Asian Journal of Chemistry; Vol. 35, No. 10 (2023), 2561-2571

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.28227

Bioisosterism based Design, Synthesis and Antifungal Evaluation of Novel Fluconazole Analogues

RANI S. KANKATE[1,*], VIKAS C. NATHE[1], SANJAY J. KSHIRSAGER[2], SUNITA N. SURSE[3], SNEHA A. BHALERAO[4], SHANTANU S. GHODKE[1] and JATIN A. SADHWANI[1]

[1]Department of Pharmaceutical Chemistry, MET's Institute of Pharmacy, Bhujbal Knowledge City, Nashik-422003, India
[2]Department of Pharmaceutics, MET's Institute of Pharmacy, Bhujbal Knowledge City, Nashik-422003, India
[3]Department of Pharmacognosy, MET's Institute of Pharmacy, Bhujbal Knowledge City, Nashik-422003, India
[4]Department of Pharmaceutical Chemistry, Indala Institute of Pharmacy, Kalyan-421301, India

*Corresponding author: E-mail: ranipharmacy@gmail.com

Received: 15 July 2023; *Accepted*: 21 September 2023; *Published online*: 28 September 2023; AJC-21411

A novel series of compounds containing *tert*.-amine moiety, piperazine triazole and triazole ring were synthesized by microwave assisted synthesis. The solubility and pharmacokinetic profiles of the compounds were improved by introducing the piperazine at the C3 position of fluconazole. The methylene bridge between the *tert*.-amine group and piperazine triazole moiety is kept constant. The *tert*.-amino moiety was chosen as a bioisostere to substitute *tert*.-alcohol group in fluconazole. In contrast to *tert*.-alcohols, *tert*.-amino groups exhibit the capacity to engage in hydrogen bonding interactions. Additionally, they have the ability to accept protons or form quaternary salts, thereby enhancing their water solubility. Furthermore, *tert*.-amino groups can coordinate with metal ions, potentially resulting in improved affinity, selectivity and potency in biological properties. In present study, homology models of the CYP51 enzymes derived from *Candida albicans* were developed using the X-ray crystal structure of CYP51 from *Saccharomyces cerevisiae*. These models were subsequently employed in a molecular docking investigation, focusing on the active site of the fungal enzymes responsible for lanosterol 14α-demethylation. The synthesized compounds were characterized using ^{1}H NMR, IR and mass spectral techniques. The evaluation of compounds using the *in vitro* broth dilution technique and disc diffusion assay shown antifungal activity against *C. albicans* and *C. tropicalis*. The results indicated that certain compounds exhibited activity similar to that of fluconazole.

Keywords: Fluconazole, Antifungal activity, *Candida albicans*, Triazole, Microwave irradiation, Lanosterol 14α-demethylase.

INTRODUCTION

Antifungal infections are as old as the existence of life itself on earth. Three decades earlier the available antifungal drug therapy was not efficient in eradicating the fungal infections. In the late 1950s, Amphotericin B became the "gold standard" for the treatment of systemic antifungal infections [1]. From 1958 to1959, a dramatic change in the status of antifungal therapy was indicated with the introduction of imidazoles. Chlormidazole, a chlorobenzyl imidazole and iodinated trichlorophenols, which acted by disruption of essential components of the fungal cell wall. The success story of imidazole antifungals continued in the decade of the 1960s with the development of antifungals agents like clotrimazole, miconazole and econazole [1]. In late 1970s, the problems associated with these imidazole antifungals stimulated the discovery of ketoconazole abroad spectrum antifungal agent. The imidazole antifungal were used against superficial skin infections caused by *Candida* and *Aspergillus* spp., but lack complete penetration in cerebrospinal fluid (CSF) and interferes with adrenal corticosteroid synthesis along with another potential side-effects [2]. Thus, ketoconazole becomes the choice of drug in non-immuno-compromised individuals. Over the three decades, significant advances have been made with triazole antifungals. The first generation triazoles fluconazole and itraconazole were launched in mid of 1980s [3].

Three novel yet broad-spectrum second-generation triazole antifungals voriconazole, posaconazole and ravuconazole were marketed from 2000-2007 and became the promising agents in antifungal drug therapy. The most recent triazole antifungal that is still under clinical trials is albaconazole indicated for vulvo vaginal candidiasis and onychomycosis [4-9]. The issue of development of resistance to currently available antifungal drugs with discovery and evolution of new strains poses a threat

and forces the worldwide scientist to make constant efforts around these issues [5,10-13]. CYP51 plays an essential role in the biosynthesis of membrane sterols (ergosterol) thus became the primary target for antifungal drug therapy. The mechanism of inhibition of CYP51 involvesbinding of amidine nitrogen atom (N-4 of triazole or N-3 of imidazole) to the heme iron atom of the enzyme [14-17].

The literature review reveals that unsubstituted triazole rings, the hydroxyl moiety and phenyl ring with a substitution of halogen (F/Cl) at a second or fourth position were the pharmacophoric groups of antifungal agents. A varied number of (3D) models of CYP51interacting with azole antifungals have been proposed [18-21]. The researchers mainly focus on the modification of the side chain at the C-3 position of these triazole antifungals, which includes the incorporation of the linkers like the substituted piperazinyl group, *N*-alkyl groups or aryloxy, *etc*. [22-25]. In this research, we also intended to change the side chain to in order improve the antifungal activity along with improved solubility and ADME properties. Recently, marketed antifungal drugs contains piperazine moiety in their structure like ketoconazole, posaconazole and itraconazole. The second approach includes increasing the carbon atom length to ethylene or benzyl to offer the flexibility in the side chain [26-28]. A novel series of (1*H*-1,2,4-triazol-3-yl)piperazine tertiary amine type of fluconazole analogues were synthesized on the basis of the *in silico* drug design approach like molecular docking studies. The docking studies were performed using homology modeled lanosterol 14α-demethylase of *Candida albicans* built using VLife MDS software and validated using online servers.

EXPERIMENTAL

The chemicals required for the experiment were obtained from HI Media Laboratories, S.D. fine chemicals, Sigma-Aldrich and Merck Pvt. Ltd. The purity of compounds confirmed using thin layer chromatography using precoated aluminum plates and column chromatographic techniques and UV cabinet was used for the visualization of the spots. KBr disc was used to record the IR spectra on a Shimadzu 1000 FTIR spectrometer in the range of 4000-200 cm^{-1}. ^1H NMR spectra recorded on Bruker Advance II 400 NMR Spectrophotometer using DMSO as solvent, at the Sophisticated Analytical Instrumentation Facility (SAIF)-CIL, Panjab University, Chandigarh, India. Mass spectra of compounds recorded on LC-MS Spectrometer Model Q-T of Micro Waters at SAIF-CIL Panjab University, Chandigarh, India.

The synthesis of triazol-3-yl piperazine *tert*.-amine type of fluconazole analogues were carried in five steps.

Synthesis of 1-(1*H*-1,2,4-triazol-3-yl)piperazine (3): *bis*(2-Chloroethylamine)hydrochloride (**1**) (40 mmol), 3-amino-1*H*-1,2,4-triazole (**2**) (10 mmol), *p*-toluene sulphonic acid (PTSA) were mixed in xylene and poured the reaction mixture in a microwave flask containing condenser. The mixture irradiated with microwaves at microwave intensity of 350 W for 15 min. TLC having chloroform:methanol (8:2) as mobile phase was used in order to check the progress of the reaction. After the reaction was over, the reaction mixture was poured into the ice-cold water till a solid product crystallizes out. Removed excess xylene by simple filtration and then the solid product was recrystallized from ethanol. The solid product was dried under anhydrous Na_2SO_4 [29-31]. White solid; yield 90%; m.p.: 156-160 ºC; IR (KBr, ν_{max}, cm^{-1}): 1666 (C=N), 1310 (C-N), 3301 (N-H), 1600 ($C=C_{arom}$), 1584 (N-N), 915 (1,2,4-trisubstituted$_{arom}$). ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 8.02 (s, 1H, triazole N-H), 8.34 (s, 1H, triazole C5-H), 3.3 (s, 1H, -NH piperazine), 2.50-3.16 (m, 8H, piperazinenn). ^{13}C NMR; (δ_C ppm, DMSO-d_6): 45.9-51.6 (d, 4C, -CH_2 of piperazine), 151.1-162.5 (d, 2C, triazine). ESI-MSMS *m/z*: 153, 85, 68.

