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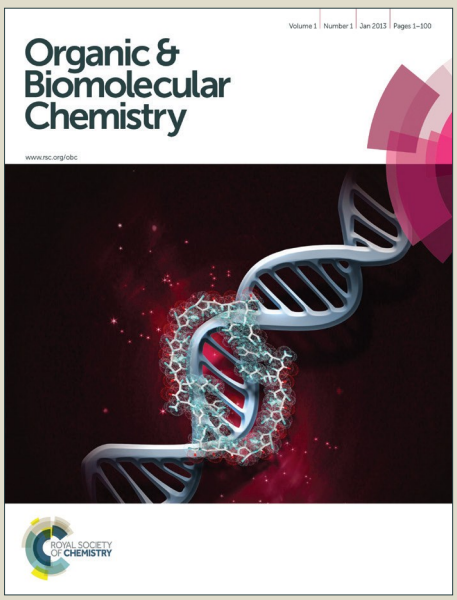

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Journal Name

ARTICLE

Design and Synthesis of new fluconazole analogues

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Abstract: We have synthesized new fluconazole analogues containing two different 1,2,3-triazole units in the side chain. Synthesis of new amide analogues by using a variety of acids is also described. All the compounds showed very good antifungal activity. Hemolysis study of the most active compounds **6e** and **13j** showed that both compounds did not cause any hemolysis at the dilutions tested. These compounds did not exhibit any toxicity to L929 cells at MIC and lower concentrations. In docking study, overall binding mode of **6e** and **13j** appeared reasonable and provided a good insight into the structural basis of inhibition of *Candida albicans* Cyp51 by these compounds.

Introduction

Invasive fungal disease continues to be a problem associated with significant morbidity and high mortality in immune compromised and, to a lesser extent, immune competent individuals[1,2]. In many cases, it is not the AIDS or cancer itself but the mycoses that are lethal to these patients. Serious fungal infections are caused mostly by *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigates*[3,4]. The common antifungal agents currently used in clinic are polyenes, allylamines, azoles and echinocandins[5]. The current antifungal drugs like amphotericin B are highly toxic. Some drugs such as flucytosine and azoles are becoming ineffective due to appearance of resistant strains.

Triazole antifungals have emerged as front-line drugs for the treatment and prophylaxis of many systemic mycoses because of their broad antifungal spectrum, high potency and low toxicity against most yeasts and fungi.[6] A large number of triazole compounds as clinical drugs or candidates have been frequently employed for the treatment of various types of diseases, which have shown their large development value and wide potential as medicinal agents. There are several reports on current developments of azole-based antifungal drugs.[7] Unfortunately, the excessive use of azoles has led to development of severe resistance, which significantly reduced the efficacy of them.[8,9] Several new

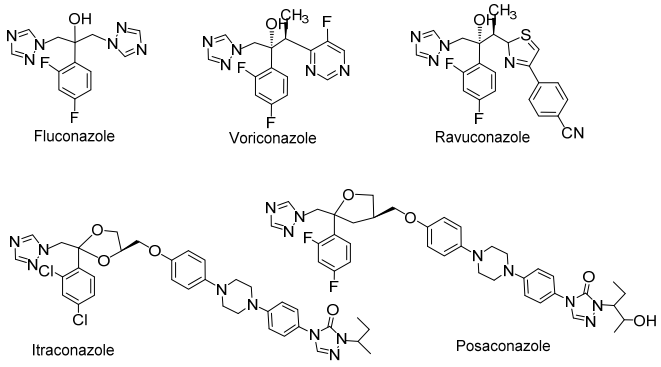

Figure 1- Presently marketed Azole antifungals containing 1,2,4-triazole

triazoles such as voriconazole, posaconazole, ravuconazole, albaconazole etc. containing 1,2,4-triazole and difluorobenzene moieties are marketed or in the late stages of clinical trials (Figure 1)[10].

Fluconazole, α-(2,4-difluorophenyl)-α-(1H-1,2,4 triazol-1-yl-methyl)-1H-1,2,4 triazol-1-ethanol (Figure 1) plays an excellent role in prophylaxis, empirical therapy, and the treatment of both superficial and invasive yeast fungal infections. It is a potent inhibitor of the cytochrome P450 (CYP)-mediated metabolism of the antiepileptic agent phenytion, a well-known human and animal tetratogen[11]. Fluconazole is well absorbed and exhibit high oral bioavailability. It is an antifungal agent of choice for the treatment of infections by *Candida albicans* and *Cryptococcus neoformans* due to its potent activity, excellent safety profile, and favourable pharmacokinetic characteristics[12]. However fluconazole is not fungicidal and is ineffective against invasive aspergillosis. Extensive use of fluconazole has increased the number of fluconazole-resistant *C. albicans* isolates[13]. Therefore, toxicity concerns, limited

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Electronic Supplementary Information (ESI) available: ^{1}H and ^{13}C NMR of all the compounds. See DOI: 10.1039/x0xx00000x

antifungal spectrum, development of resistant strains and observed nephrotoxicity to fluconazole have created a need to modify its activity. Several reports on the synthesis and biological activity of structurally modified new analogues of fluconazole are available in the literature[14].

The cytochrome P450 (CYP) enzymes play an essential role in the metabolism of xenobiotics by catalyzing the monooxygenation of a broad diversity of substrates. Some nitrogen-containing heteroaromatic xenobiotics such as pyridine, imidazole, and triazole derivatives are known to inhibit CYP enzymes by direct coordination with the heme iron (type II ligands).[15] Based on the structure of the active site of CYP51 and the extensive investigation of the structure activity relationships of azole antifungals, we designed and synthesized a novel series of 1-(1H-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-(substituted or unsubstituted 1,2,3-triazol-1yl)-propan-2-ols containing a 1,2,4-triazole ring, a difluorobenzene group and a 1,2,3-triazole ring having long side chain or bile acid. Some of these molecules showed much better activity than fluconazol and amphotericin B and were found to be less toxic at lower dose (0.001mg/mL) to human cancer cells Hep3B and A431.[14f,16]

Results and Discussion

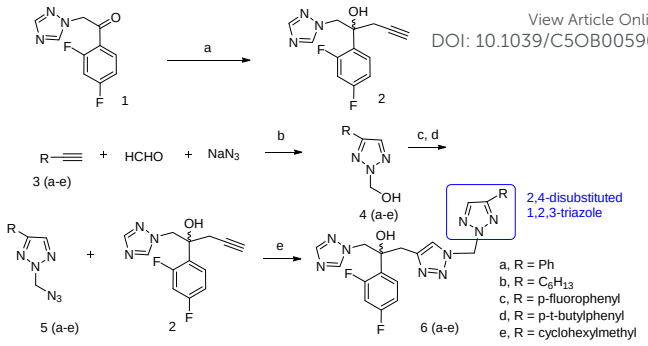
Though the 1,2,4-triazole ring, the difluorophenyl group, and the hydroxyl group are the pharmacophores of antifungal agents,[17] the side chain also plays an important role and the optimization of the side chain attached to the pharmacophore remains attractive to the current research.[18] Application of the isosterism concept for the development of new compounds with therapeutic potential leads to significant advances in molecular diversity and allows covering chemical space in the important areas of medicinal chemistry. It is reported that triazoles and amide group are isosteres.[19] It is found that the antifungal activity of fluconazole can be enhanced by replacing one of the 1,2,4-triazole ring of fluconazole by isosteres 1,2,3-triazole, thiocarbamate, 1,2,4-triazolone and by incorporating amide group or 1,2,3-triazole in the side chain.[14,18]

Chemistry:

In continuation of our work on fluconazole analogues[20] we designed and synthesized new fluconazole analogues featuring complex side chain having one 1,4-disubstituted 1,2,3-triazole ring and the one 2,4-disubstituted 1,2,3-triazole ring as shown in Schemes 1 and 2.

