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ORIGINAL ARTICLE

DESIGN AND SYNTHESIS OF SOME NOVEL 1,2,4-TRIAZOLE-3-YL-MERCAPTO DERIVATIVES AS POTENTIAL ANTI-*CANDIDA* AGENTS

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Abstract

In the context of the alarming incidence of the multidrug-resistant *Candida sp.* based infections, a new series of 1,2,4-triazole-3-yl-mercapto derivatives were synthesized and evaluated as potential antifungal agents. The affinity of the synthesized compounds towards the catalytic site of the lanosterol 14α-demethylase (CYP51) was evaluated *in silico*, by molecular docking studies. The antifungal activity of the titled compounds was evaluated *in vitro* against pathogenic strains of *Candida sp.,* by measuring the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC). The data obtained from the docking simulation showed that the new synthesized compounds might act as non-covalent inhibitors of fungal CYP51. The results of the *in vitro* antifungal screening support their potential anti-*Candida* activity, compound **3b** exhibiting a similar effect as fluconazole, used as antifungal reference drug.

Rezumat

În contextul creşterii alarmante a numărului de infecţii cauzate de specii de *Candida* multirezistente, au fost sintetizaţi şi evaluaţi ca potenţiali agenţi antifungici noi compuşi cu structură 3-mercapto-1,2,4-triazolică. A fost evaluată *in silico*, prin studii de andocare moleculară, afinitatea de legare a noilor derivaţi triazolici de situsul catalitic al lanosterol 14α-demetilazei fungice (CYP51). Activitatea antifungică a compuşilor sintetizaţi a fost evaluată *in vitro*, pe tulpini standardizate de *Candida sp.,* prin determinarea concentraţiei minime inhibitorii (CMI) şi a concentraţiei minime fungicide (CMF). Rezultatele testării *in silico* au confirmat potenţialul anti-*Candida* al compuşilor noi sintetizaţi, aceştia acţionând ca inhibitori ai lanosterol 14α-demetilazei fungice, legarea de enzimă fiind mediată de interacţiuni non-covalente. Potenţialul antifungic al noilor derivaţi triazolici este susţinut şi de rezultatele testării *in vitro*, compusul **3b** având CMI egală cu cea a fluconazolului, folosit ca compus de referinţă.

Keywords: antifungal, azole derivatives, molecular docking, lanosterol 14α-demethylase

Introduction

Candida sp. based infections, ranging from superficial and mucosal to systemic and bloodstream ones, are the most frequently reported fungal infections in Europe and USA [3], being associated with significant morbidity and mortality, as a result of the increasing resistance of the pathogenic fungal strains to the currently available antifungals [28]. Azoles have been widely used as potent anti-*Candida* drugs [21]. Their molecular mechanism of action is based on the inhibition of lanosterol 14α-demethylase (CYP51), a key enzyme involved in the fungal ergosterol bio-synthesis [7]. Due to the high affinity of the nucleophilic nitrogen of the azole heterocycle towards the heme Fe^{2+}, these compounds are responsible for the cross-over inhibition of human CYP51 and other CYP450 enzymes [22]. Safety issues related to the azole drugs, such as hepatotoxicity, endocrine disorders, drug interactions, unfavourable pharmacokinetic properties and fungistatic activity, corroborated with the increasing resistance to treatment of the fungal strains, require the development of new CYP51 inhibitors, with greater selectivity towards this fungal enzyme [6, 26, 27].

A particular interest in the field of antifungal drug design was assigned to the 1,2,4-triazole heterocyclic compounds [9]. Their aromaticity and electron-rich property [25] enable them to bind lanosterol 14α-

demethylase, both through covalent coordination of the heme Fe^{2+} by the N4 nitrogen atom of the triazole ring and non-covalent interactions, including π-π stacking [23]. 3-*S*-substituted-1,2,4-triazole ring systems have also been studied during the last years and the antifungal activity has been reported for a large number of derivatives [10, 12]. Moreover, S-linker was reported to improve important drug-like parameters, such as lipophilicity and water solubility [13].

Considering all the above, we synthesized a new series of 1,2,4-triazole-3-yl-mercapto derivatives starting from thymol, a terpenoid phenol, based on the evidences related to its anti-*Candida* properties [1, 2, 17, 20]. A molecular docking study was carried out in order to evaluate the interaction of the synthesized compounds with the catalytic site of fungal lanosterol 14α-demethylase, as well as their ability to trigger the cross-over inhibition of the human target enzyme. The antifungal activity of the synthesized compounds was evaluated *in vitro,* against pathogenic strains of *Candida sp*.