Synthesis of 1-(bromomethyl)-4-(1*H*-1,2,4-triazol-3-yl)-piperazine (4): 1-(1*H*-1,2,4-Triazol-3-yl)piperazine (**3**) (20 mmol) in absolute ethanol (25 mL) was added to dibromomethane (10 mmol) under the continuous stirring at room temperature. Further, continued the stirring for next 24-72 h at the same reflux temperature. Close the mouth of round bottom flask with a plug of cotton at neck to prevent the loss of ethanol during stirring and then allowed to evaporate ethanol slowly. The obtained product was recrystallized from methanol. An eluent mixture of chloroform:methanol (9:1) used as mobile phase. Colour: creamish white solid; yield 81%; m.p.: 177-180 ºC; IR (KBr, ν_{max}, cm^{-1}): 1650 (C=N), 1341 (C-N), 3321 (N-H), 1598 ($C=C_{arom}$), 1564 (N-N), 942 (1,2,4-trisubstituted$_{arom}$). ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 7.82 (s, 1H, triazole N-H), 8.09 (s, 1H, triazole C5-H), 3.6 (s, 2H, -CH_2-Br), 2.31-3.28 (m, 8H, piperazine-8H). ^{13}C NMR; (δ_C ppm, DMSO-d_6): 48.8-50.9 (d, 4C, -CH_2 of piperazine), 56.2 (s, 1C, -CH_2), 151.1-162.5 (d, 2C, triazine). ESI-MSMS *m/z*: 245, 177, 152, 68.

General procedure for synthesis of N-(bromomethyl)-di/mono-substituted aniline (6a-f): The synthesis was executed with di/mono halogen-substituted arylamine (**5a-f**, 2 mmol) and dibromomethane (4 mmol) in water (2-3 mL) in the microwave reaction flask and subjected to microwave irradiation for 25 min at 150 W. The reaction mixture was then cooled to room temperature followed by dilution with excess quantity (10-20 mL) of cold water followed by the addition of $NaHCO_3$ in order to neutralize the reaction mixture. The solution was extracted with CH_2Cl_2 (3 × 10) and then the organic phase was allowed to evaporate under vacumm pressure. Finally, collect the residue and its purity was confirmed by TLC using CH_2Cl_2: methanol mixture (9:1) as mobile phase [32].

6a: Faint brown solid; yield 86%; m.p.: 162-166 ºC; IR (KBr, ν_{max}, cm^{-1}): 3314 (N-H), 1620 ($C=C_{arom}$), 1299 (C-N), 889 ($C-F_{arom}$). ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 7.52 (s, 1H, Ar-NH), 7.10-6.97 (m, 3H, Ar-H), 3.1 (s, 2H, -CH_2-Br). ^{13}C NMR; (δ_C ppm, DMSO-d_6): 54.1 (s, 1C, -CH_2), 116.6-126.1 (m, 4C, aromatic), 152.9 & 156.4 (s, 2C, aromatic C-F). ESI-MSMS *m/z*: 220, 142, 113, 70.

General procedure for synthesis of *N*-((1*H*-1,2,4-triazol-1-yl)methyl)-di/mono halogen substituted aniline (8a-f): Accurately weighed 1*H*-1,2,4-triazole (**7**) (10 mmol), K_2CO_3 (4 mmol), dry N-(bromo methyl)-di/monohalo substituted aniline derivative (**6a-f**, 6 mmol) and tetraethylammonium iodide (TEAI) in acetonitrile (10 mL) were added into the flask provided with calcium chloride guard and subjected to stirring for 24-

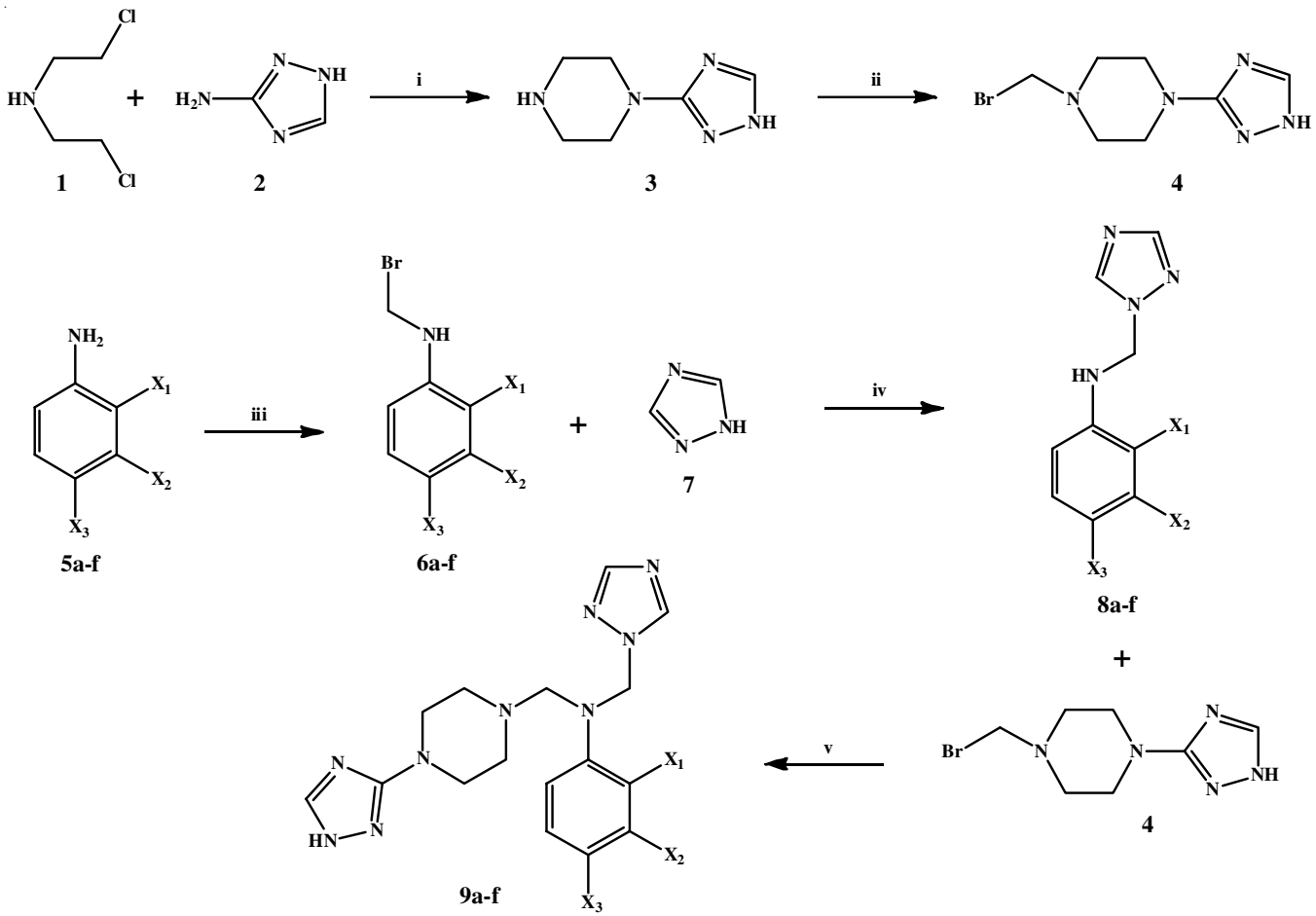
72 h on magnetic stirrer coupled with heating mantle at reflux temperature. The reaction mixture was then poured into 50 mL chilled water to get the solid precipitate. Collected solid precipitate washed with polar solvents like water or ethanol. An eluting solvent mixture of chloroform:methanol (8:2) used as the mobile phase for TLC [24,33]. In the alternative method, acetonitrile can be replaced with DMF containing powdered sodium hydroxide [34,35].

8a: White solid; yield 93%; m.p.: 192-196 ºC; IR (KBr, ν_{max}, cm^{-1}): 3299 (N-H), 1670 (C=N), 1588 (C=C_{arom}), 1579 (N-N), 1320 (C-N), 908 (1,2,4-trisubstituted$_{arom}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.09 (s, 1H, triazole N-H), 8.34 (s, 1H, triazole C5-H), 3.5 (s, 2H, -CH_2-), 7.21 (s, 1H, Ar-NH), 7.01-6.37 (m, 3H, Ar-H), ^{13}C NMR; (δ_C ppm, DMSO-d_6): 53.6 (s, 1C, -CH_2), 105.6-126.9 (m, 4C, arom.), 152.5 & 156.3 (d, 2C, arom. C-F), 158.6-159.5 (d, 2C, triazine). ESI-MSMS *m/z*: 210, 128, 113, 68.

General procedure for synthesis of *N*-((1*H*-1,2,4-triazol-1-yl)methyl)-*N*-((4-(1*H*-1,2,4-triazol-3-yl)piperazin-1-yl)-methyl)-di or mono halogen substituted aniline (9a-f): In a typical experiment, (10 mmol) of 1-(bromomethyl)-4-(1*H*-1,2,4-triazol-3-yl)piperazine (**4**, 10 mmol) of *N*-(1*H*-1,2,4-triazol-1-yl)methyl)-2,4-difluoroaniline (**8a-f**) dissolved in separate dry DMF in separate beakers and mixed in a round bottom flask. To this reaction mixture, 0.76 mL of added triethylamine (TEA) and the reaction mixture exposed to microwave radiations at 350 W for 20-25 min. Added ice-cold water into the reaction mixture with vigorous stirring and allow the solid to precipitate which was further separated under reduced pressure at the suction pump and finally recrystallize the solid product from ethanol (**Scheme-I**).