Synthesis of fluconazole analogues in which 2, 4-disubstituted 1,2,3-triazole ring is in side chain component:

Ketone **1**[18d] was reacted with propargyl bromide in the presence of zinc to get acetylenic compound **2** as per our earlier reported[16] procedure (**Scheme 1**). One pot reaction of alkynes **3** with formaldehyde solution and sodium azide in glacial AcOH/ 1,4-dioxane[21] afforded alcohols **4**. Mesylation of alcohols **4** followed by reaction with sodium azide gave azides **5**. Click reaction of azido compounds **5** with alkyne **2** under microwave irradiation afforded 1,4 and 2,4-disubstituted 1,2,3-triazole containing compounds **6**. All



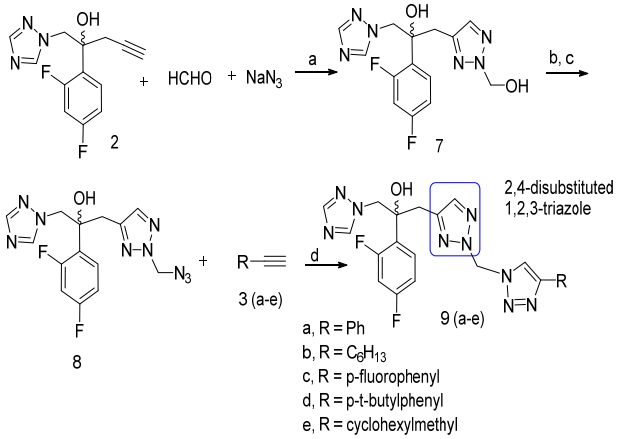
Reagents and conditions: (a) Zn, propargyl bromide, DMF/THF, 25 °C, 5 h, 95%; (b) i) AcOH, 1,4-dioxane; ii) $CuSO_4 \cdot 5H_2O$ (5 mol%), Sodium ascorbate (40 mol%), 16-18h, (R= Ph 92%, R= C_6H_{13} 84%, R = *p*-fluorophenyl 87%, R = *p*-*t*-butylphenyl 83%, R = cyclohexylmethyl 81%); (c) Triethyl amine, Mesyl chloride, dry DCM, 82-97%; (d) Sodium azide, DMF, 65 °C, (87-94%); (e) $CuSO_4 \cdot 5H_2O$ (5 mol%), Sodium ascorbate (40 mol%), DMF:H_2O, (4:1), Microwave (245 W), 10 min., (53-58%).

Scheme 1: Synthesis of compounds **6 (a-e)**

the compounds in Scheme 1 were fully characterized by spectroscopic method and tested for antifungal activity. The results of biological activity of these compounds are summarized in Table 1.

Synthesis of fluconazole analogues in which 2, 4-disubstituted 1,2,3-triazole ring is in fluconazole component:

By using alkyne **2** we synthesized azido compound **8** using similar reaction protocol and click reaction of compound **8** with different alkynes **3** under microwave conditions afforded compounds **9** which are new analogues of fluconazole containing 1,4 and 2,4-disubstituted-1,2,3-triazole units in the side chain (**Scheme 2**).



Reagents and conditions: (a) i) AcOH, 1,4-dioxane; ii) $CuSO_4 \cdot 5H_2O$ (5 mol%), Sodium ascorbate (40 mol%), 18h, 83%); (b) Triethyl amine, Mesyl chloride, dry DCM, (82-99%); (c) Sodium azide, DMF, 65 ºC, 96%; (d) $CuSO_4 \cdot 5H_2O$ (5 mol%), Sodium ascorbate (40 mol%), DMF:H_2O, (4:1), Microwave (245 W), 10 min., (66-75%).

Scheme 2: Synthesis of compounds **9 (a-e)**

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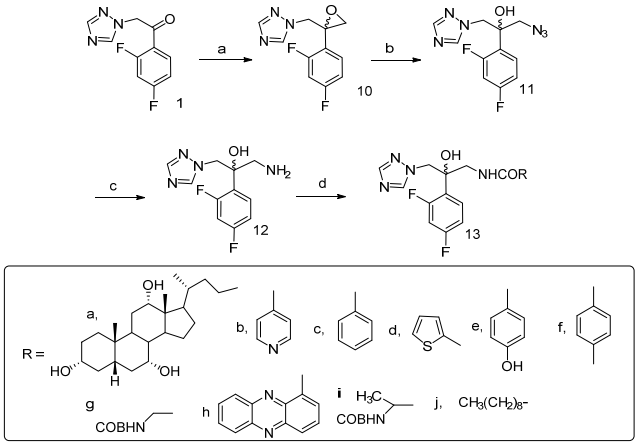
Table 1. *In Vitro* antifungal activity of test compounds **4**-**9** and standard antifungal agents

Comp.No.	Minimum inhibitory concentration (MIC) µg/mL					
	Ca	Cn	Ss	Tm	Af	Cp
4a	>50	>50	50	50	>50	>50
4b	>50	50	>50	>50	>50	>50
4c	50	50	50	50	>50	>50
4d	50	50	50	25	>50	>50
4e	>50	50	50	50	>50	>50
5a	>50	>50	50	50	>50	>50
5b	>50	50	>50	>50	>50	>50
5c	>50	50	50	50	>50	>50
5d	>50	50	50	25	>50	>50
5e	>50	50	50	50	>50	>50
6a	**0.19**	**0.012**	**1.56**	3.12	12.5	**0.19**
6b	**0.012**	**0.00038**	**0.09**	**0.39**	**1.56**	**0.024**
6c	**0.39**	**0.012**	**0.39**	1.56	12.5	**0.09**
6d	FP	FP	50	0.39	**1.56**	FP
6e	**0.09**	**0.00015**	**0.19**	6.25	12.5	**0.003**
7	6.25	**0.39**	12.5	50	>50	**1.56**
8	**0.39**	**0.19**	3.12	50	50	**0.19**
9a	**0.78**	**0.39**	50	50	>50	**0.78**
9b	**0.39**	**0.19**	6.25	50	>50	**0.19**
9c	**1.56**	**0.39**	25	50	>50	**0.39**
9d	**0.39**	**0.39**	6.25	50	>50	**0.09**
9e	**0.39**	**0.39**	3.12	50	>50	**0.39**
Fluconazole	0.5	1.0	2.0	1.0	2.0	1.0
Amphotericin B	0.12	0.06	0.12	0.12	0.5	0.12

Ca = *Candida albicans*; Cn = *Cryptococcus neoformans*; Ss = *Sporothrix schenckii*; Tm = *Trichophyton mentagrophytes*; Af = *Aspergillus fumigatus*; Cp = *Candida parapsilosis* (ATCC-22019)

Synthesis of amide analogues of fluconazole:



Reagents and conditions: (a) Trimethylsulfoxonium iodide, NaH, DMSO/THF, 25 °C, 2 h, 91%; (b) NaN_3, DMF, 60-65 °C, 12 h, 75%; (c) H_2/Pd-C, methanol, 25 °C, 16 h, 94%; (d) acids, EDC.HCl, HOBt, Dry DMF, 0-25 °C, 10 h, (Cholic acid 92%, Isonicotinic acid 94%, Benzoic acid 94%,[22] Thiophene-2- carboxylic acid 96%, *p*-hydroxybenzoic acid 93%, Terphthalic acid 71%, N-boc Glycine 93%, Phenazine carboxylic acid 96%, N- boc alanine 97%, Decanoic acid 93%).

Scheme 3: Synthesis of compounds **13 (a-j)**

Racemic intermediate azide **11** was synthesized from ketone **1** by our earlier reported[14f] procedure through epoxide **10** as shown in **Schemes 3**. Reduction of Azide **11** by using hydrogen on Pd/C to amine **12** and its coupling with different types of acids (**Schemes 3**) afforded a variety of amides **13**. These amides were fully characterized by spectroscopic analysis and were tested for their antifungal activity. The results of biological activity are summarized in Table 2.

Biology:

***In Vitro* antifungal activity of compounds 4-9 and compound 13:**

All the newly synthesized compounds were tested for their antifungal activity. The results of biological activity are summarized in Tables 1 and 2.

The biological activity results in Table 1 show that intermediate compounds **4** and **5** were inactive against all the tested fungal strains.

