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Article

# 1,3,4-Thiadiazole and 1,2,4-triazole-3(4*H*)-thione bearing salicylate moiety: synthesis and evaluation as anti-*Candida albicans*

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Dramatically increased occurrence of both superficial and invasive fungal infections has been observed. *Candida albicans* appear to be the main etiological agent of invasive fungal infections. The anti-*C. albicans* activity of thiosemicarbazide, 1,3,4-Thiadiazole, and 1,2,4-triazole-3(4*H)*-thione compounds (compounds **3-23**) were investigated. The MIC values of thiadiazole and triazole derivatives **10-23** were in the range of 0.08-0.17 μmol $mL^{-1}$, while that of fluconazole was 0.052 μmol $mL^{-1}$. Compound **11** (5-(2-(4-chlorobenzyloxy)phenyl)-*N*-allyl-1,3,4-thiadiazol-2-amine) and compound **18** (5-(2-(4-chlorobenzyloxy)phenyl)-4-allyl-2*H*-1,2,4-triazole-3(4H)-thione) were found to be the most active compounds, with MIC values of 0.08 μmol $mL^{-1}$. The newly synthesized thiadiazole and triazole compounds (compounds **10-23)** showed promising anti-*Candida* activity. The allyl substituent-bearing compounds **11** and **18** exhibited significant anti-*Candida albicans* activity and showed a binding mode as well as the fluconazole x-ray structure.

**Uniterms**: Anti-*Candida*/docking studies. Thiadiazole. Triazole.

## INTRODUCTION

Over the last two decades, dramatically increased occurrence of both superficial and invasive fungal infections has been observed. In organ transplant cases and in immune compromised individuals, including patients with cancer or AIDS, fungal infections are the major cause of morbidity and mortality (Romani, 2008). The invasive *Candida* infections are mainly caused by *Candida albicans* and account for 30-40% of fungal infection-related mortality (Pfaller, Diekema, 2010).

A major obstacle in the treatment of *Candida* infections is the spread of anti-*Candida* drug resistance following long-term regular administration of antimycotic therapy in severely immunocompromised subjects, such as cancer patients, transplant recipients, and patients undergoing surgery. In spite of the development of new drugs, such as new azole or echinocandin derivatives, there has been a dramatic increase in both anti-*Candida* resistance and the frequency of invasive *Candida* infections (Pfaller, 2012). Among enormous classes of heterocyclic compounds, the synthesis of new derivatives of 1,2,4-triazole-3-thiones and 2-amino-1,3,4-thiadiazoles has been attracting considerable attention because of their broad biological activity, including antibacterial and antifungal (Güzeldemirci, Küçükbasmaci, 2010; Plec *et al.*, 2011; Camoutsis *et al.*, 2010; Küçükgüzel *et al.*, 2007; Kadi *et al.*, 2007), anti-tubercular (Küçükgüzel *et al.*, 2001; Küçükgüzel *et al.*, 2008), antiviral (Abdel-Aal *et al.*, 2003; Küçükgüzel *et al.*, 2008), antioxidant (Ayhan-Kilcigil *et al.*, 2004; Khan *et al.*, 2010), antitumoral (Duran, Dogan, Rollas, 2002; Rzeski, Matysiak, Kandefer-Szerszen, 2007; Mavrova *et al.*, 2009), anti-inflammatory (Amir, Shikha, 2004; Kadi *et al.*, 2007; Küçükgüzel *et al.*, 2007; Kumar *et al.*, 2008), and anticonvulsant activity (Kane *et al.*, 1994; Sharma *et al.*, 2011; Dogan *et al.*, 2002). Clinically-used antifungal azole drugs containing either an imidazole (e.g., econazole, miconazole, clotrimazole, and ketoconazole) or a 1,2,4-triazole moiety (e.g., fluconazole and itraconazole) have a high therapeutic index and significant safety

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profile (Wang *et al.*, 2009). Azoles were broadly used as first-line treatment of invasive fungal infections, which led to the emergence of high resistance (Hoffman, Ernst, Klepser, 2000; Casalinuovo, Di Francesco, Garaci, 2004) that largely decreased their efficacy. Azoles exert their antifungal activity by inhibiting the synthesis of sterols in fungal cells. This may be achieved through its inhibitory effect on cytochrome P450-dependent 14a-lanosterol demethylase, exerted through binding to the heme cofactor of the cytochrome CYP51". (Odds, Brown, Gow, 2003; Hamdan, Hahn, 2006). As a result, ergosterol is depleted, and subsequent accumulation of lanosterol inside the fungal cells occurs, leading to inhibition of their growth. Because of the vital role of the CYP51 enzyme in the biosynthetic processes of fungal cells, CYP51 is considered the main target of antifungal azole agents (Lamb *et al.*, 1999). In view of the potential biological activity of derivatives of 1,2,4-triazole and 1,3,4-thiadiazole and in continuation of our ongoing research on the synthesis of heterocycles with potential antimicrobial activity (Radwan, Hussein, 2006; Mostafa *et al.*, 2008; Aboul-Fadl *et al.*, 2012; Attia *et al.*, 2013; Radwan, 2013; Ghorab *et al.*, 2013a, b; Radwan *et al.*, 2014; Radwan, Abdel-Mageed, 2014), we synthesized a new series from 1,2,4-triazole and 1,3,4-thiadiazole in order to examine their antifungal activity. Hydrocarbon substituents with variable size were included at position 2 of the thiadiazole ring and at position 1 of the triazole ring in order to enhance the lipophilicity and subsequently the biological activity of the compounds and to study the steric and electronic effect of these substituents on biological activity.

## MATERIAL AND METHODS

Methyl salicylate (1), chlorobenzyl chloride (2), and isothiocyanate derivatives were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Measurements of melting points were done on a Barnstead 9001 Electrothermal apparatus using open capillary tubes; these measurements were uncorrected. FTIR spectra were obtained using KBr discs on a Perkin-Elmer (USA) spectrophotometer at the research center of the College of Pharmacy, King Saud University, Saudi Arabia. $^{1}H$ and $^{13}C$ NMR spectra were recorded in DMSO-$d_6$ on a Bruker NMR spectrophotometer operating at either 500 MHz or 125.76 MHz. Elemental analysis was performed on a Perkin-Elmer CHNSO analyzer, model no. 2400. Reaction monitoring and purity testing of the final products were carried out by thin layer chromatography (TLC) using silica gel-precoated aluminum sheets (60 F254, Merck) and visualization with ultraviolet light (UV) at 365 and 254 nm.