***N*-((1*H*-1,2,4-triazol-1-yl)methyl)-*N*-((4-(1*H*-1,2,4-triazol-3-yl)piperazin-1yl)methyl)-2,4-dichloroaniline (9a):** Yellow solid; yield 87%; m.p.: 205-208 ºC; IR (KBr, ν_{max}, cm^{-1}): 3325 (N-H), 1340 (C-N), 1674 (C=N), 1616 (C=C_{arom}), 1584 (N-N), 1568 (C-C_{arom}), 902 (1,2,4-trisubstituted$_{arom}$), 707 (s, C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.10-6.97 (m, 4H, Ar-H), 8.02 (s, 1H, triazole C3-H), 8.34 (s, 1H, triazole C5-H), 5.68 (s, 2H, -CH_2-triazole), 3.6 (s, 2H, -CH_2-piperazine), 2.50-3.16 (m, 8H, piperazine-8H), 8.3 (s, 1H, triazole C5′-H). ^{13}C NMR (δ_C ppm, DMSO-d_6): 49.4-50.3 (d, 4C, -CH_2 of piperazine), 60.5 (s, 1C, -CH_2), 73.4 (s, 1C, -CH_2), 117.1-148.8 (m, 6C, aromatic), 151.1-162.5 (m, 4C, triazine). ESI-MS *m/z*: 408, 372, 336, 268, 194, 74. Anal. calcd. (found) % for $C_{16}H_{19}N_9Cl_2$ (*m.w.* 408): C, 47.07 (47.02); H, 4.69 (4.65); Cl, 17.37 (17.20); N, 30.88 (30.72).

***N*-((1*H*-1,2,4-Triazol-1-yl)methyl)-*N*-((4-(1*H*-1,2,4-triazol-3-yl)piperazin-1-yl)methyl)-2,4-difluoroaniline (9b):** Brown solid; yield 65%; m.p.: 227-230 ºC; IR (KBr,



Scheme-I: Synthesis of triazol-3-yl piperazine *tertiary* amine type fluconazole analogues. Chemical reagents and reaction conditions: **9a-f**: X_1, X_2, X_3 = Cl, F (i) PTSA, xylene/(DMF); MWI 350 W, 15 min (ii) dibromomethane, ethanol, reflux at room temperature 24-72 h, (iii) water, $NaHCO_3$; MWI 150 W, 25 min; (iv) K_2CO_3, TEAI, acetonitrile (ACN), stirring 24-72 h

ν_{max}, cm^{-1}): 3317 (N-H), 1640 (C=N), 1635 (C=C_{arom}), 1580 (N-N), 1520 (C-C_{arom}), 1348 (C-N), 970 (C-F), 740 (1,2,4-trisubstituted$_{arom}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.06-6.72 (m, 4H, Ar-H), 8.04 (s, 1H, triazole C3-H), 8.06 (s, 1H, triazole C5-H), 5.6 (s, 2H, -CH_2-triazole), 3.6 (s, 2H, -CH_2-piperazine), 2.50-3.16 (m, 8H, piperazine-H), 8.44 (s, 1H, triazole C5′-H). ^{13}C NMR (δ_C ppm, DMSO-d_6): 48.7-51.0 (d, 4C, -CH_2 of piperazine), 61 (s, 1C, -CH_2), 75.1 (s, 1C, -CH_2), 105.6-132.9 (m, 4C, aromatic), 154.0 & 157.3 (s, 2C, aromatic C-F), 143.6-159.5 (m, 4C, triazine). ESI-MS *m/z*:375, 356, 337, 269, 194, 75. Anal. calcd. (found) % for $C_{16}H_{19}N_9F_2$ (*m.w.* 375): C, 51.19 (51.12); H, 5.10 (5.09); N, 33.58 (33.38); F, 10.12 (10.10).

N-((1*H*-1,2,4-Triazol-1-yl)methyl)-*N*-((4-(1*H*-1,2,4-triazol-3-yl)piperazin-1-yl)methyl)-3,4-dichloroaniline (9c): White solid; yield 70%; m.p.: 202-205 ºC; IR (KBr, ν_{max}, cm^{-1}): 3330 (N-H), 1645 (C=N), 1640 (C=C_{arom}), 1585 (N-N), 1530 (C-C_{arom}), 1340 (C-N), 750 (C-Cl), 740 (1,3,4-trisubstituted$_{arom}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.07-6.77 (m, 4H, Ar-H), 8.05 (s, 1H, triazole C3-H), 8.0 (s, 1H, triazole C5-H), 5.56 (s, 2H, -CH_2-triazole), 3.6 (s, 2H, -CH_2-piperazine), 2.5-3.16 (m, 8H, piperazine-8H), 8.54 (s, 1H, triazole C5′-H). ^{13}C NMR (δ_C ppm, DMSO-d_6): 48.9-50.3 (d, 4C, -CH_2 of piperazine), 61.5 (s, 1C, -CH_2), 71.4 (s, 1C, -CH_2), 116.1-134.8 (m, 6C, aromatic), 146.5-161.5 (m, 4C, triazine). ESI-MS *m/z*: 408, 372, 336, 268, 194, 74. Anal. calcd. (found) for $C_{16}H_{19}N_9Cl_2$ (*m.w.* 408): C, 47.07 (47.02); H, 4.69 (4.65); Cl, 17.37 (17.20); N, 30.88 (30.72).

***N*-((1*H*-1,2,4-Triazol-1-yl)methyl)-*N*-((4-(1*H*-1,2,4-triazol-3-yl)piperazin-1-yl)methyl)-2-fluoroaniline (9d):** Brown solid; yield 68%; m.p.: 190-192 ºC; IR (KBr, ν_{max}, cm^{-1}): 3319 (N-H), 1651 (C=N), 1616 (C=C_{arom}), 1585 (N-N), 1506 (C-C_{arom}), 1344 (C-N), 975 (C-F), 900 (1,2-disubstituted$_{arom}$). ^{1}H NMR (400 MHz, DMSO-d_6): 6.58-7.38 (m, 4H, Ar-H), 8.03 (s, 1H, triazole C3-H), 8.44 (s, 1H, triazole C5-H), 5.56 (s, 2H, -CH_2-triazole), 3.6 (s, 2H, -CH_2-piperazine), 2.50-3.16 (m, 8H, piperazine-H), 8.44 (s, 1H, triazole C5′-H). ^{13}C NMR (δ_C ppm, DMSO-d_6): 49.9-50.8 (d, 4C, -CH_2 of piperazine), 61.0 (s, 1C, -CH_2), 73.3 (s, 1C, -CH_2), 115.7-129.6 (m, 6C, aromatic), 149.7-156.5 (m, 4C, triazine). ESI-MS *m/z*: 357, 338, 270, 194, 76. Anal. calcd. (found) % for $C_{16}H_{20}N_9F$ (*m.w.* 357): C, 53.77 (53.61); H, 5.64 (5.62); N, 35.27 (35.28); F, 5.32 (5.29).

***N*-((1*H*-1,2,4-Triazol-1-yl)methyl)-*N*-((4-(1*H*-1,2,4-triazol-3-yl)piperazin-1-yl)methyl)-4-chloroaniline (9e):** Yellow solid; yield 78%; 125-127 ºC; IR (KBr, ν_{max}, cm^{-1}): 3334 (N-H), 1695 (C=N), 1624 (C=C), 1580 (N-N), 1500 (C-C_{arom}), 1498 (C-Cl), 1344 (C-N), 740 (1,4-disubstituted$_{arom}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 6.59-7.09 (m, 4H, Ar-H), 8.02 (s, 1H, triazole C3-H), 8.34 (s, 1H, triazole C5-H), 5.23 (s, 2H, -CH_2-triazole), 3.71 (s, 2H, -CH_2-piperazine), 2.50-3.16 (m, 8H, piperazine-H), 8.3 (s, 1H, triazole C5′-H). ^{13}C NMR (δ_C ppm, DMSO-d_6): 49.3-51.3 (d, 4C, -CH_2 of piperazine), 60.1 (s, 1C, -CH_2), 72.4 (s, 1C, -CH_2), 119.7-127.8 (m, 6C, aromatic), 151.0-159.7 (m, 4C, triazine). ESI-MS *m/z*: 372, 338, 270, 196, 127, 74. Anal. calcd. (found) % for $C_{16}H_{20}N_9Cl$ (*m.w.* 372): C, 51.40 (51.36); H, 5.39 (5.15); N, 33.72 (33.62); Cl, 9.48 (9.38);

N-((1*H*-1,2,4-Triazol-1-yl)methyl)-*N*-((4-(1*H*-1,2,4-triazol-3-yl)piperazin-1-yl)methyl)-4-nitroaniline (9f): Yellowish green solid; yield 77%; 230-232 ºC; IR (KBr, ν_{max}, cm^{-1}): 3334 (N-H), 1695 (C=N), 1624 (C=C), 1580 (N-N), 1500 (C-C_{arom}), 1344 (C-N), 975 (C-F), 740 (1,2-disubstituted$_{arom}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 6.71-8.04 (m, 4H, Ar-H), 8.04 (s, 1H, triazole C3-H), 8.06 (s, 1H, triazole C5-H), 5.34 (s, 2H, -CH_2-triazole), 3.89 (s, 2H, -CH_2-piperazine), 2.50-3.16 (m, 8H, piperazine-H), 8.8 (s, 1H, triazole C5′-H). ^{13}C NMR (δ_C ppm, DMSO-d_6): 49.4-50.3 (d, 4C, -CH_2 of piperazine), 61.1 (s, 1C, -CH_2), 73.9 (s, 1C, -CH_2), 115.9-137.3 (m, 5C, arom.), 147.4 (s, 1C, aromatic C-F), 151.0-162.5 (m, 4C, triazine). ESI-MS *m/z*: 357, 338, 270, 194, 76. Anal. calcd. (found) % for $C_{16}H_{20}N_9F$ (*m.w.* 357): C, 53.77 (53.71); H, 5.64 (5.59); N, 35.27 (35.18); F, 5.32 (5.28).