Materials and Methods

Chemistry

All chemicals and reagents used for synthesis were of analytical grade purity and were obtained from Alfa Aesar (Karlsruhe, Germany) and Merck (Darmstadt, Germany). Analytical thin layer chromatography (TLC) carried out on Silica Gel $60F_{254}$ sheets was used for monitoring the reaction progress and to confirm the purity of the newly synthesized compounds. A mixture of ethyl acetate:n-hexane 3:1 was used as elution system, UV light (254 nm) being employed for visualization. The uncorrected melting points were determined with an Electrothermal melting point meter through the open glass capillary method. In order to confirm the structures of the synthesized compounds, spectral analytical methods (mass spectrometry [MS], infrared spectroscopy [IR], nuclear magnetic resonance [NMR]) and elemental analysis were used. IR spectra were recorded on a Jasco FT/IR 6100 spectrometer (Jasco, Easton, MD), using anhydrous potassium bromide for sample preparation. MS analyses were performed in positive ionization at 70 eV, using an Agilent 1100 series and an Agilent Ion Trapp SL mass spectrometer (Agilent, Santa Clara, CA). ^{1}H-NMR spectra were recorded on a Bruker Advance NMR spectrometer (Karlsruhe, Germany), operating at 500 MHz, using DMSO-d_6 as solvent and tetra-methylsilane (TMS) as internal standard. ^{13}C-NMR analyses were performed on a Bruker Advance NMR spectrometer, operating at 125 MHz, in DMSO-d_6, using a Waltz-16 decoupling scheme, with TMS as internal standard. Chemical shift (δ) values were expressed in parts *per* million (ppm). For the elemental analyses, a Vario El CHNS instrument was used.

*General procedure for the synthesis of 2-(2-(2-iso-propyl-5-methylphenoxy)acetyl)-N-substituted-hydrazinecarbothioamide (**2a-c**)*

Equimolar quantities of acetohydrazide (**1**) (10 mmol, 2.22 g) and *N*-substituted isothiocyanate (10 mmol) in absolute ethanol (30 mL) were refluxed for 6 hours. After cooling to the room temperature, the reaction mixture was poured into ice cold water. The obtained precipitate was filtered and crystallized from water:ethanol 3:1 mixture.

*General procedure for the synthesis of 5-(2-iso-propyl-5-methylphenoxy)-4-substituted-4H-1,2,4-triazole-3-thiol (**3a-c**)*

The alcoholic solution of the **2a-c** (1 mmol in 3 mL of absolute ethanol) was treated with triethylamine (15 drops) and the reaction mixture was refluxed for 5 hours. The solvent was removed under *vacuum* and the obtained precipitate was filtered, dried and recrystallized from ethanol.

*General procedure for the synthesis of 1-((5-(2-iso-propyl-5-methylphenoxy)-4-substituted-4H-1,2,4-triazol-3-yl)thio)ethers (**4a-o**)*

A mixture of **3a-c** (1 mmol) and potassium hydroxide (1.5 mmol, 0.084 g) in 5 mL *N,N*-dimethylform-amide:methanol 1:1 was stirred at room temperature for 1 hour. Then, an equimolar quantity (1 mmol) of α-halocarbonyl compound was added and the stirring was continued for other 4 hours. After the reaction was complete (according to the TLC detection) the mixture was poured over ice cold water. The formed precipitate was filtered, dried and recrystallized from ethanol, to afford the target compounds.

Molecular Docking Study

In order to evaluate the affinity of the synthesized compounds towards *Candida albicans* lanosterol 14α-demethylase, as well as to assess their ability for cross-over inhibition of the human homologous, a molecular docking study was carried out using Auto-Dock 4.2.6. [15]. Fluconazole was used as reference drug. The sequence homologous (PBD ID: 5EQB) used as template for the building of the fungal lanosterol 14α-demethylase was chosen from the Protein Data Bank based on the results of a BLAST search. The fungal target enzyme (5EQBCA), was built through homology modelling based on the UniProt P10613 sequence from *Candida albicans* (www.uniprot.org), using SWISS-MODEL (www.swissmodel.expasy.org). The crystal structure of human CYP51 was taken from the Protein Data Bank (PDB ID: 3JUS) [4]. Dataset files containing the ligands and target enzymes (5EQBCA and 3JUS) were prepared using a previously reported protocol [5, 14].

