Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 2225–2238
Bioorganic & Medicinal Chemistry

Optically active antifungal azoles: synthesis and antifungal activity of (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl/1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol ☆

Ram Shankar Upadhayaya,[a] Neelima Sinha,[a] Sanjay Jain,[a] Nawal Kishore,[a] Ramesh Chandra[b] and Sudershan K. Arora[a,*]

[a]*Medicinal Chemistry Division, New Chemical Entity Research, Lupin Research Park, 46/47 A, At Village Nande, Taluka Mulshi, Pune 411042, India*
[b]*Bundelkhand University, Jhansi, India*

Received 21 November 2003; accepted 10 February 2004

Abstract—A series of (2*R*,3*S*)-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 (**11a–n**) and (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazole-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12a–n**) has been synthesized. The antifungal activity of compounds was evaluated by in vitro agar diffusion and broth dilution assay. Compounds **11d** and its positional isomer **12d** having 3-trifluoromethyl substitution on the phenyl ring of piperazine demonstrated significant antifungal activity against variety of fungal cultures (*Candida* spp. *C. neoformans* and *Aspergillus* spp.). The compound **12d** showed MIC value of 0.12 μg/mL for *C. albicans*, *C. albicans* V-01-191A-261 (resistant strain); 0.25 μg/mL for *C. tropicalis*, *C. parapsilosis* ATCC 22019 and *C. krusei* and MIC value of 0.5 μg/mL for *C. glabrata*, *C. krusei* ATCC 6258, which is comparable to itraconazole and better than fluconazole. Further, compound **11d** showed significant activity (MIC; 0.25–0.5 μg/mL) against *Candida* spp. and strong anticryptococcal activity (MIC; 0.25 μg/mL) against *C. neoformans*.

1. Introduction

The incidence of systemic fungal infections such as Candidosis, Cryptococcosis and Aspergillosis has been increasing recently due to an increase in the number of immunocompromised hosts. For the treatment of these infections, the new antifungal azoles have been developed for clinical use. Attention has been paid to triazole derivatives because of their generally broad antifungal spectrum and low toxicity.[1] Triazole derivatives displace lanosterol from lanosterol 14-demethylase (14 DM), a cytochrome P-450-dependent enzyme, and block the biosynthesis of an essential component of fungal cell membrane, ergosterol.[2] Fluconazole has relatively low antifungal activity in vitro, but it is water soluble, and has excellent pharmacokinetic properties.[3,10] It is effective against candidiasis after both oral administration and injection. However, its activity against *Aspergillus* seems limited. Itraconazole has an excellent and broader antifungal spectrum.[4] Newer triazole agents such as voriconazole,[5] posaconazole[6] and ravuconazole,[7] are active against *Aspergillus* and currently under clinical trials (Fig. 1).

In order to seek new triazole antifungal agents we designed a series of tetrazole–triazole compounds depicted by the formula **11** and **12**. We presumed that the left half portion of the molecule is essential to the high antifungal activity. It is a common substructure seen in many other triazole antifungals. The 1-aryl-4-(2-[*1H*-tetrazol-5-yl]-ethyl)-piperazine ring was introduced in the expectation that: (1) since salt of aryl piperazine moieties will make molecule more water soluble than simple hydrocarbon moieties, the compound could be more easily delivered to the target enzyme; (2) since many other triazole antifungal agents have heteroatoms at the corresponding part of the molecule, complementary structure of the target enzyme would be implied; and (3) a variety of aryl piperazines should be available

Keywords: Antifungal activity; Tetrazole derivatives; Chiral synthesis; Tetrazole–triazole compounds.
☆ Taken in part from the PhD dissertation of Ram Shankar Upadhayaya, Bundelkhend University, 2003.
* Corresponding author. Tel.: +91-20-25126689; fax: +91-20-25126175; e-mail addresses: sudershanarora@hotmail.com, sudershanarora@lupinpharma.com

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.02.014

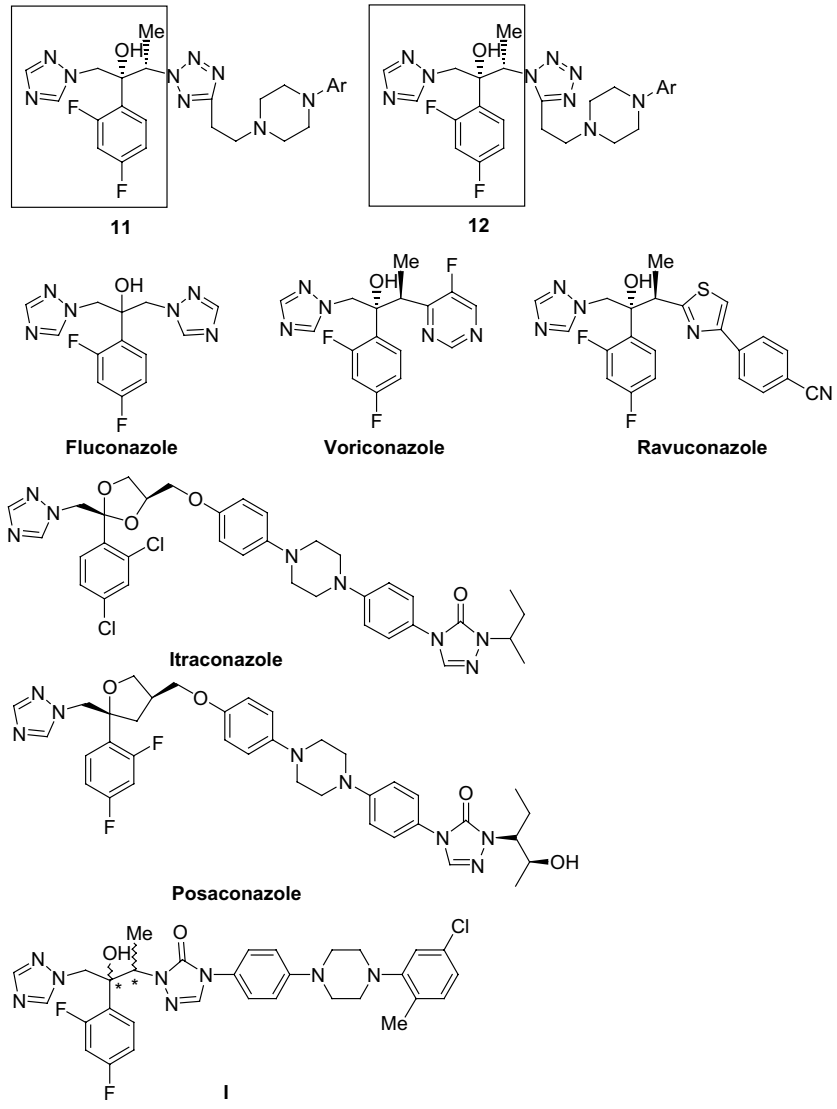


Figure 1. Structures of compounds **11**, **12**, fluconazole, itraconazole, voriconazole, posaconazole, ravuconazole and **I**.

as the side chain (Fig. 1). The methyl group at the 3-position mimics the 13β-methyl group of lanosterol.[8,9] The two fluorine atoms in the benzene ring in the left half of the molecule aim at strengthening the antifungal activity.[10,11]

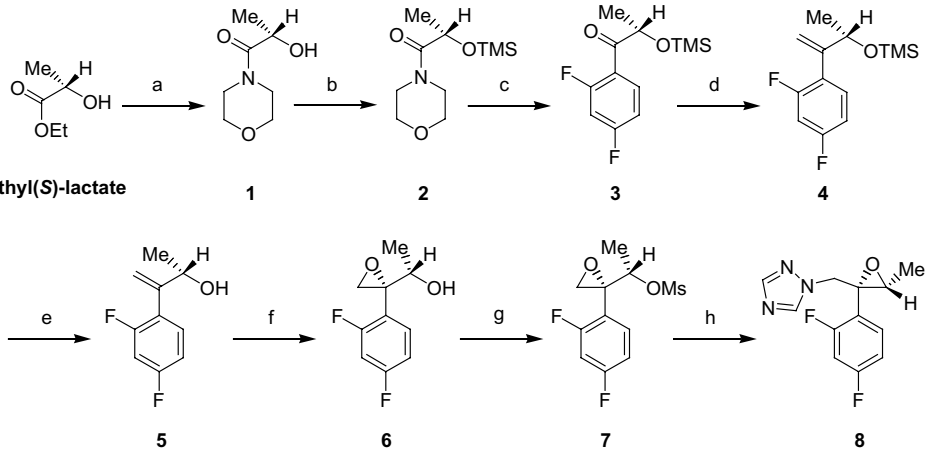
Furthermore, the stereoselective interaction of the enzyme with stereoisomeric azoles has become an intriguing field of study. The relationship between stereochemistry and antifungal activity showed that the target enzyme, cytochrome P-450_{14DM}, recognized the configuration of the chiral centres: (1*R*,2*R*)-enantiomers showed the most potent activity but findings support the assumption that the (1*R*,2*S*) and (1*S*,2*R*) also fit in the same pocket as the lanosterol D ring in the target enzyme, cytochrome P-450_{14DM}.[12,13] To further prove this rule in our series and examine the influence of each centre's absolute configuration upon activity, we had synthesized four stereoisomers of compound **I** (Fig. 1) [i.e., (1*R*,2*R*), (1*S*,2*S*), (1*R*,2*S*) and (1*S*,2*R*)], and its recemate were tested in vitro and in vivo and found that (1*R*,2*R*) and (1*S*,2*R*) have same antifungal activity.[14] Similar findings have been observed by us in our other series of azole antifungals.[15]

In this paper, we will present our recent findings on synthesis and antifungal activities of the compounds **11** and **12**.