### Synthesis of methyl 2-(4-chlorobenzyloxy) benzoate (1)

Methyl 2-hydroxybenzoate (1.3 g, 0.01 mol) was dissolved in dry acetone (120 mL). Dry potassium carbonate (4.14 g, 0.03 mol) was added and the mixture was stirred at room temperature for 10 min. Chlorobenzyl chloride (3.86 mL, 0.03 mol) was added to the mixture and the solution was heated under reflux for 18 h under a nitrogen atmosphere. The cooled reaction mixture was filtered, the solvent was removed under reduced pressure, and the residue was dissolved in dichloromethane (25 mL). The organic layer was washed consecutively with 5% aqueous NaOH (25 mL), brine (20 mL), and distilled water (20 mL). The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue was purified by column chromatography with 5%-20% ethyl acetate/hexane as eluent to afford the product. Yield 2.5 g, 90%.

### Synthesis of 2-(4-chlorobenzyloxy) benzohydrazide (2)

Methyl 2-(4-chlorobenzyloxy)benzoate (**1**) (2.76 g, 0.01 mol), ethanol (20 mL), and 64% hydrazine hydrate (4 mL) were mixed together and heated under reflux at 80°C for 8 h. TLC eluted with 1:4 ethanol/benzene showed the development of a new spot at Rf = 0.65. Ethanol was evaporated under reduced pressure and a white solid product was recrystallized from $H_2O$/MeOH to yield compound **2** (2.6 g, 95% yield), melting point 111-112°C, IR (KBr, $cm^{-1}$) 3246 (NH), 1583 (CO-N). $^{1}H$ NMR (DMSO$d_6$, δ ppm): 4.53 (s, 2H, $NH_2$), 5.24 (s, 2H, Ar–$CH_2$–O), 7.02–7.65 (8H, m, Ar-Hs), 9.24 (s, 1H, -CONH). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 69.4 (Ar–$CH_2$–O), 113.82, 121.27, 123.38, 128.97, 129.81, 130.18, 132.12, 132.94, 136.3, 156.97 (C-Ar), 165.54 (C=O). Anal. calcd. for $C_{14}H_{13}ClN_2O_2$: C, 60.77; H, 4.74; N, 10.12. Found: C, 60.63; H, 4.85; N, 10.01.

### Synthesis of 1-(2-(4-chlorobenzyloxy)benzoyl)-4-substituted-thiosemicarbazide (3-9)

The compound 2-(4-chlorobenzyloxy) benzohydrazide **(2)** (2.76 g, 0.01 mol) was dissolved in absolute ethanol (20 mL). The isothiocyanate reagents (0.011 mol) were separately dissolved in absolute ethanol (10 mL). Then, the solution of isothiocyanate was poured into the solution of hydrazide, with continuous stirring. The reaction mixture was refluxed for 2 h, and then cooled to room temperature. The crude solid was then filtered

and recrystallized from appropriate solvent to yield compounds **3-9**.

*1-(2-(4-chlorobenzyloxy)benzoyl)-4-phenylthiosemicarbazide **(3)***

Yield 4.02 g, 98%, melting point 176–178 °C; IR (KBr) 3320, 1664, 1618, 833, 750, 691 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 5.32 (2H, s, (Ar–$CH_2$–O), 7.08–7.87 (13H, m, Ar-Hs), 9.42, 9.93, 10.15 (3s, 3H, 3NH). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 69.69 (Ar–$CH_2$–O), 114.05, 121.4, 122.1, 123.4, 125.2, 126.4, 128.63, 128.09, 130.1, 131.22, 133.07, 133.25, 135.93, 139.48, 156.38 (C-Ar), 165.15 (C=O), 182.23 (C=S). Anal. calcd. for $C_{21}H_{18}ClN_3O_2S$: C, 61.23; H, 4.40; N, 10.20; S, 7.78. Found: C, 61.01; H, 4.58; N, 10.03; S, 7.63.

*1-(2-(4-chlorobenzyloxy)benzoyl)-4-allylthiosemicarbazide **(4)***

Yield 3.2 g, 85%, melting point 157–158 °C; IR (KBr) 3314, 1648, 1622, 852, 772, 672 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 4.04 (s, 2H, $CH_2$=CH-$\underline{CH_2}$-), 5.07 (d, 1H, J=10, *trans*C$\underline{H_2}$=CH-), 5.11 (d, 1H, J=17, *cis*C$\underline{H_2}$=CH-), 5.28 (s, 2H, Ar–$CH_2$–O), 5.79 (m, 1H, $CH_2$=$\underline{CH}$-$CH_2$-), 7.06–7.97 (8H, m, Ar-Hs), 9.37, 9.71, 10.09 (3s, 3H, 3NH). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 46.19 (allyl-$CH_2$), 69.57 (Ar–$CH_2$–O), 113.93, 119.71, 121.32, 128.95, 130.05, 131.03, 133.03, 133.05, 135.01, 136.05, 156.26 (C-Ar), 165.02 (C=O), 181.95 (C=S). Anal. calcd. for $C_{18}H_{18}ClN_3O_2S$: C, 57.52; H, 4.83; N, 11.18; S, 8.53. Found: C, 57.43; H, 4.91; N, 11.03; S, 8.61.

*1-(2-(4-chlorobenzyloxy)benzoyl)-4-(m-tolyl) thiosemicarbazide **(5)***

Yield 4.08 g, 96%, melting point 170–171 °C; IR (KBr) 3294, 1640, 1608, 816, 746, 694 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 2.28 (s, 3H, $CH_3$), 5.31 (s, 2H, Ar–$CH_2$–O), 6.96–7.86 (m, 12H, Ar-Hs), 9.36, 9.82, 10.18 (3s, 3H, 3NH). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 21.44 ($CH_3$), 69.7 (Ar–$CH_2$–O), 114.04, 121.42, 125.05, 128.45, 128.89, 130.08, 131.18, 133.07, 133.23, 135.91, 139.32, 156.37 (C-Ar), 165.23 (C=O), 180.76 (C=S). Anal. calcd. for $C_{22}H_{20}ClN_3O_2S$: C, 62.04; H, 4.73; N, 9.87; S, 7.53. Found: C, 61.92; H, 4.80; N, 9.76; S, 7.38.

*1-(2-(4-chlorobenzyloxy)benzoyl)-4-(p-tolyl) thiosemicarbazide **(6)***

Yield 4.16 g, 98%, melting point 162–163 °C; IR (KBr) 3294, 1640, 1608, 816, 746, 694 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 2.29 (s, 3H, $CH_3$), 5.31 (s, 2H, Ar–$CH_2$–O), 7.07–7.86 (m, 12H, Ar-Hs), 9.42, 9.88, 10.12 (3s, 3H, 3NH). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 21.01 ($CH_3$), 69.69 (Ar–$CH_2$–O), 114.03, 121.4, 128.89, 129.14, 130.09, 131.19, 133.08, 133.21, 135.91, 136.85, 156.37 (C-Ar), 165.78 (C=O), 180.93 (C=S). Anal. calcd. for $C_{22}H_{20}ClN_3O_2S$: C, 62.04; H, 4.73; N, 9.87; S, 7.53. Found: C, 61.88; H, 4.79; N, 9.93; S, 7.61.

