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ARTICLE TYPE

Re-engineering of PIP3-Antagonist triazole PITENIN's chemical scaffold: development of novel antifungal leads†

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A novel 4-(1-phenyl-1-hydroxyethyl)-1-(*o*-hydroxyphenyl)-1*H*-1,2,3-triazole was designed by integrating the structural features of triazole PITENIN anticancer agents and azole class of antifungal drugs. A two-step protocol comprising the Barbier propargylation and Cu-catalyzed azide-alkyne cycloaddition was established to synthesise a diverse set of compounds of this class. Their screening against a wide range of human fungal pathogens led to identification of several potential antifungal hits and some of them displayed better antifungal activity than fluconazole against *Candida glabrata, Cryptococcus neoformans, Aspergillus fumigatus* and *Aspergillus niger*. Mode of action studies revealed that their antifungal activity was resulting either from the inhibition of lanosterol 14 α-demethylase enzyme (leading to ergosterol depletion) or by the generation of reactive oxygen species (ROS).

1. Introduction

Fungal infections cause a continuous and momentous threat to human health, especially in immuno-compromised patients. Drug resistance among fungal pathogens is an increasing problem, hence there is continuing demand for safe and effective broad spectrum anti-fungal agents.[1] Among the several conventional anti-fungal agents used, azoles are the most frequently employed antifungal drugs, due to their high therapeutic index, broad spectrum of activity and more favourable safety profile.[2] Structurally, one of the most important groups of azole antifungals is triazole alcohols. Fluconazole, a bis-triazolylpropan-2-ol derivative was the first triazole alcohol antifungal drug which was introduced in to the market.[3] It has initiated remarkable progress in the treatment of candidiasis. Its widespread use has limited its clinical efficacy. Hence, the replacement of 1,2,4-triazole with 1,2,3-triazole as well as the incorporation of several functional groups on the triazole ring have provided a much extended chemical and biological functionalities on the triazole scaffold.[4] In recent years, studies have revealed that many of the cancer drugs have the potential to be antifungal agents conversely, antifungal compounds also show potential anticancer properties.[5] For example, Thiabendazole, an orally available antifungal drug in clinical use for 40 years, which also potently inhibits angiogenesis in animal models and in human cells and thus acts as a potential complementary therapeutic agent for use in combination with current anti-angiogenic therapies.[6] Similarly, recent Phase II studies have revealed the use of antifungal azole drug itraconazole for advanced prostate cancer treatment.[7] We have also developed a new class of anticancer agents which interfere with the interaction between phosphatidylinositol (3,4,5)-trisphosphate (PIP3) and Pleckstrin homology (PH) domains and are termed PITENIN analogs (PIT-1, PIT-2 and DM-PIT-1 having acylthiourea unit) and subsequent investigations have led to the identification of 1,4-disubstituted 1,2,3-triazoles (Triazole PIT) as promising anticancer agents *in vivo* and *in vitro*.[8] Moreover, we have also been working on the development of novel antifungal agents employing the 1,2,3-triazole moiety as an alternative pharmacophore in the search for a new azole class of antifungal drugs.[9]

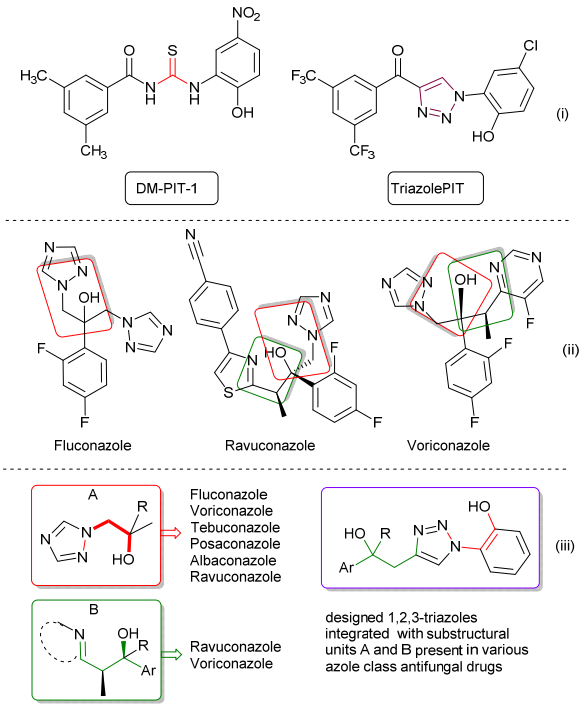


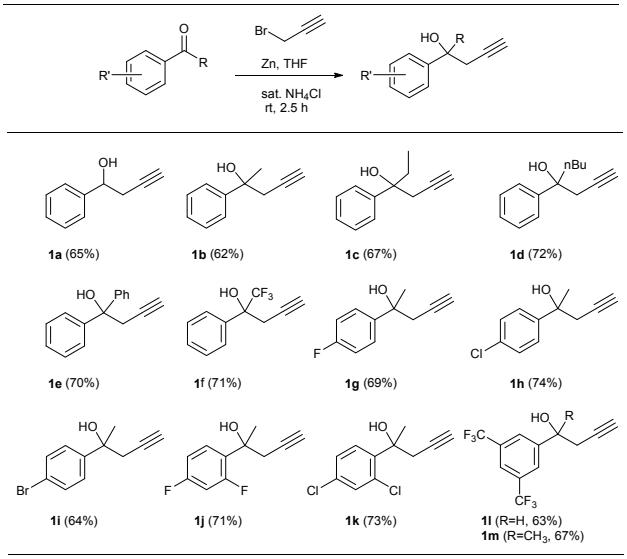
Figure 1: Structures of (i) (Triazole-)PITENIN anticancer agents; (ii) selected antifungal drugs Fluconazole, Ravuconazole and Voriconazole indicating structural elements A (red box) and B (green box); and (iii) novel anticancer pharmacophore integrating the substructures A and B present in various azole class of antifungals.

2. Results and Discussion

2.1. Synthesis of compounds

Having these newly developed 1,2,3-triazole anticancer hits in hand, we reasoned that a slight structural/functional group modification of Triazole PIT by incorporating some of the key aspects of 1,2,4-triazole antifungals, shall lead to new anti-fungal compounds. Figure 1 reveals the salient features of our design. A dissection of the 1,2,4-traizole antifungals revealed two key central structural elements **A** (**N-N-C-C-OH**) and **B** (**N=C-C-C-OH**). A novel hybrid triazole scaffold was then designed by incorporating the key central structural units in the Azole PIT scaffold.[10]

Scheme 1. Synthesis of alkynol precursors



With this idea, we proceeded further for the synthesis of a collection of compounds having the designed hybrid triazole scaffol. The synthesis of these triazoles has been planned from the Cu-catalyzed [3+2]-cycloaddition of a suitably functionalized 1-aryl-but-3-yn-1-ol (**1**) with a 2-azidophenol (**2**). A large set of alkynol intermediates (**1a**–**1m**) having functional group diversity on aryl rings and also varying the substitution such as $–CH_3$, –Et, -^{n}Bu, –Ph and finally $–CF_3$ at the central hydroxyl bearing 3°-carbon have been synthesized by the Zn-mediated Barbier reaction using propargyl bromide (Scheme 1).[11]

In case of anticancer DM-PIT analogues and Triazole PIT hits, the best activities were seen when the N-aryl group brings a chlorine substituent along with the *o*-OH group.[8] Considering this, 2-azido-4/5-chlorophenols (**2a**/**2b**) have been selected as the key azidophenols partners for building the library along with 2-azido-phenol (**2c**) and 2-azido-5-fluorophenol (**2d**) for preparing additional series. These 2-azidophenols **2a**–**2d** have been synthesized from the corresponding 2-aminophenols following established procedures. The targeted 1,2,3-triazole derivatives **1** were synthesized by the traditional Cu(I)-catalyzed azide alkyne cycloaddition approach employing $CuSO_4$-sodium ascorbate-proline combination in ^{t}BuOH-water as solvent.[12] The cycloaddition reactions proceeded smoothly at rt within 5–6 h and provided the requisite 1,2,3-triazoles in very good yields. The structures of all the final compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS methods and the purity was determined (> 98%) with the help of analytical HPLC.

2.2. Antifungal screenings

2.2.1. Antifungal activity of the triazoles. Next, we proceeded for the screening of these novel azoles against different human pathogenic yeasts and filamentous fungi.[13] The minimum inhibitory concentrations (MIC) of all the compounds against tested human fungal pathogens are presented in Table 1. Many of these compounds exhibited potent antifungal activity against several of the tested pathogens. Compounds **3kb**, **3kd** were most potent against all the fungal pathogens, with MIC in the range of 4–32 μg/mL. Compounds **3lb**, **3mb**, **3la**, **3ma** and **3da** also showed very good antifungal activity except against *A. niger* (MIC >128 μg/mL). Compounds **3kc**, **3id**, **3ha** and **3ga** showed moderate antifungal activity. Among the pathogens, *A. niger* was least susceptible with an MIC of >128 μg/mL for 12 out of 35 compounds. Ten compounds exhibited antifungal activity against filamentous *Aspergilli*, for which fluconazole was not effective (MIC >128 μg/mL). All the compounds showed better activity than fluconazole against *Candida glabrata* NCYC 388. The minimum fungicidal concentration (MFC) of selected active compounds was also estimated. It can be noted from Table 2 that compounds **3kb**, **3kd** and **3ia** exerted strong fungicidal effect with MFC values being the same as MIC or 2 X MIC. Whereas, for compound **3ja** complete killing was not observed (MFC >256 μg/mL) indicating its fungi-static action.