Most of the other compounds **6** to **9** were active against *C. albicans*, *C. neoformans* and *C. parapsilosis (ATCC-22019)* than

Table 2: *In Vitro* antifungal activity of compounds **13a- 13j** and standard antifungal agents

Comp. No.	Minimum inhibitory concentration (µg/mL)					
	Ca	Cn	Ss	Tm	Af	Cp
13a	**3.12**	**1.56**	50	>50	>50	6.25
13b	**1.56**	**0.78**	50	50	>50	3.12
13c[a]	**0.09**	**0.39**	**3.12**	**3.12**	>50	0.19
13d	**0.09**	**0.39**	**6.25**	12.5	>50	0.39
13e	**0.225**	**6.25**	50	50	>50	0.19
13f	25	**6.25**	50	>50	>50	>50
13g	12.5	**6.25**	50	>50	>50	>50
13h	**3.12**	**1.56**	50	50	>50	>50
13i	**6.25**	**3.12**	50	50	>50	50
13j	**0.007**	**0.00001**	**0.78**	**0.19**	**12.5**	**0.39**
Fluconazole	0.5	1.0	2.0	1.0	2.0	1.0
AmphotericinB	0.12	0.06	0.12	0.12	0.5	0.12

Ca = *Candida albicans*; Cn = *Cryptococcus neoformans*; Ss = *Sporothrix schenckii*; Tm = *Trichophyton mentagrophytes*; Af = *Aspergillus fumigatus*; Cp = *Candida parapsilosis* (ATCC-22019)
[a] Known compound[22]

fluconazole. Some compounds were found to be more active than amphotericin B also. Specifically compound **6b** (MIC 0.00038 µg/mL for *C. Neoformans*) and **6e** (MIC 0.00015 µg/mL for *C. Neoformans*) showed very good antifungal activity and compound **6e** was found to show highest activity against *Cryptococcus neoformans* (MIC 0.00015 µg/mL) and *Candida parapsilosis (ATCC*-22019) (MIC 0.003 µg/mL) and hence hemolysis and docking study was carried out on this compound.

The biological activity results in Table 2 show that all the amides **13** (**a-j**) are active against *C. albicans* and *C. neoformans*. Compounds **13c** and **13d** showed good activity against all the strains except *A. fumigatus*. Some compounds showed better activity than standard drugs fluconazole and amphotericin B. Compound **13j** showed highest activity against all the strains, particularly against *C. albicans* and *C. neoformans* (70 fold more active than fluconazole and 17 fold more active than amphotericin B against *C. albicans* while highly active than fluconazole and amphotericin B against *C. neoformans*) and hence hemolysis and docking study was carried out on this compound.

Among all the compounds tested for the antifungal activity, compounds **6a**, **6b**, **6c**, **6e** and **13j** were found to exhibit the best activity particularly against yeast like fungi. These compounds were tested for their toxicity using mouse fibroblast cell line L929.[23] Morphological anomalies in L929 cells exposed to compounds **6a**, **6b**, **6c**, **6e** and **13j** (25 µg/mL) were evident under phase contrast microscope. L929 cells in control were fairly transparent and attached to the surface of the wells of tissue culture plate (**Figure 2A**). The compounds did not exhibit any toxicity to L929 cells at MIC and lower concentrations, as was evident from their normal morphology (**Figure 2B**) however when exposed to higher concentrations (50 and 25 µg/mL), the L929 cells lost their normal morphology (**Figure 2C**) whereas the MTT assay revealed >90% viability of L929 cells even at higher concentrations.

Among all the compounds **6e** and **13j** were found to be most active and may be treated as lead molecules and hence hemolysis and docking study of these molecules was carried out.

Toxicity Study:

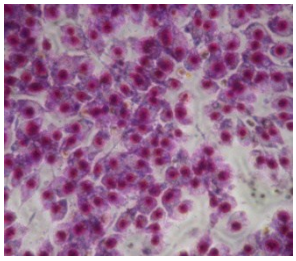


Figure 2A

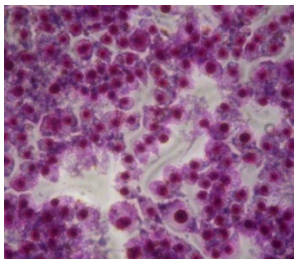


Figure 2B

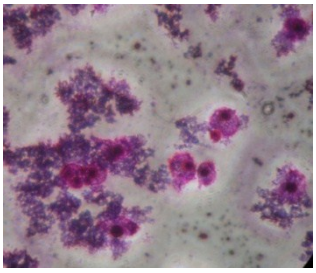


Figure 2C

The cytotoxicity study of lead compounds **6a**, **6b**, **6c**, **6e** and **13j** was performed against mammalian fibroblast cell line (L929) by MTT assay. Figure: 2A-Control showing normal morphology of L929 cells at resting stage, Figure 2B- Morphology of L929 cells treated at MIC of compound **6a** (similar morphology was observed for compounds **6b**, **6c**, **6e** and **13j** at their MIC)**,** Figure 2C-Morphology at higher concentration (50 and 25 µg/ml) of compound **6a** which was similar to compounds **6b**, **6c**, **6e** and **13j.**

Hemolysis Study:

Hemolytic assay results were evaluated by observing the effect of test compounds on mammalian RBCs (**Figure 3**). Hemolytic assay of compounds **6e** and **13j** was determined at 560 nm by using SOFT-max Pro 4.3 Software (Molecular Devices, Sunnyvale, USA)[24] and both compounds did not cause any hemolysis at the dilutions tested.

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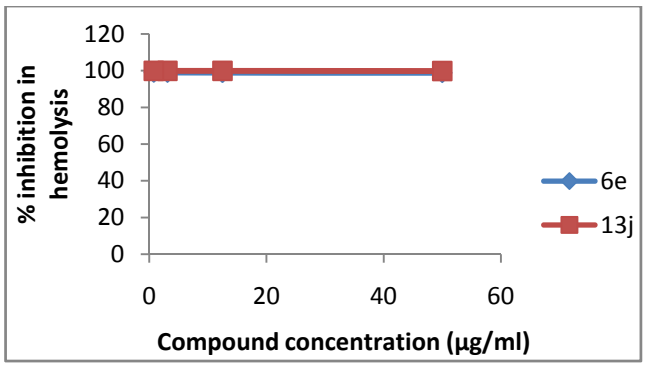

Figure 3: Hemolytic assay of compounds **6e** and **13j**

Docking Study

Analyses of 50 possible binding poses of **6e** and **13j** each in the binding site of *Candida albicans* Cyp51 revealed that many of the conformations of the bound ligand are in good agreement with the reported binding mode of flucanazole and its analogues. Proposed binding mode of **6e** and **13j** in the active site of *Candida albicans* Cyp51 (**Figure 4**) showed that in both the molecules azole ring is optimally oriented to make coordination bond with Fe of heme group. In docked conformation of **6e** (**Figure 4A**), diflurophenyl group was placed near His310 and Val509. However, in case of **13j** (**Figure 4B**), difluorophenyl group was found to orient towards Tyr118 and Tyr132. Long hydrophobic tails of **6e** and **13j** occupied the hydrophobic channel of Cyp51. We observed that binding is mostly stabilized by hydrophobic interactions contributed by Cyp51. This stabilization was due to the residues Phe126, Ile131, Phe228 and Leu300. Overall binding mode of **6e** and **13j** appeared reasonable and provided a good insight into the structural basis of inhibition of *Candida albicans* Cyp51 by these compounds. For image generation UCSF Chimera 1.7 was used.[25]

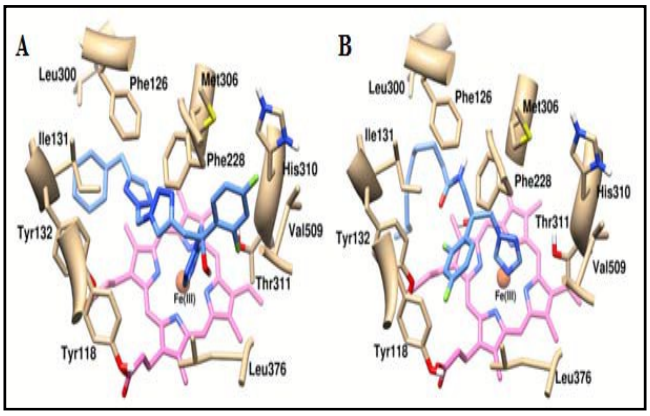

Figure 4: Putative binding mode of **6e** (A) and **13j** (B) in the active site of *Candia albicans* Cyp51. Protein is shown in mixed ribbon and stick representation with heme prosthetic group in hot pink colour.