*1-(2-(4-chlorobenzyloxy)benzoyl)-4-(o-tolyl) thiosemicarbazide **(7)***

Yield 4.0 g, 95%, melting point 148–149 °C; IR (KBr) 3323, 1662, 1628, 869, 772, 732 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 2.14 (s, 3H, $CH_3$), 5.24 (s, 2H, Ar–$CH_2$–O), 7.07–7.85 (m, 12H, Ar-Hs), 9.15, 9.72, 10.16 (3s, 3H, 3NH). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 18.06 ($CH_3$), 69.69 (Ar–$CH_2$–O), 113.93, 121.32, 126.26, 126.97, 128.87, 130.04, 130.53, 131.13, 133.06, 135.87, 156.35 (C-Ar), 165.62 (C=O), 181.06 (C=S). Anal. calcd. for $C_{22}H_{20}ClN_3O_2S$: C, 62.04; H, 4.73; N, 9.87; S, 7.53. Found: C, 62.14; H, 4.8; N, 9.75; S, 7.46.

*1-(2-(4-chlorobenzyloxy)benzoyl)-4-(3-chlorophenyl) thiosemicarbazide **(8)***

Yield 4.35 g, 98%, melting point 151–152 °C; IR (KBr) 3314, 1683, 1612, 876, 778, 674 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 5.24 (s, 2H, Ar–$CH_2$–O), 7.08–7.87 (m, 12H, Ar-Hs), 9.52, 10.04, 10.14 (3s, 3H, 3NH). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 69.67 (Ar–$CH_2$–O), 114.06, 121.4, 124.98, 128.88, 129.81, 130.05, 131.27, 132.62, 133.07, 133.32, 135.92, 141.06, 156.43 (C-Ar), 165.3 (C=O), 181.58 (C=S). Anal. calcd. for $C_{21}H_{17}Cl_2N_3O_2S$: C, 56.51; H, 3.84; N, 9.41; S, 7.18. Found: C, 56.43; H, 3.71; N, 9.52; S, 7.25.

*1-(2-(4-chlorobenzyloxy)benzoyl)-4-isopropylthiosemicarbazide **(9)***

Yield 3.54 g, 94%, melting point 163–164 °C; IR (KBr) 3335, 1672, 1628, 853, 764, 679 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 1.06 (d, 6H, J=6, $2CH_3$), 4.35 (m, 1H, CH), 5.31 (s, 2H, Ar–$CH_2$–O), 7.06–7.75 (m, 8H, Ar-Hs), 9.25, 9.48, 10.12 (3s, 3H, 3NH). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 22.34 ($CH_3$), 46.01 (>CH), 69.61 (Ar–$CH_2$–O), 113.97, 118.32, 121.4, 127.81, 128.97, 129.82, 129.25, 133.04, 133.08, 156.06 (C-Ar), 165.1 (C=O), 180.52 (C=S). Anal. calcd. for $C_{18}H_{20}ClN_3O_2S$: C, 57.21; H, 5.33; N, 11.12; S, 8.49. Found: C, 57.08; H, 5.41; N, 11.19; S, 8.56.

## Synthesis of 5-(2-(4-chlorobenzyloxy)phenyl)-*N*-substituted-1,3,4-thiadiazol-2-amine (10-16)

Concentrated sulfuric acid (10 mL) was added to the thiosemicarbazide compounds **(3-9)** (0.01 mol)

with stirring at 0 °C. The reaction mixture was stirred for 3 h at room temperature, allowed to stand overnight, and neutralized with diluted sodium hydroxide. The precipitated crude solid was filtered and washed with water. The crude product was then recrystallized from a mixture of acetic acid and water (1:1 or 1:2) to yield disubstituted 1,3,4-thiadiazole **(10-16)**.

*5-(2-(4-chlorobenzyloxy)phenyl)-N-phenyl-1,3,4-thiadiazol-2-amine **(10)***

Yield 3.35 g, 85%, melting point 154–156 °C; IR (KBr) 3278, 838, 751, 695 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 4.6 (s, 2H, Ar–$CH_2$–O), 4.8–5.52 (bs, 1H, NH), 6.94-7.69 (m, 13H, Ar-Hs). $^{13}C$ NMR ($DMSO-d_6$, δ ppm): 70.42 (Ar–$CH_2$–O), 117.69, 118.12, 122.1, 123.4, 125.2, 126.4, 128.63, 128.85, 129.56, 130.18, 133.07, 133.25, 135.93, 139.97, 154.05, 150.1, 169.04. Anal. calcd. for $C_{21}H_{16}ClN_3OS$: C, 64.03; H, 4.09; N, 10.67; S, 8.14. Found: C, 63.88; H, 4.15; N, 10.51; S, 8.06.

*5-(2-(4-chlorobenzyloxy)phenyl)-N-allyl-1,3,4-thiadiazol-2-amine **(11)***

Yield 2.67 g, 75%, melting point 142–144 °C; IR (KBr) 3305, 883, 752, 672 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 4.36 (s, 2H, $CH_2$=CH-$\underline{CH_2}$-), 4.92 (s, 2H, Ar–$CH_2$–O), 5.11 (d, 1H, J=10, *trans*C$\underline{H_2}$=CH-), 5.16 (d, 1H, J=17, *cis*C$\underline{H_2}$=CH-), 5.67 (m, 1H, $CH_2$=$\underline{CH}$-$CH_2$-), 5.8–6.34 (bs, 1H, NH), 7.13–7.72 (8H, m, Ar-Hs). $^{13}C$ NMR ($DMSO-d_6$, δ ppm): 45.86 (allyl-$CH_2$), 69.17 (Ar–$CH_2$–O), 114.23, 116.04, 121.25, 122.4, 128.05, 128.6, 129.1, 129.42, 133.62, 134.83, 139.65, 157.02, 164.3, 168.06. Anal. calcd. for $C_{18}H_{16}ClN_3OS$: C, 60.41; H, 4.51; N, 11.74; S, 8.96. Found: C, 60.28; H, 4.64; N, 11.61; S, 8.82.

*5-(2-(4-chlorobenzyloxy)phenyl)-N-m-tolyl-1,3,4-thiadiazol-2-amine **(12)***

Yield 3.33 g, 82%, melting point 143–145 °C; IR (KBr) 3319, 884, 791, 697 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 2.31 (s, 3H, $CH_3$), 4.95 (s, 2H, Ar–$CH_2$–O), 5.61–6.37 (bs, 1H, NH), 7.15–7.68 (m, 12H, Ar-Hs). $^{13}C$ NMR ($DMSO-d_6$, δ ppm): 23.02 ($CH_3$), 70.16 (Ar–$CH_2$–O), 112.61, 114.52, 118.3, 119.22, 120.81, 121.65, 126.41, 127.91, 128.86, 129.32, 130.66, 131.54, 138.62, 139.35, 141.55, 150.11, 155.54, 167.31. Anal. calcd. for $C_{22}H_{18}ClN_3OS$: C, 64.78; H, 4.45; N, 10.30; S, 7.86. Found: C, 64.62; H, 4.42; N, 10.38; S, 7.78.