***In vitro* antifungal activity:** Clinical Laboratory and Standards Institute (CLSI) approved methods M27-A3 for broth dilution technique and for disc diffusion assay M44-A was employed in this study [36]. In present study, *C. albicans* (MTCC 227), *C. tropicalis* (ATCC 750) and *A. fumigatus* (MTCC 870) was used to investigate the antifungal activity. Fluconazole and N,N-dimethyl formamide (DMF) were used as standard and solvent, respectively. The data obtained after performing the *in vitro* antifungal activity show that all the synthesized compounds show moderate to excellent activity against the fungal pathogens. The parameters for testing of antifungal activity include the inoculum size of 1-5 × 10^4 CFU/mL prep-ared spectrophotometrically, Sabouraud Dextrose Broth as the test medium for inoculation and incubation at 24, 48 or 72 h; and the end point was determined with 50% reduction in the fungal growth with test compounds.

Broth dilution technique: The dilutions of test triazoles were prepared ranged from 2-320 µg/mL while the dilutions of reference standard fluconazole ranged from 0.125-64 µg/mL. Streaks loopful of previously maintained *Candida albicans* colonies were incubated overnight in a BOD incubator at 35 ºC on fresh SDA plate. With the help of sterile nichrome wire loop pick, five distinct yet isolated colonies that show similarity in colony morphology at least 1 mm in diameter further added to 5 mL of sterile NaCl saline 8.5 g/L solution. Form the homogenous suspension of inoculum by vertexing in vortex mixture for 15 to 20 s. The suspension was adjusted to 85% transmittance at 530 nm spectrophotometrically with the addition of 0.85% NaCl. Resulting suspension became 1-5 × 10^6 CFU/mL. A 1 mL of above suspension was added to 9 mL of Sabouraud Dextrose Broth (Sabouraud liquid medium). In order to prepare 1 mL of diluted antifungal agent solution, first pipette out 0.9 mL of liquid medium into 11 sterile test tubes. All the tubes were incubated for 48 h in BOD incubator and the experiments were conducted in triplicate [37-48].

Disc diffusion assay: Glass petri dishes were sterilized and then added 10 mL of sterilized melted SDA. The procedure for inoculum preparation was similar as mentioned in the broth dilution technique. Allowed the suspension to rest for 15 min after adjusting the turbidity. Then dipped a sterile cotton swab in the suspension of inoculum and remove the excess of liquid by pressing against the inner wall test tube above the level of

suspension in the test tube. Now the dried surface of SDA in the plates was streaked evenly and thoroughly over the entire surface of the agar medium. Prepared the sterile disc of the test samples and press down against the solidified agar surface to make sure complete contact with the agar surface. The plates were placed in the inverted position inside the incubator set at 35 ºC (± 2 ºC) within 15 min of applying the disc. Examined the plates for 24 to 48 h and if the plates were streaked correctly then uniform fungal growth is observed with distinct circular zone of inhibition and measured the zone of inhibition in mm.

Molecular docking studies: The molecular docking studies were carried out on Intel Windows PC using the VLifeMDS 4.3 software package.

Docking of the ligands: The lowest energy conformers were obtained by performing a systematic conformational search. The conformers generated were optimized until the rms gradient is achieved upto the 0.01 kcal/mol energy level. The binding mode of the novel fluconazole analogues in the active site of homology model of CYP450 from *C. albicans* was studied using the GA based docking method.

RESULTS AND DISCUSSION

Present work, the modifications were attem-pted in the structure of fluconazole by considering the three aspects (Fig. 1) *i.e.* (i) enhance the compound's solubility and pharmacokinetic profile through the incorporation of triazol-3-yl piperazine moiety at the C-3 position of fluconazole. The log P values of the proposed compound and fluconazole were found to be 2.49 and 0.99, respectively as reported previously [49-55]. (ii) the principle of bioisosterism was applied in this study, namely by selecting a *tert*.-amino moiety as a bioisostere to substitute *tert*.-alcohol present in fluconazole. The rationale behind this bioisosteric substitution is based on the observation that similar to *tert*.-alcohol group in fluconazole, *tert*.-amine moiety possesses the capability to engage in hydrogen bonding, either by accepting a proton or by forming quaternary salts. As a result, this substitution leads to an enhancement in the water solubility of the compound. This methodology not only enhances the potential for coordination with metals, but also impacts the biological properties by increasing affinity, selectivity and potency [56,57]; and (iii) the substitution of difluorophenyl group with the dihalogen substituted aniline group leads to an elevated absorption rate and enhanced lipid solubility [56].

The use of microwave technology in synthesis resulted in drastically reduced reaction times and increased yields. The designed compounds were synthesized by a five-step reaction process (**Scheme-I**). Initially, compound **3** was synthesized using *bis*(2-chloroethyl) amine (**1**) and 1*H*-1,2,4-triazol-3-amine (**2**) by microwave-assisted method. Different combinations of microwave energy and irradiation times were tried however the best result was obtained when the reaction mixture was irradiated for 15min at 350W powers. Within the next step, the product obtained in the previous step was treated with dibromomethane to give 1-(bromomethyl)-4-(1*H*-1,2,4-triazol-3-yl)piperazine (**4**) where both the reactants were dissolved in ethanol and stirred for next 24-72 h.The further step was most critical of the entire scheme as it required *N*-monoalkylation of anilines **5a-f**. Similar to the first step, several combinations of microwave power and time were tried, but monoalkylated products were obtained at 150 W with the irradiation for 20-25 min and water as solvent, thus can be considered as new efficiently green method ascompared to tedius reaction of N-monoalkylation. The next step includes selective N-1 alkylation of 1,2,4-triazoles (**7**), which was achieved with the additionof catalyst tetraethylammonium iodide (TEAI) + powdered NaOH in DMF with continuous stirring for 24-72 h. After stirring, a homogenous mixture was further irradiated in microwave at 420 W for 10 min. Finally, compounds **4** and **8a-f** were reacted to give final designed molecule (**9a-f**) in the presence of triethylamine using the microwave energy of 350 W for 15-20 min

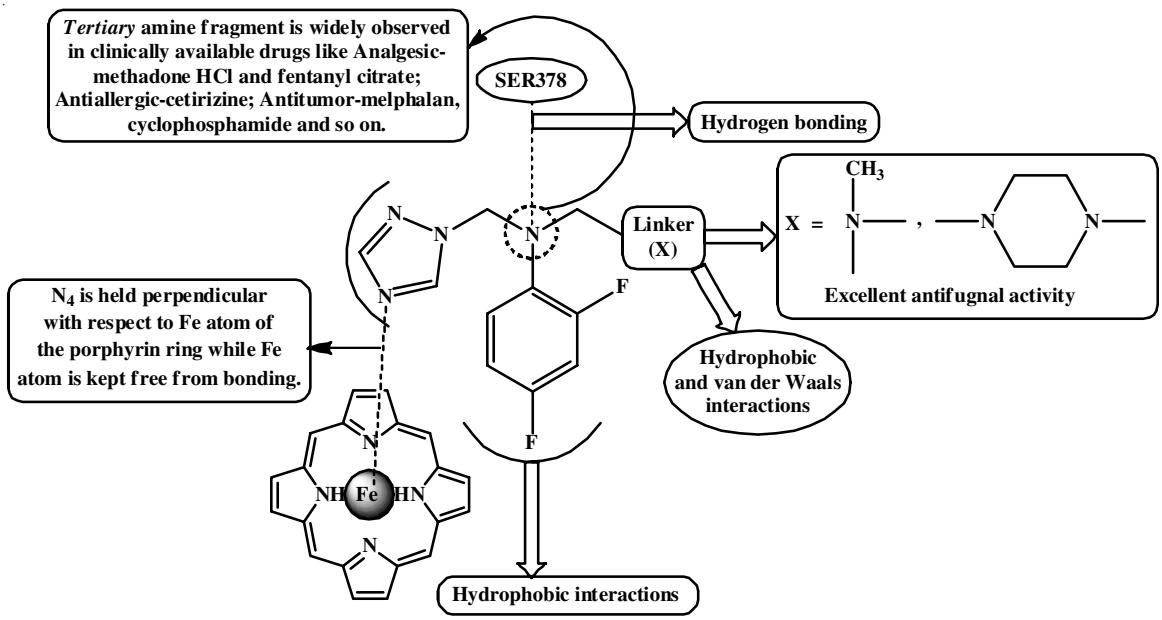


Fig. 1. Basic interaction model of lead azole antifungals

where yield was found to be higher when compared with conventional synthesis.