2. Results and discussion

2.1. Chemistry

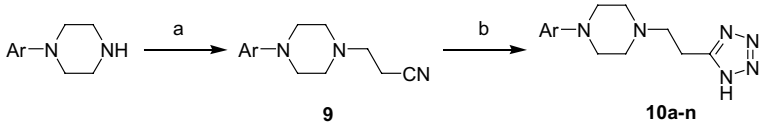
The title compounds **11a**–**n** and **12a**–**n** were prepared from the optically active key intermediate (2*R*,3*R*)-2-(2,4-diflourophenyl)-3-methyl-2-[1,2,4]-triazol-1-yl-methyl-oxirane (**8**),[16] which was derived from ethyl (*S*)-lactate in eight steps as shown in Scheme 1. The chiral intermediate **3** was prepared by following the literature methods.[16] The intermediate **3** was then converted to the exomethylene intermediate **4** by reacting with methyl



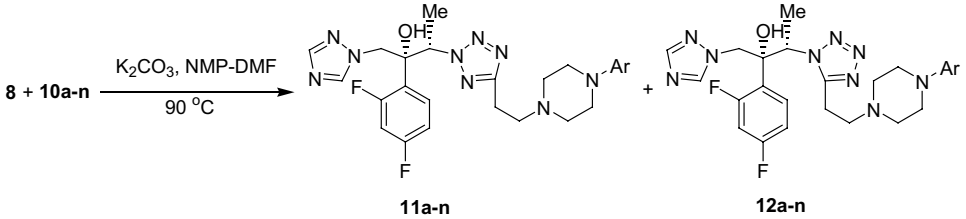
Scheme 1. Synthesis of key intermediate **8**. Reagents and conditions: (a) morpholine, 80 °C, 4 days; (b) HMDS, Py·HBr, DCM, rt; (c) 2,4-$F_2C_6H_3MgBr$, THF; (d) *t*-BuOK, PPh_3MeI, toluene, 80 °C, 2 h; (e) HCOOH, EtOH, rt, 1 h; (f) Ti(*i*-PrO)$_3$, L-(+)-DET, *t*-BuOOH; (g) MsCl, TEA, 50 °C, 30 min; (h) NaH, 1,2,4-triazole, 50 °C, 30 min.

triphenyl phosphonium iodide in presence of potassium *tert*-butoxide in dry toluene. The compound **4**, after deprotection, provided the substituted allyl alcohol (**5**), which was then reacted with *tert*-butyl hydroperoxide in the presence of titanium isopropoxide and (L)-(+)-diethyl tartarate, following the 'Sharpless' stereoselective epoxidation method,[16] in an aprotic solvent at a temperature of −20 °C to generate an asymmetric centre with high diastereomeric yields. The compound **6** thus produced was treated with methanesulfonyl chloride in presence of triethylamine in DCM at 0 °C to afford methylsulfonate (**7**).[17] This compound on reaction with 1,2,4-triazole in DMF in the presence of sodium hydride at 55 °C produced oxirane (**8**). The chiral intermediates **4**–**8** were prepared by following the literature methods and their spectral and analytical data were also same as given in the literature.[16] Methodology to obtain each of the above compounds **1**–**8** is given in experimental section. Having obtained the oxirane (**8**), the next task was to couple it with a number of substituted tetrazoles (**10a**–**n**) as given in Scheme 3. The method of preparation of 5-substituted tetrazoles is same as given in literature (Scheme 2).[18–21] The method involves the reaction of a nitrile with an inorganic azide, using an amine salt in an aromatic solvent in a facile work-up procedure to produce tetrazoles (**10a**–**n**) with higher purity in greater yield.

The oxirane **8** so obtained was reacted with various tetrazoles (**10a**–**n**) in presence of K_2CO_3 in aprotic co-solvents DMF and NMP at 90 °C to give the condensation product (Scheme 3).[22] TLC showed it to be a mixture of two main products; one that is of lower polarity designated as **11a**–**n** and the other **12a**–**n**. These (2*R*,3*S*)-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 (**11a**–**n**) and (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazole-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**12a**–**n**) were separated by flash column chromatography over silica gel (230–400 mesh) using 2%



Scheme 2. Synthesis of substituted tetrazoles **10a**–**n**. Reagents and conditions: (a) acrylonitrile, TEA, MeOH, −5 to 10 °C; (b) NaN_3, TEA·HCl, toluene, reflux.



Scheme 3. Synthesis of (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl/1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (**11a**–**n** and **12a**–**n**).

methanol–chloroform as eluent. Compounds **11a**–**n** and **12a**–**n** showed the same molecular ion peak in their mass spectrum indicating them to be the positional isomers.

The structural data of compounds **12a**–**n** were consistent with compounds **11a**–**n** except that the ^{1}H NMR spectra of **11a**–**n** showed up-field shifting of C*H*CH$_3$ protons. The ratio of isolated yields of the above two isomers suggested that the (**11a**–**n**) isomers are the predominated one. Steric factors can also play a role in the ratio of isomers formed; such types of observation have been reported earlier also.[17,23–29]

2.2. Antifungal activity

The antifungal activities of compounds **11a**–**n** and **12a**–**n** were evaluated by in vitro agar diffusion and broth dilution assay and the results of which are presented in Table 1. Most of the compounds showed activity in the initial screen against fungal cultures when tested at 500 μg/mL concentration of compound by agar diffusion assay. Compounds **11d**, **12d**, **11m**, **12m**, **11g**, **12g**, **11l** and **12l** inhibited the growth of drug resistant species of *Candida* (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. krusei*), and *C. neoformans*, additionally compounds **11d** and **12d** showed activity against *Aspergillus* cultures. These compounds were further tested for antifungal activity against different species of *Candida*, *Cryptococcus* and *Aspergillus* by microbroth dilution assay to determine the minimum inhibitory values of the compounds against fungal cultures (Table 1).

Compound **12d** having 3-trifluoromethyl substitution on the phenyl ring of piperazine was the most active of all the compounds (Table 1) with MIC value of 0.12 μg/mL for *C. albicans*, *C. albicans* V-01-191A-261 (resistant strain); 0.25 μg/mL for *C. tropicalis*, *C. parapsilosis* ATCC 22019 and *C. krusei* and MIC value of 0.5 μg/mL for *C. glabrata*, *C. krusei* ATCC 6258. Compound **12d** further demonstrated similar MIC value of 0.12 μg/mL for *Cryptococcus neoformans* strain LA314. This strong wide spectrum of activity demonstrated by compound **12d** was better than fluconazole against *C. albicans* and better than fluconazole for resistant *Candida* cultures and was equivalent to the activity of itraconazole. Similar strong antifungal activity was also shown by its positional isomer **11d**, which has MIC value of 0.25 μg/mL for *C. albicans*, *C. albicans* V-01-191A-261 (resistant strain), *C. tropicalis*, *C. krusei* and *C. parapsilosis* ATCC

Table 1. In vitro susceptibility of compounds **11a**–**n** and **12a**–**n** on clinical isolates of the fungal cultures

Compd	Ar	MIC (μg/mL) 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
11a	2-MeOC$_6$H$_4$	8.0	8.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0	>8.0
11b	4-MeOC$_6$H$_4$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
11c	4-NO$_2$C$_6$H$_4$	2.0	2.0	4.0	4.0	4.0	8.0	8.0	4.0	>8.0	>8.0
11d	3-CF$_3$C$_6$H$_4$	**0.25**	**0.5**	**0.5**	**0.25**	**0.5**	**0.25**	**0.25**	**0.25**	**1.0**	**1.0**
11e	2-ClC$_6$H$_4$	4.0	4.0	8.0	8.0	8.0	8.0	8.0	8.0	>8.0	>8.0
11f	3-ClC$_6$H$_4$	2.0	4.0	8.0	4.0	>8.0	8.0	>8.0	4.0	>8.0	>8.0
11g	4-ClC$_6$H$_4$	1.0	2.0	2.0	4.0	8.0	4.0	>8.0	4.0	>8.0	>8.0
11h	CH$_2$C$_6$H$_5$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
11i	4-FC$_6$H$_4$	2.0	2.0	4.0	2.0	4.0	8.0	8.0	2.0	>8.0	>8.0
11j	2,4-(Cl)$_2$C$_6$H$_3$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
11k	2-Pyridyl	4.0	4.0	8.0	8.0	>8.0	>8.0	>8.0	8.0	>8.0	>8.0
11l	CHPh$_2$	4.0	4.0	8.0	8.0	8.0	8.0	8.0	8.0	>8.0	>8.0
11m	2-*n*-BuOC$_6$H$_4$	**0.5**	**1.0**	**0.5**	**0.5**	**2.0**	**1.0**	**1.0**	**1.0**	**>8.0**	**>8.0**
11n	C$_6$H$_5$	8.0	8.0	8.0	8.0	>8.0	>8.0	>8.0	8.0	>8.0	>8.0
12a	2-MeOC$_6$H$_4$	4.0	4.0	>8.0	>8.0	>8.0	>8.0	>8.0	8.0	>8.0	>8.0
12b	4-MeOC$_6$H$_4$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
12c	4-NO$_2$C$_6$H$_4$	2.0	2.0	4.0	4.0	8.0	4.0	>8.0	4.0	>8.0	>8.0
12d	3-CF$_3$C$_6$H$_4$	**0.12**	**0.12**	**0.5**	**0.25**	**0.5**	**0.25**	**0.25**	**0.12**	**0.5**	**1.0**
12e	2-ClC$_6$H$_4$	4.0	4.0	8.0	8.0	8.0	8.0	8.0	8.0	>8.0	>8.0
12f	3-ClC$_6$H$_4$	2.0	4.0	4.0	8.0	>8.0	8.0	>8.0	4.0	>8.0	>8.0
12g	4-ClC$_6$H$_4$	**0.5**	**0.5**	**1.0**	**1.0**	**1.0**	**1.0**	**2.0**	**1.0**	**>8.0**	**>8.0**
12h	CH$_2$C$_6$H$_5$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
12i	4-FC$_6$H$_4$	2.0	2.0	4.0	2.0	4.0	8.0	8.0	2.0	>8.0	>8.0
12j	2,4-(Cl)$_2$C$_6$H$_3$	16.0	16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0	>16.0
12k	2-Pyridyl	4.0	4.0	8.0	8.0	>8.0	8.0	>8.0	8.0	>8.0	>8.0
12l	CHPh$_2$	4.0	4.0	4.0	4.0	4.0	4.0	8.0	8.0	>8.0	>8.0
12m	2-*n*-BuOC$_6$H$_4$	**0.5**	**0.5**	**0.5**	**0.5**	**1.0**	**1.0**	**0.5**	**0.25**	**>8.0**	**>8.0**
12n	C$_6$H$_5$	8.0	8.0	>8.0	8.0	>8.0	>8.0	>8.0	8.0	>8.0	>8.0
11d·HCl	3-CF$_3$C$_6$H$_4$	0.25	0.5	0.5	0.25	0.5	0.25	0.25	0.25	1.0	1.0
12d·HCl	3-CF$_3$C$_6$H$_4$	0.12	0.12	0.5	0.25	0.5	0.25	0.25	0.12	0.5	1.0
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 strain), C. t: *C. tropicalis*, C. k I: *C. krusei* ATCC 6528, C. k II: *C. krusei*, C. p: *C. parapsilosis* ATCC 22019, C. g: *C. glabrata*, C. n: *Cryptococcus neoformans* LA314, A. f: *Aspergillus fumigatus*, A. n: *Aspergillus niger*.