*5-(2-(4-chlorobenzyloxy)phenyl)-N-p-tolyl-1,3,4-thiadiazol-2-amine **(13)***

Yield 3.25 g, 80%, melting point 154–156 °C; IR (KBr) 3318, 819, 762, 684 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 2.38 (s, 3H, $CH_3$), 5.13 (s, 2H, Ar–$CH_2$–O), 5.74–6.51 (bs, 1H, NH), 7.26–7.81 (m, 12H, Ar-Hs). $^{13}C$ NMR ($DMSO-d_6$, δ ppm): 21.32 ($CH_3$), 69.75 (Ar–$CH_2$–O), 113.06, 114.32, 116.01, 118.98, 120.01, 120.89, 123.98, 125.88, 128.31, 131.01, 133.05, 134.87, 135.92, 138.33, 146.01, 151.04, 168.03. Anal. calcd. for $C_{22}H_{18}ClN_3OS$: C, 64.78; H, 4.45; N, 10.30; S, 7.86. Found: C, 64.71; H, 4.48; N, 10.26; S, 7.95.

*5-(2-(4-chlorobenzyloxy)phenyl)-N-o-tolyl-1,3,4-thiadiazol-2-amine **(14)***

Yield 3.0 g, 75%, melting point 146–148 °C; IR (KBr) 3299, 846, 749, 669 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 2.26 (s, 3H, $CH_3$), 5.08 (s, 2H, Ar–$CH_2$–O), 5.82–6.38 (bs, 1H, NH), 7.02–7.73 (m, 12H, Ar-Hs). $^{13}C$ NMR ($DMSO-d_6$, δ ppm): 18.58 ($CH_3$), 70.04 (Ar–$CH_2$–O), 113.46, 114.52, 114.89, 116.04, 116.53, 121.08, 124.05, 125.12, 127.81, 130.76, 132.65, 133.65, 137.53, 138.09, 149.31, 152.3, 166.14. Anal. calcd. for $C_{22}H_{18}ClN_3OS$: C, 64.78; H, 4.45; N, 10.30; S, 7.86. Found: C, 64.76; H, 4.51; N, 10.38; S, 7.72.

*5-(2-(4-chlorobenzyloxy)phenyl)-N-(3-chlorophenyl)-1,3,4-thiadiazol-2-amine **(15)***

Yield 3.33 g, 78%, melting point 150–152 °C; IR (KBr) 3296, 861, 788, 681 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 5.16 (s, 2H, Ar–$CH_2$–O), 5.48–6.28 (bs, 1H, NH), 7.23–7.84 (m, 12H, Ar-Hs). $^{13}C$ NMR ($DMSO-d_6$, δ ppm): 70.33 (Ar–$CH_2$–O), 112.35, 114.58, 116.25, 116.63, 120.02, 122.87, 126.25, 126.91, 128.36, 130.57, 130.22, 136.35, 138.39, 146.24, 151.05, 166.08. Anal. calcd. for $C_{21}H_{15}Cl_2N_3OS$: C, 58.89; H, 3.53; N, 9.81; S, 7.49. Found: C, 58.81; H, 3.42; N, 9.93; S, 7.59.

*5-(2-(4-chlorobenzyloxy)phenyl)-N-isopropyl-1,3,4-thiadiazol-2-amine **(16)***

Yield 2.65 g, 74%, melting point 156–158 °C; IR (KBr) 3295, 873, 775, 693 $cm^{-1}$; $^1H$ NMR ($DMSOd_6$, δ ppm): 1.13 (d, 6H, J=6, $2CH_3$), 4.48 (m, 1H, CH), 5.08 (s, 2H, Ar–$CH_2$–O), 5.62–6.51 (bs, 1H, NH), 7.11–7.69 (m, 8H, Ar-Hs). $^{13}C$ NMR ($DMSO-d_6$, δ ppm): 21.66 ($CH_3$), 46.8 (>CH), 69.04 (Ar–$CH_2$–O), 113.31, 114.12, 114.01, 118.42, 119.09, 120.96, 124.14, 124.54, 127.33, 128.97, 133.12, 133.88, 137.63, 148.01, 151.11, 167.13. Anal. calcd. for $C_{18}H_{18}ClN_3OS$: C, 60.07; H, 5.04; N, 11.68; S, 8.91. Found: C, 60.11; H, 5.17; N, 11.61; S, 8.82.

## Synthesis of 5-(2-(4-chlorobenzyloxy)phenyl)-4-substituted-2H-1,2,4-triazole-3(4H)-thione (17-23)

Each of the thiosemicarbazides (**3-9**) (0.01 mol)

was dissolved in ethanol (200 mL). Then, 20 mL of an ethanolic solution of KOH (0.85 g, KOH, 0.015 mol) was added and the mixture was heated under reflux at 80 °C for 4 h. Excess ethanol was evaporated to dryness and the bulk of the solid was washed with 2 M hydrochloric acid, filtered, dried, and recrystallized from ethanol to yield the corresponding compounds **(17-23)**.

*5-(2-(4-chlorobenzyloxy)phenyl)-4-phenyl-2H-1,2,4-triazole-3(4H)-thione* ***(17)***

Yield 3.25 g, 83%, melting point 223–225 °C; IR (KBr) 3315, 871, 752, 699 $cm^{-1}$; $^{1}H$ NMR ($DMSOd_6$, δ ppm): 3.38 (bs, 1H, SH), 4.89 (s, 2H, Ar–$CH_2$–O), 6.91–7.51 (m, 13H, Ar-Hs). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 69.04 (Ar–$CH_2$–O), 113.1, 115.74, 122.24, 128.2, 128.28, 129.02, 129.32, 129.51, 132.33, 132.9, 132.96, 134.57, 135.92, 149.76, 156.35 (C5), 168.04 (C3). Anal. calcd. for $C_{21}H_{16}ClN_3OS$: C, 64.03; H, 4.09; N, 10.67; S, 8.14. Found: C, 63.92; H, 4.13; N, 10.52; S, 8.23.

*5-(2-(4-chlorobenzyloxy)phenyl)-4-allyl-2H-1,2,4-triazole-3(4H)-thione* ***(18)***

Yield 2.65 g, 74%, melting point 231–233 °C; IR (KBr) 3308, 873, 756, 663 $cm^{-1}$; $^{1}H$ NMR ($DMSOd_6$, δ ppm): 3.25 (bs, 1H, SH), 4.47 (d, 2H, J=5, $CH_2$=CH-$\underline{CH_2}$-), 4.7 (d, 1H, J=17, *trans*C$\underline{H_2}$=CH-), 4.95 (d, 1H, J=10 *cis*C$\underline{H_2}$=CH-), 5.19 (s, 2H, Ar–$CH_2$–O), 5.62 (m, 1H, $CH_2$=$\underline{CH}$-$CH_2$-), 7.08–7.57 (m, 8H, Ar-Hs). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 69.04 (Ar–$CH_2$–O), 46.06 (Allyl-$CH_2$), 69.14 (Ar–$CH_2$–O), 113.59, 115.83, 117.31, 124.13, 128.97, 129.78, 132.02, 132.18, 133.08, 133.1, 135.94, 149.73, 156.62 (C5), 167.18 (C3). Anal. calcd. for $C_{18}H_{16}ClN_3OS$: C, 60.41; H, 4.51; N, 11.74; S, 8.96. Found: C, 60.32; H, 4.59; N, 11.66; S, 9.08.