The spectral analyses were consistent with the assigned structures of the synthesized compounds. The mass spectra of all the novel compounds showed M+1 peak. In IR spectra, all the synthesized triazolo-piperazinyl compounds show two broad absorptions between 3317 and 3301 cm^{-1}, which are attributed to the secondary NH group. A characteristic C=N bands of triazole ring in all the derivatives appeared in the region between 1635 and 1604 cm^{-1}, while rest of the absorption bands were also observed at the expected regions. In ^1H NMR spectra, all the compounds gave singlets at 5.23 and 5.50 ppm assigned to the CH_2 protons linked with triazole ring, while the N-CH_2 protons of piperazine-triazole in the compound gave lower shift signals at 3.6-4.0 ppm. The protons of piperazine moiety showed multiplet in the range of 2.50-3.16 ppm. The protons of triazole ring C-3H and C-5H gave singlets at 8.01 and 8.30 ppm.

Biological activity: For the determination of MIC (µg/mL), the fluoro substituted derivatives shows comparable and excellent antifungal activity. Compound **9b** showed higher activity when whereas compounds **9f** and **9d** revealed to be equipotent when compared with standard (Table-1). The chloro substituted derivatives give the mild response to the inhibition of fungal growth at MIC ranged from 16-256 µg/mL. As the susceptibility was not observed at concentrations < 30 µg/mL therefore *in vitro* disc diffusion antifungal activity assayed at concentrations 32 and 64 µg/mL against *Candida* species, While in case of *Aspergillus fumigatus*, the sensitivity tested at 64 and 128 µg/L, due to lack of sensitivity below 64 µg/mL (Table-2). Compounds **9b**, **9d** and **9f** showed the superior activity at 64 µg/mL compared to standard fluconazole.

Homology modeling of CYP450 lanosterol 14α-demethylase *C. albicans*: The FASTA sequence of the target was obtained from Universal Protein Sequence (Uniprot Accession code: P10613) and homologous template was identified using Blast search (http://blast.ncbi.nlm.nih.gov/) while its three dimensional structure was retrieved from Protein Data Bank (PDB ID: 4LXJ: *Saccharomyces cerevisiae* lanosterol 14α-demethylase with lanosterol bound; Resolution 1.9 Å). The sequence alignment of target (Query 92750) and template (4LXJ) showed 65% identities, 78% positives and 1% gaps (Fig. 2). Upon building homology model, it was subjected to energy minimization until the rms gradient energy of 0.1 kcal/mol reached (Fig. 3).

Followed by energy minimization, an optimized energy model was subjected to validation with the Ramachandran plot and other suitable validation servers and parameters. Rampage a free onlinevalidation server was used for checking geometrical properties of the homology model in the form of Ramachandran plot [42,59].The Ramachandran plot gives the percentage of amino acids present in favoured region, allowed region, and outliers thus this can be understood with the Ramachandran analysis of template 4LXJ.

The results of the Ramachandran plot statistics are shown in Table-3. MolProbity provides the complete analysis concerned with all-atom contact of steric issues within the molecule as well as deals with diagnosis of updated dihedral-angle. MolProbity generates results in different forms: downloadable PDB, 3Dkinemage images viewed on-line in the KiNG viewer, overall scores, all criterion table and so on [43,46]. The comparative Ramachandran plot statistics by Rampage and MolProbity servers indicate close resemblance with the template 4LXJ (Fig. 4), thus a correct homology model is developed (Fig. 5).

TABLE-1
ANTIFUNGAL ACTIVITIES OF THE TEST COMPOUNDS **9a-f** *in vitro* MINIMUM INHIBITORY CONCENTRATION (µg/mL) BY SERIAL DILUTION METHOD AND RESULTS OF DOCKING STUDY

Compound	*Candida albicans* MTCC227	*Candida tropicalis* ATCC 750	*Aspergillus fumigatus* MTCC 870	Dosck score	Distance between N_4 nitrogen of azole and Fe atom of hemering (Å)
9a	16	32	128	-5.29	2.4
9b	2	4	32	-5.88	2.5
9c	16	64	256	-4.63	2.3
9d	8	32	64	-5.18	3.2
9e	256	128	320	-3.98	3.2
9f	8	32	64	-5.25	2.6
Fluconazole	8	32	64	-4.96	2.6

TABLE-2
ZONE OF INHIBITION OF TEST COMPOUNDS **9a-f** IN mm at MINIMUM INHIBITORY CONCENTRATION (32, 64 AND 128 µg/mL) BY DISC DIFFUSION ASSAY

Compound	*Candida albicans* MTCC 227		*Candida tropicalis* ATCC 750		*Aspergillus fumigatus* MTCC 870	
	32	64	32	64	64	128
9a	7	10	10	6	7	13
9b	20	24	24	12	11	18
9c	8	12	12	8	6	10
9d	18	22	22	10	10	16
9e	9	10	10	7	4	12
9f	17	20	20	11	9	15
Fluconazole	8	22	22	10	9	16

E-value: 0e+00, bit-score: 689, aligned-length: 520, Identity to query: 65%

```
                    10        20        30        40        50        60        70        80
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
Query_92750     4 VETVIDGINYFLSLSVTQQISILLGVPFVYNLVWQYLYSLRKDRAPLVFYWIPWFGSAASYGQQPYEFFESCRQKYGDVF  83
4LXJ_A         12 LEYVNIGLSHFLALPLAQRISLIIIIPFIYNIVWQLLYSLRKDRPPLVFYWIPWVGSAVVYGMKPYEFFEECQKKYGDIF  91

                    90        100       110       120       130       140       150       160
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
Query_92750    84 SFMLLGKIMTVYLGPKGHEFVFNAKLSDVSAEDAYKHLTTPVFGKGVIYDCPNSRLMEQKKFAKFALTTDSFKRYVPKIR 163
4LXJ_A         92 SFVLLGRVMTVYLGPKGHEFVFNAKLADVSAEAAYAHLTTPVFGKGVIYDCPNSRLMEQKKFVKGALTKEAFKSYVPLIA 171

                    170       180       190       200       210       220       230       240
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
Query_92750   164 EEILNYFVTIDESFKLKEKIHGVANVMKTQPEITIFTASRSLFGDEMRRIFDRSFAQLYSDLDKGFTPINFVFPNLPLPHY 243
4LXJ_A        172 EEVYKYFRDSKNFRLNERITGTIDVMVTQPEMTIFTASRSLLGKEMRAKLDTDFAYLYSDLDKGFTPINFVFPNLPLEHY 251

                    250       260       270       280       290       300       310       320
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
Query_92750   244 WRRDAAQKKISAIYMKEIKSRPERGDIDpNRDLIDSLLIHSTYKDGVKMTDQEIANLLIGILMGGQHTSASTSAWFLLHL 323
4LXJ_A        252 RKRDHAQKAISGTYMSLIKERRKNNDIQ-DRDLIDSLMKNSTYKDGVKMTDQEIANLLIGVLMGGQHTSAATSAWILLHL 330

                    330       340       350       360       370       380       390       400
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
Query_92750   324 GEKPHLQDVIYQEVVELLKekgGDLNDLTYEDLQKLPSVNNTIKETLRMHMPLHSIFRKVTNPLRIPETNYIVPKGHYVL 403
4LXJ_A        331 AERPDVQQELYEEQMRVLD---GGKKELTYDLLQEMPLLNQTIKETLRMHHPLHSLFRKVMKDMHVPNTSYVIPAGYHVL 407

                    410       420       430       440       450       460       470       480
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
Query_92750   404 VSPGYAHTSERYFDNPEDFDPTRWDTAAAKANSVsfnsSDEVDYGFGKVSKGVSSPYLPFGGGRHRCIGEQFAYVQLGTI 483
4LXJ_A        408 VSPGYTHLRDEYFPNAHQFNIHRWNNDSASSYSV----GEEVDYGFGAISKGVSSPYLPFGGGRHRCIGEHFAYCQLGVL 483

                    490       500       510       520
                    ....*....|....*....|....*....|....*....|.
Query_92750   484 LTTFVYNLRWTI-DGYKVPDPDYSSMVVLPTEPAEIIWEKR 523
4LXJ_A        484 MSIFIRTLKWHYpEGKTVPPPDFTSMVTLPTGPAKIIWEKR 524
```