Table 2. Comparison with standards

Compd	MIC (μg/mL) of **11d** and **12d** 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
11d	0.25	0.5	0.5	0.25	0.5	0.25	0.25	0.25	1.0	1.0
12d	0.12	0.12	0.5	0.25	0.5	0.25	0.25	0.12	0.5	1.0
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
Voriconazole	0.015	0.5	0.25	0.12	0.5	0.5	0.25	0.25	0.12	0.5
Posaconazole	0.25	0.25	0.5	0.12	0.5	0.5	0.12	1.0	0.5	1.0
Ravuconazole	0.03	0.03	0.06	16	0.5	0.5	0.5	0.12	>16	>16

[a] C. a I: *C. albicans*, C. a II: *C. albicans* V-01-191A-261 (resistant strain), C. t: *C. tropicalis*, C. k I: *C. krusei* ATCC 6528, C. k II: *C. krusei*, C. p: *C. parapsilosis* ATCC 22019, C. g: *C. glabrata*, C. n: *Cryptococcus neoformans* LA314, A. f: *Aspergillus fumigatus*, A. n: *Aspergillus niger*.

22019 and MIC of 0.5 μg/mL against *C. glabrata*, *C. krusei* ATCC 6258 (Table 1). Against *Cryptococcus neoformans* strain LA314 the compound **11d** had MIC value of 0.25 μg/mL. The antifungal activity of **11d** and **12d** was comparable and better than fluconazole against resistant *Candida* species. These compounds (**11d** and **12d**) also showed significant inhibitory activity against *A. fumigatus* (MIC; 0.5–1.0 μg/mL) and *A. niger* 1.0 μg/mL.

Compounds **11m** and **12m** having 2-butoxy group on the phenyl ring of piperazine (Table 1) also showed significant activity against all the fungal cultures. A MIC value of 0.5 μg/mL for *C. albicans*, *C. albicans* V-01-191A-261 (resistant strain), *C. glabrata* and *C. tropicalis*; 1.0 μg/mL for *C. krusei* and, against *C. parapsilosis* ATCC 22019 and 2.0 μg/mL for *C. krusei* ATCC 6528, was shown by both the compounds. The compounds also inhibited the growth of (MIC; 0.25 μg/mL) *Cryptococcus neoformans* strain LA314. This activity of the compounds **11m** and **12m** was better than fluconazole and comparable to itraconazole against resistant *Candida* cultures. Compound **12g** having 4-Cl group on the phenyl ring of piperazine demonstrated good activity against fungal cultures as is evident from (Table 1) MIC value of 0.5 μg/mL for *C. albicans*, *C. albicans* V-01-191A-261 (resistant strain), 1.0 μg/mL for *C. tropicalis*, *C. glabrata*, *C. krusei* ATCC 6528, *C. krusei*; 2.0 μg/mL for *C. parapsilosis* ATCC 22019. The compounds also showed moderate activity of 2.0 μg/mL against *Cryptococcus neoformans* strain LA314. Similar activity was seen with compound **11g** having 4-Cl group on the phenyl ring of piperazine against fungal cultures (Table 1) MIC value of 1.0 μg/mL for *C. albicans*; 2.0 μg/mL for *C. albicans* V-01-191A-261 (resistant strain), and *C. glabrata*; 4.0 μg/mL against *C. tropicalis*, *C. krusei*; 8.0 μg/mL for *C. krusei* ATCC 6528, and >8.0 μg/mL for *C. parapsilosis* ATCC 22019. The compounds had a MIC value of activity of 4.0 μg/mL against *Cryptococcus neoformans* strain LA314.

Compounds **11i**, **12i**, **11c** and **12c** having 4-F and 4-NO_2 group on the phenyl ring of piperazine also demonstrated mild activity against fungal cultures (Table 1) with a MIC value of 2.0 μg/mL for *C. albicans*, *C. albicans* V-01-191A-261 (resistant strain) and *C. tropicalis*; 4.0 μg/mL for *C. glabrata*, and *C. krusei* ATCC 6528; and 8.0 μg/mL for *C. krusei*, and *C. parapsilosis* ATCC 22019. These compounds also showed moderate activity of 2.0–4.0 μg/mL against *Cryptococcus neoformans* strain LA314. In addition moderate to weak activity was also shown by compounds **11a**,**f**,**k**,**l**,**n** and **12a**,**f**,**k**,**l**,**n** against fungal cultures (Table 1) MIC value of 2.0–4.0 μg/mL for compounds **11f**,**k**,**n** and **12f**,**k**,**n**; and 4.0–8.0 μg/mL for compounds **11a**,**n** and **12a**,**n** against *C. albicans*, *C. albicans* V-01-191A-261 (resistant strain); 4.0–8.0 μg/mL for *C. tropicalis*, *C. glabrata* and *C. krusei* ATCC 6528 by compounds **11f**,**k**,**l** and **12f**,**k**,**l** and 8.0–>8.0 μg/mL for *C. krusei*, and *C. parapsilosis* ATCC 22019 for compounds **11a**,**f**,**k**,**l**,**n** and **12a**,**f**,**k**,**l**,**n**. These compounds inhibited the growth of *Cryptococcus neoformans* strain LA314 at concentration of 4.0–>8.0 μg/mL.

These structure activity relationship clearly suggest that 3-trifluoromethyl group in compounds **11d** and **12d** is responsible for broad-spectrum antifungal activity. The presence of the groups like 2-butoxy (**11m** and **12m**), 4-Cl (**11g** and **12g**), 4-NO_2 (**11c** and **12c**) and 4-F (**11i** and **12i**) are also play a significant role in imparting antifungal activity to the compounds.

2.3. Comparison with reference compounds

Table 2 shows the in vitro activity of compounds **11d** and **12d** against *Candida*, *Aspergillus* and *Cryptococcus* species compared to those of fluconazole, itraconazole, voriconazole, posaconazole and ravuconazole. Compounds **11d** and **12d** had comparable low MICs and broad spectra of activity against most of the fungal pathogen tested. They are active against all species of *Candida*, *Aspergillus* and *Cryptococcus*.

3. Conclusion

In conclusion, a novel series of antifungal agents has been designed and synthesized, that demonstrated significant wide spectrum activity against *Candida*, *Aspergillus* and *Cryptococcus* species. These compounds demonstrated activity against fluconazole resistant *Candida* species. The antifungal activity of these compounds was better than fluconazole and was equivalent to itraconazole, voriconazole, posaconazole and ravuconazole.

4. Experimental

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 vapour or UV lamp or by spraying the plates with Dragendorff or $KMnO_4$ reagents. IR spectra (λ_{max} in cm^{-1}) were recorded either on Perkin–Elmer spectrum RX FT-IR model and ^1H 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.

4.1. (2*S*)-2-Hydroxy-1-morpholin-4-yl-propan-1-one (1)

A mixture of ethyl (*S*)-lactate (40 g, 338 mmol) and morpholine (100 mL, 1017 mmol) was heated at 80 °C for 4 days. The reaction mixture was evaporated under reduced pressure to obtain crude product. The crude product was purified by column chromatography over silica gel (100–200 mesh). Elution with hexane–ethyl acetate (1:1) gave **1** as pale yellow oil 30.5 g (55%). IR (neat): ν 1635 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.34 (d, $J = 6.5$ Hz, 3H), 2.43 (t, $J = 5.0$ Hz, 2H), 3.55–3.80 (m, 6H), 3.82 (d, $J = 7.5$ Hz, 1H), 4.44–4.53 (m, 1H). MS: m/z 160 (M+1).

4.2. (2*S*)-2-(1,1-Dimethyl-1-silaethoxy)-1-morpholin-4-yl-propan-1-one (2)

To a mixture of **1** (30.5 g, 191.8 mmol) and pyridinium hydrobromide (3.84 g, 23.98 mmol) in CH_2Cl_2 (180 mL), hexamethyldisilazane (0.07 g, 124.68 mmol) was added at ambient temperature, the mixture was stirred over a period of 3 h. After completion of reaction, the organic layer was washed with water (2 × 100 mL), brine (20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to yield the **2** as oil. Yield: 43.2 g (97.5%). IR (neat): ν 1662 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.18 (s, 9H), 1.34 (d, $J = 6.5$ Hz, 3H), 3.43–3.80 (m, 8H), 4.44–4.53 (m, 1H). MS: m/z 232 (M+1).