*5-(2-(4-chlorobenzyloxy)phenyl)-4-(m-tolyl)-2H-1,2,4-triazole-3(4H)-thione* ***(19)***

Yield 3.0 g, 73%, melting point 185–187 °C; IR (KBr) 3293, 871, 787, 693 $cm^{-1}$; $^{1}H$ NMR ($DMSOd_6$, δ ppm): 2.14 (s, 3H, $CH_3$), 4.15 (bs, 1H, SH), 4.87 (s, 2H, Ar–$CH_2$–O), 6.83–7.45 (m, 12H, Ar-Hs). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 21.17 ($CH_3$), 69.12 (Ar–$CH_2$–O), 113.06, 116.98, 121.15, 125.03, 128.55, 128.75, 128.82, 129.24, 132.37, 132.41, 134.76, 135.34, 136.16, 138.04, 149.51, 156.29 (C5), 166.38 (C3). Anal. calcd. for $C_{22}H_{18}ClN_3OS$: C, 64.78; H, 4.45; N, 10.30; S, 7.86. Found: C, 64.61; H, 4.52; N, 10.23; S, 7.94.

*5-(2-(4-chlorobenzyloxy)phenyl)-4-(p-tolyl)-2H-1,2,4-triazole-3(4H)-thione* ***(20)***

Yield 3.1 g, 76%, melting point 177–179 °C; IR (KBr) 3308, 872, 809, 754 $cm^{-1}$; $^{1}H$ NMR ($DMSOd_6$, δ ppm): 2.26 (s, 3H, $CH_3$), 3.7 (bs, 1H, SH), 4.84 (s, 2H, Ar–$CH_2$–O), 6.84–7.38 (m, 12H, Ar-Hs). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 21.14 ($CH_3$), 69.83 (Ar–$CH_2$–O), 113.04, 119.15, 121.01, 127.12, 128.7, 128.79, 129.38, 131.31, 132.29, 132.62, 134.53, 135.39, 136.7, 149.31, 156.01 (C5), 167.81 (C3). Anal. calcd. for $C_{22}H_{18}ClN_3OS$: C, 64.78; H, 4.45; N, 10.30; S, 7.86. Found: C, 64.72; H, 4.58; N, 10.41; S, 7.73.

*5-(2-(4-chlorobenzyloxy)phenyl)-4-(o-tolyl)-2H-1,2,4-triazole-3(4H)-thione* ***(21)***

Yield 2.9 g, 70%, melting point 200–202 °C; IR (KBr) 3311, 810, 754, 719 $cm^{-1}$; $^{1}H$ NMR ($DMSOd_6$, δ ppm): 1.99 (s, 3H, $CH_3$), 3.49 (bs, 1H, SH), 5.0 (s, 2H, Ar–$CH_2$–O), 6.89–7.42 (m, 12H, Ar-Hs). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 18.17 ($CH_3$), 69.51 (Ar–$CH_2$–O), 113.17, 120.91, 126.4, 128.36, 129.12, 129.38, 129.43, 130.36, 132.01, 132.37, 132.79, 134.98, 136.2, 136.64, 149.33, 156.47 (C5), 166.86 (C3). Anal. calcd. for $C_{22}H_{18}ClN_3OS$: C, 64.78; H, 4.45; N, 10.30; S, 7.86. Found: C, 64.71; H, 4.36; N, 10.39; S, 7.72.

*5-(2-(4-chlorobenzyloxy)phenyl)-4-(m-chlorophenyl)-2H-1,2,4-triazole-3(4H)-thione* ***(22)***

Yield 3.5 g, 84%, melting point 225–226 °C; IR (KBr) 3327, 858, 776, 684 $cm^{-1}$; $^{1}H$ NMR ($DMSOd_6$, δ ppm): 4.26 (bs, 1H, SH), 5.01 (s, 2H, Ar–$CH_2$–O), 7.08–7.61 (m, 12H, Ar-Hs). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 70.29 (Ar–$CH_2$–O), 112.22, 118.64, 124.31, 125.01, 127.54, 128.12, 129.26, 131.47, 131.16, 132.22, 133.06, 135.18, 138.01, 148.31, 158.32 (C5), 169.11 (C3). Anal. calcd. for $C_{21}H_{15}Cl_2N_3OS$: C, 58.89; H, 3.53; N, 9.81; S, 7.49. Found: C, 58.72; H, 3.58; N, 9.73; S, 7.58.

*5-(2-(4-chlorobenzyloxy)phenyl)-4-(isopropyl)-2H-1,2,4-triazole-3(4H)-thione* ***(23)***

Yield 2.7 g, 75%, melting point 212–213 °C; IR (KBr) 3328, 871, 809, 755 $cm^{-1}$; $^{1}H$ NMR ($DMSOd_6$, δ ppm): 1.17 (d, 6H, J=6, $2CH_3$), 4.25 (m, 1H, CH), 4.06 (bs, 1H, SH), 5.28 (s, 2H, Ar–$CH_2$–O), 6.72–7.65 (m, 8H, Ar-Hs). $^{13}C$ NMR (DMSO-$d_6$, δ ppm): 23.18 ($CH_3$), 42.86 (>CH), 69.84 (Ar–$CH_2$–O), 113.34, 116.81, 121.05, 125.35, 127.67, 128.33, 129.14, 130.45, 136.16, 132.68, 133.06, 136.18, 139.01, 141.52, 152.08 (C5), 166.63 (C3). Anal. calcd. for $C_{18}H_{18}ClN_3OS$: C, 60.07; H, 5.04; N, 11.68; S, 8.91. Found: C, 59.91; H, 5.11; N, 11.62; S, 8.88.

## Anti-*Candida albicans* screening

The initial screening of antifungal activity and

determination of minimum inhibitory concentration (MIC) for different synthetic compounds were performed using the cup plate diffusion method and macro-broth dilution method, respectively (Bauer *et al.*, 1966; Arendrup *et al.*, 2012).

