring were flipped compared to the previous series, placing the nitrogens at *meta*- to 2º amine linkage. The unsubstituted compound **11** showed relatively poor activity compared to compound **1** (the phenyl compound), particularly against *C. auris* strains, where reductions in activity of 32 to 64-fold were observed. Introducing fluorine at the 4 position of this compound restored some activity, but it remained significantly lower compared to both the unsubstituted phenyl ring compound **1** and the unsubstituted pyrimidine compound **6**. Finally, when a methyl substitution was made at the 4 position of this ring, as seen in compound **12**, a further reduction in MIC was observed. The structure-activity relationship observed for the Library A compounds suggests that the effects of fluorine and methyl substitutions on activity are highly dependent on the core aromatic or heteroaromatic ring. These substitutions are well tolerated in phenyl and pyridyl rings, while their effects on pyrimidine rings are more variable.

Synthesis of the library B

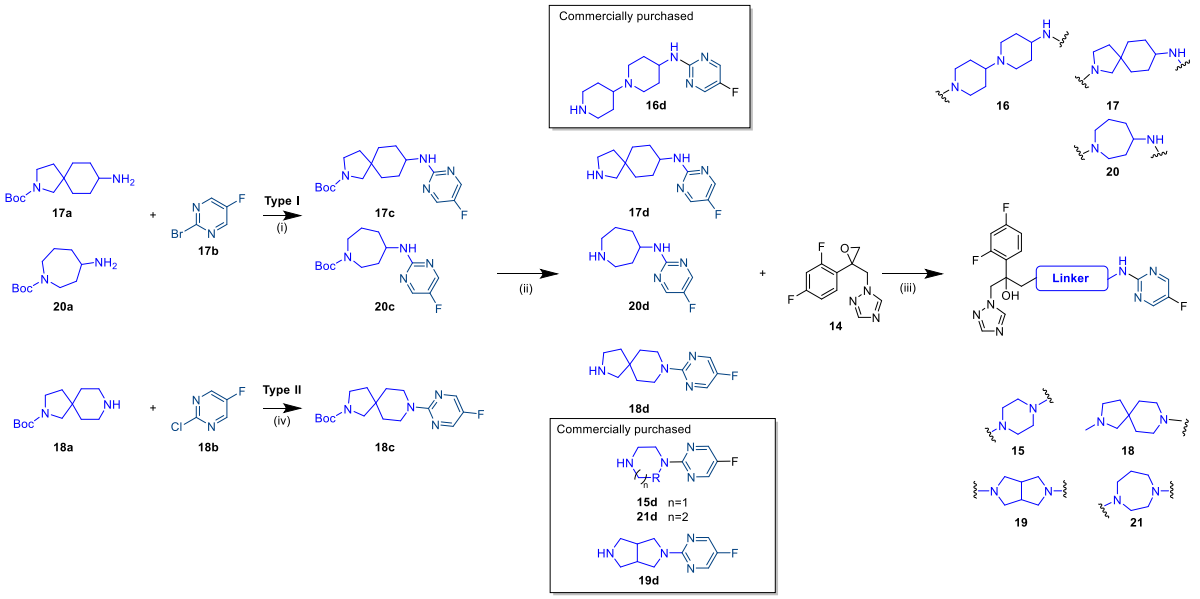
One interesting observation was the hydrophobicity, as measured by LogP, of the members of Library A. Except for compounds **11** and **12**, all other compounds had a higher LogP, indicating they are more hydrophobic compared to fluconazole (Table 2). Almost all of the compounds, except for compound **13**, showed significantly superior activity compared to fluconazole. This suggests that the hydrophobicity of the azole compounds potentially plays an important role in their interaction with the target enzyme Erg11 and in the *in vitro* activity of this compound series against azole resistant Candida strains.

After reviewing the MIC activity data of Library A compounds, compound **7**, which contains a pyrimidine ring with nitrogens at the ortho positions relative to the amine linker and a fluorine atom at the 4-position, appeared to have the best overall activity profile. Therefore,

the terminal heteroaromatic fragment present in compound **7** was selected to design the next set of compounds, where the linker for aminopiperidine was varied. This was done specifically to explore the potential of introducing different types of linkers in the design and development of modified azole compounds that are active against resistant Candida strains—a strategy that has not been previously reported in the literature.

We selected seven different ring structures to modify compound **7**. These included various types of linkers, such as spiro and fused linkers, as well as six- and eight-membered linkers to introduce different types of ring strains. For compound **15**, we selected a piperazine linker. A [1,4'-bipiperidin]-4-amine was used as the linker for compound **16**, while 2-azaspiro[4.5]decan-8-amine was chosen for compound **17**. For compound 18, we used 2,8-diazaspiro[4.5]decane as the linker. A fused ring like octahydropyrrolo[3,4-c]pyrrole was selected for compound **19**, azepan-4-amine was chosen for compound **20**, and finally, a 1,4-diazepane ring, which is a seven-membered ring, was selected for compound **21**.

The linker-heteroaromatic fragments, **15a** to **21a** were either commercially obtained or synthesised according to Scheme 2. The final modified azole compounds **15** to **21** were synthesised following Scheme 2.



Scheme 2. General synthetic routes for library B, where terminal heteroaromatic fragment of 7 was connected to fluconazole core via different linkers. Conditions: (i) NaOtBu, JohnPhos, $Pd_2(dba)_3$, dioxane, 80 °C overnight; (ii) 4 M HCl in 4-dioxane, 2 h; (iii) Et_3N, EtOH, 80 °C, overnight; (iv) K_2CO_3, DMSO, 70 °C, overnight or $EtN(Pr\text{-}i)_2$, DMF, 130 °C, overnight;

Antifungal activity of library B

The synthesized compounds were evaluated against the same Candida panel, which included clinically important Candida strains and drug-resistant *C. auris* strains. From the activity data shown in Table 3, it can be seen that all linkers, except for the bipiperidine-4-amine linker used in compound **16** and the fused linker octahydropyrrolo[3,4-c]pyrrole used in compound **19**, were generally well tolerated. Compounds containing other linkers either maintained their activity against these Candida strains, or in some cases, like compound **18**, showed improved activity compared to compound **7** against most strains tested.

Compound **18**, which contains a 2,8-diazaspiro[4.5]decane linker demonstrated a 4- to 8-fold improvement in activity across various Candida strains and had the best overall activity profile among all the compounds synthesized. The compound was highly active against *C. albicans* NCPF3281 (MIC ≤0.008 µg/mL), *C. parapsilosis* NCPF3209 (MIC 0.004 µg/mL) and *C. auris* NCPF8977 (0.002 µg/mL). The MIC of this compound was less than 0.5 µg/mL against all other strains. However, compound **15**, which contained a piperidine linker, also showed good activity across the board, except against *C. auris* NCPF8984, where an approximately 256-fold reduction in activity was observed and *C. auris* TDG2506 with a 64 fold reduction in activity. The compound had an MIC of 0.5 µg/mL or less against all other strains. Similarly, compound **17** maintained activity for most strains, but generally exhibited a 2- to 8-fold reduction in activity (Table 3).

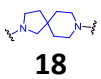
Compound **20**, on the other hand, showed some variability in activity. Its activity against *C. tropicalis NCPF8760* was 16-fold lower compared to compound **7**. Interestingly, against two *C. auris* strains, TDG2211 and NCPF8984, compound **20** also showed significantly reduced activity, with a more than 512-fold reduction observed. However, it showed comparable activity against all other Candida strains.

Compound **21**, which had a 7-membered diazepane ring, generally maintained good activity across all Candida strains tested. However, the activity was lower against *C. auris* strains, with a 4- to 64-fold reduction observed for most strains, except for TDG2506, which showed a more than 256-fold reduction in activity. It showed comparable activity against other clinically important Candida strains with MICs ranging from 0.015 to 4 µg/mL (Table 3).

From the activity profile of these second-generation compounds, it appears that linker length did not play a specific role on activity. Compounds **16**, **17**, **18**, and **19**, which had either fused or spiro linkers, generally had longer linkers compared to compounds **7**, **15**, **20**, and **21**, but these lengths did not correlate with activity. For example, compound **18**, with a longer spiro linker, and compound **7**, with a relatively short 4-aminopiperidine linker showed the overall best activity profile and can be considered as lead compounds. These two

compounds showed a more than 128- to 512-fold increase in activity compared to fluconazole against *C. auris* strains. This suggests that the nature of the linker is more important than its length.

Table 3. Antifungal activity (µg/mL) of modified -fluconazole compounds against *Candida* from library B where different linker types were utilised.

Strains	Fluconazole	 7	 15	 16	 17	 18	 19	 20	 21
C. albicans NCPF3281	0.06–0.125	≤0.125	≤0.016	0.015	0.0313	≤0.008	0.12–0.25	0.06–0.125	≤0.016
C. glabrata NCPF8018	4	0.25	0.125	64	2	0.25–0.5	1–2	1	0.25–0.5
C. tropicalis NCPF8760	16	0.25	0.5	64	2	0.01 – 0.5	8	4	1 – 2
C. parapsilosis NCPF3209	0.25	≤0.125	0.008	0.125	0.03	0.004–0.008	0.125	0.12–0.25	0.016–0.06
C. auris TDG1912	>128	0.25	0.06–0.12	4	0.5	0.12–0.25	8–16	1	2
C. auris TDG2512	8	0.06	0.25	0.5	0.06–0.12	0.03	4	0.25–0.5	2
C. auris TDG1102	64	4	0.5	4	2	0.25	8–16	2	1–2
C. auris TDG2211	64	0.5	0.5	64	8	0.25	4	0.5	1
C. auris TDG2506	128	≤0.03	2	8	2	0.5	>128	16	8
C. auris NCPF8984	128	≤0.03	8	0.5	2	0.125	32	16	0.125
C. auris NCPF8971	32	0.25	0.125	0.5	0.25	0.125	1	0.25	0.125
C. auris NCPF8977	16	≤0.03	0.03	1	0.5	0.002–0.03	1–4	0.25	0.12–0.25

To further explore the differences in activity observed between fluconazole and the modified compound 7, particularly in *C. auris* strains, we evaluated the binding of these two compounds to the lanosterol 14α-demethylase (LDM) enzyme in *C. auris* using molecular docking. As shown in Figure 2, interesting differences were observed in the 2D and 3D binding of both compounds. Fluconazole and compound 7 were found to bind to adjacent binding pockets. However, while fluconazole interacted with a more hydrophilic overall binding pocket, compound 7 interacted with a predominantly hydrophobic pocket and engaged with more

amino acid residues within the binding site. The length of the molecule may have contributed to these interactions, but the nature of the binding and the differences observed between compound 7 and fluconazole likely played a role in their ability to inhibit the target enzyme, and, their effectiveness in killing *C. auris* strains. The Chemscore and binding affinity values (Table 1) suggest that compound 7 binds more tightly to the LDM enzyme, and this tight binding, along with the differences in interactions, particularly in the hydrophobicity of key binding residues, may explain compound 7's superior ability to kill *C. auris* strains.