4.3. (2*S*)-1-(2,4-Difluorophenyl)-2-(1,1-dimethyl-1-silaethoxy)-propan-1-one (3)

To a suspension of magnesium turnings (4.94 g, 205.7 mmol) in dry THF (110 mL), 1-bromo-2,4-difluorobenzene (39.7 g, 25.7 mmol) was added dropwise under vigorous stirring at such a rate that temperature of the reaction mixture was maintained at 35–40 °C. After completion of addition, the reaction mixture was stirred at ambient temperature for 1 h to get the 2,4-difluorobenzene magnesium bromide and then cooled to 20 °C. A solution of **2** (43.2 g, 18 mmol) in THF (40 mL) was added dropwise to the reaction mixture. The reaction mixture was then stirred at ambient temperature for 4 h, then a saturated aqueous solution of NH_4Cl (100 mL) and water (100 mL) were added, and the resulting mixture was extracted with ethyl acetate (2 × 500 mL). The combined organic phase was washed with water (2 × 50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated under reduced pressure to give crude product. The crude product was purified by column chromatography over silica gel (100–200 mesh) using hexane–ethyl acetate (15:75) as eluent to give 40.83 g (84.80%) **3** as a pale yellow oil. ^1H NMR ($CDCl_3$): δ 0.18 (s, 9H), 1.34 (d, $J = 7.0$ Hz, 3H), 4.45–4.53 (m, 1H), 6.68–7.05 (m, 2H), 7.84–8.00 (m, 1H). MS: m/z 259 (M+1).

4.4. 1-[(1*S*)-2-(2,4-Difluorophenyl)-1-methylprop-2-enyloxy]-1,1-dimethyl-1-silaethane (4)

To a solution of trimethylsulfonium methyl iodide (29.70 g, 73.64 mmol) in toluene (160 mL) was added potassium *tert*-butoxide (10.72 g, 95.73 mmol) under nitrogen over a period of 2 h. To this solution was added **3** (18.9 g, 73.64 mmol) in dry toluene (30 mL). The reaction mixture was again heated at 80 °C for 1 h. After completion of the reaction, product was extracted with toluene (3 × 50 mL). The combined organic layer was concentrated under reduced pressure to afford crude product as oil. Hexane (120 mL) was added and stirred for 4 h, followed by cooling to −30 °C under stirring. Filtered the solid thus separated out and washed with chilled hexane. Organic layer was concentrated under reduced pressure to obtain compound **4** as oil. Yield: 17.83 g (95.0%). ^1H NMR ($CDCl_3$): δ 0.16 (s, 9H), 1.34 (d, $J = 6.5$ Hz, 3H), 4.40–4.50 (m, 1H), 5.13–6.15 (m, 2H), 6.82–7.00 (m, 2H), 7.84–8.00 (m, 1H). MS: m/z 257 (M+1).

4.5. (2*S*)-3-(2,4-Difluorophenyl)-but-3-en-2-ol (5)

To a solution of **4** (17.83 g, 69.65 mmol) in ethanol (50 mL) was added formic acid (4.67 mL, 123.98 mmol) dropwise over a period of 0.5 h and reaction mixture was neutralized with aqueous solution of sodium bicarbonate (pH = 8.0). The aqueous layer was extracted with DCM (2 × 100 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to obtain crude product. The crude product was purified over silica gel (100–200 mesh) using hexane–ethyl acetate (9:1) as eluent to obtain **5** as oil. Yield: 7.0 g (54.60%). ^1H NMR ($CDCl_3$): δ 1.32 (d, $J = 6.5$ Hz, 3H), 4.42–4.28 (m, 1H), 4.94 (br s, 1H), 5.10–5.60 (m, 2H), 6.80–6.95 (m, 2H), 7.90–8.00 (m, 1H). MS: m/z 185 (M+1).

4.6. 1-[(2*R*)-2-(2,4-Difluorophenyl)-oxirane-2-yl]-(1*S*)-ethan-1-ol (6)

To a mixture of molecular sieves (4 Å, 15 g) in anhydrous DCM (200 mL), titanium isopropoxide (2.70 mL, 9.07 mmol) was added at −20 °C. Then L-(+)-diethyl

tartarate in anhydrous DCM (300 mL) was added under vigorous stirring. To this, *tert*-butylhydroperoxide (2.62 mmol, 3.2 molar solution) in toluene (82 mL) was added dropwise over a period of 1 h, followed by **5** (16.70 g, 90.76 mmol) in DCM (75 mL). The reaction mixture was stirred for 2 h at −20 °C and then for 6 h at −10 °C. The reaction mixture was quenched with 100 mL of water at 0 °C and stirred for 1 h at ambient temperature. Sodium hydroxide solution (30%, 80 mL) was added to hydrolyze the tartarate and the solid separated out was filtered off by passing through a Celite pad. Filtrate was washed with brine (200 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to obtain crude product. The crude product was purified over silica gel (100–200 mesh) using dichloromethane–hexane (1:1) as eluent to obtain **6** as oil. Yield: 10.91 g (60%). ^{1}H NMR ($CDCl_3$): δ 1.16 (d, $J = 6.5$ Hz, 3H), 2.69 (br s, 1H), 2.88–2.96 (m, 1H), 3.27–3.30 (m, 1H), 4.08–4.12 (m, 1H), 6.79–6.94 (m, 2H), 7.32–7.44 (m, 1H). MS: m/z 201 (M+1).

4.7. (2*R*,3*R*)-2-(2,4-Difluorophenyl)-3-methyl-2-[1,2,4]-triazol-1-yl-methyl-oxirane (8)

To a solution of **6** (2.0 g, 10 mmol), methanesulfonyl chloride (1.52 g, 15.0 mmol) in DCM (40 mL) was added triethylamine (1.52 g, 15 mmol) at 5 °C under stirring. The stirring was continued at same temperature for 1 h. Water (20 mL) was added to the reaction mixture and washed the organic layer with a saturated $NaHCO_3$ solution (20 mL). The organic layer was dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated at reduced pressure to get **7**. In another 100 mL round bottom flask NaH (1.44 g, 60.0 mmol) pre-washed with hexane was taken in DMF (50 mL) and 1,2,4-triazole (2.76 g, 69.0 mmol) was added at 0 °C under stirring. The stirring was continued for 15 min followed by the addition of the compound **7**. The reaction mixture was stirred for 20 min at 0 °C and then at 50–55 °C for 1 h. The reaction mixture was taken in ethyl acetate (100 mL), washed with water (2×25 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to obtain the (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-methyl-2-[1,2,4]-triazol-1-yl-methyl-oxirane (**8**) as oil. Yield: 2.04 g (81.27%). ^{1}H NMR ($CDCl_3$): δ 1.62 (d, $J = 5.5$ Hz, 3H), 3.16 (q, $J = 5.5$ Hz, 1H), 4.39 (d, $J = 15.0$ Hz, 1H), 4.62 (d, $J = 15.0$ Hz, 1H), 6.63–6.80 (m, 2H), 6.91–7.03 (m, 1H), 8.01 (s, 1H), 8.15 (s, 1H). MS: m/z 252 (M+1).

4.8. 3-[4-(2-Methoxyphenyl)-piperazin-1-yl]-propionitrile (9a)

To a solution of 1-(2-methoxyphenyl)-piperazine (1.67 g, 8.7 mmol) in methanol (10 mL) was added TEA (1.33 mL, 9.1 mmol) at 0 °C followed by the addition of acrylonitrile (0.5 mL, 8.74 mmol). After complete addition, the reaction mixture was stirred at ambient temperature for 24 h. Excess TEA was removed under reduced pressure and the residue was dissolved in DCM (100 mL). The organic phase was washed with water (2×20 mL), brine (20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give **9a** as oil. Yield: 84.30%. ^{1}H NMR ($CDCl_3$): δ 2.57–2.70 (m, 8H), 3.03–3.11 (m, 4H), 3.74 (s, 3H), 6.61–6.85 (m, 2H), 6.94–6.99 (m, 2H). MS: m/z 246 (M+1).

The other 3-(4-aryl-piperazin-1-yl)-propionitrile (**9b**–**n**) were also prepared by the same procedure as described above and their spectral and analytical data are given below.

4.9. 3-[4-(4-Methoxyphenyl)-piperazin-1-yl]-propionitrile (9b)

This was obtained in 86.56% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.57–2.70 (m, 8H), 3.09–3.17 (m, 4H), 3.76 (s, 3H), 6.61–6.71 (m, 4H). MS: m/z 246 (M+1).

4.10. 3-[4-(4-Nitrophenyl)-piperazin-1-yl]-propionitrile (9c)

This was obtained in 91.04% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.55–2.72 (m, 8H), 3.10–3.18 (m, 4H), 6.58–6.62 (d, 2H), 8.04–8.10 (d, 2H). MS: m/z 261 (M+1).

4.11. 3-[4-(3-Trifluoromethylphenyl)-piperazin-1-yl]-propionitrile (9d)

This was obtained in 89.43% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.57–2.73 (m, 8H), 3.08–3.16 (m, 4H), 6.03–6.86 (m, 1H), 7.00–7.13 (m, 1H), 7.31–7.55 (m, 2H). MS: m/z 284 (M+1).

4.12. 3-[4-(2-Chlorophenyl)-piperazin-1-yl]-propionitrile (9e)

This was obtained in 89.40% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.56–2.70 (m, 8H), 3.07–3.15 (m, 4H), 6.46–6.80 (m, 3H), 7.50–7.54 (m, 1H). MS: m/z 250 (M+1).

4.13. 3-[4-(3-Chlorophenyl)-piperazin-1-yl]-propionitrile (9f)

This was obtained in 85.20% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.55–2.70 (m, 8H), 3.09–3.17 (m, 4H), 6.48–6.83 (m, 3H), 7.52–7.55 (m, 1H). MS: m/z 250 (M+1).

