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Original article

Synthesis of novel substituted tetrazoles having antifungal activity

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Abstract

In an effort to find potent antifungal agents, a variety of triazole derivatives with a 5-substituted tetrazole structure **6, 7, 12** and **14** were prepared and evaluated for antifungal activity against *Candida* spp., *Cryptococcus neoformans*, and *Aspergillus* spp. in vitro. The location of the methyl group at the C-3 of compounds **12** and **14** has been demonstrated to be a key structural element of antifungal potency.

Keywords: Antifungal activity; 5-Substituted tetrazole derivatives; Synthesis

1. Introduction

Over the past 25 years, the incidence of systemic fungal infections has been increasing dramatically due to an increase in the number of immunocompromised hosts. Patients undergoing anticancer chemotherapy, organ transplants or long treatment with antimicrobial agents and patients with AIDS are immunosuppressed and very susceptible to life threatening systemic fungal infections like candidiasis, cryptococcosis and aspergillosis. Antifungal azoles, fluconazole and itraconazole, which are strong inhibitors of lanosterol 14α-demethylase (cytochrome P-450$_{14DM}$) and orally active have been widely used in antifungal chemotherapy. In recent years the developments of resistance to currently available antifungal azoles in *Candida* spp., as well as clinical failures in the treatment of fungal infections have been reported [1–4]. Furthermore most of the present antifungal drugs are not effective against invasive aspergillosis and the only drug of choice in such patients is the injectable amphotericin B. Therefore, there is an urgent need of new and more effective antifungal agents with a broad antifungal spectrum.

In the course of our search for therapeutically useful antifungal azoles, we designed tetrazole based triazole derivatives depicted by general formula **6** and **7** (Fig. 1). These compounds revealed strong growth inhibitory activity against *Candida* spp. As an extension of our study on the tetrazole based antifungal triazoles, we planned to increase the antifungal activity as well as to improve the physicochemical properties, stability and water solubility; some chemical modifications have been done in the structure of formula **6** and **7**. Thus by introducing in compounds **6** and **7** the methyl group at C-3 position, a series of new compounds **12** and **14** has been designed and synthesized (Fig. 1). The location of the methyl group at the C-3 of compounds **12** and **14** has been demonstrated to be a key structural element of antifungal potency.

2. Chemistry

In order to prepare a variety of derivatives of 2-(2,4-difluorophenyl)-1-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6a–n**) and 2-(2,4-difluorophenyl)-1-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7a–n**) in an efficient manner, the 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1H-[1,2,4]-triazole (**4**) was planned as a pivotal precursor [5]. Preparation of oxirane (**4**) was accomplished as given in Scheme 1. According to this scheme, Friedel–Craft acylation of 1,3-difluorobenzene (**1**) using chloroacetyl chloride in presence of $AlCl_3$ as Lewis acid in 1,2-dichloroethane

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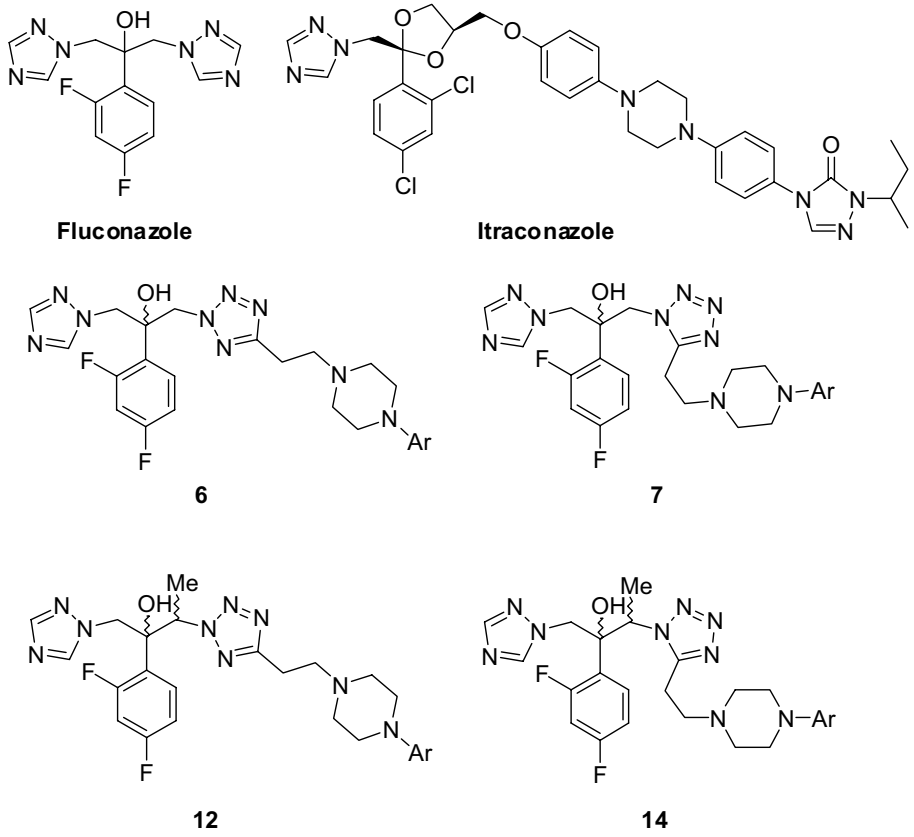
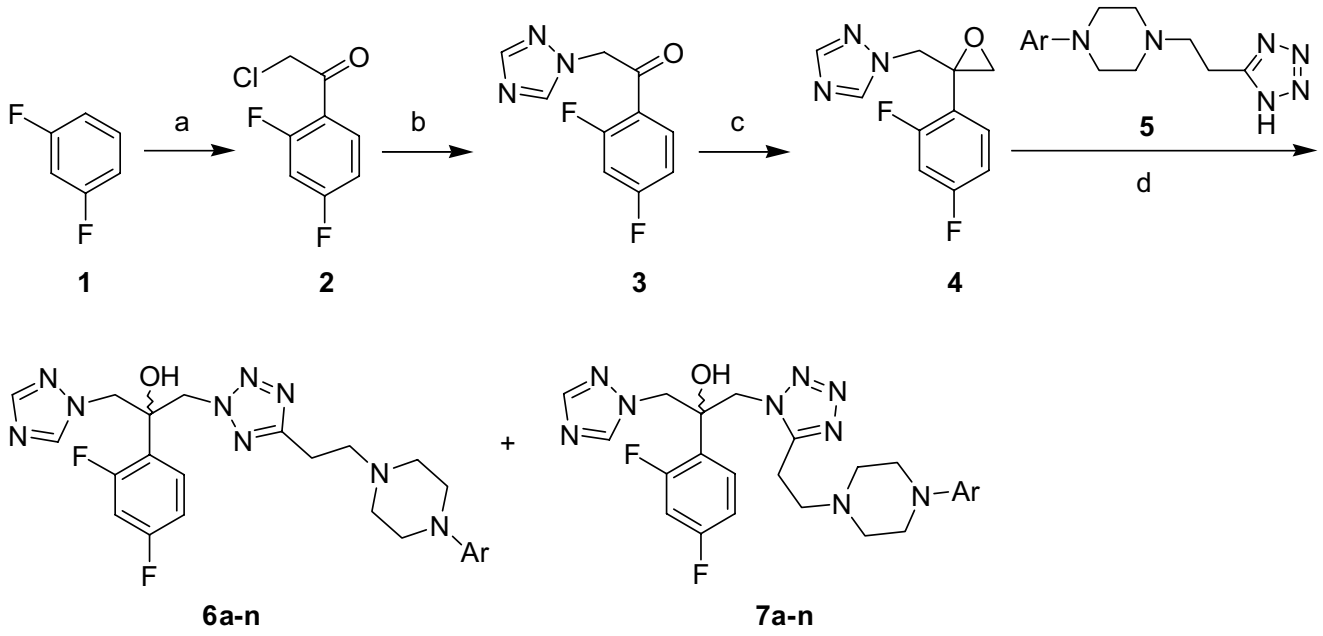
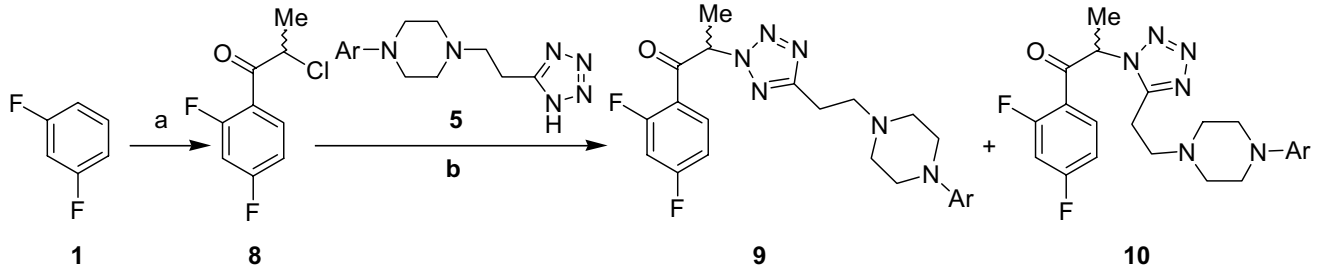


Fig. 1. Structure of fluconazole, itraconazole, and compounds **6, 7, 12, 14**.



Scheme 1. Synthesis of compounds **6a–n** and **7a–n**. (a) $AlCl_3$, 1,2-dichloroethane, chloroacetyl chloride, 25–30 °C; (b) 1,2,4-triazole, $NaHCO_3$, toluene, reflux; (c) TMSI, NaOH, toluene, 60 °C; (d) **5**, NaH, DMF, 80 °C.

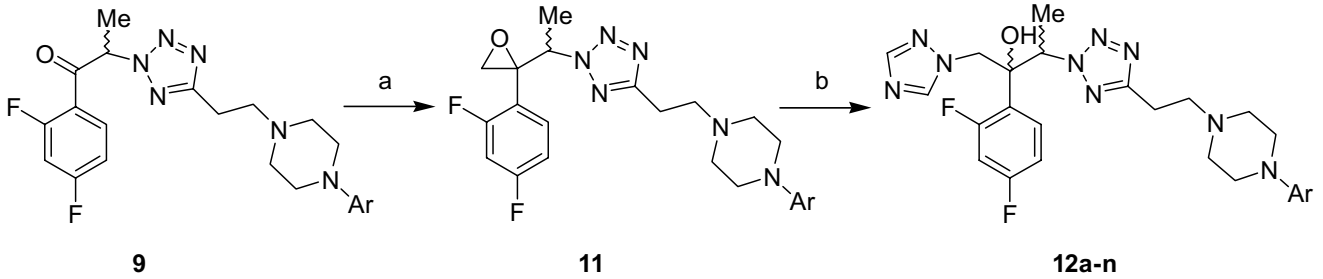
(DCE) provided the 2-chloro-1-(2,4-difluorophenyl)-ethanone (**2**) in quantitative yield [5]. In turn, compound **2** was reacted with 1,2,4-triazole in presence of $NaHCO_3$ to give a very useful intermediate 1-(2,4-difluorophenyl)-2-[1,2,4]-triazol-1-yl-ethanone (**3**) [5]. Further activation of compound **3** using the Corey ylide (trimethylsulfoxonium iodide, TMSI) in toluene at 60 °C yielded the 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4]-triazole (**4**) [5]. Having obtained the compound **4**, the next task was to couple with a number of 5-substituted tetrazoles (**5**). These



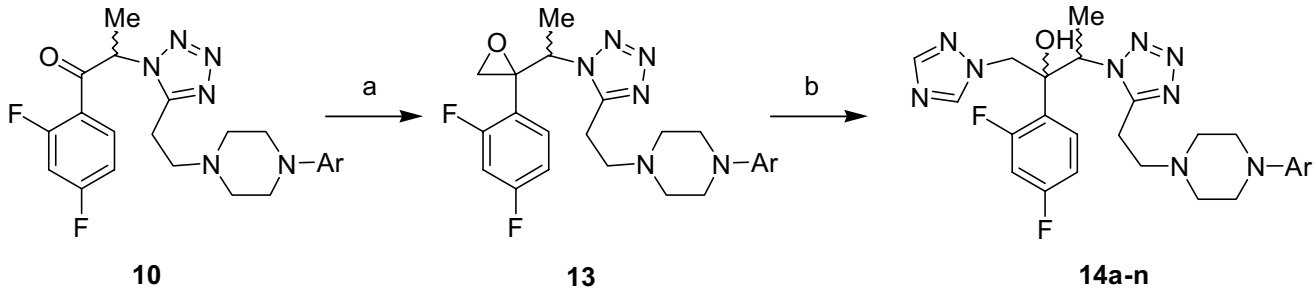
Scheme 2. Synthesis of compounds **9a–n** and **10a–n**. (a) $AlCl_3$, 1,2-dichloroethane, (±)-2-chloropropionyl chloride, 25–30 °C; (b) **5**, NaH, DMSO, 60 °C.