AutoDock uses a Lamarckian genetic algorithm [15], based on the random modifications of structural parameters of the ligand, in order to identify the most likely binding conformation, corresponding to a solution of minimum energy. The binding energy of possible ligands poses is evaluated through a grid-

based method. The conformational search space is configured as a cubic area, with edges x = y = z = 76 points and centre Cartesian coordinates set to x = 39.048, y = 4.888, z = 51.406 for the human target enzyme and to x = 23.134, y = 13.943, z = 19.959 for the fungal homologous. The space between the grid points was set to 0.375 Å. Visualization and analysis of the docking results were performed using UCSF Chimera 1.10.2 [16] and AutoDockTools 1.5.6. [15]. The values of the inhibition constants (K_i) were calculated through the conversion of the binding energy values (ΔG), using formula $K_i = e^{\frac{\Delta G \times 1000}{R \times T}}$, where R represents the Regnault constant = 1.98719 $\frac{kcal}{K \times mol}$ and T = 298.15 K.

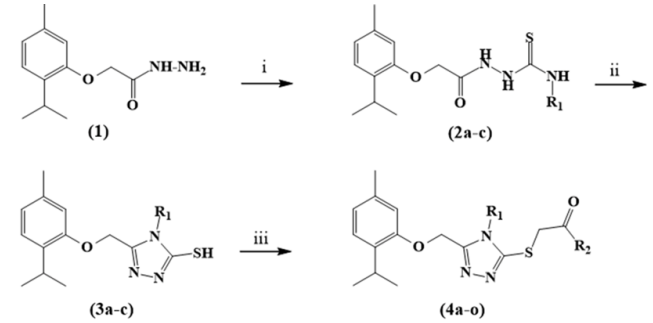
Antifungal activity assay

The antifungal activity of the synthesized compounds was evaluated *in vitro* against three pathogenic strains of *Candida sp*. The microorganisms used for this assay were *Candida albicans* ATCC 10231, *Candida albicans* ATCC 18804 and *Candida krusei* ATCC 6258 and were obtained from the Food Biotechnology Laboratory, Life Sciences Institute, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania. The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) were determined according to the Clinical and Laboratory Standards Institute guidelines, following a previously reported protocol [14, 18]. Fluconazole was used as reference antifungal drug. All experiments were performed in triplicate.

Results and Discussion

Chemistry

The synthesis of the titled compounds was carried out performing a stepwise reaction protocol, as outlined in Figure 1. The key intermediate hydrazide (**1**) was obtained using a previously reported synthesis protocol [19]. The 1,2,4-triazole-3-yl-mercapto intermediates **3a-c** were synthesized, according to the literature [11], through the condensation of the hydrazide compound **1** with various *N*-substituted isothiocyanates, followed by intramolecular cyclization, in basic media, of the corresponding *N*-substituted acyl-thiosemicarbazide derivatives **2a-c**. The target 1,2,4-triazole-3-yl-mercapto derivatives were obtained through a mild *S*-alkylation reaction, by stirring, at room temperature, the compounds **3a-c** with different α-halocarbonyl compounds, using a mixture of methanol:*N,N*,-dimethylformamide 1:1 as solvent. The progress of the reaction was monitored by TLC. The synthesized compounds were purified by recrystallization from the appropriate solvents and their physico-chemical and spectral properties were investigated.



	R_1	R_2		R_1	R_2
2a, 3a	$-C_6H_5$	-	**4g**	$-CH_2-CH=CH_2$	$-CH_3$
2b, 3b	$-CH_2-CH=CH_2$	-	**4h**	$-CH_2-CH=CH_2$	$-C_6H_5$
2c, 3c	$-CH_2-CH_3$	-	**4i**	$-CH_2-CH=CH_2$	$-C_6H_4$-4-Cl
4a	$-C_6H_5$	$-NH_2$	**4j**	$-CH_2-CH=CH_2$	$-C_6H_3$-3-$CONH_2$-4-OH
4b	$-C_6H_5$	$-CH_3$	**4k**	$-CH_2-CH_3$	$-NH_2$
4c	$-C_6H_5$	$-C_6H_5$	**4l**	$-CH_2-CH_3$	$-CH_3$
4d	$-C_6H_5$	$-C_6H_4$-4-Cl	**4m**	$-CH_2-CH_3$	$-C_6H_5$
4e	$-C_6H_5$	$-C_6H_3$-3-$CONH_2$-4-OH	**4n**	$-CH_2-CH_3$	$-C_6H_4$-4-Cl
4f	$-CH_2-CH=CH_2$	$-NH_2$	**4o**	$-CH_2-CH_3$	$-C_6H_3$-3-$CONH_2$-4-OH

Figure 1.