### Cup plate experiment

Three to five pure colonies of *Candida albicans* ATCC 2091 were taken from overnight culture of Sabauraud's 2% dextrose agar medium (Merck®, Darmstadt, Germany) and suspended in 5 mL of Sabauraud's 2% dextrose broth medium. The inoculum was evenly suspended by vigorous shaking on a vortex mixer for 15 s. The yeast strain was measured using a spectrophotometer (LKB® Ultrospec) at 530 nm to give absorbance of 0.2-0.15 (1-5x$10^6$ CFU/mL). The suspension was diluted 1:10 in Sabauraud's 2% dextrose broth medium to obtain 1x$10^5$ CFU/mL. The suspension was swabbed onto a Sabauraud's 2% dextrose agar plate and allowed to dry completely. Three ditches per agar plate were made using a cork borer. Then, 100 µL (512 µg) of stock solution (5120 µg $mL^{-1}$) was transferred into the cup using a sterile pipette. The plates were refrigerated at 4°C for 30 min for diffusion, and then incubated at 37°C for 24 h. After the incubation period, the diameter of the inhibition zone (including the diameter cup) was measured and recorded in mm. Fluconazole (50 µg $mL^{-1}$) was used as a positive control. The assay was carried out in duplicate.

### Determination of MIC

MIC was determined with the broth dilution method. The test solution and all standard drugs were prepared at a concentration of 2048 µg $mL^{-1}$ in distilled dimethylsulfoxide and treated as stock solutions. Briefly, 1 mL of RPMI 1640 medium was dispensed into a sterile 7 mL bijou tube (Sterilin Limited, UK). Ten tubes were required for each experiment and each experiment was done in duplicate. Tubes #9 and #10 were used as the positive growth control (no tested compound) and the negative control for the medium sterility (no microorganism), respectively. A 1-mL (2048 µg $mL^{-1}$) aliquot of the tested compound was pipetted into tube #1 and mixed well for a concentration of 1024 µg $mL^{-1}$. Then, 1 mL was transferred from tube #1 to tube #2 for a twofold dilution (512 µg $mL^{-1}$). This procedure was repeated down to tube #8, for a concentration of 8 µg $mL^{-1}$ in tube #8. One milliliter was discarded from tube #8. Then, 1 mL of inoculum (1-5x$10^5$ CFU/mL) was added to each of the eight tubes to give a final concentration of 0.5-2.5x$10^5$ CFU/mL and to make a two-fold dilution of the tested compound. In addition, tube #9 (growth control tube), containing 1 mL of sterile drug-free distilled water, was inoculated with 1 mL of the same inoculum suspension. One milliliter of drug-free medium was added to tube #10 as a sterility control for medium. The inoculated tubes were incubated at 35°C for 20 h. The tubes were inoculated with prepared yeast suspension within 30 min in order to maintain the viable cell concentration. After the incubation period, the results of MIC were recorded manually and interpreted according to the guidelines of the European Committee for Antimicrobial Susceptibility Testing (EUCAST).

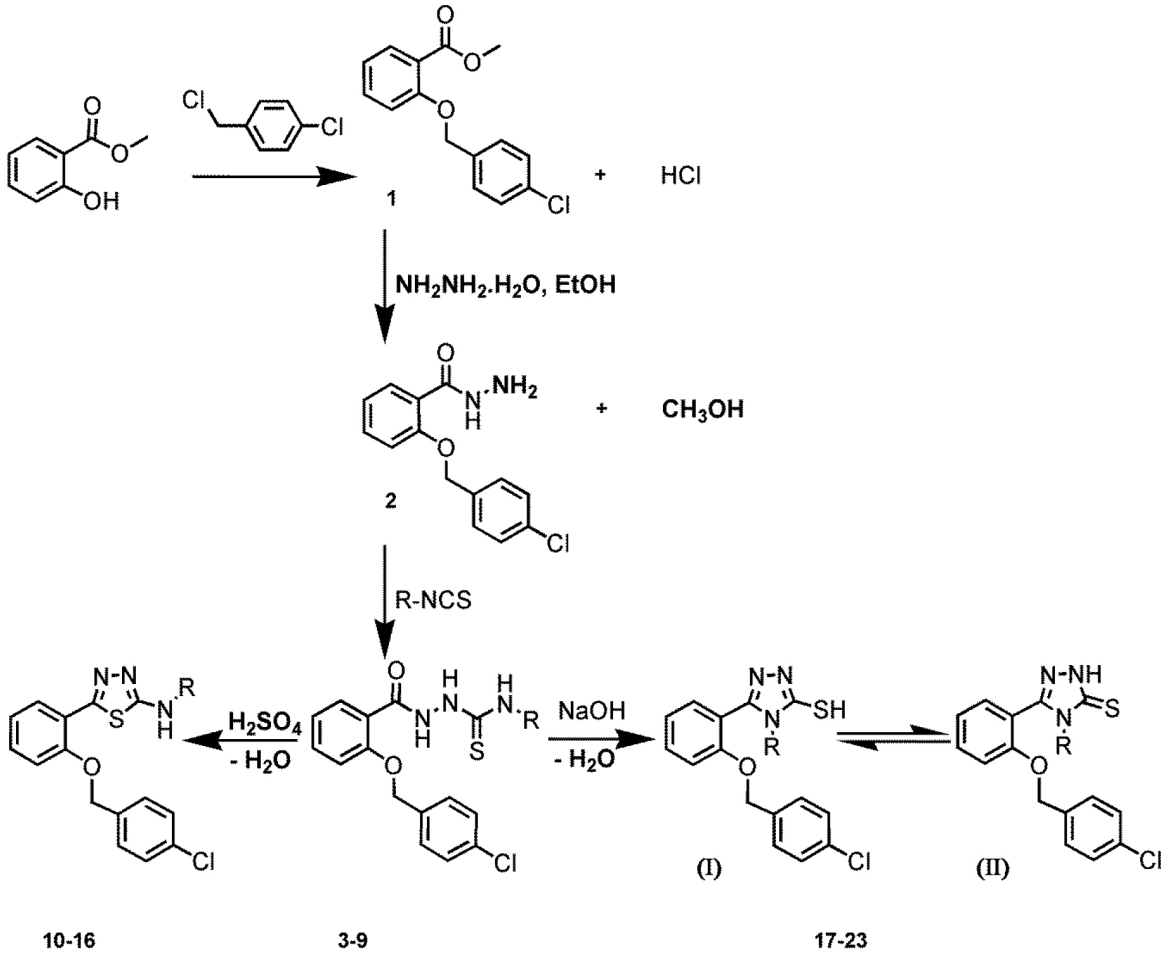
### Docking procedure

The docking studies were performed on a PC with Windows Vista Home Premium Intel(R) Core(TM)2 Duo, 1.83GHz using Autodock vina program (Trott, Olson, 2010). The chemical structures of the compounds under study were prepared with a protonation state similar to that found under physiological conditions. The X-ray crystal structure of the 14-α-sterol demethylase (CYP51) enzyme complexed with fluconazole (1EA1) was obtained from the Protein Data Bank (Podust, Poulos, Waterman, 2001). The cocrystallized fluconazole structure was docked in its target macromolecule structure using Autodock vina program with its default settings.