5-substituted tetrazoles (**5**) were prepared as described in literature [6–10]. The condensation of **4** with substituted tetrazole (**5**) gave the condensed products. TLC of these compounds showed them to be a mixture of two products, which was separated by silica gel column chromatography using chloroform/methanol as eluent. In their mass spectra, both of these compounds, the low (**7a–n**) and the high (**6a–n**) moving, showed the same molecular ion peak indicating them to be the positional isomers (Scheme 1). In ^{1}H-NMR spectra of 2-(2,4-difluorophenyl)-1-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6a–n**) the appearance of resonance signal for C**H**$_2$ attached to tetrazole ring at δ 4.65–4.72 is relatively at higher field than the resonance signals at δ 4.70–4.84 for C**H**$_2$ attached to tetrazole ring in 2-(2,4-difluorophenyl)-1-(5-{2-[4-aryl-piperzin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7a–n**) further supporting their assigned structures. The ratio of isolated yields of the above two isomers suggested that the **6a–n** isomers are the predominant ones. Steric factors also play an important role in the ratio for formation of isomers [11–18].

Next, a series of 2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12a–n**) and 2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14a–n**) were synthesized by introducing the methyl group at C-3 position in compounds **6** and **7**. According to Scheme 2; 1,3-difluorobenzene (**1**) was subjected to Friedel–Craft acylation with (±)-2-chloropropionyl chloride to afford the desired compound 2-chloro-1-(2,4-difluoro phenyl)-propan-1-one (**8**) [19]. Since we planned to introduce the methyl group at C-3 position, we first reacted compound **8** with various tetrazoles (**5**) in presence of sodium hydride at 60 °C to give desired condensation product. TLC showed it to be a mixture of two main products, one with lower mobility designated **9a–n** and the other **10a–n**. These 1-(2,4-difluorophenyl)-2-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9a–n**) and 1-(2,4-difluorophenyl)-2-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10a–n**) were separated by flash chromatography using hexane/ethyl acetate as eluent. Compounds **9a–n** and **10a–n** showed the same molecular ion peak in their mass spectrum indicating them to be positional isomers. In the ^{1}H-NMR spectra in $CDCl_3$, the appearance of C**H**CH$_3$ resonance signal in case of compounds **9a–n** as quartet between δ 5.57 and 5.63 relatively up field than the resonance signals between δ 5.85 and 5.92 for C**H**CH$_3$ in compounds **10a–n** were in conformity with their assigned structures. In these compounds also, it appeared from the ratio of isolated yields that **9a–n** isomers are the predominant one. Our next aim was to perform the epoxidation by activation of **9a–n** and **10a–n** separately by using Corey ylide, yielded the 1-[2-(2-{1-[2-(2,4-difluorophenyl)-oxiranyl]-ethyl}-2*H*-tetrazol-5-yl)-ethyl]-4-aryl-piperazine (**11a–n**) and 1-[2-(2-{1-[2-(2,4-difluorophenyl)-oxiranyl]-ethyl}-2*H*-tetrazol-5-yl)-ethyl]-4-aryl-piperazine (**13a–n**), respectively (Schemes 3 and 4). These epoxy compounds **11a–n** and **13a–n** were found to be rather unstable and deterioed even on column chromatography, so without much purification these epoxy compounds **11a–n** and **13a–n** were treated separately with 1,2,4-triazole in the presence of NaH to yield the 2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12a–n**) and 2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14a–n**), respectively (Schemes 3 and 4). The ^{1}H-NMR spectra of the compounds **14a–n** in general were almost similar to that of the compounds **12a–n** but the only



Scheme 3. Synthesis of compounds **12a–n**. (a) NaH, DMSO, TMSI, 80–90 °C; (b) NaH, 1,2,4-triazole, DMF, 100 °C.



Scheme 4. Synthesis of compounds **14a–n**. (a) NaH, DMSO, TMSI, 80–90 °C; (b) NaH, 1,2,4-triazole, DMF,100 °C.

difference was in chemical shift of C**H**CH_3 protons, which were shifted considerably down field in case of compounds **14a–n**.

3. Results and discussion

The antifungal activity of these new compounds **6a–n, 7a–n, 12a–n** and **14a–n** was evaluated by in vitro agar diffusion and micro broth dilution assay, and their antifungal activities are summarized in Tables 1 and 2. Most of the compounds showed activity against fungal cultures when tested at 500 μg ml^{-1} concentration of compounds in the agar diffusion assay. These compounds were evaluated by micro broth dilution assay to determine the minimum inhibitory concentration (MIC) values. In this assay serial twofold dilutions of the compounds were made to which the fixed volume ($1.5 \pm 1.0 \times 10^3$ cell per ml) of fungal cells (*Candida* species, *Cryptococcus neoformans* and *Aspergillus* species) were added and incubated at 25 °C for 48–72 h. The MIC values are expressed as the reciprocal of the highest dilution of the compounds showing 80% inhibition of the growth of the fungal culture. The results are expressed as mean ± S.D. of

Table 1
In vitro susceptibility of compounds **6a–n** and **7a–n** on clinical isolates of the fungal cultures

Compound	Ar	MIC (μg ml^{-1}) of compounds and standard drugs against fungal culture [a]									
		C. a I	C. a II	C. g	C. t	C. k I	C. k II	C. p	C. n	A. f	A. n
6a	2-$MeOC_6H_4$	8.0	8.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0
6b	4-$MeOC_6H_4$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
6c	4-$NO_2C_6H_4$	**4.0**	**8.0**	**8.0**	**8.0**	**8.0**	**8.0**	**8.0**	**8.0**	**>8.0**	**>8.0**
6d	3-$CF_3C_6H_4$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
6e	2-ClC_6H_4	**2.0**	**4.0**	**8.0**	**4.0**	**8.0**	**4.0**	**4.0**	**4.0**	**>8.0**	**>8.0**
6f	3-ClC_6H_4	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
6g	4-ClC_6H_4	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
6h	$CH_2C_6H_5$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
6i	4-FC_6H_4	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
6j	2,4-$(Cl)_2C_6H_3$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
6k	2-Pyridyl	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
6l	$CHPh_2$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
6m	2-*n*$BuOC_6H_4$	**1.0**	**2.0**	**4.0**	**4.0**	**8.0**	**1.0**	**2.0**	**2.0**	**8.0**	**>8.0**
6n	C_6H_5	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7a	2-$MeOC_6H_4$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7b	4-$MeOC_6H_4$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7c	4-$NO_2C_6H_4$	**4.0**	**8.0**	**8.0**	**8.0**	**8.0**	**8.0**	**8.0**	**8.0**	**>8.0**	**>8.0**
7d	3-$CF_3C_6H_4$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7e	2-ClC_6H_4	**4.0**	**4.0**	**8.0**	**4.0**	**8.0**	**4.0**	**4.0**	**8.0**	**>8.0**	**>8.0**
7f	3-ClC_6H_4	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7g	4-ClC_6H_4	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7h	$CH_2C_6H_5$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7i	4-FC_6H_4	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7j	2,4-$(Cl)_2C_6H_3$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7k	2-Pyridyl	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7l	$CHPh_2$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7m	2-*n*$BuOC_6H_4$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
7n	C_6H_5	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
Fluconazole		0.12	8.0	>8.0	8.0	>8.0	0.5	8.0	2.0	>8.0	>8.0
Itraconazole		0.007	0.25	0.25	0.12	0.25	0.007	0.25	0.06	0.12	0.5

[a] C. a I: *C. albicans*, C. a II: *C. albicans* V-01-191A-261 (resistant stain), C. t: *C. tropicolis*, C. k I: *C. krusei* ATCC6528, C. k II: *C. krusei*, C. p: *C. parapsilosis* ATCC 22019, C. g: *C. glabrata*, C. n: *C. neoformans* LA314, A. f: *A. fumigatus*, A. n: *A. niger*.

Table 2
In vitro susceptibility of compounds **12a–n** and **14a–n** on clinical isolates of the fungal cultures

Compound	Ar	MIC (μg ml^{-1}) of compounds and standard drugs against fungal culture [a]									
		C. a I	C. a II	C. g	C. t	C. k I	C. k II	C. p	C. n	A. f	A. n
12a	2-MeOC_6H_4	8.0	8.0	>8.0	>8.0	8.0	>8.0	>8.0	8.0	>8.0	>8.0
12b	4-MeOC_6H_4	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
12c	4-$NO_2C_6H_4$	4.0	4.0	8.0	8.0	8.0	4.0	8.0	4.0	>8.0	>8.0
12d	3-$CF_3C_6H_4$	**1.0**	**1.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**1.0**	**8.0**	**4.0**
12e	2-ClC_6H_4	4.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	>8.0	>8.0
12f	3-ClC_6H_4	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
12g	4-ClC_6H_4	**2.0**	**4.0**	**8.0**	**4.0**	**8.0**	**4.0**	**>8.0**	**8.0**	**>8.0**	**>8.0**
12h	$CH_2C_6H_5$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
12i	4-FC_6H_4	**2.0**	**4.0**	**4.0**	**8.0**	**8.0**	**4.0**	**8.0**	**2.0**	**>8.0**	**>8.0**
12j	2,4-$(Cl)_2C_6H_3$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
12k	2-Pyridyl	8.0	8.0	>8.0	>8.0	8.0	>8.0	>8.0	8.0	>8.0	>8.0
12l	$CHPh_2$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
12m	2-*n*BuOC_6H_4	**1.0**	**2.0**	**4.0**	**4.0**	**4.0**	**4.0**	**8.0**	**4.0**	**>8.0**	**>8.0**
12n	C_6H_5	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
14a	2-MeOC_6H_4	8.0	8.0	8.0	8.0	8.0	>8.0	>8.0	4.0	>8.0	>8.0
14b	4-MeOC_6H_4	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
14c	4-$NO_2C_6H_4$	**2.0**	**2.0**	**4.0**	**4.0**	**8.0**	**2.0**	**>8.0**	**4.0**	**>8.0**	**>8.0**
14d	3-$CF_3C_6H_4$	**0.5**	**0.5**	**1.0**	**0.5**	**1.0**	**1.0**	**1.0**	**0.5**	**2.0**	**2.0**
14e	2-ClC_6H_4	4.0	8.0	4.0	4.0	4.0	8.0	8.0	8.0	>8.0	>8.0
14f	3-ClC_6H_4	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
14g	4-ClC_6H_4	**2.0**	**2.0**	**4.0**	**4.0**	**8.0**	**2.0**	**8.0**	**4.0**	**>8.0**	**>8.0**
14h	$CH_2C_6H_5$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
14i	4-FC_6H_4	**2.0**	**2.0**	**4.0**	**4.0**	**4.0**	**2.0**	**>8.0**	**2.0**	**>8.0**	**>8.0**
14j	2,4-$(Cl)_2C_6H_3$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
14k	2-Pyridyl	8.0	8.0	8.0	8.0	8.0	>8.0	>8.0	4.0	>8.0	>8.0
14l	$CHPh_2$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
14m	2-*n*BuOC_6H_4	**1.0**	**1.0**	**2.0**	**2.0**	**4.0**	**2.0**	**4.0**	**4.0**	**>8.0**	**>8.0**
14n	C_6H_5	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
Fluconazole		0.12	8.0	>8.0	8.0	>8.0	0.5	8.0	2.0	>8.0	>8.0
Itraconazole		0.007	0.25	0.25	0.12	0.25	0.007	0.25	0.06	0.12	0.5