Coming to the structure activity relationship, the antifungal activity was not significant when there was no substituent on the left side aromatic ring for both the 4-chloro and 5-chloro series. The presence of a single halo-substituent on this aryl ring, as in the case of the triazoles **3ga**, **3ha**, **3ia**, **3gb**, **3hb**, **3ib**, was seen to lead to a moderate improvement in the antifungal activity. Interestingly, when two chlorine/fluorine atoms were placed on this ring, as in the case of **3jb** and **3kb**, it resulted in significant enhancement in the activity for the 5-chloro series. Similarly, the compounds **3la**, **3ma**, **3lb** and **3mb**, where there are two triflurometyl groups present on this aromatic motif (that are important in case of the PITENIN analogues), showed excellent antifungal activity. The addition of dichloro or bis-trifluoromethyl groups on the scaffold is expected to increase the lipophilicity and also strongly polarize the parent molecule. In the azido part of the structure, as evident from the MIC values, the activity improved from no substitution<5-fluoro<4-chloro<5-chloro with respect to –OH. Based on MIC values exhibited, the compounds **3da**, **3kb**, **3kd**, **3la**, **3lb** and **3ma** were identified as promising molecules (Figure 2). After having identified these potential hits, we proceeded for finding their mode of action.

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Scheme 2. Synthesis of diverse triazoles

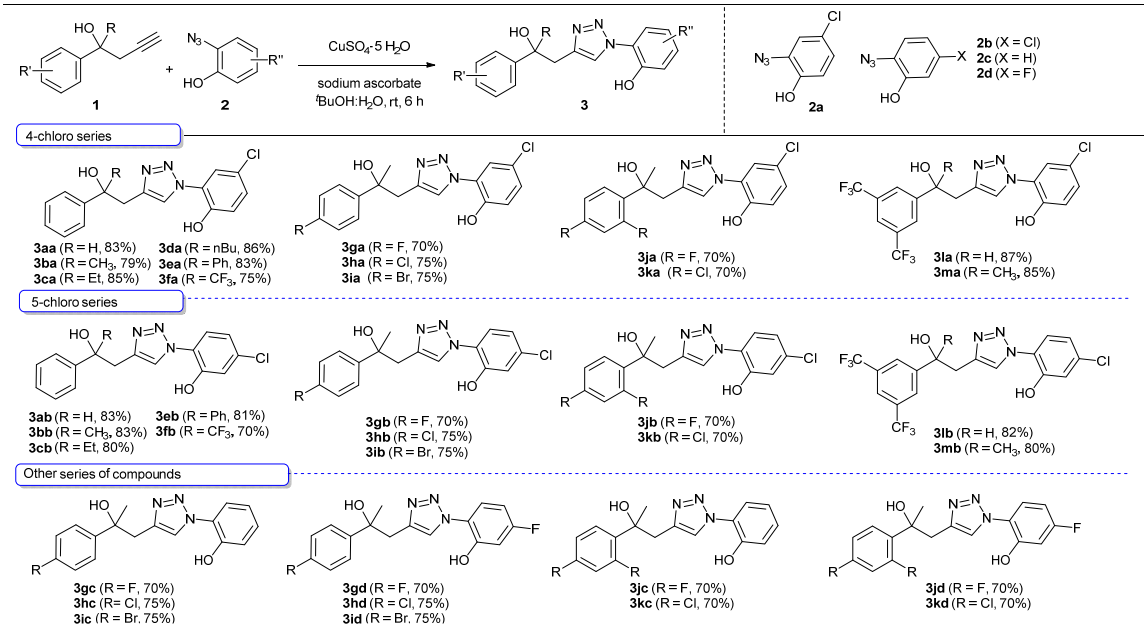

Table 1: Minimium Inhibitory Concentration (MIC_{90}) of triazoles (in μg/mL) against different human pathogenic fungi.

Compound	*C. albicans* ATCC 24433	*C .albicans* ATCC 10231	*C. neoformans* ATCC 34664	*C. glabrata* NCYC 388	*A. niger* ATCC 10578	*A. fumigatus* NCIM 902	*S. cerevisiae* ATCC 201390	
							wild strain	*erg11* mutant
3aa	64	128	>128	128	64	128	128	128
3ba	128	128	>128	128	64	128	>128	>128
3ca	64	32	32	32	128	32	128	64
3da	16	16	16	8	>128	8	32	32
3ea	64	>128	64	64	>128	>128	64	64
3fa	4	32	64	64	32	64	32	16
3ga	32	32	64	64	64	64	32	16
3ha	16	8	32	16	64	64	128	128
3ia	32	32	32	16	64	64	64	64
3ja	32	32	64	32	32	>128	>128	>128
3ka	64	>128	>128	128	>128	>128	128	128
3la	16	16	16	8	>128	>128	32	16
3ma	16	16	16	8	>128	32	32	16
3ab	64	128	>128	128	>128	128	128	128
3bb	>128	128	128	64	128	128	128	128
3cb	32	32	32	64	64	128	64	64
3eb	64	>128	32	64	>128	>128	32	32
3fb	32	64	16	64	64	64	16	16
3gb	32	128	64	16	128	64	16	16
3hb	32	16	32	32	64	64	128	128
3ib	16	16	32	32	32	64	128	128
3jb	16	32	32	32	64	64	128	128
3kb	4	16	8	16	16	16	16	16
3lb	16	32	16	8	>128	128	32	32
3mb	16	16	8	8	>128	8	16	16
3gc	128	>128	>128	128	>128	>128	16	16
3hc	16	128	128	32	128	128	128	128
3ic	32	128	128	>128	128	128	32	128
3jc	64	128	128	64	>128	128	128	128
3kc	8	32	32	16	64	32	64	64
3gd	128	>128	>128	64	>128	>128	32	32
3hd	128	128	64	32	128	64	64	64
3id	8	16	64	64	64	64	64	64
3jd	128	128	128	64	>128	128	128	128
3kd	16	8	16	16	32	64	32	32
Fluconazole	2	8	32	>128	>128	>128	32	16

Table 2: Minimum fungicidal concentration (MFC) of the selected compounds (in µg/mL)

Organism	3fa	3kb	3kd	3ia	3ja	3jb
C. albicans ATCC 24433	128	16	32	32	>256	>256
C. albicans ATCC 10231	128	32	32	64	>256	>256
C. neoformans ATCC 34664	128	32	64	32	>256	64
C. glabrata NCYC 388	64	32	32	32	>256	64
A. niger ATCC 10578	256	256	64	256	>256	64
A. fumigatus NCIM 902	256	32	64	64	>256	64

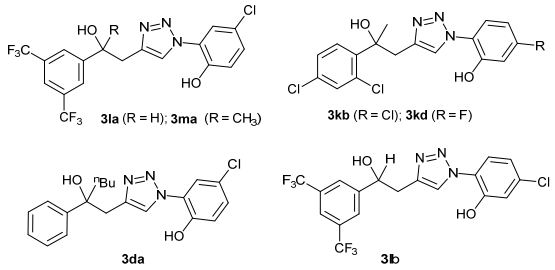

Figure 2. Selected triazoles for their mode of action studies

2.2.2. Haploinsufficiency assay for identification of ergosterol synthesis inhibitors. A yeast genome-wide drug induced haploinsufficiency screen using *Saccharomyces cerevisiae* as a model organism has been developed by Giaever *et al.*[14] The screen employs a diploid *S. cerevisiae* mutant strain which has only one copy of a specific gene, whereas there are two copies of all other genes in it. The resulting heterozygote mutant strain becomes sensitive to any drug that acts on the product of this gene as compared to the wild type strain. This haploinsufficient phenotype thereby identifies the gene product of the heterozygous locus as the likely drug target.[15] In the present study, a diploid wild type *S. cerevisiae* strain and its various mutants (including mutants haploid for the genes *erg11* – lanosterol 14 α-demethylase; *CHS2* – chitin synthase; *FAB1* - fructose 1,6-bis-phosphate aldolase; *FAS2* – Fatty acid synthase; *ILV5* – Acedohydroxyacid reductoisomerase etc.) were used for the screening of the lead compounds. For instance, in case of mutant with only one copy of *erg11,* the effect of azole compounds (those having lanosterol 14 α-demethylase enzyme as the target) on growth will be more pronounced on it as compared to the wild type, whereas other agents will exert similar effect on both the strains. All the synthesized compounds were checked for antifungal activity against *S. cerevisiae* wild and mutant strains. For all the mutants tested except for *erg11*, the MIC values of all compounds were similar to the MICs against *S. cerevisiae* wild strain (data not shown). In case of *erg11* mutant, the concentration of compounds **3fa**, **3ga**, **3la** and **3ma** required to inhibit its growth was half than the MIC for wild type *S. cerevisiae* suggesting lanosterol 14 α-demethylase as their target (Table 1). Standard fluconazole also exhibited half MIC (16 µg/mL) for the mutant as compared to the wild type.