4.14. 3-[4-(4-Chlorophenyl)-piperazin-1-yl]-propionitrile (9g)

This was obtained in 84.23% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.55–2.70 (m, 8H), 3.09–3.17 (m, 4H), 6.46 (d, $J = 8.2$ Hz, 2H), 6.89 (d, $J = 8.2$ Hz, 2H). MS: m/z 250 (M+1).

4.15. 3-[4-Benzylpiperazin-1-yl]-propionitrile (9h)

This was obtained in 89.46% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.30–3.12 (m, 12H), 3.52 (s, 2H), 7.24–7.27 (m, 5H). MS: m/z 230 (M+1).

4.16. 3-[4-(4-Fluorophenyl)-piperazin-1-yl]-propionitrile (9i)

This was obtained in 92.30% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.58–2.72 (m, 8H), 3.09–3.18 (m, 4H), 6.50–6.73 (m, 4H). MS: m/z 234 (M+1).

4.17. 3-[4-(2,4-Dichlorophenyl)-piperazin-1-yl]-propionitrile (9j)

This was obtained in 90.24% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.55–2.70 (m, 8H), 3.07–3.15 (m, 4H), 6.91–7.02 (m, 2H), 7.36–7.39 (m, 2H). MS: m/z 284 (M+1), 286 (M+3).

4.18. 3-[4-(2-Pyridyl)-piperazin-1-yl]-propionitrile (9k)

This was obtained in 84.60% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.45–2.62 (m, 8H), 3.24–3.32 (m, 4H), 6.46 (m, 2H), 7.54–7.57 (m, 1H), 8.08 (m, 1H). MS: m/z 217 (M+1).

4.19. 3-[4-Diphenylmethyl-piperazin-1-yl]-propionitrile (9l)

This was obtained in 89.32% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.42–3.15 (m, 12H), 4.18 (s, 1H), 7.12–7.23 (m, 10H). MS: m/z 306 (M+1).

4.20. 3-[4-(2-Butoxyphenyl)-piperazin-1-yl]-propionitrile (9m)

This was obtained in 90.60% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.97 (t, $J = 7.0$ Hz, 3H), 1.58–1.65 (m, 4H), 2.57–2.70 (m, 8H), 3.03–3.11 (m, 4H), 4.03 (t, $J = 7.0$ Hz, 2H), 6.60–6.83 (m, 2H), 6.90–7.12 (m, 2H). MS: m/z 288 (M+1).

4.21. 3-[4-(Phenyl)-piperazin-1-yl]-propionitrile (9n)

This was obtained in 93.00% yield as a solid, mp 70–72 °C (lit.[21] mp 71.3–72.3 °C). ^{1}H NMR ($CDCl_3$): δ 2.56–2.71 (m, 8H), 3.12–3.20 (m, 4H), 6.77–7.21 (m, 5H). MS: m/z 216 (M+1).

4.22. 1-(2-Methoxyphenyl)-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10a)

A mixture of **9a** (2.0 g, 8.3 mmol), triethylamine hydrochloride (4.60 g, 33.46 mmol) and sodium azide (2.17 g, 33.46 mmol) in toluene (50 mL) was refluxed at boiling point. After 48 h, the reaction mixture was cooled and toluene was removed under reduced pressure. The residue was dissolved in water (20 mL), acidified with aqueous HCl (10%) and washed it with chloroform (2×20 mL). The chloroform layer was discarded and the aqueous layer was basified with solid $NaHCO_3$ (pH 10), washed it with ether (2×20 mL). The aqueous layer was concentrated under reduced pressure to get a solid. The solid was taken in MeOH–$CHCl_3$ (1:9, 50 mL) and stirred for 1 h, then filtered off, residue was washed with $CHCl_3$ and filtrate was evaporated to get **10a**. Yield: 82.00%, oil. ^{1}H NMR ($CDCl_3$): δ 2.57–2.67 (m, 8H), 3.00–3.11 (m, 4H), 3.64 (s, 3H), 6.14–6.62 (m, 4H), 7.90 (br s, 1H). MS: m/z 401 (M+1).

The other 1-aryl-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazines (**10b**–**n**) were also prepared by the same procedure as described above and their spectral and analytical data are given below.

4.23. 1-(4-Methoxyphenyl)-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10b)

This was obtained in 84.20% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.57–2.65 (m, 8H), 3.09–3.17 (m, 4H), 3.62 (s, 3H), 6.22 (d, $J = 8.0$ Hz, 2H), 6.60 (d, $J = 8.0$ Hz, 2H), 7.90 (br s, 1H). MS: m/z 289 (M+1).

4.24. 1-(4-Nitrophenyl)-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10c)

This was obtained in 86.32% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.58–2.74 (m, 8H), 3.10–3.17 (m, 4H), 7.10 (d, $J = 8.0$ Hz, 2H), 7.78 (d, $J = 8.0$ Hz, 2H), 7.90 (br s, 1H). MS: m/z 304 (M+1).

4.25. 1-(3-Trifluoromethylphenyl)-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10d)

This was obtained in 89.20% yield as a solid, mp 183–185 °C (lit.[18] mp 184–186 °C). ^{1}H NMR ($CDCl_3$): δ 2.58–2.66 (m, 8H), 3.09–3.18 (m, 4H), 6.68–6.86 (m, 4H), 7.90 (br s, 1H). MS: m/z 327 (M+1).

4.26. 1-(2-Chlorophenyl)-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10e)

This was obtained in 88.20% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.57–2.74 (m, 8H), 3.06–3.14 (m, 4H), 6.50–6.95 (m, 4H), 7.90 (br s, 1H). MS: m/z 293 (M+1), 295 (M+3).

4.27. 1-(3-Chlorophenyl)-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10f)

This was obtained in 86.30% yield as a solid, mp 163–164 °C (lit.[18] mp 165–166 °C). ^{1}H NMR ($CDCl_3$): δ

2.58–2.74 (m, 8H), 3.10–3.18 (m, 4H), 6.60–6.93 (m, 4H), 7.89 (br s, 1H). MS: m/z 293 (M+1), 295 (M+3).

4.28. 1-(4-Chlorophenyl)-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10g)

This was obtained in 83.26% yield as a solid, mp 218–221 °C (lit.[18] mp 220–221 °C). ^{1}H NMR ($CDCl_3$): δ 2.58–2.72 (m, 8H), 3.10–3.18 (m, 4H), 6.71 (d, $J = 8.0$ Hz, 2H), 6.80 (m, $J = 8.0$ Hz, 2H), 7.90 (br s, 1H). MS: m/z 293 (M+1), 295 (M+3).

4.29. 1-Benzyl-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10h)

This was obtained in 87.90% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.32–2.40 (m, 8H), 2.56–2.67 (m, 4H), 3.52 (s, 2H), 6.70–6.81 (m, 5H), 7.09–7.28 (br s, 1H). MS: m/z 273 (M+1).

4.30. 1-(4-Fluorophenyl)-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10i)

This was obtained in 88.60% yield as a solid, mp 193–195 °C (lit.[18] mp 194–196 °C). ^{1}H NMR ($CDCl_3$): δ 2.58–2.67 (m, 8H), 3.09–3.17 (m, 4H), 6.48 (d, $J = 8.0$ Hz, 2H), 6.88 (d, $J = 8.0$ Hz, 2H), 7.90 (br s, 1H). MS: m/z 277 (M+1).

4.31. 1-(2,4-Dichlorophenyl)-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10j)

This was obtained in 88.30% yield as a solid, mp 206–208 °C (lit.[18] mp 207–209 °C). ^{1}H NMR ($CDCl_3$): δ 2.56–2.72 (m, 8H), 3.09–3.17 (m, 4H), 6.92–7.03 (m, 2H), 7.36–7.39 (m, 1H), 7.90 (br s, 1H). MS: m/z 327 (M+1), 329 (M+3).

4.32. 1-(2-Pyridyl)-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10k)

This was obtained in 87.40% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.58–2.72 (m, 8H), 3.24–3.32 (m, 4H), 6.46–6.51 (m, 2H), 7.56–7.58 (m, 1H), 7.91 (br s, 1H), 8.00–8.08 (m, 1H). MS: m/z 260 (M+1).

4.33. 1-Diphenylmethyl-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10l)

This was obtained in 86.29% yield as oil. ^{1}H NMR ($CDCl_3$): δ 2.57–2.74 (m, 8H), 2.34–2.42 (m, 4H), 4.18 (s, 1H), 7.03–7.20 (m, 10H), 7.90 (br s, 1H). MS: m/z 345 (M+1).

4.34. 1-(2-Butoxyphenyl)-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10m)

This was obtained in 83.70% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.98 (t, $J = 7.0$ Hz, 3H), 1.59–1.61 (m, 4H), 2.58–2.72 (m, 8H), 3.12–3.20 (m, 4H), 4.04 (t, $J = 7.0$ Hz, 2H), 6.38–6.46 (m, 2H), 6.66–6.91 (m, 3H), 7.91 (br s, 1H). MS: m/z 331 (M+1).

4.35. 1-Phenyl-4-[2-(*1H*-tetrazol-5-yl)-ethyl]-piperazine (10n)

This was obtained in 86.80% yield as a solid, mp 200–203 °C (lit.[18] mp 200–201 °C). ^{1}H NMR ($CDCl_3$): δ 2.57–2.72 (m, 8H), 3.10–3.17 (m, 4H), 6.82–7.22 (m, 5H), 7.90 (br s, 1H). MS: m/z 259 (M+1).

4.36. (2*R*,3*S*)-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 (11a) and (2R,3S)-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 (12a)

A mixture of **8** (0.50 g, 1.99 mmol), K_2CO_3 (1.37 g, 9.96 mmol), **10a** (0.55 g, 2.29 mmol) in NMP (5 mL) and DMF (4 mL) was stirred at 90 °C for 10 h. The reaction mixture was cooled to room temperature and poured into water (20 mL). The water layer was extracted with ethyl acetate (50 mL), washed with water (2 × 20 mL), brine (2 × 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give crude product. The crude product was purified by column chromatography over silica gel (230–400 mesh) using MeOH–$CHCl_3$ (2:98) as eluent to obtain **11a** and **12a**.