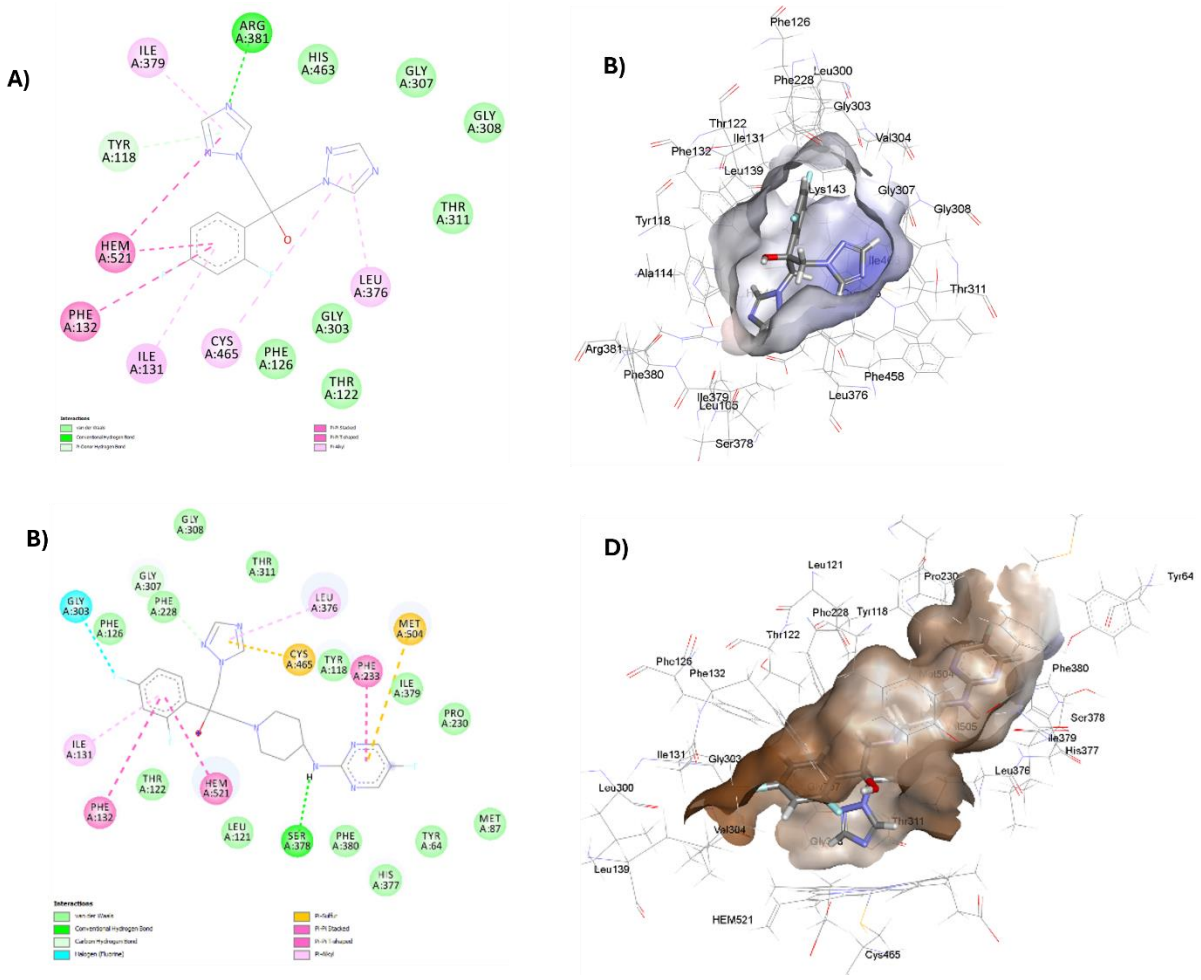


Figure 2: Differences in binding interactions observed for fluconazole (A & B), and compound 7 (C & D) with lanosterol 14α-demethylase (LDM) enzyme in *C. auris*

Efficacy study in *G. mellonella* model

After showing excellent *in vitro* activity against drug resistant *C. auris* strains, compound **7** was selected to test the *in vivo* efficacy of these modified azoles in a *G. mellonella* model [44] infected with *C. auris* (TDG1912). At first, the toxicity of the compound was tested using this

non-anmial model, and compound **7** did not show any toxicity at 50 mg/Kg dose level suggesting this modified azole scaffold is non-toxic at the doses studied. Compound **7** expressed good protection over the infected larvae compared to fluconazole, which failed to show any protection. The protection from the fungal infection recorded with the doses of 50 mg/kg and 20 mg/kg of **7** showed statistically significant protection (<0.0001 and 0.0025 with Mantel-Cox log-rank test, respectively). Even at a low dose of 5 mg/kg, treated larvae showed a reasonable survival rate of around 50% after 5 days of infection. As the dosage of **7** increased, there was a progressive enhancement in larval survival rates. At the highest tested dosage of 50 mg/kg, the final protection rate exceeded 70%, surpassing the approximately 75% larval mortality rate in the untreated group (Figure 3).

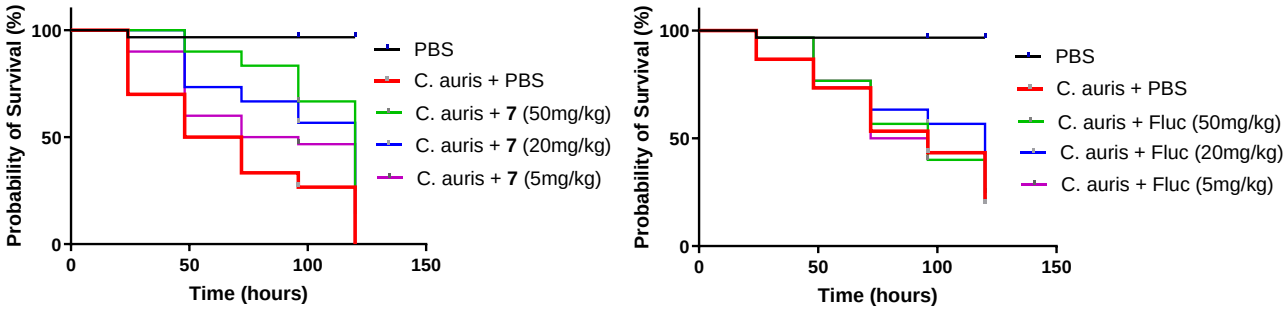


Figure 3. Efficacy of modified fluconazole compound **7** and fluconazole against *C. auris* TDG1912 infection in *G. mellonella* model.

Time-kill assay of Compound 7

Time-kill analysis, a dynamic assay using static MIC data, evaluates the kinetics and efficacy of antifungal compounds over time. It monitors fungal growth at specific intervals after treatment with antifungals at concentrations relative to the MIC. This method reveals differences in the behavior of compounds with identical MICs, providing insights into their mode of action. A reduction of colonies by ≤3-log indicates fungistatic activity, while >3-log reduction suggests fungicidal action. Azoles are typically fungistatic, but the higher *in vitro* activity observed in modified azoles warranted an evaluation of their mode of action using the time-kill kinetics assay. In this study, Compound **7**, fluconazole, and voriconazole were tested against *C. auris* TDG1912 over 24 hours at 4x MIC_{50} (Figure 4).

Compared to the untreated control sample, time-kill curves for all drug-treated samples exhibited a slightly smaller fungal population size. Notably, compound **7** stood out among the three tested compounds, demonstrating inhibition of fungal growth for at least six hours of incubation. However, regrowth of *C. auris* TDG1912 was observed after the 6-hour time point. It is important to note that the MIC value used for the time-kill assay corresponds to drug concentrations inhibiting 50% of fungal growth, as defined by EUCAST guidelines for azoles.

All compounds, whether modified or commercially available, exhibited a fungistatic nature, inhibiting fungal growth at varying efficacies, but not killing the fungi (Figure 4).

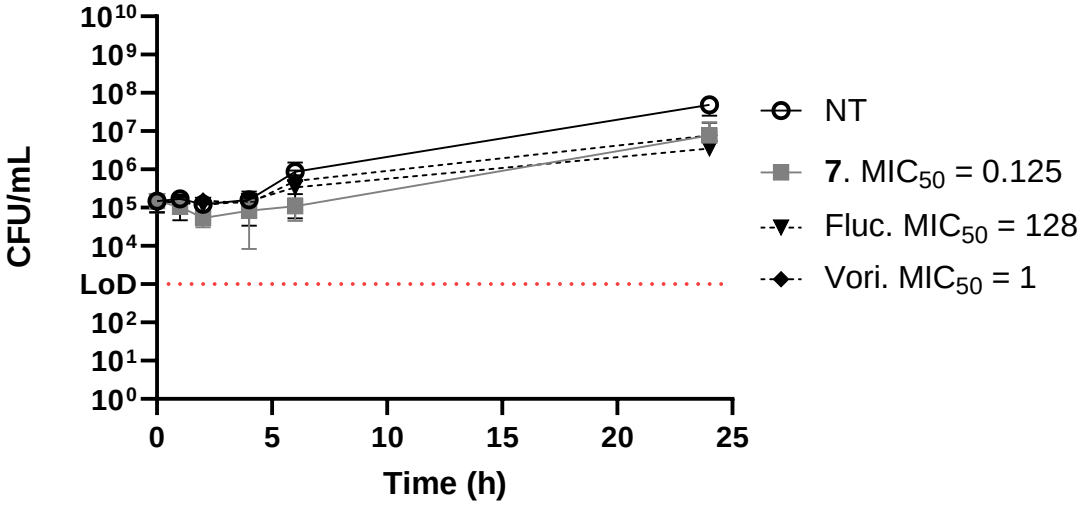


Figure 4. Time-kill of compound **7**, alongside two commercial reference antifungal drugs, fluconazole (Fluc) and voriconazole (Vori) against *C. auris* TDG1912. The limit of the detection (LoD) is 1000 CFU/mL. MIC_{50} results are in µg/mL.

Conclusions

In summary, this study focussed on the synthesis and evaluation of a novel library of modified azole compounds. The initial approach involved the use of a six-membered cyclic piperidine linker, followed by the exploration of various heteroaliphatic linkers. Systematic investigation of substitution patterns on the terminal aromatic or heteroaromatic fragment identified key structural features that enhance antifungal activity against drug-resistant *C. auris* strains, notably the impact of hydrophobicity, presence of heteroatom within the ring structure and para-positioned substitutions on the aromatic ring. Compound **7** emerged as a promising candidate, exhibiting potent activity against a broad panel of Candida strains. Further exploration of different linkers helped establish the structure-activity relationship of this modified azole series. The retention of activity across a wide range of linkers presents opportunities for additional medicinal chemistry optimization during the lead optimization phase. This research introduces a new azole-based chemical scaffold with enhanced activity against all clinically important Candida strains, particularly drug-resistant *C. auris* strains.

Experimental Section

Chemistry

General Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Spectrospin Spectrometer (400 MHz, 25 degrees) equipped with a SampleXpress autosampler system. Chemical shifts δH and δC are recorded in parts per million (ppm) and referenced to the residual solvent peak. Coupling constants (J) are recorded to the nearest 0.01 Hz. Assignment of ^{1}H and ^{13}C NMR spectra was made using the aid of TopSpin 3.5 software from Bruker, ACD/Labs or MestReNova from Mestrelab Research. Purity is calculated using the HPLC-LCMS peak areas.

LC-MS system used is a Waters Alliance 2695 system with an elution in gradient. Low-resolution mass spectra were analyzed and recorded on a Waters QZ instrument using electrospray ionization (ESI) and coupled to a High-performance liquid chromatography (HPLC) system. Selected mass-to-charge ratio peaks (m/z) are quoted in Daltons. HPLC grades solvents were used for mobile phase and a Phenomenex Monolithic C18 50*4.60 mm column was used for stationary phase. Detect method used UV detection performed on a Waters 2996 photo array detector.

Thin layer chromatography (TLC) was performed using Merck aluminium foil-backed sheets precoated with 0.2 mm Kielselgel 60 F254. Product spots were visualized by UV irradiation (λmax = 254 nm or 365 nm). Manual flash column chromatography was carried out using Aldrich silica gel 60 Å, 40 – 63 µm (230 – 400 mesh). Eluting solvents and retention factors (Rf) were indicated in the text.

The initial optical rotation of compounds was measured with a single wavelength polarimeter ADP440 by Bellingham & Stanley at λ = 589 nm. Further optical rotatory dispersion (ORD) measurements of selected compounds were measured with Chirascan spectrometers by Applied Photophysics and were analysed with Pro-Data Chirascan.

All reactions were carried out in oven-dried glassware and all reagents were obtained commercially either from Aldrich Chemicals Ltd., Alfa Aesar Ltd, Fluorochem, Fisher Scientific or Apollo Chemicals Ltd.