2.2.3. Effect of the compounds on sterol profile of *Candida albicans* ATCC 24433. The inhibition of lanosterol 14 α-demethylase by azoles lead to the depletion of ergosterol and the accumulation of methylated sterol intermediates such as eburicol and obtusifoliol.[16] In spectrophotometric sterols analysis, the presence of ergosterol and the late sterol intermediate 24(28)dehydroergosterol (24(28)DHE) in a sample without any compound resulted in a characteristic four-peaked curve. In the presence of **3la**, **3ma**, **3kb**, **3kd** and fluconazole, a dose-dependent decrease in the height of the absorbance peaks was observed and corresponded to the decrease in the ergosterol concentration in *C. albicans* ATCC 24433 cells (Figure 3, data shown for **3kb** and fluconazole only). The % ergosterol decrease was calculated and depicted in Figure 4. The results indicated that compounds **3la**, **3ma**, **3kb** and **3kd** are potent inhibitors of lanosterol 14 α-demethylase, and that they exerted their antifungal action through ergosterol depletion. No ergosterol depletion was observed for **3da** and **3lb** indicating a different mode of action. As ergosterol is the major sterol component of the fungal cell membrane Inhibition of lanosterol 14 α-demethylase and subsequent ergosterol depletion by the compounds leads to loss in cell integrity and function.

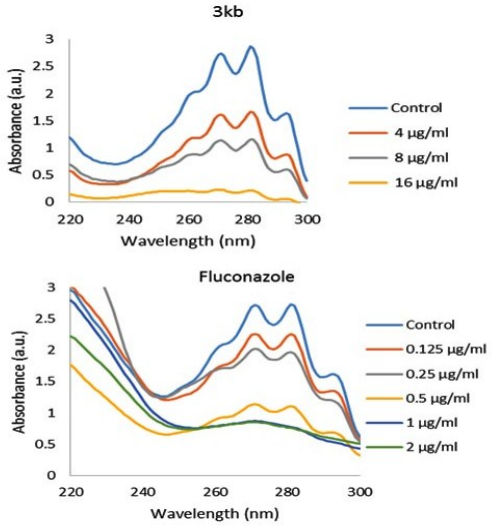

Figure 3. Spectrophotometric sterol analysis of *C. albicans* ATCC 24433 exposed with different concentrations of compound **3kb** and fluconazole.

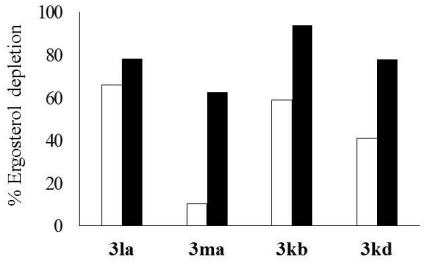

Figure 4. Depletion of total ergosterol content of *C. albicans* ATCC24433 exposed to 8 µg/ml (open bars) and 16 µg/ml (filled bars) concentrations of compounds.

2.2.4. Detection of Reactive Oxygen Species (ROS) generation. Apart from lanosterol 14 α-demethylase inhibition, few azoles like miconazole are known to exert their antifungal action by reactive oxygen species (ROS) generation.[17] In fungal cells, mitochondria are the source of reactive oxygen species (ROS). Chemical compounds may be involved in the release of intracellular ATP and the inhibition of membrane ATPase activity leading to ROS production.[17] Therefore, ROS production by the lead compounds in *C. albicans* ATCC 24433 was evaluated by dichlorofluorescin diacetate (DCFH-DA) staining. DCFH-DA is a non-fluorescent dye that undergoes intracellular deacetylation followed by ROS mediated oxidation to a fluorescent 2′,7′-dichlorofluorescein and is generally used to measure ROS generation in the cytoplasm and cellular organelles.[18]

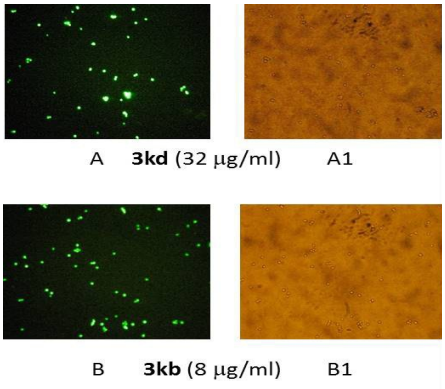

Figure 5. DCFH-DA staining of *Candida albicans* ATCC 24433 cells exposed to A) 32 µg/ml **3kd**; B) 8 µg/ml **3kb**; A1 and B1 - respective bright field images.

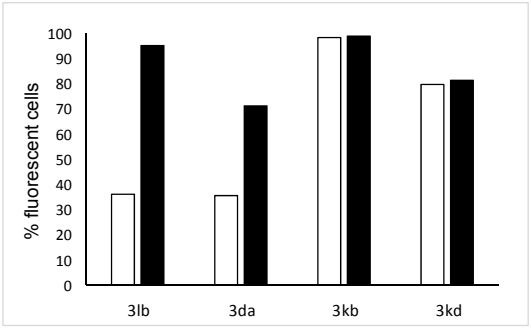

Figure 6. Reactive oxygen species generation in *C. albicans* ATCC 24433 exposed to MIC (open bars) and 2 X MIC (filled bars) of compounds.

The results indicated that **3lb** and **3da** caused fungal death through ROS generation. ROS generation was not observed for **3la** and **3ma**, while compounds **3kb** and **3kd** apart from ergosterol inhibition also showed ROS generation in *C. albicans* ATCC 2443 (Figure 5). The level of ROS production increased in a dose-dependent manner and >80% fluorescent cells were observed at 2X MIC for all the compounds except for **3da** (Figure 6). The fungicidal nature of **3kb** and **3kd** was confirmed by ROS generation which is in correlation with the MFC values. ROS can damage a wide range of molecules, including nucleic acids, proteins and lipids, and with these wide range of targets, it is difficult to determine which events lead to loss of viability of cells following damage.[19] ROS are also implicated in apoptosis and necrotic death. It is one of the important hallmarks of apoptosis in which DNA fragmentation, chromatin condensation, externalisation of phosphatidyl serine, membrane damage takes place.

2.2.5. Haemolysis assay. Cellular toxicity of the compounds was checked by haemolysis assay.[20] The concentrations tested were in the range of 4–512 µg/mL. None of the compounds except **3ma** caused haemolysis at the concentrations tested. Even for **3ma**, the haemolysis was observed at much higher concentrations than MIC i.e. 3.3% and 37.4% at 256 and 512 µg/mL, respectively.

3. Conclusions

In conclusion, founded upon anticancer leads and integrating the structural features of azole anti-fungal drugs, a novel 4-(1-phenyl-1hydroxyethyl)-1-(*o*-hydroxyphenyl)-1*H*-1,2,3-triazole was designed and further led to synthesis of corresponding series of compounds employing various functional group modifications around it. The antifungal activities and mode of action of the compounds were also studied. From a total of 35 synthesized compounds, six compounds (**3da**, **3kb**, **3kd**, **3la**, **3lb** and **3ma**) showed promising antifungal activity against human pathogenic fungi. Some of these new entities exhibited better antifungal activity than fluconazole against *C. glabrata, C. neoformans, A. fumigatus* and *A. niger*. Mode of action studies revealed that compounds **3ma** and **3la** inhibited the lanosterol 14 α-demethylase enzyme, thus exerting antifungal action through ergosterol depletion. Compounds **3lb** and **3da** caused fungal death through ROS generation. In case of compounds **3kb** and **3kd**, generation of ROS and associated ergosterol depletion led to the death of the fungal pathogen. The observed structure-activity relationship will be useful in lead optimization. With simple two/three-step synthesis, high overall yield, purity and ease of functional group diversification, the new series offers an attractive template for lead discovery. This series of compounds hold promise for the control of pathogenic fungi and need to be further investigated for increasing activity and therapeutic potential.

4. Experimental

4.1. General experimental procedure

All the cycloaddition reactions were carried out employing 100 mg of the alkyne. A representative procedure as follows: to a solution of alkyne **1a** (100 mg, 0.68 mmol) and azide **2a** (116 mg, 0.68 mmol) in ^{t}BuOH:H_2O (4 mL, 3:1) at rt, sodium ascorbate (97 mg, 0.59 mmol) and $CuSO_4 \cdot 5H_2O$ (31 mg, 0.14 mmol) were added and the resulting brick reddish mixture was stirred vigorously for 6 h. The reaction mixture was diluted with water (5 mL) and extracted with EtOAc (2 x 10 mL). The organic layer was dried (Na_2SO_4), concentrated under reduced pressure. The crude product was purified by column chromatography with their respective solvent systems.