[a] C. a I: *C. albicans*, C. a II: *C. albicans* V-01-191A-261 (resistant stain), C. t: *C. tropicolis*, C. k I: *C. krusei* ATCC6528, C. k II: *C. krusei*, C. p: *C. parapsilosis* ATCC 22019, C. g: *C. glabrata*, C. n: *C. neoformans* LA314, A. f: *A. fumigatus*, A. n: *A. niger*.

the MIC values obtained in duplicate assay. The MIC values (in μg ml^{-1}) against *Candida* species, *C. neoformans* and *Aspergillus* species in comparison with fluconazole and itraconazole are given in Table 1. Compounds **6c, 7c, 6e, 7e** and **6m** were active against most of the fungi. The compound **6m** having 2-butoxy substitution on the phenyl ring of piperazine was the most active compound among **6** and **7** with MIC value of 1.0–2.0 μg ml^{-1} for *Candida albicans, Candida tropicalis, Candida parapsilosis* and *C. neoformans*. The antifungal activity of this compound was better than fluconazole against drug resistant *Candida* species. Other compounds **6c, 7c, 6e, 7e** also showed moderate activity against most of the *Candida* species having the MIC value of (2.0–8.0 μg ml^{-1}), however, none of the compounds showed significant activity against *Aspergillus* species up to 8.0 μg ml^{-1} concentration in the micro broth dilution assay.

Improvement in the spectrum and antifungal activity was observed in compounds **12a–n** and **14a–n** having methyl group at C-3 position (Table 2). Majority of these compounds demonstrated inhibitory activity against fungal cultures in the agar diffusion assay at 500 μg ml^{-1}. Compound **14d** having 3-CF_3 group on the phenyl ring of piperazine moiety was found to be the most active compound in micro broth dilution assay. This compound demonstrated strong antifungal activity better than fluconazole and comparable to itraconazole against *Candida* species and *C. neoformans* with an MIC value of 0.5–1.0 μg ml^{-1}. Similar antifungal activity against all the *Candida* species and *C. neoformans* culture was also shown by compound **12d**, which is positional isomer of compound **14d**. Furthermore, these compounds also demonstrated significant activity against *Aspergillus fumigates* (2.0 μg ml^{-1}) and *Aspergillus niger* (2.0–4.0 μg ml^{-1}).

Compounds **12c, 14c, 12g, 14g, 12i, 14i, 12m** and **14m** having different substitutions on the phenyl ring of piperazine also demonstrated good to moderate antifungal activity against different *Candida* species and *C. neoformans* with a MIC value of 2.0–8.0 μg ml^{-1}. However, these compounds did not show activity against *Aspergillus* species up to 8.0 μg ml^{-1} concentrations in the micro broth dilution assay.

4. Conclusions

We have synthesized tetrazole-based triazole derivatives bearing an ethyl chain linked with an aryl-piperazine. Some of these compounds possessed good antifungal activity

against the different fungal cultures such as *Candida* species, *C. neoformans* and *Aspergillus* species.

5. Experimental protocols

5.1. Chemistry

Melting points were taken in an electrically heated instrument and are uncorrected. Compounds were routinely checked for their purity on silica gel 60 F_{254} TLC plates and their spots were visualized by exposing them to iodine vapor or UV lamp or by spraying the plates with Dragendorff or $KMnO_4$ reagents. IR spectra (λ_{max} in cm^{-1}) were recorded on Perkin–Elmer Spectrum RX FT-IR model and ^{1}H-NMR spectra were recorded on Bruker Advance DRX 200 MHz instrument as solutions in $CDCl_3$ otherwise mentioned, using TMS as internal reference and chemical shifts values are expressed in δ units. Mass spectra were run on Applied Biosystems API 3000 instrument using direct inlet system. Elemental analyses were carried out with a Perkin–Elmer 2400 analyzer and the values found were within ±0.4% of theoretical values.

5.1.1. 2-Chloro-1-(2,4-difluorophenyl)-ethanone (**2**)

To a solution of 1,3-difluorobenzene (5.7 g, 50 mmol) in 1,2-dichloroethane (DCE, 30 ml), anhydrous aluminum chloride (7.98 g, 60 mmol) was added at 25–30 °C and stirred for 30 min. The reaction mixture was then cooled to 0 °C and chloroacetyl chloride (6.21 g, 54 mmol) in DCE (15 ml) was added into it over a period of 30 min at 0–10 °C. Then the reaction mixture was stirred at 25–30 °C for 7 h and diluted with the DCE (30 ml) and poured into 5% hydrochloric acid (50 ml) at 0–5 °C. The product was extracted with DCE (2 × 50 ml) and the combined organic layer was washed with 5% aqueous $NaHCO_3$ solution (20 ml), water (2 × 20 ml), brine (20 ml) and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to yield the product **2**, as yellow solid; yield 7.60 g (80%); m.p. 46–48 °C (lit [5] m.p. 46.5 °C). ^{1}H-NMR δ (ppm): 4.41 (s, 2H); 6.95–7.08 (m, 2H); 7.96–7.99 (m, 1H). MS: *m/z* 191 (M + 1) and 193 (M + 3).

5.1.2. 2-Chloro-1-(2,4-difluorophenyl)-propan-1-one (**8**)

Compound **8** [10] was prepared using same method as described for compound **2** using (±)-2-chloropropionyl chloride in 97% yield. ^{1}H-NMR δ (ppm): 1.60–1.64 (d, 3H); 5.02–5.12 (q, 1H); 6.74–6.94 (m, 2H); 7.79–7.91 (m, 1H). MS: *m/z* 205 (M + 1), 207 (M + 3).

5.1.3. 1-(2,4-Difluorophenyl)-2-[1,2,4]-triazol-1-yl-ethanone (**3**)

A mixture of **2** (9.05 g, 47.5 mmol), 1,2,4-triazole (3.93 g, 57.01 mmol), sodium bicarbonate (4.80 g, 57.00 mmol) in toluene (50 ml) was refluxed for 4 h. After the reaction was completed, the reaction mixture was poured into crushed ice and extracted with toluene (2 × 50 ml). The combined organic layer was washed with H_2O (2 × 20 ml), brine (20 ml), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to yield compound **3** as a brown solid; yield 7.30 g (69%), m.p. 104–106 °C (lit [5] m.p. 103–105 °C). ^{1}H-NMR δ (ppm): 5.60 (s, 2H); 7.15–7.22 (m, 2H); 8.04–8.10 (m, 2H); 8.22 (s, 1H). MS: *m/z* 224 (M + 1).

5.1.4. 1-[2-(2,4-Difluorophenyl)-oxiranylmethyl]-1H-[1,2,4]-triazole (**4**)

To a solution of **4** (7.30 g, 32.70 mmol) in toluene (60 ml) was added trimethylsulfoxonium iodide (8.64 g, 39.30 mmol) followed by the addition of 20% sodium hydroxide solution (8 ml). The reaction mixture was then heated at 60 °C for 4 h. After the reaction was over, it was diluted with toluene (40 ml) and poured into chilled water. The organic layer was washed with water (2 × 20 ml), brine (20 ml) dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give **4** as light brown oil; yield 4.60 g (60%). ^{1}H-NMR δ (ppm): 3.06–3.16 (m, 2H); 4.49–4.60 (m, 2H); 6.95–6.97 (m, 2H); 7.70–7.82 (m, 1H); 8.17 (s, 1H); 8.22 (s, 1H). MS: *m/z* 238 (M + 1).

5.1.5. General procedure for the synthesis of 2-(2,4-difluorophenyl)-1-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6a–n**) and 2-(2,4-difluorophenyl)-1-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7a–n**)

To a stirred solution of 1-aryl-4-[2-(1*H*-tetrazol-5-yl)-ethyl]-piperazine (**5**, 1.0 mmol) in DMF (10 ml) under nitrogen atmosphere, sodium hydride pre-washed with hexane (1.1 mmol) was added and stirred the reaction mixture for 30 min at the room temperature. After 30 min, a solution of 1-[2-(2,4-difluorophenyl)-oxiranylmethyl]-1*H*-[1,2,4] triazole (**4**, 1.5 mmol) in DMF (15 ml) was added dropwise into the reaction mixture at 40 °C, and then the reaction mixture was stirred at 80 °C for 4 h. The reaction mixture was cooled to room temperature and was poured into chilled water (100 ml); and extracted with ethyl acetate (2 × 100 ml). The combined organic layer was washed with water (3 × 50 ml), brine (30 ml), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give a mixture of two isomers **6a–n** and **7a–n**. The isomers were separated on flash silica gel column using $CHCl_3$/MeOH as eluent, the **6a–n** was eluted first with $CHCl_3$/MeOH (98.5:1.5), while **7a–n** was eluted later with $CHCl_3$/MeOH (98.25:1.75).

5.1.5.1. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(2-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6a**).

Compound **6a** was obtained as oil in 43% yield. ^{1}H-NMR δ (ppm): 2.60–2.65 (m, 4H); 2.86 (t, 2H); 3.13 (t, 2H); 3.43–3.48 (m, 4H); 3.72 (s, 3H); 4.22 (d, 1H); 4.52 (d, 1H); 4.72 (s, 2H); 5.82 (brs, 1H); 6.22–6.55 (m, 4H); 6.96–7.02 (m, 2H); 7.92–8.00 (m, 1H); 8.04 (s, 1H); 8.20 (s, 1H). MS: *m/z* 526 (M + 1). *Anal.* ($C_{25}H_{29}F_2N_9O_2$): C, H, N.

*5.1.5.2. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(2-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7a**).* Compound **7a** was obtained as oil in 7% yield. ^{1}H-NMR δ (ppm): 2.55–2.63 (m, 4H); 2.80 (t, 2H); 3.05 (t, 2H); 3.36–3.40 (m, 4H); 3.70 (s, 3H); 4.15 (d, 1H); 4.52 (d, 1H); 4.80 (s, 2H); 5.90 (brs, 1H); 6.20–6.58 (m, 4H); 6.96–6.99 (m, 2H); 7.92–8.00 (m, 1H); 8.02 (s, 1H); 8.18 (s, 1H). MS: *m/z* 526 (M + 1). *Anal*. ($C_{25}H_{29}F_2N_9O_2$): C, H, N.

*5.1.5.3. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(4-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6b**).* Compound **6b** was obtained as oil in 52% yield. ^{1}H-NMR δ (ppm): 2.60–2.65 (m, 4H); 2.81 (t, 2H); 3.11 (t, 2H); 3.42–3.47 (m, 4H); 3.70 (s, 3H); 4.18 (d, 1H); 4.59 (d, 1H); 4.65 (s, 2H); 5.90 (brs, 1H); 6.18 (d, 2H); 6.57 (d, 2H); 6.96–7.04 (m, 2H); 7.93–7.97(m, 1H); 8.06 (s, 1H); 8.19 (s, 1H). MS: *m/z* 526 (M + 1). *Anal*. ($C_{25}H_{29}F_2N_9O_2$): C, H, N.