Fig. 2. Target-template sequence alignment

Fig. 3. Homology model of *Candida albicans* lanosterol 14α-demethylase

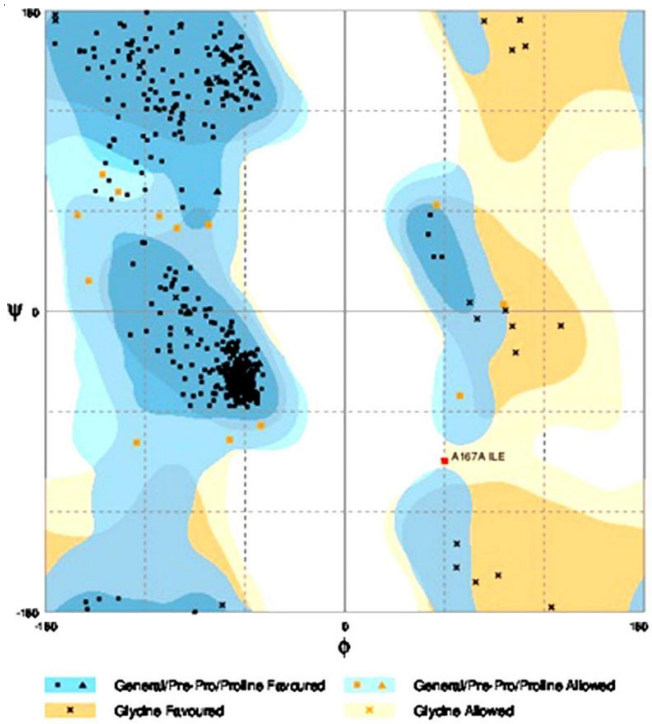


Fig. 4. Ramachandran plot of template 4LXJ

TABLE-3
RAMACHANDRAN PLOT SHOWING PERCENTAGE OF RESIDUES IN FAVORED, ALLOWED AND OUTLIER REGION

Structure	Validation server	Number of residues in favored region (%)	Number of residues in allowed region (%)	Number of residues in outlier region (%)/amino acid
4LXJ	Rampage	97.1	2.7	0.2
MODEL BUILT	Rampage	97.3	2.5	0.2
MODEL BUILT	Molprobity	96.9	99.6	167AILE; 383AASP

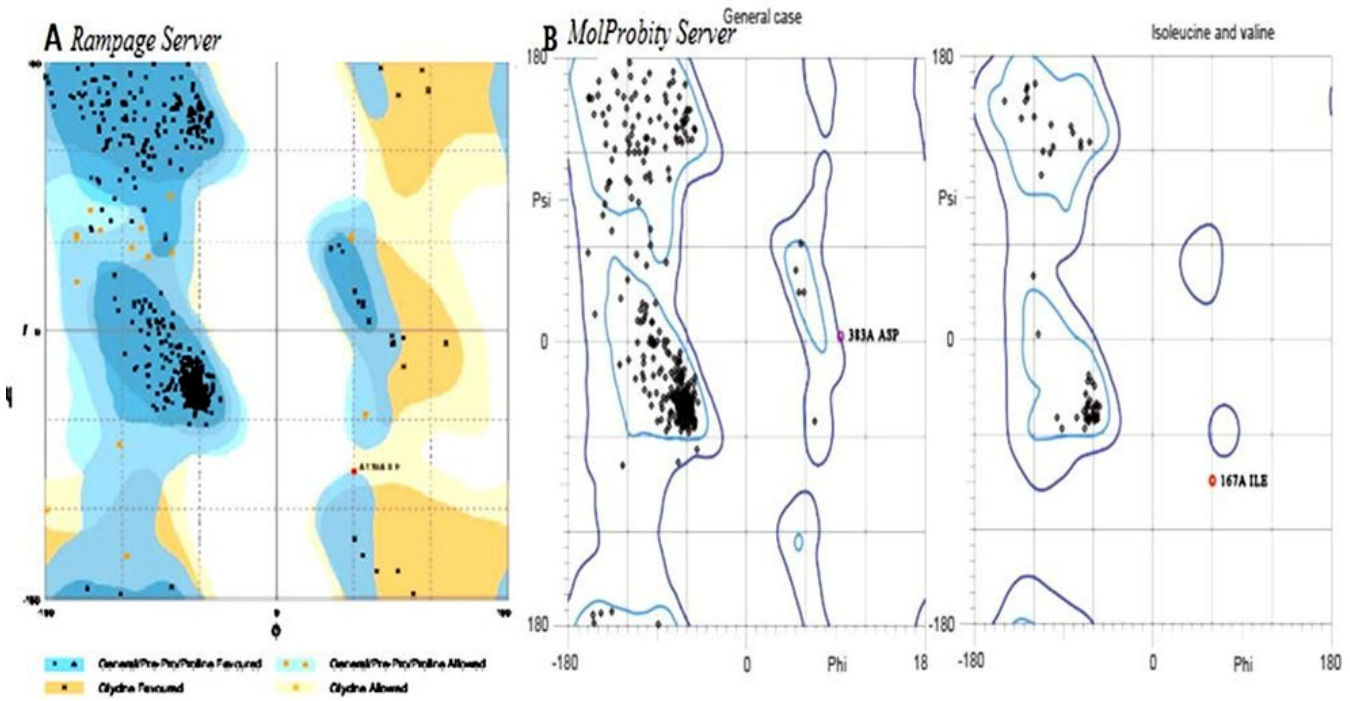


Fig. 5. Ramachandran plot of modeled structure by (a) Rampage server

Among the other validation servers ProSA was utilized to obtain the Z-score value. It is mainly used for the fold reliability analysisor comparison of compatibility. The plot shows two distinct regions (i) NMR region represented by dark blue colour and (ii) X ray region shown by light blue colour. The Z-score plot shows a black spot an indicative of the corresponding Z-score value of the template and modeled structure. For the template utilized and homology modeled structure of *C. albicans* CYP450 Z-score value is revealed to be -8.68 and -8.67, respectively (Fig. 6) [44-46].

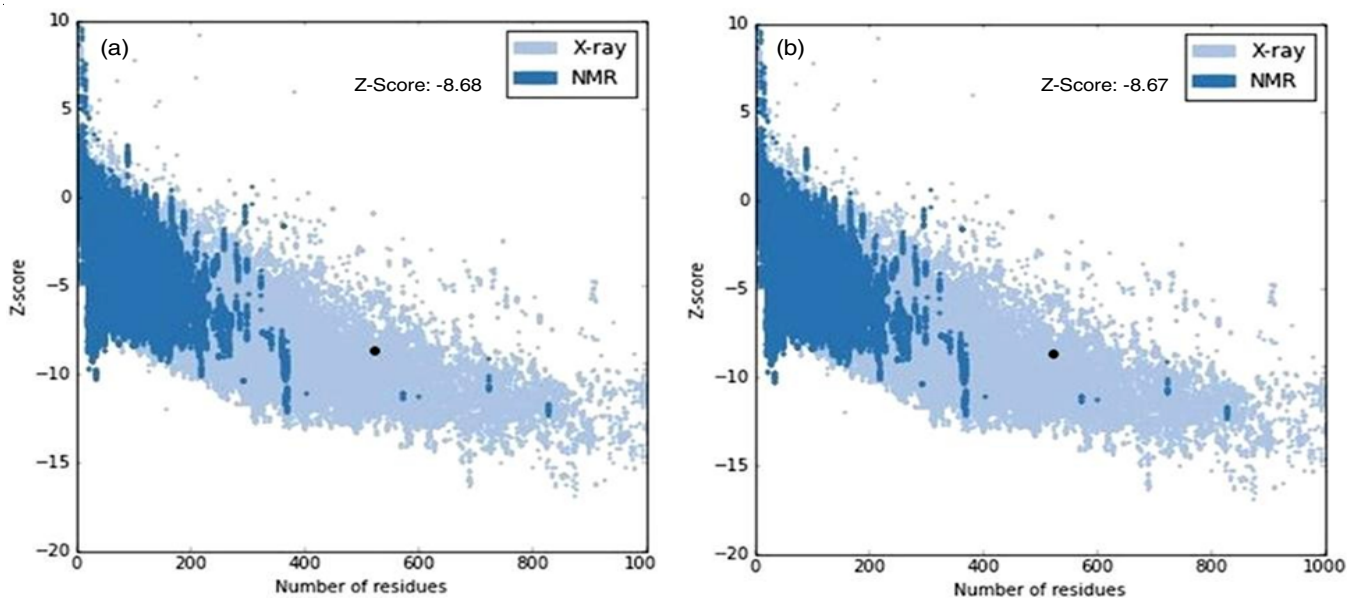


Fig. 6. ProSA plot of (a) 4LXJ and (b) modeled structure

G-factor was the next factor used to validate the model. PROCHECK analysis, an online server was used to calculate the G-factor. A property values below -0.5 is unusual, whereas the values below -1.0 is highly unusual.TheoverallaverageofG-factorsforthehomology modeledstructure of enzyme lanosterol 14α-demethylase of *C. albicans* is -0.15 indicating the model is unusual [47].

The TM-align(Version20150914) an freely downloadable online server was used to calculate RMSD (0.36Å) where 523 amino acids as backbone atoms from both template 4LXJ and modeled structure of lanosterol 14α-demethylase were superimposed on each other. This RMSD value clearly indicated that homology model closely resemble the template (Fig. 7) [48].