4.1.1. 4-Chloro-2-(4-(2-hydroxy-2-phenylethyl)-1H-1,2,3-triazol-1-yl)phenol (3aa). 178 mg (83% yield); R_f = 0.3 (petroleum ether/EtOAc = 7:3); Colourless solid; Mp: 148–150 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 3.22 (d, J = 5.3 Hz, 2H), 5.04 (t, J = 6.6 Hz, 1H), 7.03 (d, J = 8.8 Hz, 1H), 7.22–7.43 (m, 6H), 7.71 (d, J = 2.5 Hz, 1H), 8.08 (s, 1H) ppm; ^{13}C

NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 34.8 (t), 72.5 (d), 117.7 (d), 123.3 (d), 123.8 (s), 123.9 (d), 124.6 (s), 125.3 (d, 2C), 126.9 (d), 127.8 (d, 2C), 129.0 (d), 143.2 (s), 143.7 (s), 147.4 (s) ppm; HRMS (ESI) calcd for $C_{16}H_{15}O_2N_3Cl$ (M^++H): 316.0847; found: 316.0847.

4.1.2. 4-Chloro-2-(4-(2-hydroxy-2-phenylpropyl)-1H-1,2,3-triazol-1-yl)phenol (3ba). 164 mg (79% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Colourless solid; Mp: 180–181 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.61 (s, 3H), 3.28 (s, 2H), 6.98 (d, J = 8.8 Hz, 1H), 7.20–7.37 (m, 4H), 7.43–7.49 (m, 2H), 7.65 (d, J = 2.5 Hz, 1H), 7.83 (s, 1H) ppm; ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 27.9 (q), 39.5 (t), 73.0 (s), 117.6 (d), 123.3 (d), 123.7 (s), 124.3 (d, 3C), 124.5 (s), 126.0 (d), 127.4 (d, 2C), 128.9 (d), 142.9 (s), 146.7 (s), 147.4 (s) ppm; HRMS (ESI) calcd for $C_{17}H_{17}O_2N_3Cl$ (M^++H): 330.1004; found: 330.0999.

4.1.3. 4-Chloro-2-(4-(2-hydroxy-2-phenylbutyl)-1H-1,2,3-triazol-1-yl)phenol (3ca). 168 mg (85% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Colourless solid; Mp: 153–155 °C; ^{1}H NMR [400 MHz, $CDCl_3$ + MeOH (D_4)]: δ 0.80 (t, J = 7.0 Hz, 3H), 1.85–2.02 (m, 2H), 3.32 (s, 2H), 6.97 (d, J = 8.0 Hz, 1H), 7.18–7.22 (m, 2H), 7.31 (t, J = 7.3 Hz, 2H), 7.37–7.42 (m, 2H), 7.57 (s, 1H), 7.71 (s, 1H) ppm; ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 7.5 (q), 34.7 (t), 38.4 (t), 76.5 (s), 118.7 (d), 122.8 (d), 123.4 (d), 124.4 (s, 2C), 125.3 (d, 2C), 126.4 (d), 127.9 (d, 2C), 129.4 (d), 143.5 (s), 144.7 (s), 147.7 (s) ppm; HRMS (ESI) calcd for $C_{18}H_{19}O_2N_3Cl$ (M^++H): 344.1160; found: 344.1164.

4.1.4. 4-Chloro-2-(4-(2-hydroxy-2-phenylhexyl)-1H-1,2,3-triazol-1-yl) phenol (3da). 158 mg (86% yield); R_f = 0.5 (petroleum ether/EtOAc = 6:4); Colourless solid; Mp: 165–167 °C; ^{1}H NMR [400 MHz, $CDCl_3$ + MeOH (D_4)]: δ 0.83 (t, J = 7.3 Hz, 3H), 1.01–1.11 (m, 1H), 1.20–1.36 (m, 3H), 1.81–1.89 (m, 1H), 1.91–1.99 (m, 1H), 3.32 (t, J = 15.3 Hz, 1H), 4.03 (bs, 2H), 6.98 (d, J = 8.8 Hz, 1H), 7.19–7.23 (m, 2H), 7.32 (t, J = 7.8 Hz, 1H), 7.40 (d, J = 7.5 Hz, 2H), 7.58 (d, J = 2.3 Hz, 1H), 7.69 (s, 1H) ppm; ^{13}C NMR [100 MHz, $CDCl_3$ + MeOH (D_4)]: δ13.4 (q), 22.6 (t), 25.2 (t), 38.7 (t), 41.7 (t), 76.0 (s), 118.2 (d), 123.2 (d), 123.9 (d), 124.1 (s), 124.6 (s), 125.1 (d, 2C), 126.1 (d), 127.7 (d, 2C), 129.2 (d), 143.2 (s), 145.0 (s), 147.6 (s) ppm; HRMS (ESI) calcd for $C_{20}H_{23}O_2N_3Cl$ (M^++H): 372.1473; found: 372.1471.

4.1.5. 4-Chloro-2-(4-(2-hydroxy-2,2-diphenylethyl)-1H-1,2,3-triazol-1-yl)phenol (3ea). 147 mg (83% yield); R_f = 0.5 (petroleum ether/EtOAc = 7:3); Colourless solid; Mp: 194–196 °C; ^{1}H NMR [200 MHz, $CDCl_3$]: δ 3.82 (s, 2H), 6.96 (d, J = 8.7 Hz, 1H), 7.17–7.35 (m, 7H), 7.46–7.51 (m, 6H), 7.58 (d, J = 2.5 Hz, 1H), 7.66 (s, 1H) ppm; ^{13}C NMR [50 MHz, $CDCl_3$]: δ37.9 (t), 77.2 (s), 118.0 (d), 123.3 (d), 124.0 (s), 124.2 (d), 124.5 (s), 125.8 (d, 4C), 126.5 (d, 2C), 127.6 (d, 4C), 129.1 (d), 143.1 (s), 146.0 (s, 2C), 147.5 (s) ppm; HRMS (ESI) calcd. for $C_{22}H_{19}O_2N_3Cl$ (M^++H): 392.1160; found: 392.1162.

4.1.6. 4-Chloro-2-(4-(3,3,3-trifluoro-2-hydroxy-2-phenylpropyl)-1H-1,2,3-triazol-1-yl)phenol (3fa). 154 mg (86% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Colourless solid; Mp: 202–206 °C; ^{1}H NMR [400 MHz, $CDCl_3$ + MeOH (D_4)]: δ 3.57 (d, J = 15.1 Hz, 1H), 3.77 (d, J = 15.1 Hz, 1H), 6.92 (td, J = 7.7, 1.1 Hz, 1H), 6.98 (dd, J = 8.2, 1.4 Hz, 1H), 7.23–7.38 (m, 4H), 7.50 (dd, J = 8.0, 1.6 Hz, 1H), 7.61 (d, J = 7.33 Hz, 2H), 7.76 (s, 1H); ^{13}C NMR [100 MHz, $CDCl_3$ + MeOH (D_4)]: δ 33.1 (t), 118.1 (d), 121.1 (s), 125.7 (s), 126.0 (s), 126.7 (s), 128.3 (s), 129.2 (s), 129.5 (s), 131.3 (s), 138.2 (s), 142.2 (s), 150.8 (s) ppm; HRMS (ESI) calcd for $C_{17}H_{14}O_2N_3ClF_3$ (M^++H): 384.0721; found: 384.0720.

4.1.7. 4-Chloro-2-(4-(2-(4-fluorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)phenol (3ga). 154 mg (79% yield); R_f = 0.3 (petroleum ether/EtOAc = 7:3); Colourless solid; Mp: 164–166 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.6 (s, 3H), 3.21 (s, 2H), 6.90–6.99 (m, 3H), 7.17 (dd, J = 8.8, 2.5 Hz, 1H),7.33–7.42 (m, 2H),7.59 (d, J = 2.5 Hz, 1H), 7.77 (s, 1H); ^{13}C NMR [100 MHz, $CDCl_3$ + MeOH (D_4)]: δ 29.0 (q), 39.9 (t), 73.3 (s), 114.2 (d), 114.7 (d), 118.3 (d), 123.1 (d), 123.8, 124.3 (s), 126.4 (d, 2C), 126.5 (d, 2C), 129.3 (d, 2C),142.7 (s), 147.5 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{16}ClFN_3O_2$(M^++H): 348.0910; found 348.0904.

4.1.8. 4-Chloro-2-(4-(2-(4-chlorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)phenol (3ha). 154 mg (82% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Orange color solid; Mp: 171–173 °C; ^{1}H NMR [400 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.54 (s, 3H), 3.21 (s, 2H), 6.94 (d, J = 8.8 Hz, 1H), 7.17 (dd, J = 8.6, 2.5 Hz, 1H), 7.23 (d, J = 8.6 Hz, 2H), 7.32–7.38 (m, 3H), 7.59 (d, J = 2.5 Hz, 1H), 7.81 (br. s., 1H); ^{13}C NMR [100 MHz, $CDCl_3$ + MeOH (D_4)]: δ 29.0 (q), 39.6 (t), 73.3 (s), 118.3 (d), 123.2 (d), 123.9 (s), 124.3 (s), 124.6 (s), 126.3 (d, 2C), 127.9 (d, 3C), 129.3 (d), 132.2 (s), 145.6 (s), 147.6 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{16}Cl_2N_3O_2$(M^++H): 364.0614; found 364.0609.