*5.1.5.4. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(4-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7b**).* Compound **7b** was obtained as oil in 8% yield. ^{1}H-NMR δ (ppm): 2.55–2.65 (m, 4H); 2.80 (t, 2H); 3.05 (t, 2H); 3.36–3.40 (m, 4H); 3.70 (s, 3H); 4.18 (d, 1H); 4.56 (d, 1H); 4.72 (s, 2H); 5.88 (brs, 1H); 6.20 (d, 2H); 6.57 (d, 2H); 6.96–7.02 (m, 2H); 7.95–8.00 (m, 1H); 8.04 (s, 1H); 8.16 (s, 1H). MS: *m/z* 526 (M + 1). *Anal*. ($C_{25}H_{29}F_2N_9O_2$): C, H, N.

*5.1.5.5. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(4-nitrophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6c**).* Compound **6c** was obtained as oil in 45% yield. ^{1}H-NMR δ (ppm): 2.56–2.65 (m, 4H); 2.84 (t, 2H); 3.13 (t, 2H); 3.42–3.47 (m, 4H); 4.20 (d, 1H); 4.59 (d, 1H); 4.72 (s, 2H); 5.82 (brs, 1H); 6.75–6.84 (m, 4H); 7.43–7.46 (m, 1H); 8.06 (s, 1H); 8.12 (d, 2H); 8.16 (s, 1H). MS: *m/z* 541 (M + 1). *Anal*. ($C_{24}H_{26}F_2N_{10}O_3$): C, H, N.

*5.1.5.6. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(4-nitrophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7c**).* Compound **7c** was obtained as oil in 9% yield. ^{1}H-NMR δ (ppm): 2.53–2.62 (m, 4H); 2.82 (t, 2H); 3.08 (t, 2H); 3.38–3.42 (m, 4H); 4.13 (d, 1H); 4.50 (d, 1H); 4.84 (s, 2H); 5.93 (brs, 1H); 6.75–6.84 (m, 4H); 7.43–7.46 (m, 1H); 8.06 (s, 1H); 8.12 (d, 2H); 8.16 (s, 1H). MS: *m/z* 541 (M + 1). *Anal*. ($C_{24}H_{26}F_2N_{10}O_3$): C, H, N.

*5.1.5.7. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(3-trifluoromethylphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6d**).* Compound **6d** was obtained as oil in 49% yield. ^{1}H-NMR δ (ppm): 2.57–2.61 (m, 4H); 2.80 (t, 2H); 3.08 (t, 2H); 3.42–3.47 (m, 4H); 4.18 (d, 1H); 4.50 (d, 1H); 4.65 (s, 2H); 5.80 (brs, 1H); 6.28–6.60 (m, 4H); 6.93–6.98 (m, 2H); 7.95–8.01 (m, 1H); 8.07 (s, 1H); 8.21 (s, 1H). MS: *m/z* 564 (M + 1). *Anal*. ($C_{25}H_{26}F_5N_9O$): C, H, N.

*5.1.5.8. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(3-trifluoromethylphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7d**).* Compound **7d** was obtained as oil in 11% yield. ^{1}H-NMR δ (ppm): 2.58–2.65 (m, 4H); 2.80 (t, 2H); 3.08 (t, 2H); 3.36–3.40 (m, 4H); 4.18 (d, 1H); 4.50 (d, 1H); 4.73 (s, 2H); 5.95 (brs, 1H); 6.28–6.63 (m, 4H); 6.93–6.98 (m, 2H); 7.97–8.00 (m, 1H); 8.07 (s, 1H); 8.17 (s, 1H). MS: *m/z* 564 (M + 1). *Anal*. ($C_{25}H_{26}F_5N_9O$): C, H, N.

*5.1.5.9. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(2-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6e**).* Compound **6e** was obtained as oil in 48% yield. ^{1}H-NMR δ (ppm): 2.55–2.63 (m, 4H); 2.82 (t, 2H); 3.11 (t, 2H); 3.40–3.43 (m, 4H); 4.22 (d, 1H); 4.50 (d, 1H); 4.70 (s, 2H); 5.85 (brs, 1H); 6.22–6.56 (m, 4H); 6.90–6.94 (m, 2H); 7.93–8.00 (m, 1H); 8.08 (s, 1H); 8.15 (s, 1H). MS: *m/z* 529 (M + 1), 531 (M + 3). *Anal*. ($C_{24}H_{26}ClF_2N_9O$): C, H, N.

*5.1.5.10. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(2-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7e**).* Compound **7e** was obtained as oil in 11% yield. ^{1}H-NMR δ (ppm): 2.56–2.64 (m, 4H); 2.81 (t, 2H); 3.07 (t, 2H); 3.38–3.42 (m, 4H); 4.18 (d, 1H); 4.55 (d, 1H); 4.82 (s, 2H); 5.96 (brs, 1H); 6.22–6.56 (m, 4H); 6.93–6.94 (m, 2H); 7.95–8.02 (m, 1H); 8.03 (s, 1H); 8.17 (s, 1H). MS: *m/z* 529 (M + 1), 531 (M + 3). *Anal*. ($C_{24}H_{26}ClF_2N_9O$): C, H, N.

*5.1.5.11. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(3-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6f**).* Compound **6f** was obtained as oil in 49% yield. ^{1}H-NMR δ (ppm): 2.59–2.65 (m, 4H); 2.84 (t, 2H); 3.12 (t, 2H); 3.42–3.46 (m, 4H); 4.20 (d, 1H); 4.58 (d, 1H); 4.68 (s, 2H); 5.93 (brs, 1H); 6.18–6.50 (m, 4H); 6.90–6.97 (m, 2H); 7.98–8.04 (m, 1H); 8.10 (s, 1H); 8.21 (s, 1H). MS: *m/z* 529 (M + 1), 531 (M + 3). *Anal*. ($C_{24}H_{26}ClF_2N_9O$): C, H, N.

*5.1.5.12. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(3-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7f**).* Compound **7f** was obtained as oil in 8% yield. ^{1}H-NMR δ (ppm): 2.55–2.60 (m, 4H); 2.82 (t, 2H); 3.05 (t, 2H); 3.36–3.42 (m, 4H); 4.17 (d, 1H); 4.55 (d, 1H); 4.80 (s, 2H); 5.92 (brs, 1H); 6.18–6.50 (m, 4H); 6.90–6.95 (m, 2H); 7.98–8.02 (m, 1H); 8.05 (s, 1H); 8.19 (s, 1H). MS: *m/z* 529 (M + 1), 531 (M + 3). *Anal*. ($C_{24}H_{26}ClF_2N_9O$): C, H, N.

*5.1.5.13. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(4-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6g**).* Compound **6g** was obtained as oil in 45% yield. ^{1}H-NMR δ (ppm): 2.59–2.63 (m, 4H); 2.84 (t, 2H); 3.08 (t, 2H); 3.40–3.46 (m, 4H); 4.20 (d, 1H); 4.58 (d, 1H); 4.65 (s, 2H); 5.90 (brs, 1H); 6.20 (d, 2H); 6.68 (d, 2H); 6.92–6.99 (m, 2H); 7.94–8.02 (m, 1H); 8.09 (s, 1H); 8.19 (s, 1H). MS: *m/z* 529 (M + 1), 531 (M + 3). *Anal*. ($C_{24}H_{26}ClF_2N_9O$): C, H, N.

5.1.5.14. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(4-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-

*1-yl-propan-2-ol (**7g**).* Compound **7g** was obtained as oil in 10% yield. ^{1}H-NMR δ (ppm): 2.51–2.62 (m, 4H); 2.83 (t, 2H); 3.02 (t, 2H); 3.34–3.41 (m, 4H); 4.16 (d, 1H); 4.54 (d, 1H); 4.75 (s, 2H); 5.95 (brs, 1H); 6.20 (d, 2H); 6.75 (d, 2H); 6.92–6.97 (m, 2H); 7.94–8.01 (m, 1H); 8.04 (s, 1H); 8.14 (s, 1H). MS: *m/z* 529 (M + 1), 531 (M + 3). *Anal*. ($C_{24}H_{26}ClF_2N_9O$): C, H, N.

*5.1.5.15. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-benzyl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6h**).* Compound **6h** was obtained as oil in 46% yield. ^{1}H-NMR δ (ppm): 2.59–2.64 (m, 4H); 2.84 (t, 2H); 3.12 (t, 2H); 3.40–3.45 (m, 4H); 3.52 (s, 2H); 4.22 (d, 1H); 4.54 (d, 1H); 4.70 (s, 2H); 5.92 (s, 1H); 6.28–6.55 (m, 5H); 6.92–6.98 (m, 2H); 7.95–8.03 (m, 1H); 8.08 (s, 1H); 8.19 (s, 1H). MS: *m/z* 510 (M + 1). *Anal*. ($C_{25}H_{29}F_2N_9O$): C, H, N.

*5.1.5.16. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-benzyl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7h**).* Compound **7h** was obtained as oil in 10% yield. ^{1}H-NMR δ (ppm): 2.52–2.65 (m, 4H); 2.80 (t, 2H); 3.05 (t, 2H); 3.40–3.44 (m, 4H); 3.53 (s, 2H); 4.15 (d, 1H); 4.50 (d, 1H); 4.80 (s, 2H); 6.00 (brs, 1H); 6.28–6.50 (m, 5H); 6.90–6.95 (m, 2H); 7.95–8.02 (m, 1H); 8.05 (s, 1H); 8.15 (s, 1H). MS: *m/z* 510 (M + 1). *Anal*. ($C_{25}H_{29}F_2N_9O$): C, H, N.

*5.1.5.17. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(4-fluorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6i**).* Compound **6i** was obtained as oil in 50% yield. ^{1}H-NMR δ (ppm): 2.57–2.65 (m, 4H); 2.82 (t, 2H); 3.10 (t, 2H); 3.40–3.44 (m, 4H); 4.20 (d, 1H); 4.56 (d, 1H); 4.68 (s, 2H); 5.85 (brs, 1H); 6.25 (d, 2H); 6.60 (d, 2H); 6.93–6.98 (m, 2H); 7.99–8.04 (m, 1H); 8.10 (s, 1H); 8.20 (s, 1H). MS: *m/z* 514 (M + 1). *Anal*. ($C_{24}H_{26}F_3N_9O$): C, H, N.

*5.1.5.18. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(4-fluorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7i**).* Compound **7i** was obtained as oil in 9% yield. ^{1}H-NMR δ (ppm): 2.57–2.65 (m, 4H); 2.83 (t, 2H); 3.05 (t, 2H); 3.34–3.40 (m, 4H); 4.18 (d, 1H); 4.56 (d, 1H); 4.81 (s, 2H); 5.85 (brs, 1H); 6.25 (d, 2H); 6.63 (d, 2H); 6.90–6.97 (m, 2H); 7.99–8.03 (m, 1H); 8.06 (s, 1H); 8.22 (s, 1H). MS: *m/z* 514 (M + 1). *Anal*. ($C_{24}H_{26}F_3N_9O$): C, H, N.

*5.1.5.19. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(2,4-dichlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6j**).* Compound **6j** was obtained as oil in 44% yield. ^{1}H-NMR δ (ppm): 2.57–2.65 (m, 4H); 2.83 (t, 2H); 3.12 (t, 2H); 3.40–3.44 (m, 4H); 4.22 (d, 1H); 4.55 (d, 1H); 4.66 (s, 2H); 5.80 (brs, 1H); 6.21–6.58 (m, 3H); 6.90–6.98 (m, 2H); 7.97–8.03 (m, 1H); 8.10 (s, 1H); 8.22 (s, 1H). MS: *m/z* 564 (M + 1). *Anal*. ($C_{24}H_{25}Cl_2F_2N_9O$): C, H, N.