## RESULTS AND DISCUSSION

### Chemistry

Methyl 2-(4-chlorobenzyloxy)benzoate (**1**) was synthesized through the reaction of methyl 2-hydroxybenzoate with *p*-chlorobenzyl chloride. Reaction of **1** and hydrazine hydrate yielded the corresponding hydrazide (**2**). Reaction of **2** with the corresponding isothiocyanate yielded the isothiocyanate derivatives (**3-9**), which were cyclized in concentrated sulfuric acid resulting in 1,3,4-thiadiazoles (**10-16**) (Daoud, Al-Obaydi, 2008). The reflux of **2** in 2 M NaOH yielded the corresponding 1,3,4-triazole-2-thione derivatives (**17-23**) (Daoud, Al-Obaydi, 2008). The synthetic route of these compounds is given in the scheme. The chemical structures of the synthesized compounds were in accordance with their $^1$H and $^{13}$C NMR spectra. The spectral data are summarized in the material and methods section. $^1$H NMR and $^{13}$C NMR spectra were measured in dimethylsulfoxide-$d_6$ for compounds **2-23** at ambient temperature. $^1$H NMR spectrum of compound **2** showed




**SCHEME -** Synthetic pathway of compounds **1-23.**

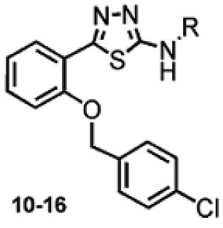
a characteristic signal at δ 5.24 ppm corresponding to the methylene protons of the *p*-chlorobenzyloxy moiety. The disappearance of the methyl ester signal and appearance of two signals at δ 4.53 and 9.24 exchangeable with deuterium oxide are consistent with the chemical structure of the hydrazide derivative **2**. $^{1}$H NMR and $^{13}$C NMR spectra of the thiosemicarbazide compounds **3–9** showed a similar trend in the chemical shift of the common part of the molecular backbone. In the $^{1}$H NMR spectra, the presence of a singlet at δ 5.24-5.32 ppm was assigned to the methylene protons of the p-chlorobenzyloxy moiety, and the three singlets exchangeable with deuterium oxide at δ 9.15-9.52, 9.48-10.04, and 10.09-10.18 ppm were assigned to the thiosemicarbazide NH protons. The $^{13}$C-NMR spectra showed characteristic signals at δ 69.37–69.7, 165.02-165.78, and 180.76-182.23 ppm due to the methylene carbon of the p-chlorobenzyloxy substituent, C=O, and C=S, respectively. The $^{1}$H NMR and $^{13}$C NMR spectra of the triazole compounds **10-16** were characterized by the absence of the amide and thioamide signals. In the 1H NMR spectra, the methylene protons of the p-chlorobenzyloxy moiety resonated at δ 4.6-5.16 ppm. In compound **11**, the allyl substituent showed a singlet at δ 4.36 due to an allylic methylene, a doublet at δ 5.11, a doublet at δ 5.16 due to *trans* and *cis* protons of vinylic methylene, and a multiplet at 5.67 due to vinylic methine. In compounds **12-14**, the methyl protons of tolyl groups resonated at δ 2.31, 2.38, and 2.26 ppm, respectively. In compound **16**, two characteristic signals of the isopropyl group appeared as a doublet at δ 1.13 and a multiplet at δ 4.48. In the $^{13}$C NMR spectra, the methylene carbon of the *p*-chlorobenzyloxy substituent resonated at δ 69.04-70.42 ppm while the C-2 and C-5 of the thiadiazole ring resonated at δ 150.1-157.02 and 164.3-169.04 ppm, respectively. The $^{1}$H NMR and $^{13}$C NMR spectra of the triazole compounds **17-23** showed two singlets at δ 3.25-4.26 and 4.47-5.28 ppm due to the SH and methylene protons, respectively, of the p-chlorobenzyloxy substituent. In addition, the methyl protons of the tolyl moiety in compounds **19-21** resonated at δ 2.14, 2.26, and 1.99 ppm, respectively. Compound **23** showed two additional characteristic signals of the isopropyl group as a doublet at δ 1.17 and a multiplet

at δ 4.25 ppm. In the $^{13}C$ NMR spectra, the methylene carbon of the p-chlorobenzyloxy substituent resonated at δ 69.04-70.29 ppm, while the C-2 and C-5 of the triazole ring resonated at δ 166.38-169.11 and 152.08-158.32 ppm, respectively.

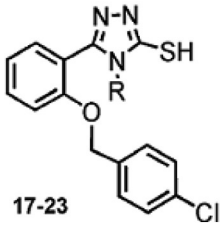
## Anti-*Candida albicans* screening

Fluconazole has been widely used for treatment of fungal infections, but its extensive clinical use has led to the development of drug resistance (Pfaller *et al.*, 2010). The *in vitro* anti-*candida* activity of the synthesized compounds **3-23** was evaluated against *C. albicans*. The obtained data, expressed as the diameter of the inhibition zone (DIZ) and minimum inhibition concentration (MIC) for the test compounds and for the reference drug fluconazole are presented in Table I. Compounds **3-9** were found to be inactive against *C. albicans*, while compounds **10-23** showed promising antifungal activity with DIZ = 13–18 mm towards *C*. This suggests that the cyclization of the biologically inactive thiosemicarbazides **3-9** into thiadiazole or triazole rings developed biologically active compounds against *C. albicans* at a concentration of 100 μg $mL^{-1}$. In other words, the heterocyclic ring in these compounds is crucial for their antifungal activity. Compounds **10-23** showed MIC values in the range of 0.08-0.17 μmol $mL^{-1}$, while the MIC value of fluconazole was 0.052 μmol $mL^{-1}$. Compounds **11** and **18** were found to be the most active, with MIC values of 0.08 μmol $mL^{-1}$. The allyl substituent at position 4 of the heterocyclic ring showed higher activity than the phenyl, tolyl, or isopropyl substituents. Basically, the small bulk size of the allyl group is proposed to be related to the high activity of the compound. For optimization of antifungal activity and in view of these results, further studies will be suggested to be undertaken starting with the two lead compounds **11** and **18**.

**TABLE I -** Anti*Candida albicans* activity of compounds **10-23** (100 μg $mL^{-1}$)




10-16




17-23

| Compd. no. | R | DIZ (mm)* | MIC (μmol $mL^{-1}$)** |
|---|---|---|---|
| **10** | Phenyl | 12 | 0.16 |
| **11** | Allyl | 16 | 0.08 |
| **12** | *m*-Tolyl | 13 | 0.15 |
| **13** | *p*-Tolyl | 12 | 0.15 |
| **14** | *o*-Tolyl | 12 | 0.15 |
| **15** | 3-Chlorophenyl | 13 | 0.14 |
| **16** | *Isopropyl* | 13 | 0.17 |
| **17** | Phenyl | 12 | 0.17 |
| **18** | Allyl | 18 | 0.08 |
| **19** | *m*-Tolyl | 13 | 0.15 |
| **20** | *p*-Tolyl | 14 | 0.15 |
| **21** | *o*-Tolyl | 14 | 0.15 |
| **22** | 3-Chlorophenyl | 13 | 0.14 |
| **23** | *Isopropyl* | 13 | 0.17 |
| **Fluconazol** | - | 18 | 0.05 |

* The arithmetic mean of the inhibition zone diameters in mean ± standard deviation. **The lowest concentration of the compound that produced 50-80% microbial growth inhibition (μmol $mL^{-1}$). Count=14, Mean=0.3, Standard Devation= 0.029, Standard Error=0.008, Sample Variance=0.001.