Conclusion:

In conclusion we have synthesized a series of new fluconazole analogues having complex side chain with two 1,2,3-triazole rings. In addition we have also synthesized amide analogues of fluconazole. All the newly synthesized molecules were found to show good antifungal activity. Compound **6e** with two 1,2,3-triazole rings with cyclohexylmethy group in the side chain and amide analogue of decanoic acid **13j** having long chain were found to show highest antifungal activity. These compounds did not exhibit any toxicity to L929 cells at MIC and lower concentrations, however when exposed to higher concentrations (50 and 25 µg/mL), the L929 cells lost their normal morphology. The MTT assay revealed >90% viability of L929 cells even at higher concentrations. Among all the compounds **6e** and **13j** were found to be the most active and may be treated as lead molecules and hence hemolysis and docking study of these molecules was carried out. Both compounds **6e** and **13j** did not cause any hemolysis at the dilutions tested. In docking study, overall binding mode of **6e** and **13j** appeared reasonable and provide a good insight into the structural basis of inhibition of *Candida albicans* Cyp51 by these compounds. This is first report of fluconazole analogues containing 2,4-disubstituted-1,2,3-triazole unit (Compounds **6** and **13**).

Experimental Protocols:

Chemistry

All chemicals were obtained from commercial sources and used as received without further purification. Solvents were dried according to literature procedures. All the reactions were carried out under nitrogen atmosphere. Column chromatography was carried out by using silica gel (60-120 mm, Merck). All reactions were monitored by TLC with silica gel coated plates; spots were visualized by UV light and/or with dipping in a phosphomolybdic acid solution or anisaldehyde solution and charring on a hot plate. ^{1}H, ^{13}C NMR were recorded in $CDCl_3$ using TMS as internal standard on AC 200 MHz or AV-400 MHz Bruker NMR spectrometers. Chemical shifts are reported in ppm. Microwave reactions were carried in CEM Discover, Model number: 908010. FTIR spectra were recorded on Shimadzu FTIR-8400 spectrophotometer on KBr plate using $CHCl_3$ or Nujol. Only diagnostic bands are reported on cm^{-1} scale. The ESI ion trap mass spectra were measured by a Finnigan MAT LCQ mass spectrometer. The HRMS spectra were acquired on thermoscientific Q exactive spectrometer.

General procedure for the Synthesis of 2-Substituted-1,2,3-triazoles

CAUTION: Any experiments which may result in the formation of hydrazoic acid should be performed in a well ventilated fume hood and behind a blast shield. Sodium azide should not be mixed with strong acids.

The mixture of 37% HCHO aq. (10 equiv), glacial AcOH (1.5 equiv), and 1, 4-dioxane (5 mL) was stirred for 15 min. To this mixture was added NaN_3 (1.5 equiv), followed by alkyne (1.5 equiv). At this point pH of the reaction mixture was 6.5. After additional 10 min of stirring, sodium ascorbate (20 mol %) was added, followed by

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$CuSO_4$ solution (5 mol %) in 4 mL of H_2O. The mixture was stirred for 18h at 25 °C, diluted with H_2O (30 mL) and it was extracted using DCM (3 x 50 mL). Combined organic layer was filtered through celite, dried over $NaSO_4$, and concentrated on a rotary evaporator to give crude product. The crude product was sufficiently pure to be used for the next step without further purification.

(4-Phenyl-1,2,3-triazol-1-yl)methanol (4 a)

Yellowish solid (92%), IR – 3276, 1640, 1456, 1296, 1077 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.94 (s, 1H), 7.84-7.75 (m, 2H), 7.50-7.34 (m, 3H), 5.87 (d, 2H) 2H, 5.54 (t, 1H) 1H;); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 148.8, 147.0, 132.11, 129.6, 129.0, 128.9, 128.8, 126.1, 76.4.

(4-hexyl-2H-1,2,3-triazol-2-yl)methanol (4b)

Yellowish liquid (84%), IR – 3310, 2929, 1519, 1081 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.46 (s, 1H), 5.75 (s, 1H), 2.69 (t, 2H), 1.69-1.62 (m, 2H), 1.32-1.30 (m, 6H), 0.88 (t, 3H); ^{13}C NMR (100 MHz, CDCl3) δ_C 150.0, 133.7, 75.7, 31.4, 29.0, 28.8, 2.3, 22.5, 14.0.

(4-(4-fluorophenyl)-2H-1,2,3-triazol-2-yl)methanol (4c)

Yellowish solid (79%), IR – 3260, 2924, 1462, 1377, 1215, 1077cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.89 (s, 1H), 7.84-7.73 (m, 2H), 7.20-7.09 (m, 2H), 5.85 (d, 2H), 4.40 (t, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.5, 160.6 148.0, 131.9, 127.9, 125.9, 125.8, 116.0, 76.4.

(4-(tert-butyl)phenyl)methanol (4d)

White solid (83 %) IR – 3227, 2924, 1647, 1462, 1073 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.92 (s, 1H), 7.75 (m, 1H), 7.71 (m, 1H), 7.49 (m, 1H), 7.45 (m, 1H), 5.85 (d, 1H), 4.92 (t, 1H), 1.36 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 152.0, 148.7, 131.9, 126.7, 128.8, 76.2,, 34.6, 31.1.

(4-(cyclohexylmethyl)-2H-1,2,3-triazol-2-yl)methanol (4e)

Colorless liquid, IR – 3111, 2924, 1516, 1449, 1080 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.47 (s, 1H), 5.56 (s, 2H), 3.28 (s, 1H), 2.58 (d, 2H), 1.72-1.65 (m, 5H), 1.33-0.88 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 148.6, 134.4, 75.8, 38.0, 33.1, 32.9, 32.7, 26.2, 26.1.

General procedure for the Synthesis of 2-azidomethyl-1,2,3-triazoles

The solution of 2-substituted-1,2,3-triazoles obtained in first step in dry DCM (5 mL) was cooled to 0 °C. Triethyl amine (1.5 equiv) was added and the reaction mixture was stirred for 5 min at 0 °C. To this mixture methane sulfonyl chloride (3 equiv) was added and the reaction mixture was stirred at 0 °C for 2h. It was then diluted with cold H_2O and extracted with DCM. Organic layer was washed with dilute $NaHCO_3$, brine and dried over Sodium sulfate. It was then filtered and concentrated under vacuum to give crude product which was used for next step without further purification.

Compound obtained in the above step was dissolve in dry DMF. Sodium azide (3 equiv) was added and the reaction mixture was stirred at 65 °C for 6h. After cooling to room temperature, the reaction mixture was diluted with cold water and was extracted with DCM (3X50 mL). Combined organic layer was washed with water and brine, dried over sodium sulfate and concentrated on rotary evaporator. The crude mixture obtained was purified by column chromatography on silica gel using EtOAc: Hexane (3:7) to give pure desired products.

2-(azidomethyl)-4-phenyl-2H-1,2,3-triazole (5a)

Yellow solid (88 %). IR – 2108, 1477, 1243 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.97 (s, 1H), 7.85-7.80 (m, 2H), 7.48-7.38 (m, 3H), 5.65 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 149.4, 132.8, 129.5, 128.9, 126.0, 67.3

2-(azidomethyl)-4-hexyl-2H-1,2,3-triazole (5b)

Yellowish liquid (89 %). IR – 2929, 2108, 1519, 1242 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.49 (s, 1H), 5.56 (s, 2H), 2.70 (t, 2H), 1.75-1.68 (m, 2H), 1.43-1.26 (m, 6H), 0.89 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 51.0, 134.6, 66.8, 31.3, 28.8, 28.7, 25.3, 22.4, 13.9.

2-(azidomethyl)-4-(4-fluorophenyl)-2H-1,2,3-triazole (5c)

Yellowish solid, (87 %). IR – 3129, 2929, 2110, 1673, 1486, 1235, 978 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.93 (s, 1H), 7.82-7.78 (m, 2H), 7.20-7.13 (m, 2H), 5.65 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 132.6, 127.9, 115.0, 67.4.