*5.1.5.20. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(2,4-dichlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7j**).* Compound **7j** was obtained as oil in 7% yield. ^{1}H-NMR δ (ppm): 2.52–2.62 (m, 4H); 2.77 (t, 2H); 3.05 (t, 2H); 3.33–3.41 (m, 4H); 4.12 (d, 1H); 4.56 (d, 1H); 4.73 (s, 2H); 5.94 (brs, 1H); 6.21–6.57 (m, 3H); 6.90–6.96 (m, 2H); 7.95–8.00 (m, 1H); 8.06 (s, 1H); 8.16 (s, 1H). MS: *m/z* 564 (M + 1). *Anal*. ($C_{24}H_{25}Cl_2F_2N_9O$): C, H, N.

*5.1.5.21. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(2-pyridyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6k**).* Compound **6k** was obtained as oil in 45% yield. ^{1}H-NMR δ (ppm): 2.50–2.66 (m, 4H); 2.86 (t, 2H); 3.14 (t, 2H); 3.42–3.48 (m, 4H); 4.22 (d, 1H); 4.56 (d, 1H); 4.66 (s, 2H); 6.00 (brs, 1H); 6.28–8.04 (m, 7H); 8.10 (s, 1H); 8.24 (s, 1H). MS: *m/z* 497 (M + 1). *Anal*. ($C_{23}H_{26}F_2N_{10}O$): C, H, N.

*5.1.5.22. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(2-pyridyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7k**).* Compound **7k** was obtained as oil in 8% yield. ^{1}H-NMR δ (ppm): 2.57–2.62 (m, 4H); 2.78 (t, 2H); 3.11 (t, 2H); 3.32–3.38 (m, 4H); 4.19 (d, 1H); 4.56 (d, 1H); 4.70 (s, 2H); 5.95 (brs, 1H); 6.30–8.00 (m, 7H); 8.05 (s, 1H); 8.20 (s, 1H). MS: *m/z* 497 (M + 1). *Anal*. ($C_{23}H_{26}F_2N_{10}O$): C, H, N.

*5.1.5.23. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(diphenylmethyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6l**).* Compound **6l** was obtained as oil in 42% yield. ^{1}H-NMR δ (ppm): 2.60–2.67 (m, 4H); 2.82 (t, 2H); 3.13 (t, 2H); 3.41–3.45 (m, 4H); 4.20 (d, 1H); 4.24 (s, 1H); 4.58 (d, 1H); 4.68 (s, 2H); 5.95 (brs, 1H); 6.24–6.50 (m, 10H); 6.90–6.97 (m, 2H); 8.00–8.05 (m, 1H); 8.08 (s, 1H); 8.21 (s, 1H). MS: *m/z* 586 (M + 1). *Anal*. ($C_{31}H_{33}F_2N_9O$): C, H, N.

*5.1.5.24. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(diphenylmethyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7l**).* Compound **7l** was obtained as oil in 9% yield. ^{1}H-NMR δ (ppm): 2.58–2.64 (m, 4H); 2.85 (t, 2H); 3.02 (t, 2H); 3.30–3.42 (m, 4H); 4.14 (d, 1H); 4.24 (s, 1H); 4.56 (d, 1H); 4.80 (s, 2H); 5.80 (brs, 1H); 6.22–6.56 (m, 10H); 6.90–6.97 (m, 2H); 8.01–8.05 (m, 1H); 8.06 (s, 1H); 8.20 (s, 1H). MS: *m/z* 586 (M + 1). *Anal*. ($C_{31}H_{33}F_2N_9O$): C, H, N.

*5.1.5.25. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(2-n-butoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6m**).* Compound **6m** was obtained as oil in 42% yield. ^{1}H-NMR δ (ppm): 0.95 (t, 3H); 1.72–1.77 (m, 4H); 2.60–2.67 (m, 4H); 2.85 (t, 2H); 3.10 (t, 2H); 3.40–3.46 (m, 4H); 4.05–4.10 (m, 2H); 4.20 (d, 1H); 4.58 (d, 1H); 4.68 (s, 2H); 5.90 (brs, 1H); 6.22–6.53 (m, 4H); 6.94–7.00 (m, 2H); 7.98–8.03 (m, 1H); 8.10 (s, 1H); 8.22 (s, 1H). MS: *m/z* 568 (M + 1). *Anal*. ($C_{28}H_{35}F_2N_9O$): C, H, N.

*5.1.5.26. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-(2-n-butoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7m**).* Compound **7m** was obtained as oil in 11% yield. ^{1}H-NMR δ (ppm): 0.96 (t, 3H); 1.70–1.75 (m, 4H); 2.58–2.67 (m, 4H); 2.77 (t, 2H); 3.08 (t, 2H); 3.38–3.41 (m, 4H); 4.05–4.10 (m, 2H); 4.15 (d, 1H); 4.50 (d, 1H); 4.77

(s, 2H); 5.83 (brs, 1H); 6.22–6.50 (m, 4H); 7.91–7.97 (m, 2H); 7.99–8.03 (m, 1H); 8.04 (s, 1H); 8.18 (s, 1H). MS: *m/z* 568 (M + 1). *Anal*. ($C_{28}H_{35}F_2N_9O$): C, H, N.

*5.1.5.27. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-phenyl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**6n**).* Compound **6n** was obtained as oil in 43% yield. ^{1}H-NMR δ (ppm): 2.59–2.65 (m, 4H); 2.84 (t, 2H); 3.10 (t, 2H); 3.42–3.45 (m, 4H); 4.20 (d, 1H); 4.56 (d, 1H); 4.68 (s, 2H); 5.90 (brs, 1H); 6.20–6.54 (m, 5H); 6.93–6.96 (m, 2H); 7.98–8.04 (m, 1H); 8.08 (s, 1H); 8.24 (s, 1H). MS: *m/z* 496 (M + 1). *Anal*. ($C_{24}H_{27}F_2N_9O$): C, H, N.

*5.1.5.28. 2-(2,4-Difluorophenyl)-1-(5-{2-[4-phenyl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-3-[1,2,4]-triazol-1-yl-propan-2-ol (**7n**).* Compound **7n** was obtained as oil in 11% yield. ^{1}H-NMR δ (ppm): 2.55–2.63 (m, 4H); 2.80 (t, 2H); 3.05 (t, 2H); 3.36–3.40 (m, 4H); 4.15 (d, 1H); 4.52 (d, 1H); 4.80 (s, 2H); 5.90 (brs, 1H); 6.20–6.54 (m, 5H); 6.93–6.96 (m, 2H); 7.95–8.00 (m, 1H); 8.03 (s, 1H); 8.16 (s, 1H). MS: *m/z* 496 (M + 1). *Anal*. ($C_{24}H_{27}F_2N_9O$): C, H, N.

*5.1.6. General procedure for the synthesis of 1-(2,4-difluorophenyl)-2-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9a–n**) and 1-(2,4-difluorophenyl)-2-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10a–n**)*

To a solution of 2-chloro-1-(2,4-difluorophenyl)-propan-1-one (**8**, 1.197 mmol) in DMSO (20 ml) under nitrogen atmosphere, NaH (2.87 mmol) pre-washed with hexane, was added at 25–30 °C under stirring. After 1 h, a solution of 1-aryl-4-[2-(1*H*-tetrazol-5-yl)-ethyl]-piperazine (**5**, 2.40 mmol) in DMSO (30 ml) was added to it at 5 °C. The reaction mixture was initially stirred at 25–30 °C for 2 h then at 60 °C for 4 h. After completion of reaction, the reaction mixture was cooled to 25–30 °C and poured into chilled water (150 ml). The reaction mixture was extracted with ethyl acetate (2 × 50 ml), the combined organic layer was washed with water (50 ml), brine (50 ml) and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give a mixture of **9a–n** and **10a–n**, which were separated by flash column chromatography over silica gel (230–400 mesh) using ethyl acetate/hexane (80:20) as eluent.

*5.1.6.1. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(2-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9a**).* Compound **9a** was obtained as oil in 62% yield. ^{1}H-NMR δ (ppm): 1.00 (d, 3H); 2.56–2.63 (m, 8H); 3.12–3.20 (m, 4H); 3.71 (s, 3H); 5.62 (q, 1H); 6.22–6.99 (m, 6H); 8.08–8.14 (m, 1H). MS: *m/z* 457 (M + 1).

*5.1.6.2. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(2-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10a**).* Compound **10a** was obtained as oil in 12% yield. ^{1}H-NMR δ (ppm): 0.98 (d, 3H); 2.54–2.61 (m, 8H); 3.05–3.15 (m, 4H); 3.72 (s, 3H); 5.87 (q, 1H); 6.22–6.95 (m, 6H); 8.10–8.15 (m, 1H). MS: *m/z* 457 (M + 1).

*5.1.6.3. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(4-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9b**).* Compound **9b** was obtained as oil in 64% yield. ^{1}H-NMR δ (ppm): 1.10 (d, 3H); 2.57–2.62 (m, 8H); 3.11–3.17 (m, 4H); 3.70 (s, 3H); 5.62 (q, 1H); 6.24 (d, 2H); 6.62 (d, 2H); 6.94–7.03 (m, 2H); 8.12–8.16 (m, 1H). MS: *m/z* 457 (M + 1).

*5.1.6.4. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(4-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10b**).* Compound **10b** was obtained as oil in 13% yield. ^{1}H-NMR δ (ppm): 1.06 (d, 3H); 2.48–2.51 (m, 8H); 3.12–3.18 (m, 4H); 3.72 (s, 3H); 5.92 (q, 1H); 6.24 (d, 2H); 6.55 (d, 2H); 6.94–7.00 (m, 2H); 8.12–8.16 (m, 1H). MS: *m/z* 457 (M + 1).

*5.1.6.5. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(4-nitrophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9c**).* Compound **9c** was obtained as oil in 61% yield. ^{1}H-NMR δ (ppm): 1.07 (d, 3H); 2.54–2.57 (m, 8H); 3.05–3.15 (m, 4H); 5.58 (q, 1H); 6.77–6.88 (m, 4H); 7.34–7.42 (m, 1H); 8.12 (d, 2H); 8.14–8.16 (m, 1H). MS: *m/z* 472 (M + 1).

*5.1.6.6. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(4-nitrophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10c**).* Compound **10c** was obtained as oil in 13% yield. ^{1}H-NMR δ (ppm): 1.00 (d, 3H); 2.51–2.57 (m, 8H); 3.09–3.18 (m, 4H); 5.85 (q, 1H); 6.75–6.82 (m, 4H); 7.41–7.44 (m, 1H); 8.10 (d, 2H); 8.12–8.18 (m, 1H). MS: *m/z* 472 (M + 1).

*5.1.6.7. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(3-trifluoromethylphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9d**).* Compound **9d** was obtained as oil in 68% yield. ^{1}H-NMR δ (ppm): 1.02 (d, 3H); 2.50–2.56 (m, 8H); 3.00–3.10 (m, 4H); 5.57 (q, 1H); 6.30–6.95 (m, 6H); 8.12–8.15 (m, 1H). MS: *m/z* 495 (M + 1).

*5.1.6.8. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(3-trifluoromethylphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10d**).* Compound **10d** was obtained as oil in 15% yield. ^{1}H-NMR δ (ppm): 0.98 (d, 3H); 2.52–2.60 (m, 8H); 3.07–3.15 (m, 4H); 5.88 (q, 1H); 6.28–6.97 (m, 6H); 8.08–8.12 (m, 1H). MS: *m/z* 495 (M + 1).

*5.1.6.9. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(2-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9e**).* Compound **9e** was obtained as oil in 66% yield. ^{1}H-NMR δ (ppm): 1.08 (d, 3H); 2.58–2.61 (m, 8H); 3.12–3.17 (m, 4H); 5.61 (q, 1H); 6.25–6.92 (m, 6H); 8.08–8.13 (m, 1H). MS: *m/z* 461 (M + 1), 463 (M + 3).