Synthesis of 1,2,4-triazole-3-yl-mercapto derivatives

i. R_1-N=C=S/EtOH abs./reflux; ii.TEA/EtOH abs./ reflux; iii. X-CH_2-CO-R_2/KOH/MeOH:DMF 1:1

The structural assignments of the synthesized compounds were based on their spectral data (MS, IR, ^{1}H-NMR and ^{13}C-NMR) and elemental analysis.

*2-(2-(2-isopropyl-5-methylphenoxy)acetyl)-N-phenyl-hydrazinecarbothioamide (**2a**)*:

Analytical calculated for $C_{19}H_{23}N_3O_2S$ (%): C, 63.84; H, 6.49; N, 11.75; S, 8.97; Found (%): C, 63.93; H, 6.38; N, 11.93; S, 8.71; white powder; m.p. 172 - 173ºC; yield 91%; IR(KBr) υ_{max} cm^{-1}: 3295 (N-H), 3095 (C-H arom.), 1674 (C=O), 1272 (C=S), 1244 (C-O-C); MS (EI, 70 eV): m/z = 358.2 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 7.90 (s, 2H, -N**H**-CS-N**H**-), 7.82 (s, 1H, -N**H**-NH), 7.43 (t, 1H ArH), 7.41 (t, 2H, ArH), 7.39 - 7.38 (d, 2H, ArH),

7.03 (d, 1H, ArH), 6.79 (s, 1H, ArH), 6.75 (d, 1H, ArH), 4.98 (s, 2H, Ar-O-CH_2), 2.95 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.24 (s, 3H, Ar-CH_3), 1.01 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 182.75 (C=S), 165.34 (C=O), 155.52, 136.41, 134.48, 133.96, 130.05, 129.81, 128.27, 126.05, 122.71, 113.51 (aromatic ring C), 60.96 (-O-**C**H_2), 25.98 (Ar-**C**H-$(CH_3)_2$), 23.18 (Ar-CH-(**C**H_3)**$_2$**), 20.99 (Ar-**C**H_3).

*N-allyl-2-(2-(2-isopropyl-5-methylphenoxy)acetyl)-hydrazinecarbothioamide (**2b**)*:

Analytical calculated for $C_{16}H_{23}N_3O_2S$ (%): C, 59.78; H, 7.21; N, 13.07; S, 9.98; Found (%): C, 59.93; H, 7.14; N, 13.21; S, 9.81; white powder; m.p. 142 - 143ºC; yield 75%; IR(KBr) υ_{max} cm^{-1}: 3268 (N-H), 3081 (C-H arom.), 3054 (=C-H), 1659 (C=O), 1266 (C=S), 1243 (C-O-C); MS (EI, 70 eV): m/z = 322.2 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 7.14 (s, 2H, -N**H**-CS-N**H**-), 7.12 (s, 1H, -N**H**-NH), 7.10 (d, 1H, ArH), 6.79 (d, 1H, ArH), 5.97 (m, 1H, -C**H**=CH_2), 5.23 (s, 2H, Ar-O-CH_2), 5.01 - 4.97 and 4.94 (d, 1H each, =CH_2), 4.92 (d, 2H, -CH_2-), 3.15 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.28 (s, 3H, Ar-CH_3), 1.11 - 1.09 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 180.09 (C=S), 166.34 (C=O), 154.15, 136.34, 133.65 (aromatic ring C), 131.26 (-CH=), 126.18, 122.52 (aromatic ring C), 118.23 (=CH_2), 113.44 (aromatic ring C), 60.38 (-O-**C**H_2), 46.37 (-CH_2-), 25.92 (Ar-**C**H-$(CH_3)_2$), 23.39 (Ar-CH-(**C**H_3)**$_2$**), 21.29 (Ar-**C**H_3).