Chemical Synthesis

General procedure for intermediate synthesis- (i) using reducing borohydride

The aniline (1.0 equiv.) synthesised in the previous step or purchased commercially was dissolved in the anhydrous DCM (0.1 mmol / mL) in the round-bottomed flask upon stirring, followed by the addition of boc-protected linker fragment (1.5 equiv.), $Na(OAc)_3BH$ (2.0 equiv.), and AcOH (2.0 equiv.) successively. After the overnight stirring at ambient temperature, the mixture was consecutively quenched with NaOH (aq.), extracted with DCM, dried with anhydrous $MgSO_4$, and evaporated *in vacuo*. The crude mixture was purified with *flash* column chromatography gradient-ly using chloroform and methanol to get the target pure product.

tert-butyl 4-(phenylamino)piperidine-1-carboxylate ***1a***

White solid (56%); ^{1}H NMR (400 MHz, 25 degree, $CDCl_3$) σ: 1.46 (9H, s), 2.04 (2H, dd, J = 13 Hz, 2 Hz), 2.44 (1H, t, J = 6 Hz), 2.88 (2H, t, J = 12 Hz), 3.42 (1H, tt, J = 10 Hz, 4 Hz), 3.72 (1H, t,

J = 6 Hz), 4.05 (2H, d, J = 8 Hz), 5.30 (1H, s), 6.68 – 6.81 (3H, m), 7.20 (2H, dd, J = 8 Hz, 7 Hz); ^{13}C NMR (101 MHz, 25 degree, $CDCl_3$) σ: 58.5, 32.0, 41.3, 79.8, 80.6, 115.3, 129.4, 129.6, 154.9, 208.0; m/z: (ESI$^+$) 277.1 ([M+H]$^+$); R_f: 0.9 (1 : 1 Hexane : Ethyl acetate); Purity: 96%.

tert-butyl 4-(p-tolylamino)piperidine-1-carboxylate ***3a***

Off-white solid (51%); ^{1}H NMR (400 MHz, 25 degree, CDCl3) σ: 1.29 – 1.39 (2H, m), 1.46 (9H, s), 2.03 (2H, dd, J = 12 Hz, 2 Hz), 2.24 (3H, s), 2.89 (2H, t, J = 12 Hz), 3.38 (1H, tt, J = 10 Hz, 4 Hz), 4.04 (2H, d, J = 9 Hz), 4.04 (1H, s, br), 6.60 (2H, d, J = 8 Hz), 7.00 (2H, d, J = 8 Hz); 13C NMR (101 MHz, 25 degree, CDCl3) σ: 20.5, 28.6, 32.3, 41.3, 51.4, 79.7, 114.6, 127.9, 130.0, 143.5, 154.9; m/z: (ESI+) 291.2 ([M+H]+); Rf: 0.03 (Ethyl acetate); Purity: ≥97%.

tert-butyl 4-((2-fluoropyrimidin-5-yl)amino)piperidine-1-carboxylate ***12a***

Off-white solid (49%); ^{1}H NMR (400 MHz, 25 degree, CDCl3) σ: 1.41 (9H, s), 1.77 – 1.83 (1H, m), 1.98 (2H, dd, J = 13 Hz, 3 Hz), 2.88 (2H, t, J = 12 Hz), 2.97 (1H, ddd, J = 13 Hz, 10 Hz, 3 Hz), 3.35 (1H, tt, J = 10 Hz, 4 Hz), 3.60 (1H, s, br), 4.03 (2H, d, J = 13 Hz), 7.97 (2H, s); 13C NMR (101 MHz, 25 degree, CDCl3) σ: 28.4, 31.7, 34.2, 50.9, 67.6, 79.6, 80.0, 139.0 (d, J = 5 Hz), 144.7 (d, J = 11 Hz), 154.7, 156.3 (d, J = 211 Hz); Rf: 0.2 (1 : 9 Ethyl acetate: DCM); Purity: ≥95%.

tert-butyl 4-((2-methylpyrimidin-5-yl)amino)piperidine-1-carboxylate ***13a***

White solid (53%); ^{1}H NMR (400 MHz, 25 degree, CDCl3) σ: 1.36 – 1.43 (2H, m), 1.45 (9H, s), 2.01 (2H, dd, J = 13 Hz, 3 Hz), 2.65 (3H, s), 2.95 (2H, t, J = 12 Hz), 3.43 (1H, tt, J = 10 Hz, 4 Hz), 4.03 – 4.07 (2H, m), 4.42 (1H, s, br), 8.20 (2H, s); 13C NMR (101 MHz, 25 degree, CDCl3) σ: 23.7, 28.6, 29.8, 31.9, 50.1, 80.0, 138.9, 141.1, 154.8, 154.8; m/z: (ESI+) 293.1 ([M+H]+); Rf: 0.5 (9 : 1 Ethyl acetate: MeOH); Purity: ≥98%.

General procedure for intermediate synthesis- (ii) using base

The starting material of pyrimidine (1.0 equiv.) in mixture of anhydrous MeCN (1.315 mmol / mL) and dimethylacetaminde (5.26 mmol / mL) was stirred under inert gas while BOC-protected linker (1.2 equiv.) and DIPEA (1.2 equiv.) was added slowly. The reaction was carried out in microwave at 160 ℃ for 1 h and at 180 ℃ for another 30 min. The crude mixture was obtained by direct solvent evaporation. The mixture was purified by gradient flash column chromatography in ethyl acetate and hexane with 1% TEA.

5-methyl-N-(piperidin-4-yl)pyrimidin-2-amine ***8a***

Pale-yellow solid (42%); ^{1}H NMR (400 MHz, 25 degree, MeOH-d4) σ: 1.34 (2H, ddd, J = 24 Hz, 12 Hz, 3 Hz), 1.92 (2H, d, J = 12 Hz), 2.10 (3H, s), 2.92 (2H, t, J = 12 Hz), 3.02 (1H, tt, J = 11 Hz, 4 Hz), 4.65 (2H, d, J = 13 Hz), 8.16 (2H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 14.4, 34.2, 44.0, 50.1, 119.7, 159.0, 151.6; m/z: (ESI+) 194.1 ([M+H]+); Rf : 0.1 (10 : 1 : 0.1 Ethyl acetate : MeOH : TEA); Purity: ≥96%.

tert-butyl 4-((4,6-dimethylpyrimidin-2-yl)amino)piperidine-1-carboxylate ***9a***

Pale-yellow solid (45%); ^{1}H NMR (400 MHz, 25 degree, CDCl3) σ: 1.20 – 1.30 (2H, m), 1.35 (9H, s), 1.91 (2H, d, J = 12 Hz), 2.15 (6H, s), 2.85 (2H, t, J = 12 Hz), 4.00 – 4.05 (1H, m), 4.59 (1H, d, J = 13 Hz), 6.18 (1H, s); 13C NMR (101 MHz, 25 degree, CDCl3) σ: 23.7, 28.3, 32.2, 42.5, 47.5, 79.3, 109.6, 154.6, 161.4, 167.3; m/z: (ESI+) 307.2 ([M+H]+); Rf : 0.3 (2 : 8 Ethyl acetate : Hexane); Purity: ≥95%.

General procedure for intermediate synthesis- (iii) using metal catalysis

In an oven-dried round-bottomed flask containing anhydrous toluene (0.6 mmol / mL), the pyrimidine fragment (1.0 equiv.), BOC-protected linker fragment (1.0 equiv.), rac-BINAP (0.04 equiv.), Pd2(dba)3 (0.02 equiv.) and KOtBu (1.2 equiv.) were added sequentially under inert gas. The mixture was then heated under microwave at 100 ℃ for 2h. After cooling to room temperature, the mixture was then filtered through celite. The filtrate was diluted with saturated brine and extracted with ethyl acetate for three times before drying over anhydrous MgSO4 and evaporated in vacuo. Crude product was then purified with gradient flash column chromatography in hexane and ethyl acetate.

tert-butyl 4-((5-fluoro-4-methylpyrimidin-2-yl)amino)piperidine-1-carboxylate ***10a***

Off-white solid (52%); ^{1}H NMR (400 MHz, 25 degree, CDCl3) σ: 1.26 – 1.40 (2H, m), 1.44 (9H, s), 1.99 (2H, d, J = 12 Hz), 2.31 (3H, d, J = 2 Hz), 2.92 (2H, t, J = 12 Hz), 3.83 – 4.01 (3H, m), 4.96 (1H, d, J = 7 Hz), 7.98 (1H, d, J = 1 Hz); ^{13}C NMR (101 MHz, 25 degree, CDCl3) σ: 17.8, 28.5, 32.2, 48.7, 79.7, 144.0 (d, J = 23 Hz), 151.1 (d, J = 246 Hz), 154.9, 155.8 (d, J = 16 Hz), 158.1 (d, J = 3 Hz); m/z: (ESI+) 255.1 ([M – tBu +H]+); Rf 0.8 (Ethyl acetate); Purity: ≥95%.

Synthesis of fluconazole core

*1-((2-(2,4-difluorophenyl)oxiran-2-yl)methyl)-1H-1,2,4-triazole (****14****)*

Trimethyl sulphoxonium iodide (2 equiv.) was added to toluene (0.12 mmol / mL) containing 1-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-yl)ethan-1-one (1 equiv.) and sodium hydroxide 30% (w/w) aqueous solution (10 equiv.). The mixture was heated under microwave (MW) radiation for 50 minutes at 80 °C. Then the mixture was diluted with water and extracted with ethyl acetate. The organic layer was combined and washed with saturated brine, dried over anhydrous magnesium sulphate, and concentrated in vacuo. Pale yellow solid (52%); ^{1}H NMR (400 MHz, 25 degree, CDCl3) σ: 2.83 (1H, d, J = 5 Hz), 2.90 (1H, d, J = 5 Hz), 4.47 (1H, d, J = 15 Hz), 4.79 (1H, d, J = 15 Hz), 6.73 – 6.82 (2H, m), 7.11 – 7.16 (1H, m), 7.81 (1H, s), 8.03 (1H, s); ^{13}C NMR (101 MHz, 25 degree, CDCl3) σ: 52.1, 53.4 (d, J = 3 Hz), 56.1 (d, J = 1 Hz), 104.0 (dd, J = 26 Hz, J = 25 Hz), 111.6 (dd, J = 21 Hz, J = 3 Hz), 119.4 (dd, J = 15 Hz, 4 Hz), 129.5 (dd, J = 15 Hz, J = 10 Hz, 5 Hz), 144.0, 151.7, 160.5 (dd, J = 249 Hz, 12 Hz), 163.0 (dd, J = 251 Hz, 12 Hz); m/z: (ESI+) 238.1 ([M+H]+); R_f: 0.24 (1 : 1 Ethyl acetate : Hexane); Purity: ≥95%.

tert-butyl 8-(5-fluoropyrimidin-2-yl)-2,8-diazaspiro[4.5]decane-2-carboxylate ***18c***

Boc-protected linker fragment (1.0 equiv.) was dissolved in anhydrous DMF under inert gas before the addition of pyrimidine fragment (1.275 equiv.) and DIPEA (3.125 equiv.). The

mixture was heated at 130 ℃ overnight and cooled to room temperature upon reaction completion. After diluted with water, the mixture was extracted by ethyl acetate for three times and dried over anhydrous $MgSO_4$ before evaporated *in vacuo*. The crude product was purified with gradient *flash* column chromatography in mixture of DCM and ethyl acetate. White solid (39%); ^{1}H NMR (400 MHz, 25 degree, $CDCl_3$) σ: 1.45 (9H, s), 1.58 (4H, dd, J = 11 Hz, 6 Hz), 1.75 (2H, t, J = 7 Hz), 3.19 (1H, s), 3.27 (1H, s), 3.38 – 3.43 (2H, m), 3.59 – 3.69 (2H, m), 3.80 – 3.92 (2H, m), 8.16 (2H, s); ^{13}C NMR (101 MHz, 25 degree, $CDCl_3$) σ: 28.7, 34.5, 36.5, 40.3, 42.1, 44.3 (d, J = 26 Hz), 55.6 (d, J = 75 Hz), 79.3, 145.2 (d, J = 22 Hz), 151.5 (d, J = 248 Hz), 154.9, 158.9; m/z: (ESI$^+$) 281.1 ([M – *t*Bu + H]$^+$); R_f: 0.8 (1 : 1 DCM : Ethyl acetate); Purity: ≥95%.

tert-butyl 4-((5-fluoropyrimidin-2-yl)amino)azepane-1-carboxylate ***20c***

Synthesis was done by following the protocol described for **18c**. White solid (36%); ^{1}H NMR (400 MHz, 25 degree, $CDCl_3$) σ: 1.46 (9H, s), 1.55 – 1.73 (3H, m), 1.83 – 1.99 (2H, m), 2.10 – 2.14 (1H, m), 3.24 (1H, tdd, J = 15 Hz, 9 Hz, 3 Hz), 3.39 – 3.48 (2H, m), 3.51 – 3.68 (1H, m), 3.94 (1H, s), 5.78 (1H, s, br), 8.20 (2H, s); ^{13}C NMR (101 MHz, 25 degree, $CDCl_3$) σ: 24.5 (d, J = 16 Hz), 33.1 (d, J = 36 Hz), 35.0 (d, J = 4 Hz), 42.7 (d, J = 41 Hz), 52.08 (d, J = 24 Hz), 79.6, 105.5 (d, J = 8 Hz), 134.1 (d, J = 64 Hz), 145.5 (d, J = 23 Hz), 155.7; m/z: (ESI$^+$) 311.2 ([M+H]$^+$); R_f: 0.6 (98 : 2 Chloroform : MeOH); Purity: ≥95%.

tert-butyl 8-((5-fluoropyrimidin-2-yl)amino)-2-azaspiro[4.5]decane-2-carboxylate ***17c***

Off-white solid (33%); ^{1}H NMR (400 MHz, 25 degree, $CDCl_3$) σ: 1.27 – 1.39 (2H, m), 1.45 (9H, s), 1.61 – 1.68 (4H, m), 1.74 (1H, t, J = 7 Hz), 1.97 (2H, t, J = 13 Hz), 3.15 (2H, t, J = 32 Hz), 3.34 – 3.40 (2H, m), 3.77 (2H, m), 5.22 (1H, s, br), 8.18 (2H, s); m/z: (ESI$^+$) 295.1 ([M – *t*Bu +H]$^+$); R_f : 0.8 (1 : 1 DCM : Ethyl acetate); Purity: ≥95%.