4.1.9. 2-(4-(2-(4-Bromophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)-4-chlorophenol (3ia). 142 mg (83% yield); R_f = 0.3 (petroleum ether/EtOAc = 7:3); Brown color solid; Mp: 183–185 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.52 (s, 3H), 3.20 (s, 2H), 6.93 (d, J = 8.8 Hz, 1H), 7.11–7.42 (m, 5H), 7.52 (d, J = 2.5 Hz, 1H), 7.77 (s, 1H); ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 29.3 (q), 39.6 (t), 73.5 (s), 118.8 (d), 120.5 (s), 122.7 (d), 123.4 (d), 124.4 (s), 124.5 (s), 126.7 (d, 2C), 129.4 (d), 131.0 (d, 2C), 146.2 (s, 2C), 147.6 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{16}BrClN_3O_2$ [M^++H]: 408.0109; found: 408.0102; calcd. For $C_{17}H_{16}Br^{37}ClN_3O_2$ [M^++H]: 410.0079; found: 410.0076; calcd. For $C_{17}H_{16}{}^{81}Br^{37}ClN_3O_2$ [M^++H]: 412.0059; found: 412.0043

4.1.10. 4-Chloro-2-(4-(2-(2,4-difluorophenyl)-2-hydroxyl propyl)-1H-1,2,3-triazol-1-yl)phenol (3ja). 154 mg (82% yield); R_f = 0.5 (petroleum ether/EtOAc = 7:3); Brown color solid; Mp: 191–193 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.60 (d, J = 1.0 Hz, 3H), 3.26 (d, J = 14.9 Hz, 1H), 3.44 (d, J = 14.8 Hz, 1H), 6.64–6.78 (m, 2H), 6.93 (d, J = 8.8 Hz, 1H), 7.15 (dd, J = 8.8, 2.5 Hz, 1H), 7.40–7.54 (m, 2H), 7.76 (s, 1H); ^{13}C NMR [125 MHz, $CDCl_3$ + MeOH (D_4)]: δ 27.4 (q), 37.4 (t), 72.1 (s), 103.5 (dd, J = 25.7, 27.7 Hz), 110.1 (dd, J = 2.9, 20.0 Hz), 117.8 (d), 123.2 (d), 124.1 (t),124.58 (s), 128.2 (dd, J = 6.5, 9.5 Hz), 129.3 (s), 143.0 (s), 147.4 (s), 157.8 (s), 159.8 (s), 160.7 (s), 162.6 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{15}ClF_2N_3O_2$(M^++H): 366.0815; found 366.0816.

4.1.11. 4-Chloro-2-(4-(2-(2,4-dichlorophenyl)-2-hydroxy propyl)-1H-1,2,3-triazol-1-yl)phenol (3ka). 142 mg (81% yield); R_f = 0.3 (petroleum ether/EtOAc = 6:4); Orange color solid; Mp: 178–180 °C; ^{1}H NMR [500 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.92 (s, 3H), 3.64 (d, J = 14.9 Hz, 1H), 3.87 (d, J = 14.9 Hz, 1H), 7.10 (dd, J = 8.9, 2.1 Hz, 1H), 7.20 (d, J = 2.1 Hz, 1H), 7.35 (dd, J = 8.5, 2.1 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.86 (d, J = 8.5 Hz, 1H), 8.09 (br. s., 1H); ^{13}C NMR [125 MHz, $CDCl_3$ + MeOH (D_4)]: δ 26.1 (q), 35.7 (t), 73.4 (s), 116.5 (d), 119.3 (d), 122.9 (s), 124.6 (d, 2C), 126.2 (d), 128.9 (d, 2C), 129.9 (d), 130.7 (s), 132.6 (s), 134.4 (s), 142.0 (s), 149.5 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{15}Cl_3N_3O_2$ (M^++H): 398.0224; found 398.0224.

4.1.12. 2-(4-(2-(3,5-Bis(trifluoromethyl)phenyl)-2-hydroxy ethyl)-1H-1,2,3-triazol-1-yl)-4-chlorophenol (3la). 139 mg (87% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Colourless solid ; Mp: 181–183 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 3.22 (d, J = 6.4 Hz, 2H), 5.19 (t, J = 6.4 Hz, 1H), 7.02 (d, J = 8.8 Hz, 1H), 7.26 (dd, J = 2.7, 8.6 Hz, 1H), 7.76 (d, J = 2.7 Hz, 1H), 7.79 (bs, 2H), 7.90 (bs, 2H), 8.17 (s, 1H) ppm; ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 34.8 (t), 72.1 (d), 117.7 (d), 120.5 (s), 120.6 (d, J = 4.0 Hz), 123.5 (d, 2C), 123.9 (s, 2C), 124.2 (d), 124.6 (s), 125.7 (d, J = 2.9 Hz), 129.1 (d), 130.6 (s, d, J = 33.3 Hz), 131.6 (s, d, J = 33.3 Hz), 142.8 (s), 146.7 (s), 147.5 (s) ppm; HRMS (ESI) calcd for $C_{18}H_{13}O_2N_3ClF_6$ (M^++H): 452.0595; found: 452.0600.

4.1.13. 2-(4-(2-(3,5-Bis(trifluoromethyl)phenyl)-2-hydroxy propyl)-1H-1,2,3-triazol-1-yl)-4-chlorophenol (3ma). 134 mg (85% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Colourless solid; Mp: 164–166 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.67 (s, 3H), 3.30 (s, 2H), 7.00 (d, J = 8.8 Hz, 1H), 7.24 (dd, J = 2.5, 8.8 Hz, 1H), 7.69 (d, J = 2.5 Hz, 1H), 7.76 (bs, 1H), 7.97 (s, 2H), 8.08 (s, 1H) ppm; ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ28.1 (q), 39.4 (t), 72.7 (s), 117.7 (d), 119.9 (d, J = 4.0 Hz), 120.3 (s), 123.4 (d, 2C), 123.9 (s, 2C), 124.5 (d), 125.1 (d, d, J = 2.9 Hz), 125.7 (s), 129.1 (d), 130.7 (s, d, J = 32.9 Hz), 143.2 (s, 2C), 147.7 (s) 150.3 (s) ppm; HRMS (ESI) calcd for $C_{19}H_{15}O_2N_3ClF_6$ (M^++H): 466.0752; found: 466.0755.

4.1.14. 5-Chloro-2-(4-(2-hydroxy-2-phenylethyl)-1H-1,2,3-triazol-1-yl)phenol (3ab). 178 mg (83% yield); R_f = 0.3 (petroleum ether/EtOAc = 7:3); Colourless solid; Mp: 171–172 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 3.19 (s, 2H), 5.01 (s, 1H), 6.95 (d, J = 8.3 Hz, 1H), 7.06 (s, 1H), 7.25–7.36 (m, 5H), 7.58 (d, J = 8.0 Hz, 1H), 8.01 (s, 1H) ppm; ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 34.7 (t), 72.4 (d), 116.4 (d), 119.3 (d), 122.9 (s), 124.7 (d), 125.2 (d, 2C), 126.8 (d), 127.6 (d, 3C), 134.4 (s, 2C), 143.2 (s), 149.5 (s) ppm; HRMS (ESI) calcd. for $C_{16}H_{15}O_2N_3Cl$ (M^++H): 316.0847; found: 316.0854.

4.1.15. 5-Chloro-2-(4-(2-hydroxy-2-phenylpropyl)-1H-1,2,3-triazol-1-yl)phenol (3bb)

171 mg (83% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Colourless solid; Mp: 189–190 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]:δ 1.61 (s, 3H), 3.28 (s, 2H), 6.95 (dd, J = 2.3, 8.6 Hz, 1H), 7.05 (d, J = 2.3 Hz, 1H), 7.22–7.36 (m, 3H), 7.44–7.49 (m, 2H), 7.55 (d, J = 8.6 Hz, 1H), 7.76 (s, 1H) ppm; ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 28.2 (q), 39.6 (t), 73.1 (s), 116.6 (d), 119.4 (d), 122.9 (s), 124.4 (d, 3C), 124.7 (d), 126.1 (d), 127.5 (d, 2C), 134.5 (s), 142.9 (s), 146.7 (s), 149.6 (s) ppm; HRMS (ESI) calcd for $C_{17}H_{17}O_2N_3Cl$ (M^++H): 330.1004; found: 330.1009.

4.1.16. 5-Chloro-2-(4-(2-hydroxy-2-phenylbutyl)-1H-1,2,3-triazol-1-yl)phenol (3cb). 158 mg (80% yield); R_f = 0.4 (petroleum ether/EtOAc = 6:4); Yellow solid; Mp: 129–131 °C; ^{1}H NMR [400 MHz, $CDCl_3$ + MeOH (D_4)]: δ 0.79 (t, J = 7.4 Hz, 3H), 1.83–2.05 (m, 2H), 3.32 (s, 2H), 6.88 (dd, J = 8.5, 2.3 Hz, 1H), 7.05 (d, J = 2.3 Hz, 1H), 7.18–7.22 (m, 1H), 7.28–7.34 (m, 3H), 7.38–7.40 (m, 2H), 7.57 (bs, 1H) ppm; ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 7.8 (q), 35.1 (t), 38.5 (t), 76.7 (s), 118.6 (d), 120.2 (d), 122.0 (d), 125.4 (d, 3C), 126.6 (d), 128.1 (d, 2C), 134.8 (s, 2C), 144.7 (s, 2C), 149.8 (s) ppm; HRMS (ESI) calcd for $C_{18}H_{19}O_2N_3Cl$ (M^++H): 344.1161; found: 344.1164.