4.37. (2*R*,3*S*)-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 (11a)

Yield: 40.80%, oil. ^{1}H NMR ($CDCl_3$): δ 1.02 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.70 (s, 3H), 3.95 (q, $J = 7.0$ Hz, 1H), 4.28 (d, $J = 14.0$ Hz, 1H), 4.46 (d, $J = 14.0$ Hz, 1H), 5.45 (br s, 1H), 6.20–6.51 (m, 4H), 6.90–6.96 (m, 2H), 7.93–7.98 (m, 1H), 8.06 (s, 1H), 8.22 (s, 1H). MS: m/z 540 (M+1). Anal. Calcd for $C_{26}H_{31}F_2N_9O_2$: C, 57.87; H, 5.79; N, 23.36. Found: C, 57.99; H, 5.84; N, 23.49.

4.38. (2*R*,3*S*)-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 (12a)

Yield: 13.50%, oil. ^{1}H NMR ($CDCl_3$): δ 0.97 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.71 (s, 3H), 4.08 (q, $J = 7.0$ Hz, 1H), 4.32 (d, $J = 14.0$ Hz, 1H), 4.53 (d, $J = 14.0$ Hz, 1H), 5.45 (br s, 1H), 6.22–6.54 (m, 4H), 6.97–7.03 (m, 2H), 7.99–8.03 (m, 1H), 8.06 (s, 1H), 8.21 (s, 1H). MS: m/z 540 (M+1). Anal. Calcd for $C_{26}H_{31}F_2N_9O_2$: C, 57.87; H, 5.79; N, 23.36. Found: C, 58.03; H, 5.96; N, 23.55.

The other compounds of this series (**11b**–**n**) and (**12b**–**n**) were also prepared by the same procedure as described above and their spectral and analytical data are given below.

4.39. (2*R*,3*S*)-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 (11b)

This was obtained in 38.00% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.03 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.72 (s, 3H), 4.00 (q, $J = 7.0$ Hz, 1H), 4.30 (d, $J = 14.0$ Hz, 1H), 4.52 (d, $J = 14.0$ Hz, 1H), 5.50 (br s, 1H), 6.20 (d, $J = 8.0$ Hz, 2H), 6.55 (d, $J = 8.0$ Hz, 2H), 6.85–6.90 (m, 2H), 7.90–7.99 (m, 1H), 8.10 (s, 1H), 8.23 (s, 1H). MS: m/z 540 (M+1). Anal. Calcd for $C_{26}H_{31}F_2N_9O_2$: C, 57.87; H, 5.79; N, 23.36. Found: C, 58.11; H, 6.04; N, 23.81.

4.40. (2*R*,3*S*)-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 (11c)

This was obtained in 36.70% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.04 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.96 (q, $J = 7.0$ Hz, 1H), 4.26 (d, $J = 14.0$ Hz, 1H), 4.47 (d, $J = 14.0$ Hz, 1H), 5.52 (br s, 1H), 6.67–6.82 (m, 4H), 7.38–7.42 (m, 1H), 8.05 (s, 1H), 8.15 (s, 1H), 8.20 (d, $J = 9.0$ Hz, 2H). MS: m/z 555 (M+1). Anal. Calcd for $C_{25}H_{28}F_2N_{10}O_3$: C, 54.15; H, 5.09; N, 25.26. Found: C, 54.39; H, 5.24; N, 25.64.

4.41. (2*R*,3*S*)-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 (11d)

This was obtained in 32.70% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.03 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.98 (q, $J = 7.0$ Hz, 1H), 4.28 (d, $J = 14.0$ Hz, 1H), 4.48 (d, $J = 14.0$ Hz, 1H), 5.50 (br s, 1H), 6.30–6.55 (m, 4H), 6.90–6.95 (m, 2H), 7.90–7.98 (m, 1H), 8.03 (s, 1H), 8.22 (s, 1H). MS: m/z 578 (M+1). Anal. Calcd for $C_{26}H_{28}F_5N_9O$: C, 54.07; H, 4.89; N, 21.83. Found: C, 54.44; H, 5.14; N, 22.12.

4.42. (2*R*,3*S*)-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 (11e)

This was obtained in 40.60% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.00 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.00 (q, $J = 7.0$ Hz, 1H), 4.29 (d, $J = 14.0$ Hz, 1H), 4.47 (d, $J = 14.0$ Hz, 1H), 5.48 (br s, 1H), 6.25–6.55 (m, 4H), 6.94-6.98 (m, 2H), 7.98–8.02 (m, 1H), 8.11 (s, 1H), 8.24 (s, 1H). MS: m/z 544 (M+1), 546 (M+3). Anal. Calcd for $C_{25}H_{28}ClF_2N_9O$: C, 55.20; H, 5.19; N, 23.17. Found: C, 55.49; H, 5.37; N, 23.44.

4.43. (2*R*,3*S*)-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 (11f)

This was obtained in 36.60% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.02 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.99 (q, $J = 7.0$ Hz, 1H), 4.27 (d, $J = 14.0$ Hz, 1H), 4.50 (d, $J = 14.0$ Hz, 1H), 5.42 (br s, 1H), 6.25–6.50 (m, 4H), 7.01–7.10 (m, 2H), 7.85–7.89 (m, 1H), 8.05 (s, 1H), 8.21 (s, 1H). MS: m/z 544 (M+1), 546 (M+3). Anal. Calcd for $C_{25}H_{28}ClF_2N_9O$: C, 55.20; H, 5.19; N, 23.17. Found: C, 55.57; H, 5.37; N, 23.22.

4.44. (2*R*,3*S*)-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 (11g)

This was obtained in 38.90% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.04 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.00 (q, $J = 7.0$ Hz, 1H), 4.26 (d, $J = 14.0$ Hz, 1H), 4.49 (t, $J = 14.0$ Hz, 1H), 5.45 (br s, 1H), 6.19 (d, $J = 8.0$ Hz, 2H), 6.66 (d, $J = 8.0$ Hz, 2H), 6.93–6.96 (m, 2H), 7.99–8.03 (m, 1H), 8.11 (s, 1H), 8.22 (s, 1H). MS: m/z 544 (M+1), 546 (M+3). Anal. Calcd for $C_{25}H_{28}ClF_2N_9O$: C, 55.20; H, 5.19; N, 23.17. Found: C, 55.32; H, 5.29; N, 23.33.

4.45. (2*R*,3*S*)-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 (11h)

This was obtained in 35.50% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.00 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.52 (s, 2H), 3.96 (q, $J = 7.0$ Hz, 1H), 4.28 (d, $J = 14.0$ Hz, 1H), 4.50 (d, $J = 14.0$ Hz, 1H), 5.50 (br s, 1H), 6.30–6.61 (m, 5H), 6.90–6.95 (m, 2H), 7.93–7.96 (m, 1H), 8.10 (s, 1H), 8.21 (s, 1H). MS: m/z 524 (M+1). Anal. Calcd for $C_{26}H_{31}F_2N_9O$: C, 59.64; H, 5.97; N, 24.08. Found: C, 59.97; H, 6.24; N, 24.41.

4.46. (2*R*,3*S*)-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 (11i)

This was obtained in 41.60% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.05 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.96 (q, $J = 7.0$ Hz, 1H), 4.28 (d, $J = 14.0$ Hz, 1H), 4.47 (d, $J = 14.0$ Hz, 1H), 5.55 (br s, 1H), 6.30 (d, $J = 8.0$ Hz, 2H), 6.64 (d, $J = 8.0$ Hz, 2H), 6.98–7.04 (m, 2H), 7.90–7.97 (m, 1H), 8.09 (s, 1H), 8.21 (s, 1H). MS: m/z 528 (M+1). Anal. Calcd for $C_{25}H_{28}F_3N_9O$: C, 56.92; H, 5.35; N, 23.90. Found: C, 57.19; H, 5.54; N, 24.14.

4.47. (2*R*,3*S*)-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 (11j)

This was obtained in 41.70% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.03 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.98 (q, $J = 7.0$ Hz, 1H), 4.32 (d, $J = 14.0$ Hz, 1H), 4.45 (d, $J = 14.0$ Hz, 1H), 5.55 (br s, 1H), 6.25–6.45 (m, 3H), 6.94–6.99 (m, 2H), 7.90–7.95 (m, 1H), 8.04 (s, 1H), 8.22 (s, 1H). MS: m/z 578 (M+1). Anal. Calcd for $C_{25}H_{27}Cl_2F_2N_9O$: C, 51.91; H, 4.70; N, 21.79. Found: C, 52.09; H, 4.85; N, 21.91.

4.48. (2*R*,3*S*)-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 (11k)

This was obtained in 39.70% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.04 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.00 (q, $J = 7.0$ Hz, 1H), 4.28 (d, $J = 14.0$ Hz, 1H), 4.51 (d, $J = 14.0$ Hz, 1H), 5.40 (br s, 1H), 6.26–6.44 (m, 3H), 6.94–6.99 (m, 2H), 7.90–7.97 (m, 2H), 8.09 (s, 1H), 8.23 (s, 1H). MS: m/z 511 (M+1). Anal. Calcd for $C_{24}H_{28}F_2N_{10}O$: C, 56.46; H, 5.53; N, 27.44. Found: C, 56.77; H, 5.91; N, 27.71.