2-(azidomethyl)-4-(4-(tert-butyl)phenyl)-2H-1,2,3-triazole (5d)

White solid (91 %), IR – 2964, 2107, 1618, 1486, 1242cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.94 (s, 1H), 7.77 (m, 1H), 7.73 (m, 1H), 7.51 (m, 1H), 7.46 (m, 1H), 5.65 (s, 2H), 1.36 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 152.2, 149.4, 132.8, 126.7, 125.8, 67.3, 34.7, 31.2.

2-(azidomethyl)-4-(cyclohexylmethyl)-2H-1,2,3-triazole (5e)

Yellowish liquid (94 %), IR – 3444, 2925, 2105, 1622, 1242 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.47 (s, 1H), 5.56 (s, 1H), 3.28 (s, 1H), 2.57 (d, 2H), 1.73-1.68 (m, 5H), 1.33-0.88 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 149.4, 135.4, 66.9, 38.0, 33.1, 33.0, 26.3, 26.1.

General method for the synthesis of the compound 6 (a-e).

2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)pent-4-yn-2-ol (alkyne) 2 (1 mmol) and azide 5 (a-e) (1.2 mmol) were dissolved in DMF/H_2O 4:1 (10 mL). To this solution, $CuSO_4.5H_2O$ (0.05 mmol) and sodium ascorbate (0.40 mmol) were added. The reaction mixture was placed in a domestic microwave reactor and irradiated for 10 min at 245 W. The reaction mixture was cooled, ice was added, and it was then extracted with ethyl acetate. The extract was washed with water and brine. Solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel using 5% MeOH/CH_2Cl_2 system to obtain 1,4-disubstituted 1,2,3-triazole compounds 6 (a-e).

2-(2,4-difluorophenyl)-1-(1-((4-phenyl-2H-1,2,3-triazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (6a)

Yellowish solid (58 %), IR – 3421, 1617, 1500, 1456, 1138, 965 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.10 (s, 1H), 7.90 (s, 1H), 7.80 (s, 1H), 7.76 (d, *J* = 7.34 Hz, 2H), 7.52 (s, 1H), 7.45 (t, *J*= 7.02, 2H), 7.40 (t, 1H), 7.31-7.26 (m, 1H), 6.72 (dd, *J* = 14.03 & 19.53 Hz, 2H), 6.69-6.64 (m, 1H), 6.61-6.57 (m, 1H), 5.25 (bs, 1H), 4.71 (d, *J* = 14.34, 1H), 4.55 (d, *J* = 14.34 Hz, 1H), 3.45 (d, *J* = 14.95 Hz, 1H), 3.15 (d, *J* = 15.26 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 163.5, 161.5, 159.4, 157.38, 145.0, 143.6, 133.3, 129.9, 129.3, 129.0, 126.1, 124.4, 122.7, 111.5, 103.9, 75.2, 64.8, 56.8, 33.9; HRMS calcd for $C_{22}H_{20}N_9OF_2$ $[M + H]^+$: 464.1753, found: 464.1749.

2-(2,4-difluorophenyl)-1-(1-((4-hexyl-2H-1,2,3-triazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (6b)

Glassy solid (55%); IR -3418, 1617, 1502, 1458, 1145, 965 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.02 (s, 1H), 7.73 (s, 1H), 7.40-7.35 (m, 2H), 7.24-7.29 (m, 2H), 6.66-6.54 (m, 2H), 5.18 (s, 1H), 4.64 (d, *J* = 14.34 Hz, 1H), 4.45 (d, *J* = 14.34 Hz, 1H), 3.37 (d, *J*= 13.84 Hz, 1H), 3.05 (d, *J* = 13.84 Hz, 1H), 2.56 (t, 2H), 1.55 (m, 2H), 1.25-1.19 (m, 6H), 0.81 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + $MEOD_4$) δ_C 164.7, 160.8, 159.7, 155.7, 150.9, 150.3, 142.9, 134.8, 129.4, 123.9, 122.8, 111.0, 103.6, 74.3, 64.05, 56.9, 33.8, 31.0, 29.3, 28.4, 24.9, 22.1, 13.5; HRMS calcd for $C_{22}H_{28}N_9OF_2$ $[M + H]^+$: 472.2379, found: 472.2377.

2-(2,4-difluorophenyl)-1-(1-((4-(4-fluorophenyl)-2H-1,2,3-triazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (6c)

Glassy solid (59%); ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.09 (s, 1H), 7.86 (s,1H), 7.79-7.70 (m, 3H), 7.52 (s, 1H), 7.36-7.28 (m, 1H), 7.19-7.10 (m, 2H), 6.72 (d, 2H), 6.68-6.55 (m, 2H), 5.23 (s, H), 4.73 (d, *J* = 14.15 Hz, 1H), 4.54 (d, *J* = 14.15 Hz, 1H), 3.46 (d, *J* = 15.79 Hz, 1H), 3.15 (d, *J* = 15.79 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C : 164.3, 163.5, 162.3, 161.6, 19.4, 157.4, 151.6, 149.1, 143.7, 133.1, 129.1, 128.0, 125.4, 124.4, 122.7, 116.1, 111.4, 104.0, 75.2, 64.8, 56.8, 33.9; HRMS calcd for $C_{22}H_{19}N_9OF_3$ $[M + H]^+$: 482.1659, found: 482.1655.

1-(1-((4-(4-(tert-butyl)phenyl)-2H-1,2,3-triazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (6d)

Gummy solid (57 %), IR – 3400, 2964, 1617, 1499, 1272, 1137 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.08 (s, 1H), 7.88 (s, 1H), 7.80 (s, 1H), 7.72(m, 1H), 7.67 (m, 1H), 7.50 (dd, *J* = 7.4Hz, 2H), 7.45 (s, 1H), 7.36-7.28 (m, 1H), 7.71 (d, *J* = 6.86 Hz, 2H), 6.68-6.55 (m, 2H), 5.23 (s, 1H), 4.63 (d, *J* = 4.43Hz, 1H), 4.54 (d, 4.5 Hz, 1H), 3.45 (d, *J* = 3.5Hz, 1H), 3.14 (d, *J* = 3.18Hz, 1H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C : 164.9, 160.7, 159.9, 155.8, 152.4, 149.8,143.4, 133.1, 129.8, 126.1, 125.8, 124.4, 122.7, 111.1, 103.8, 77.2, 75.0, 64.7, 71.7, 34.6, 33.9, 31.1; HRMS calcd for $C_{26}H_{28}N_9OF_2$ $[M + H]^+$: 520.2379, found: 520.2379.

1-(1-((4-(cyclohexylmethyl)-2H-1,2,3-triazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (6e)

Glassy solid (58 %) IR – 3422, 3019, 2927, 1618, 1500, 1215 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.09 (s, 1H), 7.80 (s, 1H), 7.46 (s, 1H), 7.41 (s, 1H), 7.38-7.29 (m, 1H), 6.76-6.65 (m, 2H), 6.62 (d, *J* = 2.15 Hz, 2H), 5.25 (s, 1H), 4.72 (d, *J* = 14.27 Hz, 1H), 4.53 (d, *J* = 14.27 Hz, 1H), 3.44 (d, *J* = 13.89 Hz, 1H), 3.14 (d, *J* = 13.89 Hz, 1H), 2.53 (d, 2H), 1.77-1.71 (m, 3H), 1.70-1.64 (m, 3H), 1.21-1.13 (m, 2H), 1.05-0.85 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 164.8 160.7, 159.8, 155.7, 149.7, 143.2, 135.2, 129.8, 124.4,122.5, 111.05, 103.7, 77.2,74.8, 64.3, 56.8, 37.6, 33.8, 32.8, 32.7, 29.4, 26.0, 25.8; HRMS calcd for $C_{23}H_{28}N_9OF_2$ $[M + H]^+$: 484.2379, found: 484.2380.

2-(2,4-difluorophenyl)-1-(3-(hydroxymethyl)cyclopenta-1,4-dien-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (7)

Yellowish solid (83 %) IR – 3420, 3020, 2927, 1617, 1499, 1216 cm^{-1},^1H NMR (400 MHz, $CDCl_3$) δ_H 8.06 (s, 1H), 7.82 (s, 1H), 7.40-7.31 (m, 2H), 6.83-6.68 (m, 2H), 5.67 (s, 2H), 4.97 (bs, 1H), 4.77 (d, *J* = 14.15 Hz, 1H), 4.56 (d, *J* = 14.15 Hz), 3.44 (d, *J* = 14.15 Hz, 1H) 3.21 (d, *J* = 14.15 Hz, 1H); HRMS calcd for $C_{14}H_{15}N_6O_2F_2$ $[M + H]^+$: 337.1219, found: 337.1046.