*N-ethyl-2-(2-(2-isopropyl-5-methylphenoxy)acetyl)-hydrazinecarbothioamide (**2c**)*:

Analytical calculated for $C_{15}H_{23}N_3O_2S$ (%): C, 58.22; H, 7.49; N, 13.58; S, 10.36; Found (%): C, 58.49; H, 7.37; N, 13.42; S, 10.55; white powder; m.p. 117 - 118ºC; yield 78%; IR(KBr) υ_{max} cm^{-1}: 3316 (N-H), 3089 (C-H arom.), 1681 (C=O), 1274 (C=S), 1253 (C-O-C); MS (EI, 70 eV): m/z = 310.3 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 7.63 (s, 1H, -N**H**-NH), 7.55 (s, 2H, -N**H**-CS-N**H**-), 7.11 (d, 1H, ArH), 6.91 (s,1H, ArH), 6.83 (d, 1H, ArH), 5.20 (s, 2H, Ar-O-CH_2), 4.06 (m, 2H, -C**H$_2$**-), 3.22 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.30 (s, 3H, Ar-CH_3), 1.25 (t, 3H, -C**H$_3$**), 1.12 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 181.54 (C=S), 167.45 (C=O), 154.95, 136.53, 133.79, 126.34, 122.60, 113.46 (aromatic ring C), 60.71 (-O-**C**H_2), 39.06 (-CH_2-), 26.07 (Ar-**C**H-$(CH_3)_2$), 23.40 (Ar-CH-(**C**H_3)**$_2$**), 21.46 (Ar-**C**H_3), 13.89 (-CH_3**)**.

*5-(2-isopropyl-5-methylphenoxy)-4-phenyl-4H-1,2,4-triazole-3-thiol (**3a**)*:

Analytical calculated for $C_{19}H_{21}N_3OS$ (%): C, 67.23; H, 6.24; N, 12.38; S, 9.45; Found (%): C, 66.91; H, 6.12; N, 12.65; S, 9.61; white powder; m.p. 174 - 175ºC; yield 69%; IR(KBr) υ_{max} cm^{-1}: 3044 (C-H arom), 2546 (S-H), 1616 (-N=CH-), 1255 (C-O-C); MS (EI, 70 eV): m/z = 340.2 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 14.05 (s, 1H, -SH), 7.55 (t, 1H, ArH), 7.54 - 7.50 (t, 2H, ArH), 7.46 - 7.45 (d, 2H, ArH), 7.01 - 6.99 (d, 1H, ArH), 6.72 (s, 1H, ArH), 6.70 (d, 1H, ArH), 4.98 (s, 2H, Ar-O-CH_2), 2.78 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.20 (s, 3H, Ar-CH_3), 0.97 - 0.96 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 167.81 (C_3-triazole ring), 155.38 (aromatic ring C), 154.46 (C_2-triazole ring), 136.32, 134.04, 129.91, 129.73, 128.25, 126.19, 122.63, 113.43 (aromatic ring C), 60.81 (-O-**C**H_2), 25.64 (Ar-**C**H-$(CH_3)_2$), 23.27 (Ar-CH-(**C**H_3)**$_2$**), 21.29 (Ar-**C**H_3).

*4-allyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazole-3-thiol (**3b**)*:

Analytical calculated for $C_{16}H_{21}N_3OS$ (%): C, 63.33; H, 6.9; N, 13.85; S, 10.57; Found (%): C, 63.71; H, 7.10; N, 13.51; S, 10.69; white powder; m.p. 134 - 135ºC; yield 72%; IR(KBr) υ_{max} cm^{-1}: 3092 (C-H arom), 3053 (=C-H), 2574 (S-H), 1616 (-N=CH-), 1248 (C-O-C); MS (EI, 70 eV): m/z = 304.1 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 13.93 (s, 1H, -SH), 7.09 - 7.08 (d, 1H, ArH), 6.98 (s, 1H, ArH), 6.78 - 6.76 (d,1H, ArH), 5.93 (m, 1H, C**H**=CH_2), 5.21 (s, 2H, Ar-O-CH_2), 4.99 - 4.95 and 4.90 (d, 1H each, =CH_2), 4.72 (d, 2H, -CH_2-), 3.14 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.27 (s, 3H, Ar-CH_3), 1.09 - 1.08 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 164.07 (C_3-triazole ring), 154.92 (aromatic ring C), 152.31 (C_2-triazole ring), 136.35, 132.21 (aromatic ring C), 131.25 (-CH=), 126.22, 122.47 (aromatic ring C), 118.28 (=CH_2), 113.40 (aromatic ring C), 60.34 (-O-**C**H_2), 46.44 (-CH_2-), 25.95 (Ar-**C**H-$(CH_3)_2$), 23.30 (Ar-CH-(**C**H_3)**$_2$**), 21.38 (Ar-**C**H_3).