4.49. (2*R*,3*S*)-2-(2,4-Difluorophenyl)-3-(5-{2-[4-(diphenylmethyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (11l)

This was obtained in 44.00% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.06 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.00 (q, $J = 7.0$ Hz, 1H), 4.20 (s, 1H), 4.30 (d, $J = 14.0$ Hz, 1H), 4.50 (d, $J = 14.0$ Hz, 1H), 5.55 (br s, 1H), 6.25–6.62 (m, 10H), 6.85–6.90 (m, 2H), 8.00–8.03 (m, 1H), 8.09 (s, 1H), 8.23 (s, 1H). MS: m/z 600 (M+1). Anal. Calcd for $C_{32}H_{35}F_2N_9O$: C, 64.09; H, 5.88; N, 21.02. Found: C, 64.27; H, 6.05; N, 21.19.

4.50. (2*R*,3*S*)-2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2-butoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (11m)

This was obtained in 42.30% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, $J = 6.0$ Hz, 3H), 1.03 (d, $J = 7.0$ Hz, 3H), 1.72–1.77 (m, 4H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.97 (q, $J = 7.0$ Hz, 1H), 4.04–4.11 (m, 2H), 4.30 (d, $J = 14.0$ Hz, 1H), 4.48 (d, $J = 14.0$ Hz, 1H), 5.52 (br s, 1H), 6.20–6.43 (m, 4H), 7.32–7.36 (m, 2H), 7.90–7.95 (m, 1H), 8.08 (s, 1H), 8.21 (s, 1H). MS: m/z 582 (M+1). Anal. Calcd for $C_{29}H_{37}F_2N_9O_2$: C, 59.88; H, 6.41; N, 21.67. Found: C, 60.12; H, 6.79; N, 21.91.

4.51. (2*R*,3*S*)-2-(2,4-Difluorophenyl)-3-(5-{2-[4-phenyl-piperazin-1-yl]-ethyl}-tetrazol-2-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (11n)

This was obtained in 43.80% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.06 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.05 (q, $J = 7.0$ Hz, 1H), 4.35 (d, $J = 14.0$ Hz, 1H), 4.65 (d, $J = 14.0$ Hz, 1H), 5.45 (br s, 1H), 6.15–6.56 (m, 5H), 6.85–6.93 (m, 2H), 7.80–7.92 (m, 1H), 8.04 (s, 1H), 8.16 (s, 1H). MS: m/z 510 (M+1). Anal. Calcd for $C_{25}H_{29}F_2N_9O$: C, 58.93; H, 5.74; N, 24.74. Found: C, 59.17; H, 5.94; N, 24.86.

4.52. (2*R*,3*S*)-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 (12b)

This was obtained in 12.60% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.98 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.72 (s, 3H), 4.00 (q, $J = 7.0$ Hz, 1H), 4.35 (d, $J = 14.0$ Hz, 1H), 4.55 (d, $J = 14.0$ Hz, 1H), 5.37 (br s, 1H), 6.20 (d, $J = 8.0$ Hz, 2H), 6.55 (d, $J = 8.0$ Hz, 2H), 6.92–6.97 (m, 2H), 7.96–7.99 (m, 1H), 8.06 (s, 1H), 8.20 (s, 1H). MS: m/z 540 (M+1). Anal. Calcd for $C_{26}H_{31}F_2N_9O_2$: C, 57.87; H, 5.79; N, 23.36. Found: C, 58.01; H, 5.93; N, 23.55.

4.53. (2*R*,3*S*)-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 (12c)

This was obtained in 14.80% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.99 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.10 (q, $J = 7.0$ Hz, 1H), 4.31 (d, $J = 14.0$ Hz, 1H), 4.52 (d, $J = 14.0$ Hz, 1H), 5.40 (br s, 1H), 6.75–6.82 (m, 4H), 7.40–7.44 (m, 1H), 8.05 (s, 1H), 8.18 (s, 1H), 8.22 (d, $J = 9.0$ Hz, 2H). MS: m/z 555 (M+1). Anal. Calcd for $C_{25}H_{28}F_2N_{10}O_3$: C, 54.15; H, 5.09; N, 25.26. Found: C, 54.42; H, 5.37; N, 25.41.

4.54. (2*R*,3*S*)-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 (12d)

This was obtained in 13.80% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.98 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.04 (q, $J = 7.0$ Hz, 1H), 4.33 (d, $J = 14.0$ Hz, 1H), 4.53 (d, $J = 14.0$ Hz, 1H), 5.42 (br s, 1H), 6.28–6.60 (m, 4H), 6.95–6.99 (m, 2H), 7.96–8.00 (m, 1H), 8.04 (s, 1H), 8.20 (s, 1H). MS: m/z 578 (M+1). Anal. Calcd for $C_{26}H_{28}F_5N_9O$: C, 54.07; H, 4.89; N, 21.83. Found: C, 54.32; H, 5.09; N, 22.21.

4.55. (2*R*,3*S*)-2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2-chlorophenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (12e)

This was obtained in 17.60% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.95 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.12 (q, $J = 7.0$ Hz, 1H), 4.30 (d, $J = 14.0$ Hz, 1H), 4.50 (d, $J = 14.0$ Hz, 1H), 5.55 (br s, 1H), 6.25–6.60 (m, 4H), 6.90–6.94 (m, 2H), 7.92–8.01 (m, 1H), 8.09 (s, 1H), 8.22 (s, 1H). MS: m/z 544 (M+1), 546 (M+3). Anal. Calcd for $C_{25}H_{28}ClF_2N_9O$: C, 55.20; H, 5.19; N, 23.17. Found: C, 55.41; H, 5.39; N, 23.39.

4.56. (2*R*,3*S*)-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 (12f)

This was obtained in 18.30% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.96 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.14 (q, $J = 7.0$ Hz, 1H), 4.30 (d, $J = 14.0$ Hz, 1H), 4.55 (d, $J = 14.0$ Hz, 1H), 5.50 (br s, 1H), 6.25–6.50 (m, 4H), 7.01–7.09 (m, 2H), 7.90–7.93 (m, 1H), 8.07 (s, 1H), 8.23 (s, 1H). MS: m/z 544 (M+1), 546 (M+3). Anal. Calcd for $C_{25}H_{28}ClF_2N_9O$: C, 55.20; H, 5.19; N, 23.17. Found: C, 55.29; H, 5.32; N, 23.35.

4.57. (2*R*,3*S*)-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 (12g)

This was obtained in 19.30% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.00 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.13 (q, $J = 7.0$ Hz, 1H), 4.30 (d, $J = 14.0$ Hz, 1H), 4.54 (d, $J = 14.0$ Hz, 1H), 5.54 (br s, 1H), 6.15 (d, $J = 8.0$ Hz, 2H), 6.70 (d, $J = 8.0$ Hz, 2H), 6.90–6.94 (m, 2H), 7.99–8.04 (m, 1H), 8.08 (s, 1H), 8.24 (s, 1H). MS: m/z 544 (M+1), 546 (M+3). Anal. Calcd for $C_{25}H_{28}ClF_2N_9O$: C, 55.20; H, 5.19; N, 23.17. Found: C, 55.41; H, 5.37; N, 23.29.

4.58. (2*R*,3*S*)-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 (12h)

This was obtained in 19.80% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.97 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 3.50 (s, 2H), 4.12 (q, $J = 7.0$ Hz, 1H), 4.34 (d, $J = 14.0$ Hz, 1H), 4.50 (d, $J = 14.0$ Hz, 1H), 5.45 (br s, 1H), 6.32–6.56 (m, 5H), 6.90–6.95 (m, 2H), 7.90–7.94 (m, 1H), 8.08 (s, 1H), 8.23 (s, 1H). MS: m/z 524 (M+1). Anal. Calcd for $C_{26}H_{31}F_2N_9O$: C, 59.64; H, 5.97; N, 24.08. Found: C, 59.89; H, 6.13; N, 24.24.

4.59. (2*R*,3*S*)-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 (12i)

This was obtained in 14.80% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.00 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.15 (q, $J = 7.0$ Hz, 1H), 4.34 (d, $J = 14.0$ Hz, 1H), 4.50 (d, $J = 14.0$ Hz, 1H), 5.50 (br s, 1H), 6.28 (d, $J = 8.0$ Hz, 2H), 6.60 (d, $J = 8.0$ Hz, 2H), 6.98–7.02 (m, 2H), 7.92–7.97 (m, 1H), 8.10 (s, 1H), 8.23 (s, 1H). MS: m/z 528 (M+1). Anal. Calcd for $C_{25}H_{28}F_3N_9O$: C, 56.92; H, 5.35; N, 23.90. Found: C, 57.27; H, 5.69; N, 24.09.

4.60. (2*R*,3*S*)-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 (12j)

This was obtained in 18.20% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.98 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.08 (q, $J = 7.0$ Hz, 1H), 4.34 (d, $J = 14.0$ Hz, 1H), 4.50 (d, $J = 14.0$ Hz, 1H), 5.50 (br s, 1H), 6.20–6.50 (m, 3H), 6.90–6.98 (m, 2H), 7.93–7.98 (m, 1H), 8.06 (s, 1H), 8.20 (s, 1H). MS: m/z 578 (M+1). Anal. Calcd for $C_{25}H_{27}Cl_2F_2N_9O$: C, 51.91; H, 4.70; N, 21.79. Found: C, 52.17; H, 4.97; N, 21.98.

4.61. (2*R*,3*S*)-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 (12k)

This was obtained in 20.00% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.00 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.11 (q, $J = 7.0$ Hz, 1H), 4.30 (d, $J = 14.0$ Hz, 1H), 4.55 (d, $J = 14.0$ Hz, 1H), 5.45 (br s, 1H), 6.23–6.40 (m, 3H), 6.94–6.99 (m, 2H), 7.96–8.00 (m, 2H), 8.08 (s, 1H), 8.21 (s, 1H). MS: m/z 511 (M+1). Anal. Calcd for $C_{24}H_{28}F_2N_{10}O$: C, 56.46; H, 5.53; N, 27.44. Found: C, 56.63; H, 5.79; N, 27.59.