*5.1.6.10. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(2-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10e**).* Compound **10e** was obtained as oil in 14% yield. ^{1}H-NMR δ (ppm): 1.01 (d, 3H); 2.50–2.55 (m, 8H); 3.07–3.13 (m, 4H); 5.88 (q, 1H); 6.28–6.90 (m, 6H); 8.10–8.14 (m, 1H). MS: *m/z* 461 (M + 1), 463 (M + 3).

*5.1.6.11. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(3-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9f**).*

Compound **9f** was obtained as oil in 67% yield. ^{1}H-NMR δ (ppm): 1.00 (d, 3H); 2.56–2.63 (m, 8H); 3.07–3.15 (m, 4H); 5.57 (q, 1H); 6.25–6.93 (m, 6H); 8.06–8.10 (m, 1H). MS: *m/z* 461 (M + 1), 463 (M + 3).

*5.1.6.12. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(3-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10f**).* Compound **10f** was obtained as oil in 12% yield. ^{1}H-NMR δ (ppm): 1.07 (d, 3H); 2.52–2.57 (m, 8H); 3.06–3.15 (m, 4H); 5.89 (q, 1H); 6.25–6.95 (m, 6H); 8.14–8.18 (m, 1H). MS: *m/z* 461 (M + 1), 463 (M + 3).

*5.1.6.13. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(4-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9g**).* Compound **9g** was obtained as oil in 68% yield. ^{1}H-NMR δ (ppm): 1.03 (d, 3H); 2.56–2.62 (m, 8H); 3.07–3.15 (m, 4H); 5.62 (q, 1H); 6.19 (d, 2H); 6.70 (d, 2H); 6.92–6.98 (m, 2H); 8.08–8.12 (m, 1H). MS: *m/z* 461 (M + 1), 463 (M + 3).

*5.1.6.14. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(4-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10g**).* Compound **10g** was obtained as oil in 14% yield. ^{1}H-NMR δ (ppm): 0.97 (d, 3H); 2.48–2.55 (m, 8H); 3.09–3.18 (m, 4H); 5.90 (q, 1H); 6.15 (d, 2H); 6.70 (d, 2H); 6.90–6.99 (m, 2H); 8.12–8.16 (m, 1H). MS: *m/z* 461 (M + 1), 463 (M + 3).

*5.1.6.15. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-benzyl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9h**).* Compound **9h** was obtained as oil in 67% yield. ^{1}H-NMR δ (ppm): 1.05 (d, 3H); 2.55–2.60 (m, 8H); 3.09–3.15 (m, 4H); 3.50 (s, 2H); 5.61 (q, 1H); 6.30–6.96 (m, 7H); 8.08–8.12 (m, 1H). MS: *m/z* 441 (M + 1).

*5.1.6.16. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-benzyl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10h**).* Compound **10h** was obtained as oil in 14% yield. ^{1}H-NMR δ (ppm): 0.99 (d, 3H); 2.47–2.57 (m, 8H); 3.04–3.15 (m, 4H); 3.52 (s, 2H); 5.90 (q, 1H); 6.30–6.93 (m, 7H); 8.12–8.15 (m, 1H). MS: *m/z* 441 (M + 1).

*5.1.6.17. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(4-fluorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9i**).* Compound **9i** was obtained as oil in 65% yield. ^{1}H-NMR δ (ppm): 1.07 (d, 3H); 2.54–2.57 (m, 8H); 3.09–3.17 (m, 4H); 5.60 (q, 1H); 6.48–7.10 (m, 6H); 8.10–8.14 (m, 1H). MS: *m/z* 445 (M + 1).

*5.1.6.18. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(4-fluorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10i**).* Compound **10i** was obtained as oil in 13% yield. ^{1}H-NMR δ (ppm): 1.05 (d, 3H); 2.50–2.57 (m, 8H); 3.09–3.18 (m, 4H); 5.90 (q, 1H); 6.50–7.00 (m, 6H); 8.12–8.18 (m, 1H). MS: *m/z* 445 (M + 1).

*5.1.6.19. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(2,4-dichlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9j**).* Compound **9j** was obtained as oil in 69% yield. ^{1}H-NMR δ (ppm): 1.07 (d, 3H); 2.53–2.58 (m, 8H); 3.11–3.18 (m, 4H); 5.61 (q, 1H); 6.20–6.52 (m, 3H); 6.95–6.99 (m, 2H); 8.10–8.15 (m, 1H). MS: *m/z* 495 (M + 1).

*5.1.6.20. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(2,4-dichlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10j**).* Compound **10j** was obtained as oil in 15% yield. ^{1}H-NMR δ (ppm): 1.00 (d, 3H); 2.48–2.55 (m, 8H); 3.09–3.15 (m, 4H); 5.90 (q, 1H); 6.20–6.95 (m, 5H); 8.10–8.15 (m, 1H). MS: *m/z* 495 (M + 1).

*5.1.6.21. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(2-pyridyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9k**).* Compound **9k** was obtained as oil in 64% yield. ^{1}H-NMR δ (ppm): 1.10 (d, 3H); 2.55–2.60 (m, 8H); 3.10–3.17 (m, 4H); 5.63 (q, 1H); 6.28–6.98 (m, 6H); 8.12–8.17 (m, 1H). MS: *m/z* 428 (M + 1).

*5.1.6.22. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(2-pyridyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10k**).* Compound **10k** was obtained as oil in 15% yield. ^{1}H-NMR δ (ppm): 1.02 (d, 3H); 2.52–2.58 (m, 8H); 3.06–3.15 (m, 4H); 5.88 (q, 1H); 6.28–6.94 (m, 6H); 8.12–8.18 (m, 1H). MS: *m/z* 428 (M + 1).

*5.1.6.23. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(diphenylmethyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9l**).* Compound **9l** was obtained as oil in 69% yield. ^{1}H-NMR δ (ppm): 1.05 (d, 3H); 2.50–2.57 (m, 8H); 3.07–3.14 (m, 4H); 4.20 (s, 1H); 5.60 (q, 1H); 6.18–7.00 (m, 12H); 8.10–8.15 (m, 1H). MS: *m/z* 517 (M + 1).

*5.1.6.24. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(diphenylmethyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10l**).* Compound **10l** was obtained as oil in 14% yield. ^{1}H-NMR δ (ppm): 0.99 (d, 3H); 2.48–2.55 (m, 8H); 3.02–3.08 (m, 4H); 4.18 (s, 1H); 5.87 (q, 1H); 6.20–6.99 (m, 12H); 8.12–8.16 (m, 1H). MS: *m/z* 517 (M + 1).

*5.1.6.25. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(2-n-butoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9m**).* Compound **9m** was obtained as oil in 68% yield. ^{1}H-NMR δ (ppm): 0.97–0.99 (m, 6H); 1.07–1.74 (m, 4H); 2.54–2.59 (m, 8H); 3.00–3.08 (m, 4H); 4.08–4.18 (m, 2H); 5.58 (q, 1H); 6.25–7.00 (m, 6H); 8.08–8.11 (m, 1H). MS: *m/z* 480 (M + 1).

*5.1.6.26. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-(2-n-butoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10m**).* Compound **10m** was obtained as oil in 12% yield. ^{1}H-NMR δ (ppm): 0.97–0.99 (m, 6H); 1.08–1.75 (m, 4H); 2.55–2.61 (m, 8H); 3.02–3.10 (m, 4H); 4.08–4.19 (m, 2H); 5.91 (q, 1H); 6.26–7.00 (m, 6H); 8.06–8.12 (m, 1H). MS: *m/z* 480 (M + 1).

*5.1.6.27. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-phenyl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9n**).* Compound **9n** was obtained as oil in 63% yield. ^{1}H-NMR δ (ppm): 1.03 (d, 3H); 2.52–2.57 (m, 8H); 3.01–3.12 (m, 4H);

5.59 (q, 1H); 6.26–6.93 (m, 7H); 8.08–8.12 (m, 1H). MS: *m/z* (M + 1) 427.

*5.1.6.28. 1-(2,4-Difluorophenyl)-2-(5-{2-[4-phenyl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10n**).* Compound **10n** was obtained as oil in 14% yield. ^{1}H-NMR δ (ppm): 0.99 (d, 3H); 2.52–2.60 (m, 8H); 3.06–3.14 (m, 4H); 5.92 (q, 1H); 6.27–6.97 (m, 7H); 8.14–8.18 (m, 1H). MS: *m/z* 427 (M + 1).

*5.1.7. General procedure for the synthesis of 2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12a–n**)*

To a suspension of NaH (0.33 mmol), pre-washed with hexane, in DMSO (5.0 ml) at 5 °C, TMSI (0.327 mmol) was added and the reaction mixture was stirred at 25–30 °C. After 1.5 h a solution of 1-(2,4-difluorophenyl)-2-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-propan-1-one (**9a–n**) (0.27 mmol) in DMSO (10 ml) was added and the reaction mixture was stirred at 80–90 °C for 6 h. After completion of reaction the reaction mixture was cooled and poured into chilled water (40 ml) and the product was extracted with ethyl acetate (2 × 25 ml). The combined organic layer was washed with water (25 ml), brine (25 ml), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give 1-[2-(2-{1-[2-(2,4-difluorophenyl)-oxiranyl]-ethyl}-2*H*-tetrazol-5-yl)-ethyl]-4-aryl-piperazines (**11a–n**) in 75–85% yields. These oxiranes were unstable, so subjected for the next step immediately without further purification.

1,2,4-Triazole (0.654 mmol) was added to a suspension of NaH (0.77 mmol), pre-washed with hexane, in DMF (10 ml) at 25–30 °C under stirring. The stirring was continued for 1 h. To this 1-[2-(2-{1-[2-(2,4-difluorophenyl)-oxiranyl]-ethyl}-2*H*-tetrazol-5-yl)-ethyl]-4-aryl-piperazines (**11a–n**) (0.654 mmol) was added and the reaction mixture was stirred at 100 °C for 5 h. The reaction mixture was cooled to 25–30 °C and poured into chilled water (20 ml) and extracted with ethyl acetate (2 × 25 ml). The combined organic layer was washed with water (15 ml), brine (15 ml), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated to give crude 2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12a–n**), which was purified by flash column chromatography over silica gel (230–400 mesh) using 10% methanol/ethyl acetate as eluent.

*5.1.7.1. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12a**).* Compound **12a** was obtained as oil in 52% yield. ^{1}H-NMR δ (ppm): 1.00 (d, 3H); 1.29 (s, 1H); 2.68–2.71 (m, 4H); 2.92–2.98 (m, 2H); 3.12–3.22 (m, 2H); 3.39–3.45 (m, 4H); 3.71 (s, 3H); 4.06 (q, 1H); 4.76 (d, 1H); 4.96 (d, 1H); 6.22–6.58 (m, 4H); 6.96–6.99 (m, 2H); 7.93–8.00 (m, 1H); 8.02 (s, 1H); 8.18 (s, 1H). MS: *m/z* 540 (M + 1). *Anal*. ($C_{26}H_{31}F_2N_9O_2$): C, H, N.

*5.1.7.2. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(4-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12b**).* Compound **12b** was obtained as oil in 53% yield. ^{1}H-NMR δ (ppm): 0.97 (d, 3H); 1.31 (s, 1H); 2.65–2.72 (m, 4H); 2.92–2.99 (m, 2H); 3.10–3.22 (m, 2H); 3.35–3.44 (m, 4H); 3.70 (s, 3H); 4.10 (q, 1H); 4.78 (d, 1H); 4.92 (d, 1H); 6.20 (d, 2H); 6.59 (d, 2H); 6.94–7.01 (m, 2H); 7.91–8.00 (m, 1H); 8.04 (s, 1H); 8.16 (s, 1H). MS: *m/z* 540 (M + 1). *Anal*. ($C_{26}H_{31}F_2N_9O_2$): C, H, N.