4.1.17. 5-Chloro-2-(4-(2-hydroxy-2,2-diphenylethyl)-1H-1,2,3-triazol-1-yl)phenol (3eb). 143 mg (81% yield); R_f = 0.5 (petroleum ether/EtOAc = 7:3); Colourless solid; Mp: 210–212 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 3.81 (s, 2H), 6.89 (dd, J = 2.2, 8.6 Hz, 1H), 7.02 (m, 1H), 7.15–7.33 (m, 6H), 7.38 (d, J = 1.6 Hz, 1H), 7.43–7.50 (m, 4H), 7.54 (s, 1H) ppm; ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 38.0 (t), 77.2 (s), 117.3 (d), 119.8 (d), 122.8 (s), 123.9 (d), 124.2 (d), 125.9 (d, 4C), 126.7 (d, 2C), 127.8 (d, 4C), 134.7 (s), 143.2 (s), 146.0 (s, 2C), 149.7 (s) ppm; HRMS (ESI) calcd for $C_{22}H_{19}O_2N_3Cl$ (M^++H): 392.1160; found: 392.1158.

4.1.18. 5-Chloro-2-(4-(3,3,3-trifluoro-2-hydroxy-2-phenylpropyl)-1H-1,2,3-triazol-1-yl)phenol (3fb). 148 mg (82% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Colorless solid; Mp: 203–205 °C; ^{1}H NMR [400 MHz, $CDCl_3$ + MeOH (D_4)]: δ 3.56 (d, J = 15.6 Hz, 1H), 3.71 (d, J = 15.6 Hz, 1H), 6.88 (dd, J = 8.5, 2.1 Hz, 1H), 6.97 (d, J = 2.3 Hz, 1H), 7.24–7.34 (m, 3H), 7.47 (d, J = 8.2 Hz, 1H), 7.57 (d, J = 7.8 Hz, 2H), 7.7 (s, 1H); ^{13}C NMR [100 MHz, $CDCl_3$ + MeOH (D_4)]: δ 32.8 (t), 117.9 (d), 120.7 (s), 124.3 (s), 125.9 (s), 126.1 (s), 127.7 (s), 128.9 (s), 129.2 (s), 135.9 (s), 137.5 (s), 141.8 (s), 150.9 (s) ppm; HRMS (ESI) calcd for $C_{17}H_{14}O_2N_3ClF_3$ (M^++H): 384.0721; found: 384.0720.

4.1.19. 5-Chloro-2-(4-(2-(4-fluorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)phenol (3gb). 157 mg (87% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Orange color solid; Mp: 176–178 °C; ^{1}H NMR [400 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.55 (s, 3H), 3.20 (s, 2H), 6.87 (d, J = 2.2 Hz, 1H), 6.90–6.92 (m, 1H), 6.94 (s, 1H), 6.97–7.00 (m, 2H), 7.32–7.40 (m, 2H), 7.46 (d, J = 8.6 Hz, 1H), 7.69 (br. s., 1H); ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 24.6 (q), 35.3 (t), 68.7 (s), 109.7 (d, J = 21.2 Hz, 2C), 112.8 (d), 115.3 (d), 119.6 (d, 2C), 121.8 (d, J = 8.1 Hz, 2C), 130.2 (s), 138.2 (s), 145.1 (s, 2C), 154.4 (s), 161.9 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{16}ClFN_3O_2$ (M^++H): 348.0910; found 348.0906.

4.1.20. 5-Chloro-2-(4-(2-(4-chlorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)phenol (3hb). 158 mg (83% yield); R_f = 0.3 (petroleum ether/EtOAc = 7:3); Orange color solid; Mp: 202–204 °C; ^{1}H NMR [400 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.53 (s, 3H), 3.20 (s, 2H), 6.89 (dd, J = 8.7, 2.1 Hz, 1H), 7.00 (d, J = 2.2 Hz, 1H), 7.22 (m, J = 8.8 Hz, 2H), 7.30–7.36 (m, 2H), 7.45 (d, J = 8.6 Hz, 1H), 7.72 (s, 1H); ^{13}C NMR [100 MHz, $CDCl_3$ + MeOH (D_4)]: δ 29.2 (q), 39.7 (t), 73.4 (s), 117.5 (d), 120.0 (d), 122.9 (s), 124.2 (d), 126.3 (d, 2C), 128.0 (d, 3C), 132.3 (s), 134.8 (s), 145.7 (s, 2C), 149.7 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{16}Cl_2N_3O_2$ (M^++H): 364.0614; found 364.0610.

4.1.21. 2-(4-(2-(4-Bromophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)-5-chlorophenol (3ib). 138 mg (81% yield); R_f = 0.5 (petroleum ether/EtOAc = 7:3); Brown color solid; Mp: 196–198 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.53 (s, 3H), 3.20 (s, 2H), 6.89 (dd, J = 8.6, 2.2 Hz, 1H), 7.00 (d, J = 2.2 Hz, 1H), 7.26–7.33 (m, 2H), 7.36 (s, 1H), 7.40 (m, 1H), 7.47 (d, J = 8.6 Hz, 1H), 7.74 (s, 1H); ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 29.3 (q), 39.6 (t), 73.5 (s), 117.7 (d), 120.1 (d), 120.5 (s), 123.9 (d), 126.7 (d, 2C), 131.0 (d, 2C), 134.9 (s), 146.2 (s, 2C), 149.7 (s, 2C), 154.7 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{16}BrClN_3O_2$ (M^++H): 408.0109; found 408.0098; calcd. For $C_{17}H_{16}Br^{37}ClN_3O_2$ (M^++H): 410.0079; found; 410.0073; calcd. For $C_{17}H_{16}{}^{81}Br^{37}ClN_3O_2$ (M^++H): 412.0059; found: 412.0051.

4.1.22. 5-Chloro-2-(4-(2-(2,4-difluorophenyl)-2-hydroxy propyl)-1H-1,2,3-triazol-1-yl)phenol (3jb). 151 mg (81% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Brown color solid; Mp: 187–189 °C; ^{1}H NMR [400 MHz, $CDCl_3$ + MeOH

(D_4)]: δ 1.59 (s, 3H), 3.26 (d, J = 14.9 Hz, 1H), 3.41 (d, J = 14.9 Hz, 1H), 6.65–6.74 (m, 2H), 6.85 (dd, J = 8.6, 2.1 Hz, 1H), 6.98 (d, J = 1.9 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.42–7.49 (m, 1H), 7.73 (s, 1H) ppm; ^{13}C NMR [125 MHz, $CDCl_3$ + MeOH (D_4)]: δ 28.2 (q), 37.4 (t), 72.8 (s), 103.7 (s), 103.9 (dd, J = 26.1, 27.7 Hz), 110.6 (s), 110.7 (dd, J = 3.4, 20.20 Hz), 117.8 (d), 120.1 (d), 122.6 (s), 123.0 (d), 123.6 (d), 128.5 (s), 134.8 (s), 143.6 (s), 149.7 (s), 157.9 (s), 159.8 (s), 160.9 (s), 162.9 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{15}ClF_2N_3O_2$(M^++H): 366.0815; found 366.0814.

4.1.23. 5-Chloro-2-(4-(2-(2,4-dichlorophenyl)-2-hydroxy propyl) -1H-1,2,3-triazol-1-yl)phenol (3kb). 145 mg (83% yield); R_f = 0.5 (petroleum ether/EtOAc = 7:3); Orange color solid; Mp: 192–194 °C; ^{1}H NMR [500 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.68 (s, 3H), 3.34 (d, J = 15.1 Hz, 1H), 3.78 (d, J = 14.7 Hz, 1H), 6.9 (dd, J = 8.7, 2.3 Hz, 1H), 6.97 (d, J = 2.3 Hz, 1H), 7.09 (dd, J = 8.7, 2.3 Hz, 1H), 7.26 (d, J = 2.3 Hz, 1H), 7.37 (d, J = 8.7 Hz, 1H), 7.61 (d, J = 8.7 Hz, 1H), 7.71 (s, 1H); ^{13}C NMR [100 MHz, $CDCl_3$ + MeOH (D_4)]: δ 27.0 (q), 35.9 (t), 74.3 (s), 117.8 (d), 120.1 (d), 122.7 (s), 123.6 (d), 126.9 (d), 129.4 (d), 130.5 (d), 130.9 (s), 133.2 (s), 134.8 (s), 142.0 (s), 149.7 (s) ppm; HRMS (ESI+) calcd for $C_{17}H_{15}O_2N_3ClF_6$ (M^++H): 398.0224; found: 398.0225.