1-(2-(azidomethyl)-2H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (8)

Yellowish solid (96 %), IR – 3132, 3016, 2112, 1618, 1501, 1216 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.02 (s, 1H), 7.83 (s, 1H), 7.40 (s, 1H), 7.37-7.32 (m, 1H), 7.80-7.70 (m, 2H), 5.49 (s, 2H), 4.94 (bs, 1H), 4.80 (d, *J* = 14.20 Hz, 1H), 4.53 (d, *J* = 14.20 Hz, 1H), 3.46 (d, *J* = 15.57 Hz, 1H), 3.19 (d, *J* = 14.20 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 151.5, 144.8, 144.2, 136.3, 129.7, 124.1, 111.5, 104.0, 75.0, 68.8, 56.6, 34.3; HRMS calcd for $C_{14}H_{14}N_9OF_2$ $[M + H]^+$: 362.1284, found: 362.1280.

General method for the synthesis of the compound 9 (a-e):

By using alkyne 8 (1 mmol) and azide 3 (a-e) (1.2 mmol) and following procedure for click reaction as for compounds **6**, compound 9 (a-e) were obtained.

2-(2,4-difluorophenyl)-1-(2-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)-2H-1,2,3-triazol-4-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (9a)

Yellowish solid (68 %), ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.02 (s, 1H), 7.85 (s, 1H), 7.83 (s, 1H), 7.79 (d, *J* = 7.27 Hz, 2H), 7.44-7.41 (m, 3H), 7.37-7.33 (m, 1H), 7.25-7.20 (m, 1H), 6.76-6.69 (m, 3H), 6.61-6.56 (m, 1H), 4.99 (bs, 1H), 4.77 (d, *J* = 14.03 Hz, 1H), 4.52 (d, *J* = 14.03 Hz, 1H), 3.43 (d, *J* = 14.80 Hz, 1H), 3.17 (d, *J* = 14.80 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 163.9, 161.4, 159.5, 157.0, 152.0, 148.8, 145.5, 137.0, 129.8, 128.9, 128.6, 125.8, 124.0, 119.2, 111.5, 104.1, 75.0, 64.2, 56.6, 34.4; HRMS calcd for $C_{22}H_{20}N_9OF_2$ $[M + H]^+$: 464.1753, found: 464.1749.

2-(2,4-difluorophenyl)-1-(2-((4-hexyl-1H-1,2,3-triazol-1-yl)methyl)-2H-1,2,3-triazol-4-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (9b)

Yellowish solid (75 %), ^1H NMR ($CDCL_3$) δ_H : 8.01 (s, 1H), 7.83 (s, 1H), 7.37 (dd, *J* = 7.96 Hz, 2H), 6.80-6.63 (m, 4H), 4.94 (s, 1H), 4.77 (d, *J* = 14.51 Hz, 1H), 4.49 (d, *J* = 14.51 Hz, 1H), 3.42 (d, *J* = 14.78 Hz, 1H), 3.15 (d, *J* =14.78 Hz, 1H), 2.67 (t, 2H), 1.62-1.58 (m, 2H), 1.29-1.25 (m, 6H), 0.87 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C : 163.8, 161.3, 159.5, 157.1, 149.4, 145.3, 136.7, 132.8, 124.1, 111.5, 104.0,77.3, 74.9, 64.4, 60.4, 56.6, 34.3, 31.4, 29.1, 28.8, 25.4, 22.4, 22.3, 14.0; HRMS calcd for $C_{22}H_{28}N_9OF_2$ $[M + H]^+$: 472.2379, found: 472.2377.

2-(2,4-difluorophenyl)-1-(2-((4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl)methyl)-2H-1,2,3-triazol-4-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (9c)

Sticky solid (71 %), IR- 3419, 2925, 1616, 1499, 1231, 966 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.01 (s, 1H), 7.84-7.70 (m, 4H), 7.43 (s, 1H), 7.22-7.08 (m, 2H), 6.80-6.54 (m, 4H), 4.96 (bs, 1H), 4.78 (d, *J* = 14.02 Hz, 1H), 4.51 (d, *J* = 14.02 Hz, 1H), 3.44 (d, *J* = 14.78 Hz, 1H), 3.16 (d, *J* = 14.78 Hz, 1H); HRMS calcd for $C_{22}H_{19}N_9OF_2$ $[M + H]^+$: 482.1659, found: 464.1655.

1-(2-((1-(4-(tert-butyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-2H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (9d)

Yellowish solid (69 %), IR – 3130, 2963, 1667, 1617, 1500, 1272, 1138 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.09 (s, 1H), 7.87 (s, 1H), 7.78 (s, 1H), 7.68 (d, *J* = 8.24 Hz, 2H), 7.50 (s, 1H), 7.47 (d, *J* = 8.24 Hz, 2H), 7.32-7.28 (m, 1H), 6.71 (dd, *J* = 13.73 & 20.45 Hz, 2H), 6.68-6.57 (m, 2H), 5.26 (s, 1H), 4.72 (d, *J* = 14.04 Hz, 1H), 4.55 (d, *J* = 14.04 Hz, 1H), 3.45 (d, *J* = 14.91 Hz, 1H), 3.15 (d, *J* = 14.95 Hz, 1H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 163.5, 161.5, 159.3, 157.4, 152.5, 151.4, 149.9, 143.6, 133.2, 129.9, 126.2, 125.9, 124.4, 122.6, 111.4, 103.9, 75.1, 64.8, 56.8, 34.7, 33.9, 31.2; HRMS calcd for $C_{26}H_{28}N_9OF_2$ $[M + H]^+$: 520.2379, found: 520.2379.

1-(2-((4-(cyclohexylmethyl)-1H-1,2,3-triazol-1-yl)methyl)-2H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (9e)

Yellowish solid (72 %), IR – 3661, 2926, 1667, 1617, 1500, 1273, 1138 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.03 (s) 1H, 7.83 (s) 1H, 7.38 (s, 1H) 7.33 (s, 1H) , 7.29-7.23 (m) 1H, 6.77-6.66 (m) 2H, 6.63 (S, 2H), 4.97 (s, 1H), 4.77 (d, *J* = 13.94 Hz), 4.51 (d, *J* = 13.94 Hz) 1H, 3.42 (d, *J* = 14.67 Hz), 3.16 (d, *J* = 14.67 Hz), 2.54 (d, *J* = 6.78 Hz) 2H, 1.67-1.57 (m) 5H, 1.20-1.08 (m) 3H, 0.96-0.82 (m) 3H; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 164.7, 160.7, 159.7, 155.7, 149.6, 143.2, 135.6, 129.8, 124.3, 122.5, 111.3, 110.8, 103.7, 74.8, 64.2, 56.8, 37.5, 33.8, 32.7, 32.7, 26.0, 25.8; HRMS calcd for $C_{22}H_{20}N_9OF_2$ $[M + H]^+$: 484.2379, found: 484.2380.

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

To a solution of 1-azido-2-(2,4-difluorophenyl)propan-2-ol (3.5 g, 12.5 mmol) in 40 mL of ethanol was added 10% active charcoal-supported palladium (350 mg). The solution was stirred overnight at room temperature under a hydrogen atmosphere (60 psi) and filtered through celite. It was then concentrated, the residue was washed with ethyl acetate-pet ether (50:50) and oven dried to get the desired product **12.**

Yellowish solid (94 %), mp. 253 °C; IR (Nujol): 3315.98, 3115.38, 2922.61, 2851.64, 1616.12, 1598.42,1455.82,1272.03 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.07 (s, 1H), 7.81 (s, 1H), 7.48-7.41 (m, 1H), 6.90-6.73 (m, 2H), 4.85 (s, 2H), 3.20 (d, *J* = 13.19 Hz, 1H), 2.97 (d, *J* = 13.19 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 164.1, 161.6, 150.9, 144.6, 130.0, 124.6, 111.5, 104.2, 74.7, 57.3, 33.8; LCMS for $C_{11}H_{13}N_4OF_2$ $[M + H]^+$: 287.4.