General procedure for final compounds synthesis

Fluconazole core (1 equiv.) and triethylamine (1.5 equiv.) was added into the solution of amine (1.5 equiv.) in ethanol (0.07 mmol / mL) under stirring. The mixture was heated under stirring at 80 oC overnight until all starting material disappeared. The solvent was removed under reduced pressure and product was purified by flash column chromatography (MeOH in EtOAc, 0 – 20%).

2-(2,4-difluorophenyl)-1-(4-(phenylamino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***1***

Light yellow liquid (58%); [α]D24: 0 (c = 1.5 in methanol); ^{1}H NMR (400 MHz, 25 degree, MeOH-d4) σ: 1.37 – 1.47 (2H, m), 1.80 (1H, d, J = 15 Hz), 1.91 (1H, d, J = 11 Hz), 2.25 (1H, td, J = 9 Hz, 3 Hz), 2.42 (1H, td, J = 2 Hz), 2.54 – 2.59 (1H, m), 2.73 – 2.78 (1H, m), 2.81 (1H, d, J = 13 Hz), 3.02 (1H, d, J = 14 Hz), 3.15 – 3.22 (1H, m), 4.58 (1H, d, J = 11 Hz), 4.68 (1H, d, J = 15 Hz), 6.56 – 6.61 (3H, M), 6.84 (1H, td, J = 9 Hz, 2 Hz), 6.90 – 6.96 (1H, m), 7.06 (2H, t, J = 8 Hz), 7.49 (1H, td, J = 8 Hz, 6 Hz), 7.75 (1H, s), 8.35 (1H, s); ^{13}C NMR (101 MHz, 25 degree, MeOH-

d4) σ: 33.2, 50.8, 54.9, 57.6, 64.4, 74.5 (d, J = 6 Hz), 104.9 (dd, J = 28 Hz, 26 Hz), 114.9, 118.2, 127.5 (dd, J = 14 Hz, 4 Hz), 130.1, 130.8 (dd, J = 10 Hz, 6 Hz), 146.1, 149.0, 151.1, 152.0, 161.1 (dd, J = 246 Hz, 12 Hz), 164.2 (dd, J = 248 Hz, 12 Hz); m/z: (ESI+) 414.2 ([M+H]+); HRMS: (ESI+) found 414.2088, ([M+H]+) requires 414.2100; Rf : 0.4 (Ethyl acetate); Purity: ≥99%.

2-(2,4-difluorophenyl)-1-(4-((4-fluorophenyl)amino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***2***

Light yellow solid (50%); M.P. = 93 – 102 °C; [α]D24: 14 (c = 0.7 in methanol); ^{1}H NMR (400 MHz, 25 degree, MeOH-d4) σ: 2.38 (3H, m), 2.47 – 2.48 (4H, m), 2.79 (1H, d, J = 14 Hz), 2.99 (1H, dd, J = 14 Hz, 1 Hz), 3.43 – 3.50 (2H, m), 4.59 (1H, d, J = 14 Hz), 4.68 (1H, d, J = 14 Hz), 6.84 (1H, td, J = 8 Hz, 3 Hz), 6.88 – 6.94 (1H, m), 6.97 (1H, td, J = 8 Hz, 2 Hz), 7.06 (1H, dt, J = 10 Hz, 2 Hz), 7.09 (1H, d, J = 8 Hz), 7.30 (1H, td, J = 8 Hz, 7 Hz), 7.46 (1H, td, J = 9 Hz, 7 Hz), 7.74 (1H, s), 8.33 (1H, s); ^{13}C NMR (101 MHz, 25 degree, MeOH-d4) σ: 54.1, 55.4, 57.5 (d, J = 5 Hz), 63.2 (d, J = 2 Hz), 64.5 (d, J = 3 Hz), 74.9 (d, J = 4 Hz), 104.9 (td, J = 28 Hz, 2 Hz), 112.0 (dd, J = 21 Hz, 3 Hz), 115.0 (d, J = 19 Hz), 117.0 (d, J = 20 Hz), 126.3 (d, J = 3 Hz), 127.2 (dd, J = 12 Hz, 4 Hz), 130.8 (dd, J = 3 Hz, 1 Hz), 131.0 (d, J = 8 Hz), 141.5 (d, J = 7 Hz), 146.1, 151.1, 159.7 (d, J = 28 Hz), 162.9 (dd, J = 246 Hz, 12 Hz), 164.3 (dd, J = 246 Hz, 12 Hz); m/z: (ESI+) 432.2 ([M+H]+); HRMS: (ESI+) found 432.1998, ([M+H]+) requires 432.2006; Rf : 0.4 (Ethyl acetate); Purity: ≥98%.

2-(2,4-difluorophenyl)-1-(4-(p-tolylamino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***3***

Transparent liquid (60%); [α]D26: -4 (c = 2.5 in methanol); ^{1}H NMR (400 MHz, 25 degree, MeOH-d4) σ: 1.25 – 1.44 (2H, m), 1.76 – 1.81 (1H, m), 1.87 – 1.91 (1H, m), 2.17 (3H, s), 2.23 (1H, td, J = 12 Hz, 3 Hz), 2.40 (1H, td, J = 12 Hz, 3 Hz), 2.53 – 2.57 (1H, m), 2.72 – 2.76 (1H, m), 2.80 (1H, d, J = 14 Hz), 3.00 (1H, dd, J = 14 Hz, 2 Hz), 3.13 (1H, tt, J = 10 Hz, 4 Hz), 4.57 (1H, d, J = 14 Hz), 4.67 (1H, d, J = 14 Hz), 6.55 (2H, ddd, J = 8 Hz, 3 Hz, 2 Hz), 6.84 (1H, tdd, J = 9 Hz, 3 Hz, 1 Hz), 6.89 – 6.95 (3H, m), 7.48 (1H, td, J = 9 Hz, 7 Hz), 7.74 (1H, s), 8.34 (1H, s); ^{13}C NMR (101 MHz, 25 degree, MeOH-d4) σ: 20.5, 33.2, 33.5, 51.4, 54.9, 55.7, 57.6 (d, J = 5 Hz), 64.3 (d, J = 4 Hz), 74.4 (d, J = 6 Hz), 104.9 (dd, J = 28 Hz, 26 Hz), 112.0 (dd, J = 21 Hz, 3 Hz), 115.6, 127.5 (dd, J = 13 Hz, 4 Hz), 127.8, 130.5, 130.8 (dd, J = 10 Hz, 6 Hz), 146.1, 146.4, 151.1, 160.7 (dd, J = 246 Hz, 12 Hz), 164.1 (dd, J = 248 Hz, 12 Hz); m/z: (ESI+) 428.2 ([M+H]+); HRMS: (ESI+) found 428.2254, ([M+H]+) requires 428.22564; Rf : 0.4 (Ethyl acetate); Purity: ≥98%.

2-(2,4-difluorophenyl)-1-(4-(pyridin-2-ylamino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***4***

Light yellow solid (52%); M.P. = 54 – 65 °C; [α]D26: 0 (c = 1.2 in methanol); ^{1}H NMR (400 MHz, 25 degree, MeOH-d4) σ: 1.33 – 1.52 (2H, m), 1.79 (1H, d, J = 12 Hz), 1.90 (1H, d, J = 12 Hz), 2.28 (1H, td, J = 12 Hz, 1Hz), 2.44 (1H, td, J = 13 Hz, 2 Hz), 2.57 (1H, d, J = 11 Hz), 2.75 (1H, d, J = 10 Hz), 2.81 (1H, d, J = 15 Hz), 3.02 (1H, d, J = 14 Hz), 3.57 (1H, tt, J = 10 Hz, 3 Hz), 4.59 (1H, d, J = 11 Hz), 4.68 (1H, d, J = 9 Hz), 6.46 – 6.50 (2H, m), 6.84 (1H, td, J = 7 Hz, 4 Hz), 6.92 (1H, td, J = 10 Hz, 2 Hz), 7.37 (1H, td, J = 8 Hz, 3 Hz), 7.49 (1H, dd, J = 12 Hz, 8 Hz), 7.75 (1H, s), 7.86 (1H, d, J = 7 Hz), 8.35 (1H, s); ^{13}C NMR (101 MHz, 25 degree, MeOH-d4) σ: 33.2, 33.4, 49.9, 54.9, 55.6, 57.6 (d, J = 7 Hz), 64.4 (d, J = 7 Hz), 74.5 (d, J = 8 Hz), 104.9 (dd, J = 28 Hz, J = 26 Hz),

110.4, 112.0 (dd, J = 21 Hz, 5 Hz), 113.0, 126.7 (dd, J = 17 Hz, 6 Hz), 130.8 (dd, J = 9 Hz, 5 Hz), 138.7, 146.1, 147.8, 151.1, 159.5, 160.7 (dd, J = 256 Hz, 12 Hz), 164.1 (dd, J = 248 Hz, 12 Hz); m/z: (ESI+) 415.2 ([M+H]+); HRMS: (ESI+) found 415.2039, ([M+H]+) requires 415.2052; Rf : 0.4 (10 : 1 Ethyl acetate : MeOH); Purity: ≥95%.

2-(2,4-difluorophenyl)-1-(4-((5-fluoropyridin-2-yl)amino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***5***

Light yellow solid (52%); M.P. = 59 – 74 ℃; [α]D24: 7.4 (c = 1.4 in methanol); (400 MHz, 25 degree, MeOH-d4) σ: 1.31 – 1.50 (2H, m), 1.79 (1H, d, J = 17 Hz), 1.90 (1H, d, J = 17 Hz), 2.26 (1H, td, J = 8 Hz, 8 Hz), 2.42 (1H, td, J = 13 Hz, 3 Hz), 2.56 (1H, d, J = 19 Hz), 2.74 (1H, d, J = 14 Hz), 2.81 (1H, d, J = 20 Hz), 3.01 (1H, d, J = 16 Hz), 3.54 (1H, tt, J = 11 Hz, 4 Hz), 4.60 (1H, d, J = 15 Hz), 4.68 (1H, d, J = 14 Hz), 6.46 (1H, dd, J = 11 Hz, 5 Hz), 6.84 (1H, td, J = 10 Hz, 4 Hz), 6.92 (1H, td, J = 11 Hz, 3 Hz), 7.22 (1H, td, J = 11 Hz, 2 Hz), 7.48 (1H, dd, J = 15 Hz, 12 Hz), 7.75 (1H, s), 7.76 (1H, m), 8.35 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 33.1, 33.4, 49.9, 54.9, 55.6, 57.6 (d, J = 7 Hz), 64.4 (d, J = 6 Hz), 74.5 (d, J = 5 Hz), 104. 9 (dd, J = 26 Hz, J = 23 Hz), 110.8 (d, J = 4 Hz), 112.0 (dd, J = 21 Hz, 5 Hz), 126.7 (d, J = 22 Hz), 127.4 (dd, J = 11 Hz, 6 Hz), 130.8 (dd, J = 12 Hz, 7 Hz), 134.0 (d, J = 19 Hz), 146.1, 151.1, 154.2 (d, J = 249 Hz), 156.7, 160.7 (dd, J = 253 Hz, 12 Hz), 164.1 (dd, J = 246 Hz, 12 Hz); m/z: (ESI+) 433.1 ([M+H]+); HRMS: (ESI+) found 433.1948, ([M+H]+) requires 433.1958; Rf : 0.1 (Ethyl acetate); Purity: ≥95%.