4.1.24. 2-(4-(2-(3,5-Bis(trifluoromethyl)phenyl)-2-hydroxyethyl)-1H-1,2,3-triazol-1-yl)-5-chlorophenol (3lb). 119 mg (82% yield); R_f = 0.3 (petroleum ether/EtOAc = 6:4); Colourless solid; Mp: 192–194 °C; ^{1}H NMR [500 MHz, $CDCl_3$ + MeOH (D_4)]: δ 3.21 (d, J = 6.4 Hz, 2H), 5.19 (t, J = 6.3 Hz, 1H), 6.98 (dd, J = 2.2, 8.6 Hz, 1H), 7.08 (d, J = 2.2 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.79 (bs, 2H), 7.88 (bs, 2H), 8.09 (bs, 1H) ppm; ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 34.9 (t), 71.2 (d), 116.7 (d), 119.6 (d, 2C), 120.3 (s), 120.6 (d, t, J = 3.3 Hz), 122.9 (s), 124.8 (d, 2C), 125.8 (d, J = 3.3 Hz), 130.3 (s, d, J = 33.3 Hz), 131.6 (s, d, J = 32.9 Hz), 134.7 (s, 2C), 146.7 (s, 2C), 149.7 (s) ppm; HRMS (ESI+) calcd for $C_{18}H_{13}O_2N_3ClF_6$ (M^++H): 452.0595; found: 452.0599.

4.1.25. 2-(4-(2-(3,5-Bis(trifluoromethyl)phenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)-5-chlorophenol (3mb). 115 mg (80% yield); Colourless solid; R_f = 0.4 (petroleum ether/EtOAc = 6:4); Mp: 156–158 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.68 (s, 3H), 3.29 (s, 2H), 6.96 (dd, J = 2.2, 8.6 Hz, 1H), 7.07 (d, J = 2.2, 1H), 7.58 (d, J = 8.6 Hz, 1H), 7.76 (bs, 1H), 7.97 (s, 2H), 8.01 (s, 1H) ppm; ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 28.1 (q), 39.4 (t), 72.8 (s), 116.6 (d), 119.4 (d), 119.9 (d, J = 4.0 Hz), 120.3 (s), 122.9 (s), 124.6 (d), 124.8 (d, 2C), 125.1 (dd, J = 2.9 Hz), 125.7 (s), 130.1 (s), 131.4 (s,), 134.6 (s), 142.2 (s), 149.6 (s), 150.2 (s) ppm; HRMS (ESI+) calcd. for $C_{19}H_{15}O_2N_3ClF_6$ (M^++H): 466.00752; found: 466.0750.

4.1.26. 2-(4-(2-(4-Fluorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)phenol (3gc). 137 mg (78% yield); R_f = 0.3 (petroleum ether/EtOAc = 6:4); Brown color solid; Mp: 156–158 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.55 (s, 3H), 3.21 (s, 2H), 6.84–7.05 (m, 4H), 7.14–7.28 (m, 1H), 7.30–7.48 (m, 3H), 7.66 (s, 1H); ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 29.4 (q), 39.9 (t), 73.4 (s), 114.6 (d, J = 21.2 Hz, 2C), 117.8 (d), 119.9 (d), 122.6 (d), 123.1 (d), 123.7 (s), 126.4 (d, J = 7.7 Hz, 2C), 129.7 (d), 142.8 (s), 149.0 (s, 2C), 159.1 (s), 163.9 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{17}FN_3O_2$ (M^++H): 314.1299; found 314.1294.

4.1.27. 2-(4-(2-(4-Chlorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)phenol (3hc). 143 mg (84% yield); R_f = 0.3 (petroleum ether/EtOAc = 7:3); Orange color solid; Mp: 159–161 °C; ^{1}H NMR [500 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.56 (br. s., 3H), 3.22 (br. s., 2H), 6.89–6.97 (m, 1H), 7.00 (d, J = 7.6 Hz, 1H), 7.20–7.31 (m, 3H), 7.38 (d, J = 7.3 Hz, 2H), 7.54 (d, J = 7.0 Hz, 1H), 7.83 (br. s., 1H); ^{13}C NMR [125 MHz, $CDCl_3$ + MeOH (D_4)]: δ 28.4 (q), 39.6 (t), 72.9 (s), 116.7 (d), 119.4 (d), 123.6 (d), 123.9 (s), 124.2 (d), 126.1 (d, 2C), 127.6 (d, 2C), 129.5 (d), 131.9 (s), 142.7 (s), 145.6 (s, 2C), 148.9 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{17}ClN_3O_2$ (M^++H): 330.1004; found 330.0998.

4.1.28. 2-(4-(2-(4-Bromophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)phenol (3ic). 132 mg (84% yield); R_f = 0.3 (petroleum ether/EtOAc = 7:3); Brown color solid; Mp: 164–166 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.53 (s, 3H), 3.21 (s, 2H), 6.86–6.96 (m, 1H), 6.97–7.04 (m, 1H), 7.16–7.24 (m, 1H), 7.27–7.33 (m, 2H), 7.34–7.46 (m, 3H), 7.71 (s, 1H); ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 29.3 (q), 39.6 (t), 73.4 (s), 117.8 (d), 120.0 (d), 120.4 (s), 122.6 (d), 123.1 (d), 126.7 (d, 2C), 129.7 (d), 131.0 (d, 2C), 146.3 (s, 2C), 149.0 (s, 2C), 160.9 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{17}BrN_3O_2$ (M^++H): 374.0499; found 374.0495.

4.1.29. 2-(4-(2-(2,4-Difluorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)phenol (3jc). 139 mg (82% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Brown color solid; Mp: 177–179 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.68 (s, 3H), 3.38 (s, 1H), 3.47 (s, 1H), 6.70–6.87 (m, 2H), 6.92–7.10 (m, 2H), 7.23–7.36 (m, 1H), 7.43–7.62 (m, 2H), 7.82 (s, 1H); ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 28.2 (q), 37.4 (t), 72.7 (s), 103.3 (dd, J = 25.6, 27.4 Hz)), 110.6 (dd, J = 3.3, 23.8 Hz), 117.8 (d), 119.9 (d), 122.5 (d), 122.9 (d), 123.7 (s), 128.4 (dd, J =6.4, 9.1 Hz), 129.7 (d), 149.0 (s, 2C), 156.3 (s), 159.2 (s), 161.4 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{16}F_2N_3O_2$ (M^++H): 332.1205; found 332.1199.

4.1.30. 2-(4-(2-(2,4-Dichlorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)phenol (3kc). 128 mg (81% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3);Brown color solid; Mp: 155–157 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.71 (s, 3H), 3.39 (d, J = 15.0 Hz, 1H), 3.77 (d, J = 14.9 Hz, 1H), 6.86–7.03 (m, 2H), 7.09–7.25 (m, 2H), 7.28–7.34 (m, 1H), 7.44 (dd, J = 8.0, 1.6 Hz, 1H), 7.64 (d, J = 8.6 Hz, 1H), 7.78 (s, 1H); ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 26.8 (q), 35.9 (t), 74.1 (s), 117.5 (d), 119.8 (d), 122.8 (d), 123.1 (d), 126.8 (d), 129.3 (d), 129.6 (d), 130.4 (d), 130.9 (s), 133.0 (s), 142.1 (s), 148.9 (s, 2C), 151.6 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{16}Cl_2N_3O_2$ (M^++H): 364.0614; found 364.0611.

4.1.31. 5-Fluoro-2-(4-(2-(4-fluorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)phenol (3gd). 154 mg (83% yield); R_f = 0.3 (petroleum ether/EtOAc = 7:3);Brown color solid; Mp: 186–188 °C; ^{1}H NMR [500 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.54 (s, 3H), 3.21 (s, 2H), 6.87–7.00 (m, 4H), 7.29 (s, 1H), 7.32–7.42 (m, 2H), 7.76 (s, 1H); ^{13}C NMR [125 MHz, $CDCl_3$ + MeOH (D_4)]: δ 29.3 (q), 39.9 (t), 73.4 (s), 109.7 (dd, J = 27.7 Hz), 114.6 (dd, J = 21.0 Hz, 2C), 116.1 (dd, J = 22.9 Hz), 118.3 (dd, J = 8.6 Hz), 123.5 (d), 126.5 (dd, J = 8.6 Hz, 2C), 142.8 (s), 144.9 (s), 154.7 (s), 156.6 (s), 160.5 (s), 162.5 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{16}F_2N_3O_2$(M^++H): 332.1205; found 332.1199.

4.1.32. 2-(4-(2-(4-Chlorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)-5-fluorophenol (3hd). 135 mg (82% yield); R_f = 0.3 (petroleum ether/EtOAc = 7:3);Light orange color solid; Mp: 179–181 °C; ^{1}H NMR [500 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.53 (s, 3H), 3.20 (s, 2H), 6.94 (d, J = 5.8 Hz, 2H), 7.30 (s, 2H), 7.25 (s, 1H), 7.31–7.38 (m, 2H), 7.80 (s, 1H); ^{13}C NMR [125 MHz,

$CDCl_3$ + MeOH (D_4)]: δ 29.6 (q), 39.7 (t), 73.5 (s), 109.2 (m, 1C), 116.1 (d, J = 22.7 Hz), 118.7 (d, J = 8.2 Hz), 122.9 (d), 123.6 (s), 126.3 (d, 2C), 128.1 (d, 2C), 132.4 (s), 143.6 (s), 145.0 (s), 145.7 (s), 154.8 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{16}ClFN_3O_2$ (M^++H): 348.0910; found 348.0903.