## Docking Procedure

The comparative study of the binding mode of the synthesized compounds and the drug fluconazole with the binding site of the CYP51 protein was done using Autodock vina (Trott, Olson, 2010). From the protein data bank, the X-ray structure of the enzyme bound with fluconazole (FCZ) was obtained; PDB code: 1EA1 (Podust, Poulos, Waterman, 2001). The docking procedure was confirmed based on the RMSD value difference of 0.432 Å of the pose of the nonrestricted redocked FCZ into the binding site of the CYP51 from the co-crystallized FCZ (Figure 1). The binding domain shows a hydrophobic pocket surrounded by the side chains of Tyr 76, Phe 78, Met 79, Phe 83, Arg 96, Met 99, Leu 100, Ser 252, Met 253, Phe 255, Ala 256, His 259, Thr 260, Leu 321, Ile 323, Met 433, and Val 434 (Figure 2). The docking results of compound **18** revealed that its triazole ring occupied the same position inside the binding site and showed the same orientation as the triazole moiety of the co-crystallized FCZ with 1EA1 (Figure 2). The triazole ring is oriented near the heme molecule inside the binding domain and is surrounded by the side chains of Phe 255, Ala 256, His 259, and Thr 260. The p-chlorophenyl moiety overlaid the second triazole ring FCZ, showing hydrophobic interaction with the side chains of Leu 321, Ile 323, Met 433, and Val 434. In addition, the allyl substituent is oriented in the same binding pocket of the difluorophenyl moiety of FCZ, showing hydrophobic interaction with the side chains of Phe 83, Arg 96, Met 99, Leu 100, Ser 252, and Met 253.

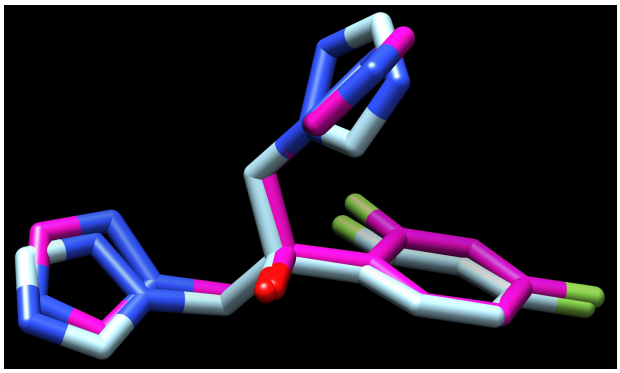


**FIGURE 1 -** The co-crystallized fluconazol FCZ (from 1EA1.pdb, colored cyan) overlaid onto the redocked fluconazol (colored magenta).

## CONCLUSION

A novel series of thiosemicarbazide derivatives of methyl o-chlorobenzylysalicylate and its cyclized forms, 1,3,4-thiadiazol-2-amine and *2H*-1,2,4-triazole-3(4H)-thione, has been synthesized and screened for

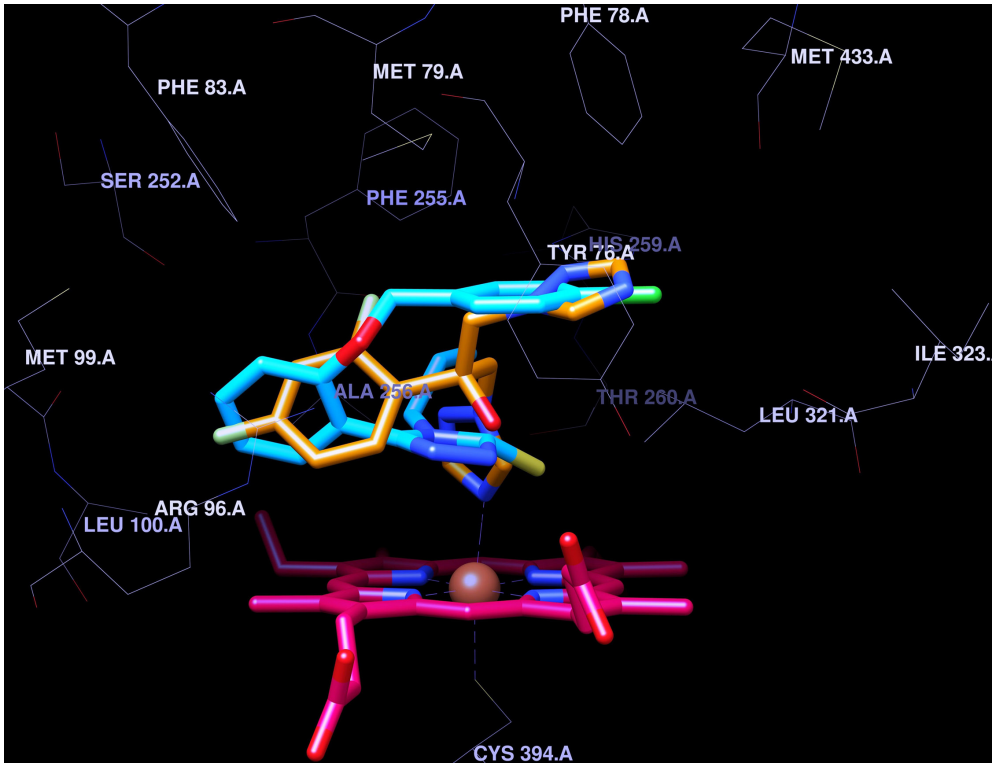



**FIGURE 2 -** The 3D diagram showing the interaction between the 1EA1.pdb structure and compound **18** (sticks, blue) overlaid on fluconazol FCZ x-ray ligand (stick, orange).

anti-*Candida albicans* activity. Variable and promising anti-*Candida albicans* activity was observed in the synthesized compounds **10-23**; these compounds had MIC values in the range of 0.08-0.17 μmol $mL^{-1}$, while the MIC value of fluconazole was 0.05 μmol $mL^{-1}$. Compounds **11** and **18** were found to be the most active, with MIC values of 0.08 μmol $mL^{-1}$. In addition, an *in silico* docking study of compound **18** with X-ray structure of 14-α-sterol demethylase (CYP51) active site domain (1EA1) postulated that the designed compound may act on the same enzyme target as the fluconazole x-ray structure. A good correlation was found between the *in silico* generated model and the reported X-ray structure, with similar hydrogen bonding and orientation inside the binding site.

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