2-(2,4-difluorophenyl)-1-(4-(pyrimidin-2-ylamino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***6***

Light orange solid (52%); M.P. = 134 – 139 ℃; [α]D24: 20 (c = 1.0 in methanol); 1H NMR (400 MHz, 25 degree, MeOH-d4) σ: 1.39 – 1.58 (2H, m), 1.84 (2H, dd, J = 49 Hz, 11 Hz), 2.26 (1H, td, J = 10 Hz, 5 Hz), 2.44 (1H, td, J = 11 Hz, 4 Hz), 2.58 (1H, d, J = 8 Hz), 2.77 (1H, d, J = 9 Hz), 2.81 (1H, d, J = 19 Hz), 3.02 (1H, d, J = 22 Hz), 3.69 (1H, tt, J = 12 Hz, 3 Hz), 4.60 (1H, d, J = 9 Hz), 4.69 (1H, d, J = 16 Hz), 6.55 (1H, t, J = 4 Hz), 6.85 (1H, td, J = 12 Hz, 3 Hz), 6.93 (1H, td, J = 10 Hz, 3 Hz), 7.49 (1H, dd, J = 16 Hz, 8 Hz), 7.75 (1H, s), 8.21 (1H, s), 8.23 (1H, s), 8.36 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 33.1, 32.9, 49.9, 54.9, 55.6, 57.6 (d, J = 5 Hz), 64.4 (d, J = 8 Hz), 74.6 (d, J = 7 Hz), 104.9 (dd, J = 33 Hz, 17 Hz), 111.2, 112.0 (dd, J = 24 Hz, 6 Hz), 127.4 (dd, J = 12 Hz, J = 4 Hz), 130.8 (dd, J = 10 Hz, 3 Hz), 146.1, 151.1, 159.3 , 160.7 (dd, J = 250 Hz, 12 Hz), 162.85, 164.14 (dd, J = 248 Hz, 12 Hz); m/z: (ESI+) 416.2 ([M+H]+); HRMS: (ESI+) found 416.1994 , ([M+H]+) requires 416.2005; Rf : 0.4 (9 : 1 Ethyl acetate : MeOH); Purity: ≥95%.

2-(2,4-difluorophenyl)-1-(4-((5-fluoropyrimidin-2-yl)amino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***7***

Pale yellow crystals (58%); M.P. = 119 ℃; [α]D23: 24 (c = 0.9 in methanol); 1H NMR (400 MHz, 25 degree, MeOH-d4) σ: 1.38 – 1.56 (2H, m), 1.79 (1H, d, J = 12 Hz), 1.90 (1H, d, J = 11 Hz), 2.25 (1H, td, J = 12 Hz, 1 Hz), 2.42 (1H, td, J = 12 Hz, 2 Hz), 2.57 (1H, d, J = 9 Hz), 2.75 (1H, d, J = 9 Hz), 2.81 (1H, d, J = 16 Hz), 3.01 (1H, d, J = 10 Hz), 3.64 (1H, tt, J = 9 Hz, 4 Hz), 4.59 (1H, d, J = 11 Hz), 4.68 (1H, d, J = 12 Hz), 6.82 – 6.96 (2H, m), 7.49 (1H, dd, J = 15 Hz, 8 Hz), 7.75 (1H, s), 8.17 (1H, s), 8.35 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 32.8, 33.1, 49.3, 54.9, 55.6, 57.6 (d, J = 5 Hz), 64.3 (d, J = 3 Hz), 74.54 (d, J = 4 Hz), 104.9 (t, J = 24 Hz), 112.0

(dd, J = 21 Hz, 3 Hz), 127.4 (dd, J = 13 Hz, 4 Hz), 130.8 (dd, J = 8 Hz, 5 Hz), 146.6 (d, J = 21 Hz), 151.1, 152.0, 154.4, 160.4 (d, J = 1 Hz), 164.2 (dd, J = 246 Hz, 12 Hz), 160.7 (dd, J = 243 Hz, 12 Hz); m/z: (ESI+) 434.2 ([M+H]+); HRMS: (ESI+) found 434.1902, ([M+H]+) requires 434.1911; Rf : 0.2 (Ethyl acetate); Purity: ≥98%.

2-(2,4-Difluorophenyl)-1-(4-((5-methylpyrimidin-2-yl)amino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***8***

Transparent liquid (51%); [α]D24: 0 (c = 1.5 in methanol); 1H NMR (400 MHz, 25 degree, methanol-d4) σ: 1.23 (2H, t, J = 10 Hz), 1.87 (2H, t, J = 13 Hz), 2.12 (3H, s), 2.62 (1H, tt, J = 9 Hz, 4 Hz), 2.92 (1H, t, J = 12 Hz), 3.08 (1H, d, J = 12 Hz), 3.22 (1H, d, J = 12 Hz), 4.52 (2H, s), 4.65 (1H, d, J = 14 Hz), 4.72 (1H, d, J = 14 Hz), 6.86 (1H, t, J = 9 Hz), 6.94 (1H, t, J = 10 Hz), 7.48 (1H, dd, J = 14 Hz, 8 Hz), 7.78 (1H, s), 8.16 (2H, s), 8.34 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 17.6, 32.9, 33.2, 54.9, 55.6, 57.6 (d, J = 5 Hz), 64.3 (d, J = 4 Hz), 74.5 (d, J = 6 Hz), 104.9 (dd, J = 28 Hz, 26 Hz), 112.0 (dd, J = 21 Hz, 3 Hz), 127.4 (dd, J = 13 Hz, 4 Hz), 130.8 (dd, J = 9 Hz, 6 Hz), 144.9 (d, J = 24 Hz), 146.1, 151.1, 151.9 (d, J = 244 Hz), 157.0 (d, J = 16 Hz), 160.7 (dd, J = 247 Hz, 12 Hz), 159.7 (d, J = 3 Hz), 164.1 (dd, J = 248 Hz, 12 Hz); m/z: (ESI+) 430.2 ([M+H]+); HRMS: (ESI+) found 430.2151, ([M+H]+) requires 430.2161; Rf : 0.4 (9 : 1 Ethyl acetate : MeOH); Purity: ≥98%.

2-(2,4-Difluorophenyl)-1-(4-((4,6-dimethylpyrimidin-2-yl)amino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***9***

Light orange solid (52%); M.P. = 160 – 162 ℃; [α]D24: -11 (c = 0.9 in methanol); 1H NMR (400 MHz, 25 degree, methanol-d4) σ: 1.36 – 1.56 (2H, m), 1.76 – 1.82 (1H, m), 1.86 – 1.93 (1H, m), 2.23 (6H, s), 2.28 (1H, td, J = 11.4 Hz, 3 Hz), 2.44 (1H, td, J = 12 Hz, 3 Hz), 2.53 – 2.59 (1H, m), 2.72 – 2.77 (1H, m), 2.81 (1H, d, J = 14 Hz), 3.02 (1H, dd, J = 14 Hz, 2 Hz), 3.78 (1H, tt, J = 11 Hz, 4 Hz), 4.59 (1H, d, J = 14 Hz), 4.69 (1H, d, J = 14 Hz), 6.37 (1H, s), 6.85 (1H, tdd, J = 8 Hz, 3 Hz, 1 Hz), 6.93 (1H, ddd, J = 12 Hz, 9 Hz, 3 Hz), 7.49 (1H, td, J = 9 Hz, 7 Hz), 7.75 (1H, s), 8.36 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 23.6, 33.1, 33.4, 54.9, 55.6, 57.6 (d, J = 5 Hz), 64.4 (d, J = 4 Hz), 75.6 (d, J = 6 Hz), 104.9 (dd, J = 28 Hz, 26 Hz), 110.3, 112.0 (dd, J = 21 Hz, 3 Hz), 127.4 (dd, J = 13 Hz, 4 Hz), 130.8 (dd, J = 9 Hz, 6 Hz), 146.1, 151.1, 160.7 (dd, J = 246 Hz, 12 Hz), 162.9, 164.2 (dd, J = 247 Hz, 12 Hz), 169.1; m/z: (ESI+) 444.2 ([M+H]+); HRMS: (ESI+) found 444.2311, ([M+H]+) requires 444.2318; Rf : 0.5 (9 : 1 Ethyl acetate : MeOH); Purity: ≥98%.

2-(2,4-Difluorophenyl)-1-(4-((5-fluoro-4-methylpyrimidin-2-yl)amino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***10***

White solid (52%); M.P. = 79 – 130 ℃; [α]D24: 17 (c = 0.6 in methanol); 1H NMR (400 MHz, 25 degree, methanol-d4) σ: 1.38 – 1.56 (2H, m), 1.80 (1H, d, J = 12 Hz), 1.90 (1H, d, J = 12 Hz), 2.26 (1H, t, J = 11 Hz), 2.30 (3H, s), 2.43 (1H, t, J = 11 Hz), 2.58 (1H, d, J = 11 Hz), 2.76 (1H, d, J = 11 Hz), 2.82 (1H, d, J = 13 Hz), 3.03 (1H, d, J = 13 Hz), 3.66 (1H, tt, J = 10 Hz, 4 Hz), 4.60 (1H, d, J = 14 Hz), 4.70 (1H, d, J = 14 Hz), 6.86 (1H, td, J = 7 Hz, 2 Hz), 6.94 (1H, td, J = 9 Hz, 2 Hz), 7.50 (1H, dd, J = 16 Hz, 9 Hz), 7.77 (1H, s), 8.01 (1H, s), 8.37 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 17.6, 32.9, 33.2, 54.9, 55.6, 57.6 (d, J = 5 Hz), 64.3 (d, J = 4 Hz), 74.5 (d, J = 6 Hz), 104.9 (dd, J = 28 Hz, 26 Hz), 112.0 (dd, J = 21 Hz, 3 Hz), 127.4 (dd, J = 13 Hz, 3 Hz),

130.8 (dd, J = 9 Hz, 6 Hz), 145.0 (d, J = 24 Hz), 146.1, 151.1, 151.9 (d, J = 244 Hz), 157.0 (d, J = 15 Hz), 159.7 (d, J = 3 Hz), 160.7 (dd. J = 246 Hz, 12 Hz), 164.2 (dd, J = 248 Hz, 12 Hz); m/z: (ESI+) 448.2 ([M+H]+); HRMS: (ESI+) found 448.2057, ([M+H]+) requires 448.2067; Rf : 0.3 (Ethyl acetate); Purity: ≥97%.

2-(2,4-difluorophenyl)-1-(4-(pyrimidin-5-ylamino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***11***

Light yellowish solid (49%); M.P. = 141 – 198 °C; [α]D26: 17 (c = 0.6 in methanol); 1H NMR (400 MHz, 25 degree, MeOH-d4) σ: 1.38 – 1.50 (2H, m), 1.87 (2H, dt, J = 37 Hz, 2 Hz), 2.31 (1H, td, J = 12 Hz, 4 Hz), 2.45 (1H, td, J = 12 Hz, 3 Hz), 2.62 (1H, d, J =11 Hz), 2.78 (1H, H, J = 12 Hz), 2.83 (1H, d, J = 14 Hz), 3.04 (1H, dd, J = 12 Hz, 3 Hz), 3.29 (1H, tt, J = 12 Hz, 3 Hz), 4.60 (1H, d, J = 10 Hz), 4.69 (1H, d, J = 14 Hz), 6.85 (1H, td, J = 9 Hz, 3 Hz), 6.93 (1H, td, J = 10 Hz, 3 Hz), 7.49 (1H, td, J = 9 Hz, 7 Hz), 7.76 (1H, s), 8.09 (1H, s), 8.32 (1H, s), 8.35 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 32.8, 54.5, 57.6, 64.3, 64.4, 74.7 (d, J = 5 Hz), 104.9 (dd, J = 28 Hz, 21 Hz), 112.0 (dd, J = 21 Hz, 4 Hz), 127.3 (dd, J = 13 Hz, 4 Hz), 130.8 (dd, J = 9 Hz, 6 Hz), 141.4, 143.7, 146.1, 146.8, 151.1, 160.7 (dd, J = 246 Hz, 12 Hz), 164.15 (dd, J = 245 Hz, 12 Hz); m/z: (ESI+) 416.2 ([M+H]+); HRMS: (ESI+) found 416.1994, ([M+H]+) requires 416.2005; Rf : 0.1 (9 : 1 Ethyl acetate : MeOH); Purity: ≥95%.