*5.1.7.3. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(4-nitrophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12c**).* Compound **12c** was obtained as oil in 62% yield. ^{1}H-NMR δ (ppm): 0.98 (d, 3H); 1.25 (s, 1H); 2.65–2.70 (m, 4H); 2.92–2.97 (m, 2H); 3.12–3.20 (m, 2H); 3.37–3.40 (m, 4H); 4.08 (q, 1H); 4.75 (d, 1H); 4.89 (d, 1H); 6.77–6.88 (m, 4H); 7.34–7.42 (m, 1H); 7.80 (s, 1H); 7.97 (s, 1H); 8.12 (d, 2H). MS: *m/z* 555 (M + 1). *Anal*. ($C_{25}H_{28}F_2N_{10}O_3$): C, H, N.

*5.1.7.4. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(3-trifluoromethylphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12d**).* Compound **12d** was obtained as oil in 61% yield. ^{1}H-NMR δ (ppm): 0.99 (d, 3H); 1.33 (s, 1H); 2.65–2.72 (m, 4H); 2.90–2.95 (m, 2H); 3.10–3.22 (m, 2H); 3.35–3.38 (m, 4H); 4.07 (q, 1H); 4.74 (d, 1H); 4.88 (d, 1H); 6.30–6.62 (m, 4H); 6.95–7.00 (m, 2H); 7.98–8.02 (m, 1H); 8.05 (s, 1H); 8.18 (s, 1H). MS: *m/z* 592 (M + 1). *Anal*. ($C_{26}H_{28}F_5N_9O$): C, H, N.

*5.1.7.5. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12e**).* Compound **12e** was obtained as oil in 59% yield. ^{1}H-NMR δ (ppm): 1.00 (d, 3H); 1.35 (s, 1H); 2.65–2.71 (m, 4H); 2.90–2.95 (m, 2H); 3.12–3.18 (m, 2H); 3.33–3.38 (m, 4H); 4.08 (q, 1H); 4.75 (d, 1H); 4.89 (d, 1H); 6.25–6.45 (m, 4H); 6.90–6.93 (m, 2H); 7.92–7.97 (m, 1H); 8.04 (s, 1H); 8.18 (s, 1H). MS: *m/z* 544 (M + 1), 546 (M + 3). *Anal*. ($C_{25}H_{28}ClF_2N_9O$): C, H, N.

*5.1.7.6. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(3-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12f**).* Compound **12f** was obtained as oil in 55% yield. ^{1}H-NMR δ (ppm): 0.99 (d, 3H); 1.28 (s, 1H); 2.60–2.65 (m, 4H); 2.90–2.95 (m, 2H); 3.08–3.16 (m, 2H); 3.30–3.35 (m, 4H); 4.06 (q, 1H); 4.77 (d, 1H); 4.86 (d, 1H); 6.25–6.50 (m, 4H); 6.90–6.94 (m, 2H); 7.90–7.97 (m, 1H); 8.04 (s, 1H); 8.15 (s, 1H). MS: *m/z* 544 (M + 1), 546 (M + 3). *Anal*. ($C_{25}H_{28}ClF_2N_9O$): C, H, N.

*5.1.7.7. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(4-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12g**).* Compound **12g** was obtained as oil in 61% yield. ^{1}H-NMR δ (ppm): 1.01 (d, 3H); 1.25 (s, 1H); 2.67–2.72 (m, 4H); 2.92–2.95 (m, 2H); 3.10–3.18 (m, 2H); 3.37–3.42 (m, 4H); 4.08 (q, 1H); 4.75 (d, 1H); 4.86 (d, 1H); 6.19 (d, 2H); 6.70 (d, 2H); 6.94–6.98 (m, 2H); 7.96–8.02 (m,

1H); 8.03 (s, 1H); 8.16 (s, 1H). MS: *m/z* 544 (M + 1), 546 (M + 3). *Anal*. ($C_{25}H_{28}ClF_2N_9O$): C, H, N.

*5.1.7.8. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-benzyl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12h**).* Compound **12h** was obtained as oil in 61% yield. ^{1}H-NMR δ (ppm): 0.99 (d, 3H); 1.28 (s, 1H); 2.63–2.68 (m, 4H); 2.90–2.95 (m, 2H); 3.12–3.18 (m, 2H); 3.37–3.40 (m, 4H); 3.50 (s, 2H); 4.08 (q, 1H); 4.75 (d, 1H); 4.88 (d, 1H); 6.30–6.56 (m, 5H); 6.92–6.97 (m, 2H); 7.95–8.02 (m, 1H); 8.04 (s, 1H); 8.18 (s, 1H). MS: *m/z* 524 (M + 1). *Anal*. ($C_{26}H_{31}F_2N_9O$): C, H, N.

*5.1.7.9. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(4-fluorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12i**).* Compound **12i** was obtained as oil in 58% yield. ^{1}H-NMR δ (ppm): 0.99 (d, 3H); 1.26 (s, 1H); 2.66–2.71 (m, 4H); 2.80–2.83 (m, 2H); 3.06–3.12 (m, 2H); 3.37–3.40 (m, 4H); 4.10 (q, 1H); 4.78 (d, 1H); 4.98 (d, 1H); 6.28 (d, 2H); 6.60 (d, 2H); 6.93–6.99 (m, 2H); 7.99–8.02 (m, 1H); 8.05 (s, 1H); 8.22 (s, 1H). MS: *m/z* 528 (M + 1). *Anal*. ($C_{25}H_{28}F_3N_9O$): C, H, N.

*5.1.7.10. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2,4-dichlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12j**).* Compound **12j** was obtained as oil in 60% yield. ^{1}H-NMR δ (ppm): 0.98 (d, 3H); 1.24 (s, 1H); 2.65–2.71 (m, 4H); 2.80–2.85 (m, 2H); 3.05–3.09 (m, 2H); 3.35–3.42 (m, 4H); 4.10 (q, 1H); 4.78 (d, 1H); 4.92 (d, 1H); 6.20–6.52 (m, 3H); 6.95–6.99 (m, 2H); 7.95–8.01 (m, 1H); 8.04 (s, 1H); 8.18 (s, 1H). MS: *m/z* 578 (M + 1). *Anal*. ($C_{25}H_{27}Cl_2F_2N_9O$): C, H, N.

*5.1.7.11. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2-pyridyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12k**).* Compound **12k** was obtained as oil in 53% yield. ^{1}H-NMR δ (ppm): 0.98 (d, 3H); 1.25 (s, 1H); 2.68–2.71 (m, 4H); 2.94–2.98 (m, 2H); 3.14–3.20 (m, 2H); 3.39–3.44 (m, 4H); 4.12 (q, 1H); 4.74 (d, 1H); 4.86 (d, 1H); 6.28–6.48 (m, 3H); 6.94–6.98 (m, 2H); 7.99–8.02 (m, 2H); 8.06 (s, 1H); 8.22 (s, 1H). MS: *m/z* 512 (M + 1). *Anal*. ($C_{24}H_{28}F_2N_{10}O$): C, H, N.

*5.1.7.12. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(4-(diphenylmethyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12l**).* Compound **12l** was obtained as oil in 60% yield. ^{1}H-NMR δ (ppm): 0.97 (d, 3H); 1.36 (s, 1H); 2.68–2.74 (m, 4H); 2.90–2.99 (m, 2H); 3.10–3.24 (m, 2H); 3.40–3.45 (m, 4H); 4.12 (q, 1H); 4.18 (s, 1H) 4.74 (d, 1H); 4.93 (d, 1H); 6.18–6.64 (m, 10H); 6.94–7.02 (m, 2H); 8.02–8.05 (m, 1H); 8.10 (s, 1H); 8.20 (s, 1H). MS: *m/z* 600 (M + 1). *Anal*. ($C_{32}H_{35}F_2N_9O$): C, H, N.

*5.1.7.13. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2-n-butoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12m**).* Compound **12m** was obtained as oil in 61% yield. ^{1}H-NMR δ (ppm): 0.97 (t, 3H); 1.00 (d, 3H); 1.28 (s, 1H); 1.70–1.74 (m, 4H); 2.57–2.64 (m, 4H); 2.81–3.10 (m, 4H); 3.38–3.42 (m, 4H); 4.08–4.12 (m, 2H); 4.12 (q, 1H); 4.63 (d, 1H); 4.88 (d, 1H); 6.25–6.54 (m, 4H); 7.40–7.45 (m, 2H); 7.96–8.00 (m, 1H); 8.04 (s, 1H); 8.18 (s, 1H). MS: *m/z* 582 (M + 1). *Anal*. ($C_{29}H_{37}F_2N_9O_2$): C, H, N.

*5.1.7.14. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(4-phenyl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12n**).* Compound **12n** was obtained as oil in 51% yield. ^{1}H-NMR δ (ppm): 0.98 (d, 3H); 1.30 (s, 1H); 2.60–2.66 (m, 4H); 2.90–2.95 (m, 2H); 3.09–3.15 (m, 2H); 3.30–3.35 (m, 4H); 4.08 (q, 1H); 4.77 (d, 1H); 4.90 (d, 1H); 6.26–6.48 (m, 5H); 6.90–6.95 (m, 2H); 7.90–8.00 (m, 1H); 8.06 (s, 1H); 8.18 (s, 1H). MS: *m/z* 510 (M + 1). *Anal*. ($C_{25}H_{29}F_2N_9O$): C, H, N.

*5.1.8. General procedure for the synthesis of 2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14a–n**)*

These compounds **14a–n** were prepared using same method as described for compound **12a–n** using 1-(2,4-difluorophenyl)-2-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-propan-1-one (**10a–n**).

*5.1.8.1. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14a**).* Compound **14a** was obtained as oil in 56% yield. ^{1}H-NMR δ (ppm): 0.99 (d, 3H); 1.28 (s, 1H); 2.57–2.63 (m, 4H); 2.78–2.83 (m, 2H); 3.05–3.09 (m, 2H); 3.37–3.40 (m, 4H); 3.71 (s, 3H); 4.14 (q, 1H); 4.37 (d, 1H); 4.58 (d, 1H); 6.22–6.55 (m, 4H); 6.95–6.99 (m, 2H); 7.97–8.00 (m, 1H); 8.02 (s, 1H); 8.17 (s, 1H). MS: *m/z* 540 (M + 1). *Anal*. ($C_{26}H_{31}F_2N_9O_2$): C, H, N.

*5.1.8.2. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(4-methoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14b**).* Compound **14b** was obtained as oil in 64% yield. ^{1}H-NMR δ (ppm): 0.99 (d, 3H); 1.27 (s, 1H); 2.57–2.63 (m, 4H); 2.64–3.01 (m, 4H); 3.09–3.17 (m, 4H); 3.72 (s, 3H); 4.15 (q, 1H); 4.38 (d, 1H); 5.16 (d, 1H); 6.21 (d, 2H); 6.55 (d, 2H); 6.94–6.99 (m, 2H); 7.97–8.01 (m, 1H); 8.04 (s, 1H); 8.17 (s, 1H). MS: *m/z* 540 (M + 1). *Anal*. ($C_{26}H_{31}F_2N_9O_2$): C, H, N.

*5.1.8.3. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(4-nitrophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14c**).* Compound **14c** was obtained as oil in 57% yield. ^{1}H-NMR δ (ppm): 0.99 (d, 3H); 1.25 (s, 1H); 2.66–2.70 (m, 4H); 2.80–2.84 (m, 2H); 3.06–3.11 (m, 2H); 3.37–3.40 (m, 4H); 4.15 (q, 1H); 4.63 (d, 1H); 4.89 (d, 1H); 6.75–6.84 (m, 4H); 7.41–7.43 (m, 1H); 7.85 (s, 1H); 8.05 (s, 1H); 8.12 (d, 2H). MS: *m/z* 555 (M + 1). *Anal*. ($C_{25}H_{28}F_2N_{10}O_3$): C, H, N.