4.1.33. 2-(4-(2-(4-Bromophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)-5-fluorophenol (3id). 143 mg (83% yield); R_f = 0.3 (petroleum ether/EtOAc = 7:3); Brown color solid; Mp: 158–160 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.63 (s, 3H), 3.32 (d, J = 1.6 Hz, 2H), 6.97–7.06 (m, 2H), 7.10–7.17 (m, 1H),7.30–7.38 (m, 2H),7.41–7.49 (m, 2H), 7.68 (s, 1H), 9.62 (s, 1H); ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 29.4 (q), 39.6 (t), 73.5 (s), 109.1 (d), 109.6 (d), 115.9 (d), 116.4 (d), 118.5 (dd, J = 8.8 Hz), 120.5 (s),123.1 (d),126.7 (d, 2C), 131.1 (d, 2C), 143.5 (s), 146.2 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{16}BrFN_3O_2$ (M^++H): 392.0404; found 392.0398.

4.1.34. 2-(4-(2-(2,4-Difluorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)-5-fluorophenol (3jd). 143 mg (81% yield); R_f = 0.3 (petroleum ether/EtOAc = 7:3);Brown color solid; Mp: 174–176 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 1.66 (s, 3H), 3.33 (d, J = 14.9 Hz, 1H), 3.52 (d, J = 14.9 Hz, 1H), 6.64–6.86 (m, 2H), 6.88–7.09 (m, 2H), 7.14–7.36 (m, 1H), 7.40–7.65 (m, 1H), 7.85 (s, 1H); ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 28.4 (q), 37.4 (t), 72.8 (s), 104.0 (dd, J = 25.3, 27.4 Hz), 108.3 (d), 108.8 (d), 110.8 (dd, J = 3.3, 20.5 Hz), 116.0 (d), 116.4 (d), 119.1 (d, J = 8.42 Hz), 122.2 (d), 128.6 (dd, J = 6.2, 9.5 Hz), 129.4 (s), 143.9 (s), 145.0 (s), 153.4 (s), 158.2 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{15}F_3N_3O_2$ (M^++H): 350.1111; found 350.1104.

4.1.35. 2-(4-(2-(2,4-Dichlorophenyl)-2-hydroxypropyl)-1H-1,2,3-triazol-1-yl)-5-fluorophenol (3kd). 144 mg (86% yield); R_f = 0.4 (petroleum ether/EtOAc = 7:3); Orange color solid; Mp: 156–158 °C; ^{1}H NMR [200 MHz, $CDCl_3$ + MeOH (D_4)]: δ 2.3 (s, 3H), 4.03 (d, J = 15.0 Hz, 1H), 4.36 (d, J = 15.0 Hz, 1H), 7.50–7.58 (m, 2H), 7.74 (dd, J = 8.6, 2.3 Hz, 1H), 7.88–8.00 (m, 2H), 8.26 (d, J = 8.6 Hz, 1H), 8.6 (s, 1H); ^{13}C NMR [50 MHz, $CDCl_3$ + MeOH (D_4)]: δ 26.6 (q), 35.9 (t), 73.9 (s), 109.7 (d), 110.2 (d), 115.6 (d), 116.1 (d), 117.9 (d), 123.7 (d), 126.6 (d), 129.1 (d), 130.3 (d), 130.9 (s), 132.9 (s), 142.0 (s), 143.2 (s), 144.8 (s), 153.1 (s), 157.9 (s) ppm; HRMS (ESI+): calcd. For $C_{17}H_{15}Cl_2FN_3O_2$ (M^++H): 382.0520; found 382.0515.

4.2. Microorganisms and growth conditions

Human pathogens *Candida albicans* ATCC 24433, *Candida albicans* ATCC 10231, *Candida glabrata* NCYC 388, *Cryptococcus neoformans* ATCC 34664, *Aspergillus fumigatus* NCIM 902, *Aspergillus niger* ATCC 10578 were maintained on YPG (yeast extract, 0.3%; peptone, 0.5%; and glucose, 1%) agar slants at 4 °C. *Saccharomyces cerevisiae* ATCC 201390 wild type and various *S. cerevisiae* haploinsufficient mutants were maintained on 2X YPG agar slants. For different assays, the inoculum of the yeasts was prepared by growing them in YPG broth at 28 °C for 24 h.

4.3. Antifungal susceptibility assay

Antifungal activities of the synthesized compounds (in terms of Minimum Inhibitory Concentration; MIC) against *C. albicans* ATCC 24433, *C. albicans* ATCC 10231, *C. glabrata* NCYC 388, *C. neoformans* ATCC 34664, (CLSI - Clinical Laboratory Standards Institute document M27-A3)[13] and *A. fumigatus* NCIM 902, *A. niger* ATCC 10578 (CLSI M38-A2)[13] were determined by CLSI broth microdilution assay. For the assay, growth medium used was YPG. Briefly, appropriate amount of compounds were dissolved in dimethyl sulfoxide to get 100X final strength. The stock was then diluted 1:50 in YPG medium and 200 µL from this was added to the first row of a 96-well microtitre plate. The compounds were serially diluted two fold in successive wells to get a range of 1–128 µg/mL. Yeast cells (~2 x 10^3 cfu/mL), freshly grown in YPG broth in logarithmic phase, were suspended in the medium and inoculated (100 µL) in the wells of the plate. For filamentous fungi, 2 x 10^4 spores/mL were added. The microtitre plate was incubated for 24 and 48 h for yeasts and filamentous fungi, respectively. The absorbance was measured at 600 nm by using microtitre plate reader (xMark™ Microplate Absorbance Spectrophotometer, Bio-Rad, CA, USA) to assess cell growth. The MIC was defined as the lowest concentration exhibiting >90% inhibition of visible growth as compared to growth of the control. For determination of MFC, medium from the MIC and above wells was spot inoculated on 1% YPG agar plates. The plates were incubated for 48 h, and the minimum concentration showing no growth was reported as MFC.

4.4. Haploinsufficiency assay

In the haploinsufficiency assay, diploid wild type *S. cerevisiae* strain and its mutants (including mutants haploid for the genes *erg11* – lanosterol 14 α-demethylase; *CHS2* – chitin synthase; *FAB1* - fructose 1,6-bis-phosphate aldolase; *FAS2* – Fatty acid synthase; *ILV5* – Acedohydroxyacid reductoisomerase etc.) were used for target identification. The synthesized compounds were checked for antifungal activity against all the *S. cerevisiae* strains in 2X YPG medium by broth microdilution assay (CLSI M27-A3).[13]

4.5. Effect of the compounds on sterol profile of *C. albicans* ATCC 24433

Depletion of ergosterol as a result of inhibition of lanosterol 14 α-demethylase by the compounds was quantified by spectrophotometry.[16] Briefly, overnight grown *C. albicans* ATCC 24433 (1 x 10^6 cfu/mL) cells were inoculated in a series of flasks with 50 mL of YPG broth containing 8 and 16 µg/mL of the compounds **3da, 3kb**, **3kd, 3la, 3lb, 3ma** and 0.125–2 µg/mL fluconazole. The flasks were incubated on rotary shaker (180 rpm, 30 °C) for 24 h. After incubation, the cells were harvested by centrifugation (3000 rpm for 5 min) and washed with sterile distilled water. The net wet weight of the cell pellet was determined. Three mL of 25% alcoholic potassium hydroxide solution (25 g of KOH and 35 mL of sterile distilled water, brought to 100 mL with 100% ethanol), was added to 125 mg of pellet and vortex mixed for 1 min. Cell suspensions were transferred to sterile borosilicate glass screw-cap tubes and were incubated in an 85 °C water bath for 1 h. Following incubation, tubes were allowed to cool to room temperature. Sterols were then extracted by addition of a mixture of 1 mL of sterile distilled water and 3 mL of *n*-heptane followed by vigorous mixing for 3 min. The heptane layer was scanned between 220 and 300 nm with a spectrophotometer (Spectrascan UV-2600 Spectrophotometer, Chemito). Ergosterol content was determined as a percentage of the wet weight of the cells using following equations:

% ergosterol + % 24(28)DHE = [($A_{281.5}$/290) x F]/pellet weight,

% 24(28)DHE = [(A_{230}/518) x F]/pellet weight,

% ergosterol = [% ergosterol + %24(28)DHE] - %24(28)DHE

Where, *F* is the dilution factor in ethanol 290 and 518 are the *E* values for ergosterol and 24(28)DHE, respectively.

4.6. Detection of Reactive Oxygen Species (ROS) production

Intracellular reactive oxygen species (ROS) generation was assessed by DCFH-DA staining.[17] After incubation of the cells of *C. albicans* ATCC 24433 with different concentrations of the compounds for 120 min, 10 µM DCFH-DA in Phosphate Buffer Saline (PBS, pH 7.4) was added and incubated further for 30 min. Cells were then harvested, washed with PBS and directly viewed using epifluorescence microscope (LeitzLaborlux S, Germany) equipped with a 50 W mercury lamp and a filter set (I3 filter block with excitation filter BP 450–490, and suppression filter LP520). The digital images were acquired with a Canon Powershot S80 camera and ZoomBrowser EX 5.5 software for image acquisition and management. The % of fluorescent cells for each treatment was determined from the number of total (>400) and fluorescent cells counted.

4.7. Hemolysis assay

All the compounds were tested for RBC hemolysis as described previously.[20] The concentrations tested were in the range of 4–512 µg/mL.

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Graphical Abstract

Re-engineering of PIP3-antagonist triazole PITENIN's chemical scaffold: development of novel antifungal leads

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