2-(2,4-difluorophenyl)-1-(4-((2-fluoropyrimidin-5-yl)amino)piperidin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***12***

Light white liquid (54%); [α]D25: 28 (c = 0.4 in methanol); 1H NMR (400 MHz, 25 degree, MeOH-d4) σ: 1.66 – 1.92 (2H, m), 2.17 – 2.34 (1H, m), 2.42 (1H, td, J = 11 Hz, 3 Hz), 2.53 – 2.60 (1H, m), 2.65 – 2.82 (2H, m), 3.02 (1H, dd, J = 14 Hz, 1 Hz), 3.15 (1H, tt, J = 10 Hz, 4 Hz), 3.87 (2H, s), 4.60 (1H, d, J = 7 Hz), 4.66 (1H, d, J = 4 Hz), 6.85 (1H, td, J = 8 Hz, 2 Hz), 6.92 (1H, ddt, J = 12 Hz, 9 Hz, 2 Hz), 7.49 (1H, dddd, J = 12 Hz, 7 Hz, 7 Hz, 3 Hz), 7.75 (1H, d, J = 4 Hz), 7.95 (2H, s), 8.35 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 32.9, 35.1, 35.3, 55.2, 57.6 (d, J = 5 Hz), 64.3 (d, J = 4 Hz), 74.6 (d, J = 6 Hz), 104.9 (dd, J = 28 Hz, 26 Hz), 112.0 (dd, J = 21 Hz, 3 Hz), 127.4 (dd, J = 9 Hz, 4 Hz), 130.8 (dd, J = 10 Hz, 6 Hz), 138.9, 145.1, 146.1, 151.1, 159.5, 160.7 (dd, J = 246 Hz, 12 Hz), 164.13 (dd, J = 248 Hz, 12 Hz); m/z: (ESI+) 446.2; Rf : 0.3 (9 : 1 Ethyl acetate : MeOH); Purity: ≥95%.

2-(2,4-difluorophenyl)-1-(4-(5-fluoropyrimidin-2-yl)piperazin-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***15***

Light yellow solid (67%); M.P. = 140 – 149 °C; [α]D26: 0 (c = 0.8 in methanol); 1H NMR (400 MHz, 25 degree, MeOH-d4) σ: 2.51 (4H, td, J = 4 Hz, 3 Hz), 2.84 (1H, d, J = 14 Hz), 3.02 (dd, J = 14 Hz, 2 Hz), 3.65 (4H, t, J = 5 Hz), 4.65 (1H, d, J = 14 Hz), 4.72 (1H, d, J = 14 Hz), 6.87 (1H, tdd, J = 8 Hz, 3 Hz, 1 Hz), 6.94 (1H, ddd, J = 12 Hz, 9 Hz, 3 Hz), 7.50 (1H, td, J = 9 Hz, 7 Hz), 7.76 (1H, s), 8.23 (2H, d, J = 6 Hz), 8.35 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 45.5, 55.6, 57.5 (d, J = 5 Hz), 64.77 (d, J = 4 Hz), 75.3 (d, J = 6 Hz), 104.9 (dd, J = 28 Hz, 26 Hz), 112.0 (dd, J = 21 Hz, 3 Hz), 127.2 (dd, J = 13 Hz, 4 Hz), 130.9 (dd, J = 10 Hz, 6 Hz), 146.1, 146.3 (d, J = 22 Hz), 151.1, 153.2 (d, J = 247 Hz), 160.2 (d, J = 1 Hz), 160.7 (dd, J = 247 Hz, 12 Hz), 164.2 (dd, J = 248 Hz, 12 Hz); HRMS: (ESI+) found 420.1750, ([M+H]+) requires 420.17542; Rf : 0.4 (Ethyl acetate); Purity: ≥99%.

2-(2,4-Difluorophenyl)-1-(4-((5-fluoropyrimidin-2-yl)amino)-[1,4'-bipiperidin]-1'-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***16***

Yellowish liquid (50%); [α]D27: 8 (c = 2.5 in methanol); 1H NMR (400 MHz, 25 degree, methanol-d4) σ: 1.45 – 1.93 (6H, m), 2.09 – 2.20 (3H, m), 2.35 (1H, td, J = 12 Hz, 2 Hz), 2.55 – 2.69 (4H, m), 2.79 (1H, d, J = 14 Hz), 2.90 (1H, m), 3.00 (1H, dd, J = 14 Hz, 1 Hz), 3.14 – 3.20 (2H, m), 3.81 (1H, tt, 10 Hz, 4 Hz), 4.60 (1H, d, J = 14 Hz), 4.7 (1H, d, J = 14 Hz), 6.86 (1H, ddd, J = 8 Hz, 8 Hz, 2 Hz), 6.93 (1H, ddd, J = 12 Hz, 9 Hz, 3 Hz), 7.49 (1H, ddd, J = 9 Hz, 9 Hz, 7 Hz), 7.76 (1H, s), 8.22 (1H, d, J = 1 Hz), 8.34 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 28.6 (d, J = 30 Hz), 31.5, 55.0, 55.7, 57.5 (d, J = 5 Hz), 63.6, 64.0 (d, J = 4 Hz), 75.0 (d, J = 5 Hz), 79.3, 104.9 (dd, J = 28 Hz, 26 Hz), 112.03 (dd, J = 21 Hz, 3 Hz), 127.3 (dd, J = 12 Hz, 4 Hz), 130.9 (dd, J = 9 Hz, 6 Hz), 146.0, 146.7 (d, J = 22 Hz), 151.1, 153.4 (d, J = 246 Hz), 160.4 (d, J = 1 Hz), 160.6 (dd, J = 246 Hz, 12 Hz), 164.2 (dd, J = 247 Hz, 12 Hz); m/z: (ESI+) 517.2 ([M+H]+); HRMS: (ESI+) found 517.2643, ([M+H]+) requires 517.2646; Rf : 0.4 (9 : 1 : 0.1 Ethyl acetate : MeOH : TEA); Purity: ≥95%.

2-(2,4-difluorophenyl)-1-(8-((5-fluoropyrimidin-2-yl)amino)-2-azaspiro[4.5]decan-2-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***17***

Transparent liquid (38%); [α]D25: 36 (c = 0.6 in methanol); 1H NMR (400 MHz, 25 degree, MeOH-d4) σ: 1.11 – 1.25 (1H, m), 1.31 – 1.39 (3H, m), 1.51 (1H, td, J = 7 Hz, 2 Hz), 1.54 – 1.64 (3H, m), 1.82 – 1.85 (2H, m), 2.27 (1H, dd, J = 14 Hz, 9 Hz), 2.40 (1H, dd, J = 24 Hz, 9 Hz), 2.51 – 2.58 (2H, m), 2.95 (1H, dd, J = 13 Hz, 10 Hz), 3.05 (1H, ddd, J = 23 Hz, 13 Hz, 2 Hz), 3.58 – 3.66 (1H, m), 4.59 (1H, dd, J = 14 Hz, 2 Hz), 4.68 (1H, d, J = 14 Hz), 6.86 (1H, td, J = 8 Hz, 2 Hz), 6.93 (1H, dddd, J = 11 Hz, 9 Hz, 2 Hz, 2 Hz), 7.46 – 7.53 (1H, m), 7.76 (1H, d, J = 3 Hz), 8.18 (2H, s), 8.35 (1H, s); m/z: (ESI+) 488.2 ([M+H]+); HRMS: (ESI+) found 488.2382, ([M+H]+) requires 488.23802; Rf : 0.3 (Ethyl acetate); Purity: ≥96%.

2-(2,4-Difluorophenyl)-1-(8-(5-fluoropyrimidin-2-yl)-2,8-diazaspiro[4.5]decan-2-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***18***

White solid (55%); M.P. = 110 – 112 °C; [α]D24: 31 (c = 0.7 in methanol); 1H NMR (400 MHz, 25 degree, methanol-d4) σ: 1.48 (4H, t, J = 6 Hz), 1.61 (2H, t, J = 7 Hz), 2.37 (1H, d, J = 9 Hz), 2.43 (1H, d, J = 9 Hz), 2.62 (2H, t, J = 7 Hz), 2.98 (1H, d, J = 13 Hz), 3.10 (1H, dd, J = 13 Hz, 2 Hz), 3.58 – 3.70 (4H, m), 6.86 (1H, tdd, J = 8 Hz, 3 Hz, 1 Hz), 6.93 (1H, ddd, J = 12 Hz, 9 Hz, 3 Hz), 7.50 (1H, td, J = 9 Hz, 7 Hz), 7.76 (1H, s), 8.22 (2H, d, J = 1 Hz), 8.35 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 37.4, 37.9 (d, J = 13 Hz), 41.7, 43.3 (d, J = 2 Hz), 55.8, 57.5 (d, J = 5 Hz), 62.8 (d, J = 4 Hz), 67.7, 75.2 (d, J = 6 Hz), 104.8 (dd, J = 28 Hz, 26 Hz), 112.0 (dd, J = 21 Hz, 3 Hz), 127.3 (dd, J = 13 Hz, 4 Hz), 131.1 (dd, J = 9 Hz, 6 Hz), 146.1, 146.2 (d, J = 22 Hz), 151.1, 152.9 (d, J = 246 Hz), 160.3 (d, J = 1 Hz), 160.8 (dd, J = 246 Hz, 12 Hz), 164.3 (dd, J = 247 Hz, 12 Hz); m/z: (ESI+) 474.2 ([M+H]+); HRMS: (ESI+) found 474.2220, ([M+H]+) requires 474.2224; Rf : 0.2 (Ethyl acetate); Purity: ≥95%.

2-(2,4-difluorophenyl)-1-(4-((5-fluoropyrimidin-2-yl)amino)azepan-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***20***

Yellowish liquid (40%); [α]D26: -14 (c = 1.5 in methanol); 1H NMR (400 MHz, 25 degree, MeOH-d4) σ: 1.46 – 1.68 (4H, m), 1.81 – 1.89 (2H, m), 2.54 – 2.76 (4H, m), 2.90 (1H, dd, J = 14 Hz, 10 Hz), 3.23 (1H, ddd, J = 14 Hz, 3 Hz, 2 Hz), 3.93 (1H, dddd, J = 17 Hz, 13 Hz, 9 Hz, 4 Hz), 4.57 (1H, dd, J = 14 Hz, 7 Hz), 4.69 (1H, dd, J = 14 Hz, 1 Hz), 6.84 (1H, td, J = 8 Hz, 3 Hz), 6.89 – 6.96 (1H, m), 7.53 (1H, tdd, J = 9 Hz, 11 Hz, 7 Hz), 7.75 (1H, s), 8.19 (2H, s), 8.37 (1H, d, J = 9 Hz); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 26.1 (d, J = 7 Hz), 34.1 (d, J = 8 Hz), 35.5 (d, J = 35 Hz), 52.3 (d, J = 30 Hz), 54.8 (d, J = 52 Hz), 57.6 (dd, J = 5 Hz, 4 Hz), 58.5 (d, J = 43 Hz), 65.3 (dd, J = 30 Hz, 3 Hz), 74.9 (dd, J = 16 Hz, 6 Hz), 104.9 (dd, J = 8 Hz, 2 Hz), 105.1 (d, J = 8 Hz), 112.0 (dd, J = 21 Hz, 3 Hz), 127.4 (dd, J = 13 Hz, 4 Hz), 131.2 (dd, J = 9 Hz, 6 Hz), 134.5 (d, J = 64 Hz), 140.2 (d, J = 116 Hz), 146.1 (d, J = 7 Hz), 146.6 (d, J = 22 Hz), 151.1 (d, J = 2 Hz), 153.1 (dd, J = 245 Hz, 1 Hz), 160.2 (dd, J = 5 Hz, 1 Hz), 164.2 (dd, J = 249 Hz, 12 Hz); m/z: (ESI+) 448.2 ([M+H]+); HRMS: (ESI+) found 448.2063, ([M+H]+) requires 448.20672; Rf : 0.2 (Ethyl acetate); Purity: ≥98%.