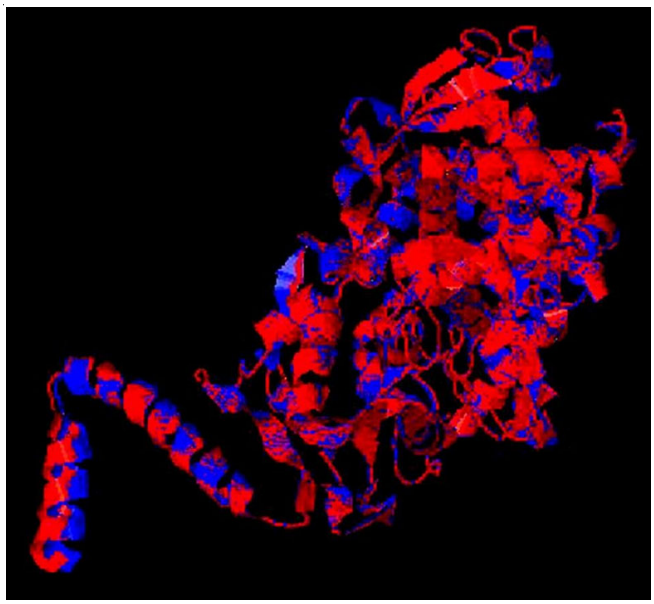


Fig. 7. RMSD calculation between template and modeled structure (0.36 Å)

Quantitative model energy analysis (QMEAN) server is used to assess and calculate the overall quality of the model. This assessment program calculates both global and local(per-residue) Quality of the model.The QMEAN server combines the result of five different structural descriptors like Cβ interaction energy, solvation energy, torsion energy, an agreement of predicted and calculated secondary structure and solvent accessibility. The global QMEAN-score for the modeled structure was revealed to be 0.6 and -1.52 was the corresponding total Z-score. The global score of the model reflect the predicted model reliability ranging from 0 to 1.

Molecular Docking studies

Binding modes of azoles: The dihalogen substituted phenyl ring occupies itself in the hydrophobic cleft above the ring and shows the hydrophobic interactions with amino acids Lys287, Ile304 and Gly450. The side chain constituted by (1*H*-1,2,4-triazol-3-yl)piperazine group showed the hydrophobic and van der Waals interactions with Phe271, Asn273, Leu274, Pro275, Phe269, Val270, Met332 and Gly344. In this study, *tert*.-alcoholic group was replaced with the *tert*.-amino group and this replacement proved to be fruitful because it shows similar hydrogen bonding interactions with the Ser378 as shown by the *tert*.-alcohol moiety of fluconazole. The fluconazole analogues showed higher dock score as compared with the standard fluconazole indicating the improved antifungal activity with the designed compounds (Fig. 8).

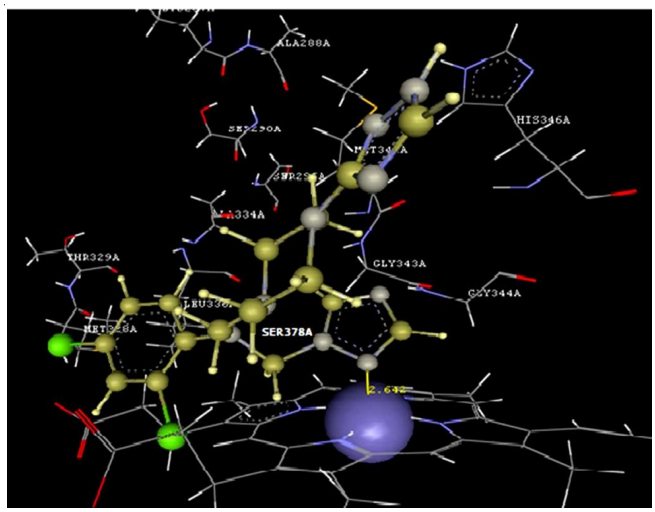


Fig. 8. Binding mode of representative compound **9a** in the active site of modeled structure of *C. albicans* CYP450

Conclusion

A series of novel triazol-3-yl piperazine tertiary amine type of fluconazole analogues was synthesized from *bis*(2-chloroethyl)amine, 3-amino-1,2,4-triazole and halogen substituted anilines as essential building blocks. The in vitro antifungal assay revealed that fluconazole analogues showed equipotent or higher activity while the rest of the compounds demonstrate intermediate to good biological activities against different species of *Candida*. As expected from the *in silico* drug design experiments results (dock score) fluoro-substituted derivatives, in particular showed good inhibition of the fungal growth. Owing to the fact that increased log P values for the chloro-substituted analogues shows the inferior activity thus decreased the water solubility of the chloro analogues, which makes them a unfavorable candidate for transport to the active site of enzyme and exert better biological activity. The possibility of development of resistance by pathogenic strains of fungi against these structurally modified fluconazole compounds is less.