*4-ethyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazole-3-thiol (**3c**)*:

Analytical calculated for $C_{15}H_{21}N_3OS$ (%): C, 61.82; H, 7.26; N, 14.42; S 11.00; Found (%): C, 62.08; H, 7.14; N, 14.67; S, 10.74; white powder; m.p. 139 - 140ºC; yield 75%; IR(KBr) υ_{max} cm^{-1}: 3044 (C-H arom), 2553 (S-H), 1613 (-N=CH-), 1253 (C-O-C); MS (EI, 70 eV): m/z = 292.1 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 13.84 (s, 1H, -SH) 7.12 - 7.10 (d, 1H, ArH), 6.97 (s, 1H, ArH), 6.80 - 6.79 (d,1H, ArH), 5.18 (s, 2H, Ar-O-CH_2), 4.08 - 4.03 (m, 2H, -C**H$_2$**-), 3.16 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.28 (s, 3H, Ar-CH_3), 1.27 (t, 3H, -CH_2-C**H$_3$**), 1.11 - 1.10 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 167.57 (C_3-triazole ring), 154.91 (aromatic ring C), 148.57 (C_2-triazole ring), 136.50, 133.85, 126.38, 122.66, 113.54 (aromatic ring C), 60.67 (-O-**C**H_2), 39.19 (-CH_2-), 26.08 (Ar-**C**H-$(CH_3)_2$), 23.31 (Ar-CH-(**C**H_3)**$_2$**), 21.41 (Ar-**C**H_3), 13.84 (-CH_3**)**.

*2-((5-(2-isopropyl-5-methylphenoxy)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (**4a**)*:

Analytical calculated for $C_{21}H_{24}N_4O_2S$ (%): C, 63.61; H, 6.10; N, 14.13; S 8.09; Found (%): C, 63.32; H, 6.29; N, 13.90; S, 8.31; white powder; m.p. 168 - 169ºC; yield 84%; IR(KBr) υ_{max} cm^{-1}: 3318 (N-H amide), 3058 (C-H arom), 1673 (C=O amide), 1610 (-N=CH-), 1252 (C-O-C); MS (EI, 70 eV): m/z = 397.2 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 7.67 (s, 2H, -CO-N**H$_2$**), 7.58 (t, 1H, ArH), 7.57 (t, 2H, ArH), 7.52 -

7.50 (d,2H, ArH), 7.00 - 6.98 (d, 1H, ArH), 6.78 (s, 1H, ArH), 6.71 - 6.70 (d, 1H, ArH), 5.10 (s, 2H, Ar-O-CH_2), 3.95 (s, 2H, -S-CH_2), 2.80 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.21 (s, 3H, Ar-CH_3), 0.94 - 0.93 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 168.92 (C=O), 155.48 (aromatic ring C), 154.67 (C_3-triazole ring), 152.26 (C_2-triazole ring), 136.29, 133.96, 133.24, 130.46, 130.28, 127.21, 126.11, 122.44, 113.42 (aromatic ring C), 60.53 (-O-**C**H$_2$), 36.36 (-S-CH_2-), 25.59 (Ar-**C**H-$(CH_3)_2$), 23.28 (Ar-CH-(**C**H$_3$)**$_2$**), 21.32 (Ar-**C**H$_3$).