General procedure for the synthesis fluconazole containing amide:

An acid (1 mmol) and Fluconazole amine 12 (1 mmol) were dissolved in dry DMF under argon atmosphere and the solution was cooled to 0 °C. HOBt (0.5 mmol) and EDC.HCl (0.5 mmol) were added and stirring was continued for 30 minute. The reaction mixture was allowed to attain room temperature and it was stirred further for 10 h. The reaction was quenched by adding ice and extracted with ethyl acetate (3 times). Organic layer was washed with water and brine dried over sodium sulfate and evaporated to get the desired product.

(4R)-N-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-4-((3R,5S,7R,8R, 9S,10S, 12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta [a]phenanthren-17-yl)pentanamide(13a)

White solid, (92 %); ^1H NMR (400 MHz, $CDCL_3$) δ_H - 8.20 (d, 1H), 7.79 (s, 1H), 7.62-7.54 (m, 1H), 7.39 (d, 1H), 6.83-6.75 (m, 2H), 4.60-4.51 (m, 2H), 3.88 (s, 1H), 3.81(s, 1H), 3.68 (bs, 2H), 3.37(s, 1H), 3.06 (m, 1H), 0.87 (d, 6H), 0.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCL_3$) δ_C 177.5, 161.5, 150.9, 144.7, 130.4, 124.0, 118.5, 111.4, 104.0, 76.1, 73.0, 71.7, 56.2, 47.4, 46.2, 45.6, 41.5, 39.3, 35.5, 34.7, 31.9, 31.4, 29.6, 29.3, 26.4, 22.6, 22.4, 17.2, 14.1, 12.4, 8.8. HRMS calcd for $C_{35}H_{51}N_4O_5F_2$ $[M + H]^+$: 645.3823, found: 645.3823.

N-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl) isonicotinamide (13b)

Brown sticky solid (94 %); ^1H NMR (400 MHz, DMSO d6) δ_H 8.69 (t, 1H), 8.63 (s, 1H), 8.28 (s, 1H), 7.70(s, 1H), 7.60-7.56 (m, 1H), 7.37-7.31 (m, 1H), 7.11-7.06 (m, 1H), 6.88-6.84 (m, 1H), 6.15 (s, 1H), 4.68 (d, *J* = 14.43 Hz, 1H), 4.55 (d, *J* = 14.43 Hz, 1H), 3.82-3.77 (dd, 2H); ^{13}C NMR (100 MHz, DMSO d6) δ_C 166.5, 163.5, 161.1, 158.4, 150.9, 150.5, 145.5, 141.6, 130.4, 124.9, 121.8, 111.2, 104.4, 79.5, 75.4, 55.5, 46.9; HRMS calcd for $C_{17}H_{16}N_5O_2F_2$ $[M + H]^+$: 360.1266, found: 360.1267.

N-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)benzamide (13c)[22]

Yellowish solid, (94 %); ^1H NMR (400 MHz, DMSO d6) δ_H 8.53 (s, 1H), 8.35 (s, 1H), 7.75-7.72 (m, 3H), 7.51-7.48 (m, 1H), 7.43-7.40 (m, 3H), 7.16-7.10 (m, 1H), 6.92-6.88 (m, 1H), 6.36 (bs, 1H), 4.68 (d, *J* = 14.43 Hz, 1H), 4.59 (d, *J* = 14.43 Hz, 1H), 3.86 (d, *J* = 6.11 Hz, 1H), 3.76 (d, *J* = 6.11 Hz, 1H); ^{13}C NMR (100 MHz, DMSO d6) δ_C 168.4, 163.5, 161.0, 158.3, 150.9, 145.5, 134.2, 132.0, 130.5, 129.8, 128.8, 127.7, 125.2, 115.3, 111.2, 104.4, 75.6, 55.7, 47.1; HRMS calcd for $C_{18}H_{17}N_4O_2F_2$ $[M + H]^+$: 359.1313, found: 359.1314.

N-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)thiophene-2-carboxamide (13d)

White solid, (96 %); ^1H NMR (400 MHz, Methanol D_4) δ_H - 8.7 (s, 1H), 7.78 (s, 1H), 7.64-7.60 (m, 1H), 7.53-7.47 (m, 1H), 7.08 (t, 1H), 6.97-6.91 (m, 1H), 6.85-6.81 (m, 1H), 5.49 (s, 1H), 4.79 (d, *J* = 14 Hz, 1H), 4.67 (d, *J* = 14 Hz, 1H), 3.89 (dd, 2H); ^{13}C NMR (100 MHz, METHANOL-D4) δ_C 166.1, 165.7, 163.2, 159.7, 151.4, 146.3, 139.2, 132.4, 131.5, 130.3, 129.0, 125.6, 112.1, 105.1, 77.1, 57.2, 54.9, 48.4; HRMS calcd for $C_{17}H_{12}N_4O_2F_2Na[M + H]^+$: 365.0881, found: 365.0821.

N-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)-4-hydroxybenzamide (13e)

Yellowish solid, (93 %); ^1H NMR (400 MHz, DMSO d6) δ_H 8.15(s, 1H), 7.96 (s, 1H), 7.76 (s, 1H), 7.63-7.54 (m, 2H), 7.49 (dd, *J* = 1.14 Hz, 1H), 7.06-7.01 (m, 1H), 6.86-6.74 (m, 2H), 6.29 (s, 1H), 5.33 (s, 1H), 4.64 (dd, *J* = 14.34 Hz, 2H), 3.97-3.76 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$+MeOD-D4) δ_C - 170.3, 160.5,150.0, 144.3, 132.2, 129.8, 128.8, 128.3, 123.5, 122.0, 115.1, 114.8, 111.9, 103.6, 75.6, 55.7, 47.1; HRMS calcd for $C_{18}H_{17}N_4O_3F_2$ $[M + H]^+$: 375.1263, found: 375.1265.

N_1,N_4-bis(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)terephthala mide (13f)

Yellowish solid, (71 %); ^{1}H NMR (400 MHz, $CDCL_3$) δ_H 8.41 (s, 2H), 7.81 (s, 2H), 7.53-7.48 (m, 2H), 6.97-6.93 (m, 2H), 6.86-6.82 (m, 2H), 4.81 (d, *J* = 14.34 Hz, 2H), 4.78 (d, *J* = 14.34 Hz, 2H), 3.98 (d, *J* = 14.34 Hz, 2H), 3.88 (d, *J* = 14.34 Hz, 2H); ^{13}C NMR (100 MHz, $CDCL_3$) δ_C 167.8, 162.3, 160.4, 158.9, 156.8, 148.5, 135.0, 128.4, 125.6, 122.4, 117.1, 109.1, 108.1, 102.1, 74.0, 54.1, 27.8; HRMS calcd for $C_{30}H_{27}N_8O_4F_4$ $[M + H]^+$: 639.2086, found: 609.2089.

tert-butyl [2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)amino)-2-oxoethyl]carbamate (13g)

Yellowish solid, (93 %); ^{1}H NMR (400 MHz, METHANOL-D4) δ_H - 8.36 (s, 1H), 7.79 (s, 1H), 7.49 (m, 1H), 6.98-6.94 (m, 1H), 6.88-6.86 (m, 1H), 4.77-4.75 (d, *J* = 14.34 Hz, 1H), 4.65-4.62 (d, *J* = 14.34 Hz, 1H), 3.88-3.86 (d, *J* = 14.34 Hz, 1H), 3.74-3.71 (d, *J* = 14.34 Hz, 1H), 3.68-3.64 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, METHANOL-D_4) δ_C 172.5, 164.0, 162.0, 160.4, 158.41, 157.0, 150.0, 144.8, 130.00, 123.9, 110.7, 103.6, 79.5, 55.4, 46.0, 43.2, 27.3; HRMS calcd for $C_{18}H_{24}N_5OF_2$ $[M + H]^+$: 412.1791, found: 412.1790.