ACKNOWLEDGEMENTS

The authors are gratefully acknowledge Principal and The Management of Bhujbal Knowledge City, MET's Institute of Pharmacy. The authors are also thankful to Dr. Kundan Ingle (Application Scientist) at VLife Sciences for his help and guidance during the homology modeling and docking studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. R. Gollapudy, S. Ajmani and S.A. Kulkarni, *Bioorg. Med. Chem.*, **12**, 2937 (2004); https://doi.org/10.1016/j.bmc.2004.03.034
2. D. Andes, K. Marchillo, T. Stamstad and R. Conklin, *Antimicrob. Agents Chemother.*, **47**, 3165 (2003); https://doi.org/10.1128/AAC.47.10.3165-3169.2003
3. R.A. Fromtling, *Clin. Microbiol. Rev.*, **1**, 187 (1988); https://doi.org/10.1128/CMR.1.2.187
4. A. Butts and D.J. Krysan, *PLoS Pathog.*, **8**, e1002870 (2012); https://doi.org/10.1371/journal.ppat.1002870
5. A. De Sarro, E. La Camera and M.T. Fera, *J. Chemother.*, **20**, 661 (2008); https://doi.org/10.1179/joc.2008.20.6.661
6. J. Heeres, L. Meerpoel and P. Lewi, *Molecules*, **15**, 4129 (2010); https://doi.org/10.3390/molecules15064129
7. V.K. Gupta, A.K. Sharma, R. Sharma, S. Diwan and S. Saini, *Nat. Prod. J.*, **4**, 82 (2014); https://doi.org/10.2174/2210315504021410090958 54
8. L. Ostrosky-Zeichner, A. Casadevall, J.N. Galgiani, F.C. Odds and J.H. Rex, *Nat. Rev. Drug Discov.*, **9**, 719 (2010); https://doi.org/10.1038/nrd3074
9. D.J. Sheehan, C.A. Hitchcock and C.M. Sibley, *Clin. Microbiol. Rev.*, **12**, 40 (1999); https://doi.org/10.1128/CMR.12.1.40
10. G. Aperis and E. Mylonakis, *Expert Opin. Investig. Drugs*, **15**, 579 (2006); https://doi.org/10.1517/13543784.15.6.579
11. A.H. Groll and J. Lumb, *Future Microbiol.*, **7**, 179 (2012); https://doi.org/10.2217/fmb.11.154
12. M.K. Kathiravan, A.B. Salake, A.S. Chothe, P.B. Dudhe, R.P. Watode, M.S. Mukta and S. Gadhwe, *Bioorg. Med. Chem.*, **20**, 5678 (2012); https://doi.org/10.1016/j.bmc.2012.04.045
13. E.C. Large, *Adv. Fungi.*, **57**, 992 (1940).
14. L.L. Frye and C.H. Robinson, *J. Chem. Soc. Chem. Commun.*, **129–131**, 129 (1988); https://doi.org/10.1039/C39880000129
15. M.A. Ghannoum and L.B. Rice, *Clin. Microbiol. Rev.*, **12**, 501 (1999); https://doi.org/10.1128/CMR.12.4.501
16. B.C. Monk, T.M. Tomasiak, M.V. Keniya, F.U. Huschmann, J.D.A. Tyndall, J.D. O'Connell III, R.D. Cannon, J.G. McDonald, A. Rodriguez, J.S. Finer-Moore and R.M. Stroud, *Proc. Natl. Acad. Sci. USA*, **111**, 3865 (2014); https://doi.org/10.1073/pnas.1324245111
17. S. Yu, X. Chai, H. Hu, Y. Yan, Z. Guan, Y. Zou, Q. Sun and Q. Wu, *Eur. J. Med. Chem.*, **45**, 4435 (2010); https://doi.org/10.1016/j.ejmech.2010.07.002
18. X. Chai, J. Zhang, S. Yu, H. Hu, Y. Zou, Q. Zhao, Z. Dan, D. Zhang and Q. Wu, *Bioorg. Med. Chem. Lett.*, **19**, 1811 (2009); https://doi.org/10.1016/j.bmcl.2009.01.048
19. S.-F. Cui, Y. Ren, S.-L. Zhang, X.-M. Peng, G.L. Damu, R.-X. Geng and C.-H. Zhou, *Bioorg. Med. Chem. Lett.*, **23**, 3267 (2013); https://doi.org/10.1016/j.bmcl.2013.03.118
20. X.-M. Peng, G.-X. Cai and C.-H. Zhou, *Curr. Top. Med. Chem.*, **13**, 1963 (2013); https://doi.org/10.2174/15680266113139990125
21. Y.-Y. Zhang, J.-L. Mi, C.-H. Zhou and X.-D. Zhou, *Eur. J. Med. Chem.*, **46**, 4391 (2011); https://doi.org/10.1016/j.ejmech.2011.07.010
22. H.B. Borate, S.P. Sawargave, S.P. Chavan, M.A. Chandavarkar, R. Iyer, A. Tawte, D. Rao, J.V. Deore, A.S. Kudale, P.S. Mahajan and G.S. Kangire, *Bioorg. Med. Chem. Lett.*, **21**, 4873 (2011); https://doi.org/10.1016/j.bmcl.2011.06.022
23. X. Chai, *Drug Des. Devel. Ther.*, **8**, 383 (2014).
24. Z. Rezaei, S. Khabnadideh, K. Zomorodian, K. Pakshir, G. Kashi, N. Sanagoei and S. Gholami, *Arch. Pharm.*, **344**, 658 (2011); https://doi.org/10.1002/ardp.201000357
25. J.N. Sangshetti, D.K. Lokwani, A.P. Sarkate and D.B. Shinde, *Chem. Biol. Drug Des.*, **78**, 800 (2011); https://doi.org/10.1111/j.1747-0285.2011.01178.x
26. K.L. Lahtchev, D.I. Batovska, S.P. Parushev, V.M. Ubiyvovk and A.A. Sibirny, *Eur. J. Med. Chem.*, **43**, 2220 (2008); https://doi.org/10.1016/j.ejmech.2007.12.027
27. Y. Luo, Y. Lu, L. Gan, C. Zhou, J. Wu, R. Geng and Y.-Y. Zhang, *Arch. Pharm.*, **342**, 386 (2009); https://doi.org/10.1002/ardp.200800221
28. Z. Nowakowska, B. Kêdzia and G. Schroeder, *Eur. J. Med. Chem.*, **43**, 707 (2008); https://doi.org/10.1016/j.ejmech.2007.05.006
29. V.K. Jain, B. Jain, U.K. Sharma and D. Saha, *Int. J. Curr. Pharm. Res.*, **3**, 66 (2011).
30. N.R. Pai, D.S. Dubhashi, S. Vishwasrao and D. Pusalkar, *J. Chem. Pharm. Res.*, **2**, 506 (2010).
31. A.Kaur and A. Chawla, *Int. J. Universal*, **3**, 113 (2014).
32. G. Marzaro, A. Guiotto and A. Chilin, *Green Chem.*, **11**, 774 (2009); https://doi.org/10.1039/B900750D
33. Z. Rezaei, S. Khabnadideh, K. Pakshir, Z. Hossaini, F. Amiri and E. Assadpour, *Eur. J. Med. Chem.*, **44**, 3064 (2009); https://doi.org/10.1016/j.ejmech.2008.07.012
34. A.R. Katritzky, W. Kuzmierkiewicz and J.V. Greenhill, *Recl. Trav. Chim. Pays Bas*, **110**, 369 (1991); https://doi.org/10.1002/recl.19911100903
35. A.R. Katritzky and J. Wu, *Synthesis*, **1994**, 597 (1994); https://doi.org/10.1055/s-1994-25530
36. NCCLS, Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline (2004).
37. M. Cuenca-Estrella, W. Lee-Yang, M.A. Ciblak, B.A. Arthington-Skaggs, E. Mellado, D.W. Warnock and J.L. Rodriguez-Tudela, *Antimicrob. Agents Chemother.*, **46**, 3644 (2002); https://doi.org/10.1128/AAC.46.11.3644-3647.2002
38. A. Espinel-Ingroff, F. Barchiesi, M. Cuenca Estrella, A. Fothergill, M.A. Pfaller, M. Rinaldi, J.L. Rodriguez-Tudela and P.E. Verweij, *J. Clin. Microbiol.*, **43**, 4535 (2005); https://doi.org/10.1128/JCM.43.9.4535-4540.2005
39. A. Espinel-Ingroff, F. Barchiesi, M. Cuenca-Estrella, M.A. Pfaller, M. Rinaldi, J.L. Rodriguez-Tudela and P.E. Verweij, *J. Clin. Microbiol.*, **43**, 3884 (2005); https://doi.org/10.1128/JCM.43.8.3884-3889.2005
40. P.B. Miniyar, A.A. Mahajan, S.N. Mokale, M.U. Shah, A.S. Kumar and G.U. Chaturbhuj, *Arab. J. Chem.*, **10**, 295 (2017); https://doi.org/10.1016/j.arabjc.2013.09.005
41. J.L. Rodriguez-Tudela, J.P. Donnelly, M. Pfaller, E. Chryssantou, P. Warn, D.W. Denning, A. Espinel-Ingroff, F. Barchiesi and M. Cuenca-Estrella, *J. Clin. Microbiol.*, **45**, 109 (2007); https://doi.org/10.1128/JCM.01969-06
42. S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson and D.C. Richardson, *Proteins*, **50**, 437 (2003); https://doi.org/10.1002/prot.10286
43. I.W. Davis, L.W. Murray, J.S. Richardson and D.C. Richardson, *Nucleic Acids Res.*, **32(Web Server)**, W615 (2004); https://doi.org/10.1093/nar/gkh398
44. M.J. Sippl, *Proteins*, **17**, 355 (1993); https://doi.org/10.1002/prot.340170404
45. F. Inagaki, *Cell Struct. Funct.*, **15**, 237 (1990); https://doi.org/10.1247/csf.15.237
46. M. Wiederstein and M.J. Sippl, *Nucleic Acids Res.*, **35**, W407 (2007); https://doi.org/10.1093/nar/gkm290
47. R.A. Engh and R. Huber, *Acta Crystallogr. A*, **47**, 392 (1991); https://doi.org/10.1107/S0108767391001071
48. Y. Zhang and J. Skolnick, *Nucleic Acids Res.*, **33**, 2302 (2005); https://doi.org/10.1093/nar/gki524
49. S. Behrouz, M.N.S. Rad, S. Rostami, M. Behrouz, E. Zarehnezhad and A. Zarehnezhad, *Mol. Divers.*, **18**, 797 (2014); https://doi.org/10.1007/s11030-014-9539-1
50. Z. Jiang, J. Gu, C. Wang, S. Wang, N. Liu, Y. Jiang, G. Dong, Y. Wang, Y. Liu, J. Yao, Z. Miao, W. Zhang and C. Sheng, *Eur. J. Med. Chem.*, **82**, 490 (2014); https://doi.org/10.1016/j.ejmech.2014.05.079

51. C. Sheng, X. Che, W. Wang, S. Wang, Y. Cao, Z. Miao, J. Yao and W. Zhang, *Eur. J. Med. Chem.*, **46**, 5276 (2011); https://doi.org/10.1016/j.ejmech.2011.03.019
52. S. Wang, G. Jin, W. Wang, L. Zhu, Y. Zhang, G. Dong, Y. Liu, C. Zhuang, Z. Miao, J. Yao, W. Zhang and C. Sheng, *Eur. J. Med. Chem.*, **53**, 292 (2012); https://doi.org/10.1016/j.ejmech.2012.04.013
53. W. Wang, S. Wang, G. Dong, Y. Liu, Z. Guo, Z. Miao, J. Yao, W. Zhang and C. Sheng, *MedChemComm*, **2**, 1066 (2011); https://doi.org/10.1039/c1md00103e
54. W. Wang, C. Sheng, X. Che, H. Ji, Z. Miao, J. Yao and W. Zhang, *Arch. Pharm.*, **342**, 732 (2009); https://doi.org/10.1002/ardp.200900103
55. Y. Xu, C. Sheng, W. Wang, X. Che, Y. Cao, G. Dong, S. Wang, H. Ji, Z. Miao, J. Yao and W. Zhang, *Bioorg. Med. Chem. Lett.*, **20**, 2942 (2010); https://doi.org/10.1016/j.bmcl.2010.03.014
56. R.S. Kankate, P.S. Gide and D.P. Belsare, *Arab. J. Chem.*, **12**, 2224 (2019); https://doi.org/10.1016/j.arabjc.2015.02.002
57. H.-Z. Zhang, G.L.V. Damu, G.-X. Cai and C.-H. Zhou, *Eur. J. Med. Chem.*, **64**, 329 (2013); https://doi.org/10.1016/j.ejmech.2013.03.049
58. H. Türkmen, N. Ceyhan, N. Ülkü Karabay Yavasoglu, G. Özdemir and B. Çetinkaya, *Eur. J. Med. Chem.*, **46**, 2895 (2011); https://doi.org/10.1016/j.ejmech.2011.04.012
59. A.M. Khallil, I.M. Daghman and A.A. Fady, *J. Microbiol. Modern Techn.*, **1**, 2575 (2015).