2-(2,4-Difluorophenyl)-1-(4-(5-fluoropyrimidin-2-yl)-1,4-diazepan-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol ***21***

Transparent liquid (52%); [α]D26: 15 (c = 0.7 in methanol); 1H NMR (400 MHz, 25 degree, methanol-d4) σ: 1.64 – 1.71(2H, m), 2.54 – 2.69 (2H, m), 2.79 (2H, td, J = 6 Hz, 1 Hz), 2.88 (1H, d, J = 14 Hz), 3.20 (1H, dd, J = 14 Hz, 2 Hz), 3.62 – 3.76 (4H, m), 4.52 (1H, d, J = 14 Hz), 4.64 (1H, d, J = 14 Hz), 6.83 (1H, tdd, J = 9 Hz, 3 Hz, 1 Hz), 6.90 (1H, ddd, J = 12 Hz, 9 Hz, 3 Hz), 7.42 (1H, td, J = 9 Hz, 7 Hz), 7.75 (1H, s), 8.24 (2H, d, J = 1 Hz), 8.31 (1H, s); 13C NMR (101 MHz, 25 degree, MeOH-d4) σ: 27.9, 47.7, 57.4 (d, J = 5 Hz), 57.4, 57.5, 63.3 (d, J = 4 Hz), 75.1 (d, J = 6 Hz), 104.8 (dd, J = 28 Hz, 26 Hz), 112.0 (dd, J = 21 Hz, 3 Hz), 127.2 (dd, J = 13 Hz, 4 Hz), 131.0 (dd, J = 10 Hz, 6 Hz), 146.0, 146.4 (d, J = 22 Hz), 151.1, 153.0 (d, J = 245 Hz), 159.9 (d, J = 1 Hz), 160.6 (dd, J = 246 Hz, 12 Hz), 164.1 (dd, J = 248 Hz, 12 Hz); m/z: (ESI+) 434.1 ([M+H]+); HRMS: (ESI+) found 434.1907, ([M+H]+) requires 434.1911; Rf : 0.4 (Ethyl acetate); Purity: ≥96%.

Molecular modelling

The protein structures of lanosterol 14-alpha demethylase from *Candida albicans* was obtained from the protein data bank (PDB ID5TZ1) and lanosterol 14-alpha demethylase from *C. auris* (Uniprot ID A0A2H4QC40) was using developed using AlphaFold3. AutoDock SMINA was initially used to identify the preferred binding pockets,[45] and Pymol was used in parallel to visualise all inhibitors or substrates' binding poses. The parameters were kept at default settings. After the locating most favoured binding sites, GOLD was used for molecular docking of the drug molecules into the selected binding sites of target proteins. GOLD was used for final experiments due to its flexible docking and more reliable and precise binding energy and scoring estimation.

MIC susceptibility tests

Microbroth dilution MICs were carried out following EUCAST guidelines. A 96-well plate was filled with 100 µL RPMI with 2% glucose in each well from column 2 to column 12. 200 µL of compound diluted down in media from a DMSO stock was then added to the first column of the 96-well plate and diluted two-fold by each column until column 11. Then, 100 µL of fungi strains from overnight cultures backdiluted to a starting concentration of ~1 x 10^5 CFU/mL was added to each well except a blank control row. The plate was incubated at 37 ℃ for 24 h in the incubator. Fungal growth was measured with a BMG plate reader (FLUOstar Microplate Reader, BMG Labtech) at OD530nm. For azole antifungals, the MIC was defined as the lowest concentration that was able to inhibit ≥ 50% of the drug-free control. DMSO and fluconazole controls were run alongside. All MICs were conducted in triplicate or more until a modal MIC value was obtained, or a range stated if a modal value was not defined.

Galleria mellonella survival test

Wax moth larvae (Galleria mellonella) were kept on wood chips at 14oC in the dark until use. For experiments, it was assumed that each Galleria had a haemolymph volume of 50 µL and can maximum tolerant 10 µL of liquid injection. Therefore, the stock solution of each compound was prepared at 6 times of the concentration they were going to be tested. For each compound at each test concentration, 10 µL of compound stock solution was injected into 10 Galleria via the foremost proleg using a Hamilton syringe. Ten of the control larvae were injected with 10 µL of PSB to control for potential lethal effects from the injection process. After injection, larvae were kept at 37oC inside the petri dishes, and the number of live larvae was recorded every 24 hrs for 5 days. This method was adapted from Wand, et al.[46]

Efficacy assay in *G. mellonella* model

G. mellonella larvae were injected with 10 µL of *C. auris* strain TDG1912 at ~1 x 10^7 CFU/mL into the first left proleg. Then *G. mellonella* were injected with antimicrobial agent/10% DMSO in PBS in the first right proleg 30 minutes after infection. Controls were injected with PBS alone. 10 larvae were treated per condition, per repeat for a total of 30 larvae per condition across 3 independent repeats. *G. mellonella* were stored at 4 ℃, allowed to come to room temperature for at least an hour before the procedure and were used within 2 weeks of the receipt date. *G. mellonella* were assessed for survival every day for 5 days.

Time-kill assay

Overnight cultures of fungal strains were back-diluted to a starting concentration of ~1 x 10^5 CFU/mL into glass universals containing 3 mL of RPMI with 2% glucose media and the drug at the concentration of 4 x MIC_{50}. The glass universals were incubated at 37 °C with shaking at

200 rpm for 24 h. Aliquots (20 µL) were taken out of the glass universals for each tested compound and non-treated (NT) control at six-time points (0, 1, 2, 4, 6 and 24 hours) and Miles-Misra performed to estimate the total number of colony forming units (CFU) per mL. The tests were conducted in triplicate and the average value of log CFU/mL was reported. Along with the synthesised compounds, commercially available antifungal drugs were also tested for comparison. Tested compounds were defined as fungicidal if the loss of fungal population is more than a 3-log reduction in CFU/mL compared to time point 0 h.

ASSOCIATED CONTENT

Supporting information.

Supporting information are available containing:

- LC-Ms method, NMR spectra and HRMS (PDF)

-Molecular formula strings (CSV)

AUTHOR INFORMATION

Corresponding author.

For KMR: e-mail k.miraz.rahman@kcl.ac.uk, phone +44 (0)207 848 1891

For JMS: e-mail mark.sutton@phe.gov.uk, phone +44 (0)198 061 2649

ORCID

Khondaker M. Rahman: 0000-0001-8566-8648

Notes

The authors declare no competing financial interest.

Acknowledgements:

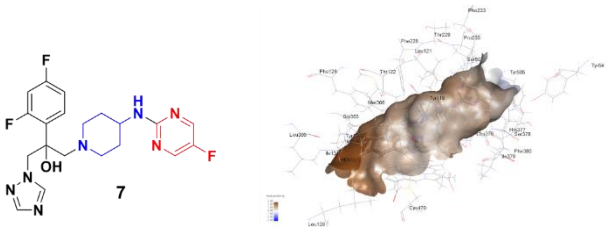
Funding was received from China Schoilarship Council (CSC) and a Medical Research Council Confidence in Concept grant (award code MC_PC_13065).

ABBREVIATIONS USED

AcOH, Acetic acid; CDC, Centers for Disease Control and Prevention; calLDM, lanosterol 14α-demethylase of *C. albicans*; carLDM, lanosterol 14α-demethylase of *C. auris*; CLSI, Clinical and Laboratory Standards Institute; DCM, Dichloromethane; DIPEA, N, N-Diisopropylethylamine; DMA, Dimethylacetamide; DMSO, Dimethylsulfoxide; DMF, N,N-

Dimethylformamide; EtN(Pr-i)$_2$, N, N-Diisopropylethylamine; ERB, Efflux resistant breaker; EtOH, Ethanol; Equiv., Equivalent; EUCAST, European Committee on Antimicrobial Susceptibility Testing; Et_3N, Triethylamine; ESI, Electrospray Ionization; Fluc, Fluconazole; HCl, Hydrochloric Acid; Hz, Hertz; HPLC, High pressure liquid chromatography; J, Coupling constants; KO*t*Bu, Potassium tert-butoxide; K_2CO_3, Potassium carbonate; LDM, lanosterol 14α-demethylase; LogP, Lipophilicity; LC-MS, Liquid chromatography-mass spectroscopy; MIC, Minimum Inhibitory Concentration; MeOH, Methanol; MeCN, Acetonitrile; $MgSO_4$, Magnesium sulfate; m/z, mass-to-charge ratio peaks; $NaBH(OAc)_3$, Sodium triacetoxyborohydride; NaO*t*Bu, Sodium tert-butoxide; NaOH, Sodium hydroxide; $Na(OAc)_3BH$, Sodium triacetoxyborohydride; NMR, Nuclear magnetic resonance; ORD, optical rotatory dispersion; $Pd_2(dba)_3$, Tris(dibenzylideneacetone)dipalladium (0); PBS, Phosphate-buffered saline; ppm, parts per million; rac-BINAP, 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl; Rf, retention factors; SAR, Structure Activity Relationship; SNAr, Nucleophilic aromatic substitution; TEA, Triethylamine; TLC, Thin layer chromatography; UV, Ultraviolet; Vori, voriconazole; WHO, World Health Organization; ΔG, Delta G.

Graphical abstract



References

(1) CDC. *Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED), U.S. Department of Health & Human Services*. https://www.cdc.gov/fungal/antifungal-resistance.html (accessed 2022 10 November).

(2) Perlin, D. S.; Rautemaa-Richardson, R.; Alastruey-Izquierdo, A. The global problem of antifungal resistance: prevalence, mechanisms, and management. *Lancet Infect Dis* **2017**, *17* (12), e383-e392. DOI: 10.1016/S1473-3099(17)30316-X From NLM Medline.

(3) Pappas, P. G.; Rex, J. H.; Sobel, J. D.; Filler, S. G.; Dismukes, W. E.; Walsh, T. J.; Edwards, J. E.; Infectious Diseases Society of, A. Guidelines for treatment of candidiasis. *Clin Infect Dis* **2004**, *38* (2), 161-189. DOI: 10.1086/380796 From NLM Medline.

(4) Jenks, J. D.; Hoenigl, M. Treatment of Aspergillosis. *J Fungi (Basel)* **2018**, *4* (3). DOI: 10.3390/jof4030098 From NLM PubMed-not-MEDLINE.

(5) Spadari, C. C.; Wirth, F.; Lopes, L. B.; Ishida, K. New Approaches for Cryptococcosis Treatment. *Microorganisms* **2020**, *8* (4). DOI: 10.3390/microorganisms8040613 From NLM PubMed-not-MEDLINE.

(6) Hendrickson, J. A.; Hu, C.; Aitken, S. L.; Beyda, N. Antifungal Resistance: a Concerning Trend for the Present and Future. *Curr Infect Dis Rep* **2019**, *21* (12), 47. DOI: 10.1007/s11908-019-0702-9 From NLM PubMed-not-MEDLINE.

(7) Stevenson, E. M.; Gaze, W. H.; Gow, N. A. R.; Hart, A.; Schmidt, W.; Usher, J.; Warris, A.; Wilkinson, H.; Murray, A. K. Antifungal Exposure and Resistance Development: Defining Minimal Selective Antifungal Concentrations and Testing Methodologies. *Front Fungal Biol* **2022**, *3*, 918717. DOI: 10.3389/ffunb.2022.918717 From NLM PubMed-not-MEDLINE.

(8) Morace, G.; Perdoni, F.; Borghi, E. Antifungal drug resistance in Candida species. *J Glob Antimicrob Resist* **2014**, *2* (4), 254-259. DOI: 10.1016/j.jgar.2014.09.002 From NLM PubMed-not-MEDLINE.