N-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)phenazine-1-carboxamide (13h)

Yellowish solid, (96 %); ^{1}H NMR (400 MHz, $CDCL_3$) δ_H – 10.57(t, 1H), 8.30 (dd, *J* = 8Hz & 1.2 Hz, 1H), 7.82 (dd, *J* = 8Hz & 1.2 Hz, 1H), 7.71 (dd, *J* = 8Hz & 1.2 Hz, 1H), 7.61 (s, 1H), 7.57 (dd, *J* = 8Hz & 1.2 Hz, 1H), 7.43-7.33 (m, 3H), 7.16-7.10 (m, 1H), 6.71 (s, 1H), 6.25-6.16 (m, 2H), 5.83 (s, 1H), 4.14 (dd, 2H), 3.62 (dd, 2H); ^{13}C NMR (100 MHz, $CDCL_3$) δ_C 167.5, 164.0, 161.5, 160.0, 157.6, 151.4, 144.6, 143.3, 141.1, 140.4, 135.5, 138.4, 132.1, 131.2, 130.6, 129.8, 128.8,,127.6, 124.3, 111.6, 103.9, 76.9, 56.5, 47.9; HRMS calcd for $C_{24}H_{19}N_6O_2F_2$ $[M + H]^+$: 461.1532, found: 461.1534.

tert-butyl (1-((2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)amino)-1-oxopropan-2-yl)carbamate (13i)

Brown sticky solid, (97 %); ^{1}H NMR (400 MHz, $CDCL_3$) δ_H 8.07 (d, 1H), 7.83 (s, 1H), 7.56-7.53 (m, 1H), 6.82-6.76 (m, 2H), 5.75(d, 1H), 4.83(bs, 1H), 4.66-4.1 (m, 2H), 4.06-4.02(m, 1H), 3.88-3.78(m, 1H), 3.67-3.57 (m, 1H), 1.42 (d, 9H), 1.22-1.19 (m, 3H); ^{13}C NMR (100 MHz, $CDCL_3$) δ_C 175.1, 151.7, 144.6, 130.2, 123.7, 111.7, 104.1, 76.3, 55.5, 50.0, 46.9, 29.7, 28.2, 17.9; HRMS calcd for $C_{19}H_{26}N_5O_4F_2$ $[M + H]^+$: 426.1947, found: 426.1942.

N-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl)decanamide (13j)

Brown sticky solid, (93 %); ^{1}H NMR (400 MHz, $CDCL_3$) δ_H 8.23 (s, 1H), 7.86 (s, 1H), 7.62-7.57 (m, 1H), 6.86-6.78 (m, 2H), 6.0 (bs, 1H), 4.55 (s, 1H), 3.76-3.67 (m, 2H), 2.12-2.00 (m, 2H), 1.47-1.41 (m, 2H), 1.29-1.19 (m, 10H), 1.13-1.11 (m, 2H), 0.88 (t, 3H); ^{13}C NMR (100 MHz, $CDCL_3$) δ_C 176.2, 163.9, 162.00, 159.7, 157.7, 150.9, 130.4, 123.7, 111.8, 104.0, 76.2, 56.2, 47.5, 36.1, 31.8 29.3, 29.2, 28.9, 25.5, 22.6, 14.0; LCMS for $C_{17}H_{16}N_5O_2F_2$ $[M + H]^+$: 385.1.

Biology:

***In Vitro* antifungal activity**

All the newly synthesized compounds were tested for their *in vitro* antifungal activity against different fungal strains such as *Candida albicans, Cryptococcus neoformans, Sporothrix schenckii, Trichophyton mentagrophytes, Aspergillus fumigatus, Candida parapsilosis (ATCC-22019)* using fluconazole and amphotericin B as standard drugs. Minimum inhibitory concentration (MIC) values were determined using standard broth microdilution technique as per NCCLS guidelines[25]. The results of biological activity are summarized in Tables 1 and 2.

Materials and Methods: MIC determination: Minimum inhibitory concentration (MIC) of compounds was tested according to standard microbroth dilution technique as per NCCLS guidelines. Briefly, testing was performed in flat bottom 96 well tissue culture plates (CELLSTAR_ Greiner bio-one GmbH, Germany) in RPMI 1640 medium buffered with MOPS (3-[N- morpholino]propanesulfonic acid) (Sigma Chem. Co., MO, USA) for fungal strains.

The concentration range of test compounds was 50–0.36 µg/mL and for standard compounds 32–0.0018 µg/mL. The plates were incubated in a moist chamber at 35 °C and observed absorbance were after 24 h for *C. albicans,* 48h for *C. parapsilosis and Cryptococcus neoformans*, 72 h for *Aspergillus fumigatus*, *S. schenckii*, and *Trichophyton mentagrophytes*. MIC was determined as 80% inhibition of growth with respect to the growth control.

Toxicity Study:

Compounds **6a**, **6b**, **6c**, **6e** and **13j** were found to exhibit the best *in vitro* activity against all the fungi. These compounds were tested for their toxicity using mouse fibroblast cell line L929.

Material and Method

To test the toxicity of lead compounds **6a**, **6b**, **6c**, **6e** and **13j** against mammalian cells, mouse fibroblast cell line L929 was used. Stock solutions (1mg/mL) of the test compounds were prepared in DMSO. The cell line L929 was grown in RPMI 1640 medium supplemented with 10% FBS and 1 X antimycotic solution (sigma, USA) at 37 °C in humidified atmosphere having 5% CO_2. One hundred µL ($1x10^3$ cells µL in RPMI) of the confluent fibroblast stock suspension ($1x10^5$ cells/mL) was dispensed in 96 well tissue culture plates. The original medium from the wells was replaced with 100 µL serum free RPMI when the cells reached 80% confluence after incubation in a CO_2 incubator at 37 °C. Various concentrations of the test compounds (25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09 µg/mL) were added to the growing cells along with control (with no compounds) and incubated for 24 hours. 200 µL of MTT solution (0.5 mg of MTT in RPMI 1640 medium) was added to each well after removing the media completely and incubated for 4 hours at 37 °C to allow MTT metabolism. An aliquot of 100 µL of DMSO solvent was added to each well and the plate was incubated for 30 minutes at room temperature. Response of L929 cells to the test compounds was determined spectrophotometrically at 570 and 630 nm. The morphology of the cells was observed using Giemsa stain under Phase contrast microscope. After fixation of the cells in the wells of 96 well tissue culture plates, Giemsa stain was added to each well and incubated for 30 min at 37 °C. The excess stain was removed by thorough washing with phosphate buffer saline

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and the culture plates were air dried and observed under a phase contrast microscope.

Hemolysis Study:

Hemolytic assay was done by observing the effect of test compounds on mammalian RBCs.

Materials and methods:

Hemolytic activity was evaluated by observing the effect of test compounds on mammalian RBCs. Briefly, fresh blood of rabbit was collected in heparinized tubes, and red blood cells (RBCs) were collected by centrifugation at 1000g for 5 min. Supernatant was discarded and sedimented RBCs were washed three times in normal saline (0.9%) by centrifugation. cell pellet

was resuspended as 5 % V/V in normal saline supplemented with 10% fetal bovine serum. For each compound, experiment was performed in duplicate with positive as well as negative controls. The test compounds were dissolved in 10% Dimethylsulphoxide (in 0.9 % saline) and diluted two fold in saline. Equal volume of diluted compounds and RBCs suspension were mixed for test solution while equal volume of saline and RBC served as negative control and 1% triton X with RBC as positive control, and incubated at 37 ºC for 30 min. After incubation mixture was centrifuged at 1000g for 5 min, supernatant was collected and diluted with normal saline in 1:1 ratio. Absorbance was taken at 560 nm by spectophotometrically and result was calculated by

$$\% \text{ Hemolysis} = \frac{(\text{Absorbance of sample}) - (\text{Absorbance of blank})}{\text{Highest absorbance of positive control}} \times 100$$

Docking Study

Putative analysis of 50 possible binding modes of the most active molecules **6e** and **13j** in the binding site of *Candia albicans* Cyp51 active site was carried out.

Materials and methods:

Previously developed homology model of *Candida albicans* Cyp51 was used to dock **6e** and **13j**. Optimized 3D coordinates of **6e** and **13j** were generated with the help of Marvin sketch (http://www.chemaxon.com). Before flexible docking, input files for Autodock 4.2 were generated using MGLtools1.5.4 (http://mgltools.scripps.edu). Docking grid was set to 75, 75 and 70 points in x, y and z directions respectively, enclosing the heme group. Lamarckian genetic algorithm was used to generate 50 conformations each of **6e** and **13j**.

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