4.62. (2*R*,3*S*)-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 (12l)

This was obtained in 17.30% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.99 (d, $J = 7.0$ Hz, 3H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.09 (q, $J = 7.0$ Hz, 1H), 4.21 (s, 1H), 4.32 (d, $J = 14.0$ Hz, 1H), 4.53 (d, $J = 14.0$ Hz, 1H), 5.40 (br s, 1H), 6.20–6.60 (m, 10H), 6.94–6.97 (m, 2H), 8.02–8.03 (m, 1H), 8.10 (s, 1H), 8.23 (s, 1H). MS: m/z 600 (M+1). Anal. Calcd for $C_{32}H_{35}F_2N_9O$: C, 64.09; H, 5.88; N, 21.02. Found: C, 64.37; H, 5.99; N, 21.31.

4.63. (2*R*,3*S*)-2-(2,4-Difluorophenyl)-3-(5-{2-[4-(2-butoxyphenyl)-piperazin-1-yl]-ethyl}-tetrazol-1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol (12m)

This was obtained in 15.70% yield as oil. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, $J = 6.0$ Hz, 3H), 0.99 (d, $J = 7.0$ Hz, 3H), 1.70–1.74 (m, 4H), 2.60–2.70 (m, 4H), 2.90–2.95 (m, 2H), 3.02–3.11 (m, 2H), 3.30–3.41 (m, 4H), 4.05–4.10 (m, 2H), 4.12 (q, $J = 7.0$ Hz, 1H), 4.38 (d, $J = 14.0$ Hz, 1H), 4.52 (d, $J = 14.0$ Hz, 1H), 5.48 (br s, 1H), 6.22–6.40 (m, 4H), 7.38–7.45 (m, 2H), 7.92–7.98 (m, 1H), 8.09 (s, 1H), 8.23 (s, 1H). MS: m/z 582 (M+1). Anal. Calcd for $C_{29}H_{37}F_2N_9O_2$: C, 59.88; H, 6.41; N, 21.67. Found: C, 60.07; H, 6.59; N, 21.72.

4.64. (2*R*,3*S*)-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 (12n)

This was obtained in 17.90% yield as oil. ^{1}H NMR ($CDCl_3$): δ 1.04 (d, $J = 7.0$ Hz, 3H), 2.50–2.57 (m, 4H), 2.76–2.82 (m, 4H), 3.00–3.30 (m, 4H), 4.00 (q, $J = 7.0$ Hz, 1H), 4.30 (d, $J = 14.0$ Hz, 1H), 4.58 (d, $J = 14.0$ Hz, 1H), 5.40 (br s, 1H), 6.15–6.60 (m, 5H), 6.89–6.94 (m, 2H), 7.85–7.94 (m, 1H), 8.00 (s, 1H), 8.14 (s, 1H). MS: m/z 510 (M+1). Anal. Calcd for $C_{25}H_{29}F_2N_9O$: C, 58.93; H, 5.74; N, 24.74. Found: C, 59.21; H, 5.89; N, 24.95.

4.65. Hydrochloride salt of compound 11d

To a solution of **11d** (144.3 mg, 0.25 mmol) in absolute ethanol (2 mL) was added 1 M ethanolic-HCl (9.13 mg, 0.25 mmol) at 5 °C under stirring. The stirring was continued at same temperature for 1 h. the solid separated out was filtered through suction, washed with diethyl ether (2 × 5 mL), dried under reduced pressure to give a off-white solid in quantitative yield. MS: m/z 578 (M+1). Anal. Calcd for $C_{26}H_{28}F_5N_9O{\cdot}HCl$: C, 50.86; H, 4.76; N, 20.53. Found: C, 51.23; H, 5.09; N, 20.82.

Using the same method as described above the hydrochloride salt of compound **12d** was also prepared. MS: m/z 578 (M+1). Anal. Calcd for $C_{26}H_{28}F_5N_9O{\cdot}HCl$: C, 50.86; H, 4.76; N, 20.53. Found: C, 50.93; H, 5.14; N, 20.66.

Acknowledgements

We wish to express our thanks to microbiology department of New Chemical Entity Research, Lupin Research Park, Pune for biological screening and analytical chemistry department for ^{1}H NMR, mass spectroscopy, elemental analyses of compounds synthesized.

References and notes

1. Sheehan, D. J.; Hitchcock, C. A.; Sibley, C. M. *Clin. Microbiol. Rev.* **1999**, *12*, 40.
2. (a) Vanden Bossche, H. In *Current Topics in Medical Mycology*; McGuinnis, M. R., Ed.; Springer: Berlin, 1985; Vol. 1, pp 313–351; (b) Aoyama, Y.; Yoshida, Y.; Sato, R. *J. Biol. Chem.* **1984**, *259*, 1661; (c) Trzaskos, J. M.; Bowen, W. D.; Shafiee, A.; Fischer, R. T.; Gaylor, J. L. *J. Biol. Chem.* **1984**, *259*, 13402.
3. Richardson, K.; Brammer, K. W.; Marriott, M. S.; Troke, P. F. *Antimicrob. Agents Chemother.* **1985**, *27*, 832.
4. Van Cutsem, J.; Van Gerven, F.; Janssen, P. A. J. In *Recent Trends in the Discovery, Development, and Evaluation of Antifungal Agents*; Fromtling, R. A., Ed.; Prous: Barcelona, 1987; pp 177–192.
5. Dickinson, R. P.; Bell, A. S.; Hitchcock, C. A.; Nayayanaswami, S.; Ray, S. J.; Richardson, K.; Troke, P. F. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2031.
6. (a) Formtling, R. A. *Drugs Future* **1996**, *21*, 160; (b) Saksena, A. K.; Girijavallabhan, V. M.; Wang, H.; Liu, Y.-T.; Pike, R. E.; Ganguly, A. K. *Tetrahedron Lett.* **1996**, *37*, 5657.
7. (a) Hata, K.; Kimura, J.; Miki, H.; Toyosawa, T.; Moriyama, M.; Katsu, K. *Antimicrob. Agents Chemother.* **1996**, *40*, 2237; (b) Hata, K.; Kimura, J.; Miki, H.; Toyosawa, T.; Moriyama, M.; Katsu, K. *Antimicrob. Agents Chemother.* **1996**, *40*, 2243; (c) Kaku, Y.; Tsuruoka, A.; Kakinuma, H.; Tsukada, I.; Yanagisawa, M.; Naito, T. *Chem. Pharm. Bull.* **1998**, *46*, 1125.
8. Konosu, T.; Tajima, Y.; Oida, S. *Chem. Pharm. Bull.* **1991**, *39*, 2241.
9. Konosu, T.; Tajima, Y.; Takeda, N.; Miyaoka, T.; Kasahara, M.; Yasuda, H.; Oida, S. *Chem. Pharm. Bull.* **1990**, *38*, 2476.
10. Richardson, K.; Cooper, K.; Marriott, M. S.; Tarbit, M. H.; Troke, P. F.; Whittle, P. J. *Ann. N.Y. Acad. Sci.* **1988**, *544*, 6.
11. Tasaka, A.; Tsuchimori, N.; Kitazaki, T.; Hiroe, K.; Hayashi, R.; Okonogi, K.; Itoh, K. *Chem. Pharm. Bull.* **1995**, *43*, 441.
12. Konosu, T.; Tajima, Y.; Takeda, N.; Miyaoka, T.; Kasahara, M.; Yasuda, H.; Oida, S. *Chem. Pharm. Bull.* **1991**, *39*, 2581.
13. Bartroli, J.; Turmo, E.; Alguero, M.; Boncompte, E.; Vericat, M. L.; Conte, L.; Ramis, J.; Merlos, M.; Garcia-Rafanell, J.; Forn, J. *J. Med. Chem.* **1998**, *41*, 1869.
14. Verma, A. K.; Arora, S. K.; Arora, J. S.; Rattan, A. PCT Application, WO 0166551, September 13, 2001.
15. Arora, S. K.; Sinha, N.; Jain, S.; Upadhayaya, R. S.; Kishore, N. Unpublished results.
16. Singh, I. P.; Sidhu, I.; Palak, B. U.S. Patent 6133485, October 17, 2000.
17. Kitazaki, T.; Tamura, N.; Tasaka, A.; Matsushita, Y.; Hayashi, R.; Okongi, K.; Itoh, K. *Chem. Pharm. Bull.* **1996**, *44*, 314.
18. Hyao, S.; Havera, H. J.; Strycker, W. G.; Leipzig, T. J.; Rodriguez, R. *J. Med. Chem.* **1967**, *10*, 400.
19. Strycker, W. J.; Hayao, S. U.S. Patent 3231574, January 25, 1966.
20. Leipzig, T. J. U.S. Patent 4097479, June 27, 1978.
21. Pollard, C. B.; Rietz, E. G.; Robbins, R. *J. Am. Chem. Soc.* **1953**, *75*, 2989.
22. Kitazaki, T.; Ichikawa, T.; Tasaka, A.; Hosono, H.; Matsushita, Y.; Hayashi, R.; Okonogi, K.; Itoh, K. *Chem. Pharm. Bull.* **2000**, *48*, 1935.
23. Huff, L.; Henry, R. A. *J. Med. Chem.* **1970**, *13*, 777.

24. Tasaka, A.; Tamura, N.; Matsushiti, Y.; Kitazaki, T.; Hayashi, R.; Okongi, K.; Itoh, K. *Chem. Pharm. Bull.* **1995**, *43*, 432.
25. Elpern, B. *J. Am. Chem. Soc.* **1953**, *75*, 661.
26. Henry, R. A. *J. Am. Chem. Soc.* **1951**, *73*, 4470.
27. Henry, R. A.; Finnegan, W. G. *J. Am. Chem. Soc.* **1954**, *76*, 923.
28. Butler, R. N.; Scott, F. L. *J. Org. Chem.* **1966**, *31*, 3182.
29. Rapp, R.; Howard, J. *Can. J. Chem.* **1969**, *47*, 813.