*5.1.8.4. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(3-trifluoromethylphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14d**).* Compound **14d** was obtained as oil in 59% yield. ^{1}H-NMR δ (ppm): 1.00 (d, 3H); 1.30 (s, 1H); 2.66–2.70 (m, 4H); 2.80–2.84 (m, 2H); 3.06–3.11 (m,

2H); 3.36–3.40 (m, 4H); 4.17 (q, 1H); 4.63 (d, 1H); 4.82 (d, 1H); 6.28–6.60 (m, 4H); 6.95–6.99 (m, 2H); 7.98–8.00 (m, 1H); 8.05 (s, 1H); 8.18 (s, 1H). MS: *m/z* 592 (M + 1). *Anal*. ($C_{26}H_{28}F_5N_9O$): C, H, N.

*5.1.8.5. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2-chlorohenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14e**).* Compound **14e** was obtained as oil in 63% yield. ^{1}H-NMR δ (ppm): 0.98 (d, 3H); 1.33 (s, 1H); 2.60–2.65 (m, 4H); 2.80–2.83 (m, 2H); 3.00–3.06 (m, 2H); 3.34–3.37 (m, 4H); 4.18 (q, 1H); 4.35 (d, 1H); 4.58 (d, 1H); 6.28–6.48 (m, 4H); 6.90–6.94 (m, 2H); 7.95–7.99 (m, 1H); 8.02 (s, 1H); 8.15 (s, 1H). MS: *m/z* 544 (M + 1), 546 (M + 3). *Anal*. ($C_{25}H_{28}ClF_2N_9O$): C, H, N.

*5.1.8.6. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(3-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14f**).* Compound **14f** was obtained as oil in 57% yield. ^{1}H-NMR δ (ppm): 1.01 (d, 3H); 1.30 (s, 1H); 2.60–2.65 (m, 4H); 2.85–2.89 (m, 2H); 3.00–3.09 (m, 2H); 3.34–3.38 (m, 4H); 4.15 (q, 1H); 4.36 (d, 1H); 4.58 (d, 1H); 6.25–6.50 (m, 4H); 6.90–6.95 (m, 2H); 7.92–8.00 (m, 1H); 8.04 (s, 1H); 8.17 (s, 1H). MS: *m/z* 544 (M + 1), 546 (M + 3). *Anal*. ($C_{25}H_{28}ClF_2N_9O$): C, H, N.

*5.1.8.7. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(4-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14g**).* Compound **14g** was obtained as oil in 55% yield. ^{1}H-NMR δ (ppm): 0.98 (d, 3H); 1.28 (s, 1H); 2.66–2.72 (m, 4H); 2.82–2.86 (m, 2H); 3.04–3.09 (m, 2H); 3.38–3.41 (m, 4H); 4.17 (q, 1H); 4.36 (d, 1H); 4.58 (d, 1H); 6.15 (d, 2H); 6.70 (d, 2H); 6.92–6.97 (m, 2H); 7.96–8.01 (m, 1H); 8.04 (s, 1H); 8.16 (s, 1H). MS: *m/z* 544 (M + 1), 546 (M + 3). *Anal*. ($C_{25}H_{28}ClF_2N_9O$): C, H, N.

*5.1.8.8. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-benzyl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14h**).* Compound **14h** was obtained as oil in 61% yield. ^{1}H-NMR δ (ppm): 0.97 (d, 3H); 1.26 (s, 1H); 2.66–2.72 (m, 4H); 2.82–2.85 (m, 2H); 3.08–3.12 (m, 2H); 3.39–3.42 (m, 4H); 3.51 (s, 2H); 4.15 (q, 1H); 4.35 (d, 1H); 4.58 (d, 1H); 6.30–6.60 (m, 5H); 6.90–6.95 (m, 2H); 7.95–8.02 (m, 1H); 8.05 (s, 1H); 8.18 (s, 1H). MS: *m/z* 524 (M + 1). *Anal*. ($C_{26}H_{31}F_2N_9O$): C, H, N.

*5.1.8.9. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(4-fluorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14i**).* Compound **14i** was obtained as oil in 56% yield. ^{1}H-NMR δ (ppm): 0.98 (d, 3H); 1.24 (s, 1H); 2.65–2.70 (m, 4H); 2.92–2.97 (m, 2H); 3.10–3.16 (m, 2H); 3.35–3.40 (m, 4H); 4.16 (q, 1H); 4.63 (d, 1H); 4.82 (d, 1H); 6.28 (d, 2H); 6.60 (d, 2H); 6.95–6.99 (m, 2H); 7.98–8.03 (m, 1H); 8.06 (s, 1H); 8.21 (s, 1H). MS: *m/z* 528 (M + 1). *Anal*. ($C_{25}H_{28}F_3N_9O$): C, H, N.

*5.1.8.10. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2,4-dichlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14j**).* Compound **14j** was obtained as oil in 55% yield. ^{1}H-NMR δ (ppm): 1.00 (d, 3H); 1.23 (s, 1H); 2.65–2.68 (m, 4H); 2.80–2.86 (m, 2H); 3.03–3.10 (m, 2H); 3.34–3.39 (m, 4H); 4.16 (q, 1H); 4.38 (d, 1H); 4.58 (d, 1H); 6.20–6.55 (m, 3H); 6.93–6.97 (m, 2H); 7.94–8.02 (m, 1H); 8.04 (s, 1H); 8.16 (s, 1H). MS: *m/z* 578 (M + 1). *Anal*. ($C_{25}H_{27}Cl_2F_2N_9O$): C, H, N.

*5.1.8.11. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2-pyridyl)-piperazin-1-yl]-ethyl)-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14k**).* Compound **14k** was obtained as oil in 63% yield. ^{1}H-NMR δ (ppm): 0.99 (d, 3H); 1.33 (s, 1H); 2.68–2.72 (m, 4H); 2.82–2.85 (m, 2H); 3.07–3.10 (m, 2H); 3.38–3.44 (m, 4H); 4.17 (q, 1H); 4.63 (d, 1H); 4.82 (d, 1H); 6.28–6.44 (m, 3H); 6.94–6.98 (m, 2H); 7.99–8.02 (m, 2H); 8.05 (s, 1H); 8.20 (s, 1H). MS: *m/z* 512 (M + 1). *Anal*. ($C_{24}H_{28}F_2N_{10}O$): C, H, N.

*5.1.8.12. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(diphenylmethyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14l**).* Compound **14l** was obtained as oil in 58% yield. ^{1}H-NMR δ (ppm): 1.00 (d, 3H); 1.29 (s, 1H); 2.55–2.60 (m, 4H); 2.80–2.82 (m, 2H); 3.08–3.10 (m, 2H); 3.34–3.38 (m, 4H); 4.16 (q, 1H); 4.22 (s, 1H); 4.62 (d, 1H); 4.82 (d, 1H); 6.20–6.60 (m, 10H); 6.94–6.99 (m, 2H); 8.01–8.03 (m, 1H); 8.08 (s, 1H); 8.22 (s, 1H). MS: *m/z* 600 (M + 1). *Anal*. ($C_{32}H_{35}F_2N_9O$): C, H, N.

*5.1.8.13. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2-n-butoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14m**).* Compound **14m** was obtained as oil in 55% yield. ^{1}H-NMR δ (ppm): 0.96 (t, 3H); 0.99 (d, 3H); 1.25 (s, 1H); 1.70–1.74 (m, 4H); 2.57–2.63 (m, 4H); 2.80–3.11 (m, 4H); 3.38–3.44 (m, 4H); 4.05–4.12 (m, 2H); 4.18 (q, 1H); 4.63 (d, 1H); 4.85 (d, 1H); 6.25–6.58 (m, 4H); 7.41–7.45 (m, 2H); 7.97–8.00 (m, 1H); 8.02 (s, 1H); 8.16 (s, 1H). MS: *m/z* 582 (M + 1). *Anal*. ($C_{29}H_{37}F_2N_9O_2$): C, H, N.

*5.1.8.14. 2-(2,4-Difluorophenyl)-3-(5-{2-[4-phenyl-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**14n**).* Compound **14n** was obtained as oil in 63% yield. ^{1}H-NMR δ (ppm): 1.02 (d, 3H); 1.33 (s, 1H); 2.66–2.72 (m, 4H); 2.80–2.86 (m, 2H); 3.08–3.11 (m, 2H); 3.32–3.36 (m, 4H); 4.12 (q, 1H); 4.35 (d, 1H); 4.58 (d, 1H); 6.26–6.46 (m, 5H); 6.90–6.94 (m, 2H); 7.90–7.94 (m, 1H); 8.04 (s, 1H); 8.18 (s, 1H). MS: *m/z* 510 (M + 1). *Anal*. ($C_{25}H_{29}F_2N_9O$): C, H, N.

5.2. Determination of antifungal activity

The compounds were evaluated for activity against fungal cultures by in vitro agar diffusion assay and micro broth dilution assay.

5.2.1. In vitro agar diffusion assay

The ability of the compounds to inhibit the growth of fungal cultures was determined by measuring the zone of inhibition in the in vitro agar diffusion assay. Briefly, the

inoculum of the individual fungal (*C. albicans, C. tropicalis, C. krusei, C. neoformans, Aspergillus fumigatus*) culture was prepared by adjusting the turbidity of the overnight grown cultures to 0.5 Mc Farland (1×10^6 yeast cells per ml). Five-hundred microliters of the 0.5 Mc Farland adjusted cultures was added to the 50 ml molten HR medium (prepared by mixing equal amount of HR medium with 2% molten agar) and poured into 150 mm sterile Petri plates, media was allowed to solidify, uniform (6–8 diameter) wells punched on the media plates. Stock solution of 1 mg ml^{-1} of each compound was prepared in DMSO in separate sterile tubes. Five serial twofold dilutions of the compounds were made in DMSO. Fifty microliters of respective dilutions were added to each well to obtain final concentration (1.56, 3.12, 6.25, 12.5, 25 and 50 μg per well. DMSO as control was added to one well per plate. The plates were incubated in upright position overnight at 25 °C. Diameter of the zone of inhibition for each dilution of the compound was determined and compared with standard drug against the respective target organism. Compounds showing equivalent or better zone of inhibition have been selected for further workup.

5.2.2. In vitro antifungal susceptibility assay

MIC of the compounds against *Candida* spp., *C. neoformans* and *Aspergillus* spp. was determined by Broth micro dilution testing in accordance with the guidelines in NCCLS document M27-A and M38-P [20,21]. Briefly, stock solutions were prepared in polyethylene glycol for itraconazole or water for fluconazole and DMSO for the compounds of the present study. Serial twofold dilution of all the compound and standard drug were made in RPMI1640 medium buffered to pH 7.0 with 0.165 M 4-morpholinepropanesulfonic acid (MOPS) buffer as outlined in NCCLS M27-A document. Aliquots of (0.1 ml) of each compound at a 2× final concentration were dispensed into the wells of plastic micro dilution micro titer plates. The final concentration of solvent did not exceed 1% in any well. An inoculum concentration of $(1.5 \pm 1.0) \times 10^3$ cells per ml was prepared by spectrometric method of inoculum preparation for each organism tested. Hundred microliters on individual fungal inoculum was added to each well of micro titer plate containing the drug/compound. The final concentration of all the compounds and drug were 0.007–16.0 μg ml^{-1}. The plates were incubated at 25 °C. MIC endpoints were read after 48 h incubation (for both *Candida* spp. and *C. neoformans*) and after 72 h for *Aspergillus* spp. After the completion of incubation, the broth micro dilution wells were examined with the aid of reading mirror; the growth in each well was compared with that of the growth control well. The MIC of each compound was defined as the lowest concentration that produced 80% inhibition in the growth of the organism compared with that of the drug free control. All assays were performed in duplicate and results were expressed as mean ± S.D.

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