(9) Whaley, S. G.; Berkow, E. L.; Rybak, J. M.; Nishimoto, A. T.; Barker, K. S.; Rogers, P. D. Azole Antifungal Resistance in Candida albicans and Emerging Non-albicans Candida Species. *Front Microbiol* **2016**, *7*, 2173. DOI: 10.3389/fmicb.2016.02173 From NLM PubMed-not-MEDLINE.

(10) Goncalves, S. S.; Souza, A. C. R.; Chowdhary, A.; Meis, J. F.; Colombo, A. L. Epidemiology and molecular mechanisms of antifungal resistance in Candida and Aspergillus. *Mycoses* **2016**, *59* (4), 198-219. DOI: 10.1111/myc.12469 From NLM Medline.

(11) Chamilos, G.; Kontoyiannis, D. P. Update on antifungal drug resistance mechanisms of Aspergillus fumigatus. *Drug Resist Updat* **2005**, *8* (6), 344-358. DOI: 10.1016/j.drup.2006.01.001 From NLM Medline.

(12) Ostrowsky, B.; Greenko, J.; Adams, E.; Quinn, M.; O'Brien, B.; Chaturvedi, V.; Berkow, E.; Vallabhaneni, S.; Forsberg, K.; Chaturvedi, S.; et al. Candida auris Isolates Resistant to Three Classes of Antifungal Medications - New York, 2019. *MMWR Morb Mortal Wkly Rep* **2020**, *69* (1), 6-9. DOI: 10.15585/mmwr.mm6901a2 From NLM.

(13) Chakrabarti, A.; Sood, P. On the emergence, spread and resistance of Candida auris: host, pathogen and environmental tipping points. *J Med Microbiol* **2021**, *70* (3). DOI: 10.1099/jmm.0.001318 From NLM Medline.

(14) Osei Sekyere, J. Candida auris: A systematic review and meta-analysis of current updates on an emerging multidrug-resistant pathogen. *Microbiologyopen* **2018**, *7* (4), e00578.

(15) Cortegiani, A.; Misseri, G.; Fasciana, T.; Giammanco, A.; Giarratano, A.; Chowdhary, A. Epidemiology, clinical characteristics, resistance, and treatment of infections by Candida auris. *J Intensive Care* **2018**, *6*, 69. DOI: 10.1186/s40560-018-0342-4 From NLM PubMed-not-MEDLINE.
(16) Chowdhary, A.; Sharma, C.; Meis, J. F. Candida auris: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. *Plos Pathog* **2017**, *13* (5), e1006290. DOI: 10.1371/journal.ppat.1006290 From NLM Medline.
(17) Rhodes, J.; Fisher, M. C. Global epidemiology of emerging Candida auris. *Curr Opin Microbiol* **2019**, *52*, 84-89. DOI: 10.1016/j.mib.2019.05.008 From NLM Medline.
(18) Lone, S. A.; Ahmad, A. Candida auris-the growing menace to global health. *Mycoses* **2019**, *62* (8), 620-637. DOI: 10.1111/myc.12904 From NLM Medline.
(19) Mishra, S. K.; Yasir, M.; Willcox, M. Candida auris: an emerging antimicrobial-resistant organism with the highest level of concern. *The Lancet Microbe* **2023**, *4* (7), e482-e483. DOI: 10.1016/S2666-5247(23)00114-3 (acccessed 2023/11/09).
(20) Maphanga, T. G.; Mpembe, R. S.; Naicker, S. D.; Govender, N. P. In Vitro Antifungal Activity of Manogepix and Other Antifungal Agents against South African Candida auris Isolates from Bloodstream Infections. *Microbiol Spectr* **2022**, *10* (1), e0171721. DOI: 10.1128/spectrum.01717-21 From NLM.
(21) Simon, S. P.; Li, R.; Silver, M.; Andrade, J.; Tharian, B.; Fu, L.; Villanueva, D.; Abascal, D. G.; Mayer, A.; Truong, J.; et al. Comparative Outcomes of Candida auris Bloodstream Infections: A Multicenter Retrospective Case-Control Study. *Clin Infect Dis* **2023**, *76* (3), e1436-e1443. DOI: 10.1093/cid/ciac735 From NLM Medline.
(22) CDC. *Antimicrobial Resistance in Candida*. Centers for Disease Control and Prevention, U.S. Department of Health & Human Services, 2020.
https://www.cdc.gov/fungal/diseases/candidiasis/antifungal-resistant.html#ref-1 (accessed.
(23) Toda, M.; Williams, S. R.; Berkow, E. L.; Farley, M. M.; Harrison, L. H.; Bonner, L.; Marceaux, K. M.; Hollick, R.; Zhang, A. Y.; Schaffner, W.; et al. Population-Based Active Surveillance for Culture-Confirmed Candidemia - Four Sites, United States, 2012-2016. *MMWR Surveill Summ* **2019**, *68* (8), 1-15. DOI: 10.15585/mmwr.ss6808a1 From NLM Medline.
(24) CDC. Antibiotic Resistance Threats in the United States. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services: Atlanta, GA, 2019.
(25) Chen, S. C. A.; Sorrell, T. C. Antifungal agents. *Medical Journal of Australia* **2007**, *187* (7), 404-409. DOI: https://doi.org/10.5694/j.1326-5377.2007.tb01313.x.
(26) Wu, J. J.; Pang, K. R.; Huang, D. B.; Tyring, S. K. Therapy of systemic fungal infections. *Dermatol Ther* **2004**, *17* (6), 532-538. DOI: 10.1111/j.1396-0296.2004.04057.x From NLM Medline.
(27) Maertens, J. A. History of the development of azole derivatives. *Clin Microbiol Infect* **2004**, *10 Suppl 1*, 1-10. DOI: 10.1111/j.1470-9465.2004.00841.x From NLM Medline.
(28) Shafiei, M.; Peyton, L.; Hashemzadeh, M.; Foroumadi, A. History of the development of antifungal azoles: A review on structures, SAR, and mechanism of action. *Bioorganic Chemistry* **2020**, *104*, 104240. DOI: https://doi.org/10.1016/j.bioorg.2020.104240.
(29) Marichal, P.; Bossche, H. Mechanisms of resistance to azole antifungals. *Acta Biochimica Polonica* **1995**, *42* (4), 509-516.
(30) Lamping, E.; Baret, P. V.; Holmes, A. R.; Monk, B. C.; Goffeau, A.; Cannon, R. D. Fungal PDR transporters: Phylogeny, topology, motifs and function. *Fungal Genetics and Biology* **2010**, *47* (2), 127-142. DOI: https://doi.org/10.1016/j.fgb.2009.10.007.

(31) Kean, R.; Delaney, C.; Sherry, L.; Borman, A.; Johnson, E. M.; Richardson, M. D.; Rautemaa-Richardson, R.; Williams, C.; Ramage, G. Transcriptome Assembly and Profiling of Candida auris Reveals Novel Insights into Biofilm-Mediated Resistance. *mSphere* **2018**, *3* (4). DOI: 10.1128/mSphere.00334-18 From NLM Medline.
(32) Tobudic, S.; Kratzer, C.; Presterl, E. Azole-resistant Candida spp.–emerging pathogens? *Mycoses* **2012**, *55*, 24-32.
(33) Morais Vasconcelos Oliveira, J.; Conceição Oliver, J.; Latércia Tranches Dias, A.; Barbosa Padovan, A. C.; Siqueira Caixeta, E.; Caixeta Franco Ariosa, M. Detection of ERG11 Overexpression in Candida albicans isolates from environmental sources and clinical isolates treated with inhibitory and subinhibitory concentrations of fluconazole. *Mycoses* **2021**, *64* (2), 220-227. DOI: https://doi.org/10.1111/myc.13208.
(34) Henry, K. W.; Nickels, J. T.; Edlind, T. D. Upregulation of ERG genes in Candida species by azoles and other sterol biosynthesis inhibitors. *Antimicrobial agents and chemotherapy* **2000**, *44* (10), 2693-2700.
(35) Nett, J. E.; Crawford, K.; Marchillo, K.; Andes, D. R. Role of Fks1p and Matrix Glucan in Candida albicans Biofilm Resistance to an Echinocandin, Pyrimidine, and Polyene. *Antimicrobial Agents and Chemotherapy* **2010**, *54* (8), 3505-3508. DOI: doi:10.1128/aac.00227-10.
(36) Bruzual, I.; Riggle, P.; Hadley, S.; Kumamoto, C. A. Biofilm formation by fluconazole-resistant Candida albicans strains is inhibited by fluconazole. *Journal of Antimicrobial Chemotherapy* **2007**, *59* (3), 441-450. DOI: 10.1093/jac/dkl521 (acccessed 11/10/2023).
(37) Williamson, B.; Wilk, A.; Guerrero, K. D.; Mikulski, T. D.; Elias, T. N.; Sawh, I.; Cancino-Prado, G.; Gardam, D.; Heath, C. H.; Govender, N. P.; et al. Impact of Erg11 Amino Acid Substitutions Identified in Candida auris Clade III Isolates on Triazole Drug Susceptibility. *Antimicrobial Agents and Chemotherapy* **2022**, *66* (1), e01624-01621. DOI: doi:10.1128/AAC.01624-21.
(38) Xiang, M. J.; Liu, J. Y.; Ni, P. H.; Wang, S.; Shi, C.; Wei, B.; Ni, Y. X.; Ge, H. L. Erg11 mutations associated with azole resistance in clinical isolates of Candida albicans. *FEMS Yeast Res* **2013**, *13* (4), 386-393. DOI: 10.1111/1567-1364.12042 From NLM Medline.
(39) Sagatova, A. A.; Keniya, M. V.; Wilson, R. K.; Monk, B. C.; Tyndall, J. D. Structural insights into binding of the antifungal drug fluconazole to Saccharomyces cerevisiae lanosterol 14α-demethylase. *Antimicrobial agents and chemotherapy* **2015**, *59* (8), 4982-4989.
(40) Emami, S.; Ghobadi, E.; Saednia, S.; Hashemi, S. M. Current advances of triazole alcohols derived from fluconazole: Design, in vitro and in silico studies. *European Journal of Medicinal Chemistry* **2019**, *170*, 173-194. DOI: https://doi.org/10.1016/j.ejmech.2019.03.020.
(41) Salehi, F.; Emami, L.; Rezaei, Z.; Khabnadideh, S.; Tajik, B.; Sabet, R. Fluconazole-Like Compounds as Potential Antifungal Agents: QSAR, Molecular Docking, and Molecular Dynamics Simulation. *Journal of Chemistry* **2022**, *2022*, 5031577. DOI: 10.1155/2022/5031577.
(42) Thamban Chandrika, N.; Shrestha, S. K.; Ngo, H. X.; Tsodikov, O. V.; Howard, K. C.; Garneau-Tsodikova, S. Alkylated Piperazines and Piperazine-Azole Hybrids as Antifungal Agents. *Journal of Medicinal Chemistry* **2018**, *61* (1), 158-173. DOI: 10.1021/acs.jmedchem.7b01138.
(43) Lebouvier, N.; Giraud, F.; Corbin, T.; Na, Y. M.; Le Baut, G.; Marchand, P.; Le Borgne, M. Efficient microwave-assisted synthesis of 1-(1H-indol-1-yl)-2-phenyl-3-(1H-1,2,4-triazol-1-yl)propan-2-ols as antifungal agents. *Tetrahedron Letters* **2006**, *47* (36), 6479-6483. DOI: https://doi.org/10.1016/j.tetlet.2006.03.199.

(44) Singkum, P.; Suwanmanee, S.; Pumeesat, P.; Luplertlop, N. A powerful in vivo alternative model in scientific research: Galleria mellonella. *Acta Microbiol Immunol Hung* **2019**, *66* (1), 31-55. DOI: 10.1556/030.66.2019.001 From NLM Medline.
(45) Koes, D. R.; Baumgartner, M. P.; Camacho, C. J. Lessons Learned in Empirical Scoring with smina from the CSAR 2011 Benchmarking Exercise. *Journal of Chemical Information and Modeling* **2013**, *53* (8), 1893-1904. DOI: 10.1021/ci300604z.
(46) Wand, M. E.; Muller, C. M.; Titball, R. W.; Michell, S. L. Macrophage and Galleria mellonella infection models reflect the virulence of naturally occurring isolates of B. pseudomallei, B. thailandensis and B. oklahomensis. *Bmc Microbiology* **2011**, *11*. DOI: 10.1186/1471-2180-11-11.