*1-((5-(2-isopropyl-5-methylphenoxy)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)propan-2-one (**4b**)*:

Analytical calculated for $C_{22}H_{25}N_3O_2S$ (%): C, 66.81; H, 6.37; N, 10.62; S, 8.11; Found (%): C, 67.03; H, 6.61; N, 10.38; S, 7.97; white powder; m.p. 121 - 122ºC; yield 41%; IR(KBr) υ_{max} cm^{-1}: 3052 (C-H arom), 1713 (C=O), 1612 (-N=CH-), 1254 (C-O-C); MS (EI, 70 eV): m/z = 396.5 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 7.63 (d, 2H, ArH), 7.44 (d, 2H, ArH), 7.39 (t, 1H, ArH), 7.02 (d,1H, ArH), 6.79 (s, 1H, ArH), 6.73 (d, 1H, ArH), 5.07 (s, 2H, Ar-O-CH_2), 4.12 (s, 2H, -S-CH_2), 3.01 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.31 (s, 3H, -CO-CH_3), 2.23 (s, 3H, Ar-CH_3), 0.95 - 0.94 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 197.65 (C=O), 155.39 (aromatic ring C), 154.81 (C_3-triazole ring), 151.97 (C_2-triazole ring), 136.40, 134.09, 133.89, 133.20, 130.17, 130.05, 126.21, 122.61, 113.49 (aromatic ring C), 60.91 (-O-**C**H$_2$), 37.01 (-S-CH_2-), 26.21 (-CH_3), 25.49 (Ar-**C**H-$(CH_3)_2$), 23.72 (Ar-CH-(**C**H$_3$)**$_2$**), 20.99 (Ar-**C**H$_3$);

*2-((5-(2-isopropyl-5-methylphenoxy)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-1-phenylethanone (**4c**)*:

Analytical calculated for $C_{27}H_{27}N_3O_2S$ (%): C, 70.87; H, 5.95; N, 9.18; S, 7.01; Found (%): C, 70.61; H, 5.83; N, 9.42; S, 7.12; light yellow powder; m.p. 104 - 105ºC; yield 61%; IR(KBr) υ_{max} cm^{-1}: 3059 (C-H arom), 1681 (C=O), 1613 (-N=CH-), 1253 (C-O-C); MS (EI, 70 eV): m/z = 458.4 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 8.06 - 8.04 (d, 2H, ArH), 7.67 (d, 2H, ArH), 7.63 - 7.62 (t, 1H, ArH), 7.61 (t,1H, ArH), 7.53 (d,2H, ArH), 7.51 (d,2H, ArH), 7.06 (d, 1H, ArH), 6.81 (s, 1H, ArH), 6.75 (d, 1H, ArH), 4.99 (s, 2H, Ar-O-CH_2), 4.33 (s, 2H, -S-CH_2), 2.78 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.22 (s, 3H, Ar-CH_3), 0.97 - 0.95 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 191.98 (C=O), 155.42 (aromatic ring C), 154.61 (C_3-triazole ring), 152.20 (C_2-triazole ring), 136.44, 134.25, 133.92, 130.47, 130.12, 129.74, 129.48, 129.04, 128.17, 127.26, 126.11, 122.50, 113.41 (aromatic ring C), 60.93 (-O-**C**H$_2$), 36.95 (-S-CH_2-), 25.54 (Ar-**C**H-$(CH_3)_2$), 23.30 (Ar-CH-(**C**H$_3$)**$_2$**), 21.14 (Ar-**C**H$_3$).

*1-(4-chlorophenyl)-2-((5-(2-isopropyl-5-methylphenoxy)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)ethanone (**4d**)*:

Analytical calculated for $C_{27}H_{26}ClN_3O_2S$ (%): C, 65.91; H, 5.33; N, 8.54; S, 6.52; Found (%): C, 65.46; H, 5.11; N, 8.87; S, 6.31; yellow powder; m.p. 108 - 109ºC; yield 89%; IR(KBr) υ_{max} cm^{-1}: 3053 (C –H arom), 1680 (C=O), 1614 (-N=CH-), 1253 (C-O-C), 627 (C-Cl); MS (EI, 70 eV): m/z = 492.7 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 8.05 - 8.03 (d, 2H, ArH), 7.65 - 7.63 (d, 2H, ArH), 7.59 - 7.58 (d, 2H, ArH), 7.52 (d, 2H, ArH), 7.50 (t, 1H, ArH), 7.00 - 6.98 (d, 1H, ArH), 6.77 (s, 1H, ArH), 6.71 - 6.70 (d, 1H, ArH), 5.09 (d, 2H, Ar-O-CH_2), 4.37 (s, 2H, -S-CH_2), 2.75 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.20 (s, 3H, Ar-CH_3), 0.95 - 0.93 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 192.25 (C=O), 155.47 (aromatic ring C), 154.78 (C_3-triazole ring), 152.36 (C_2-triazole ring), 136.31, 134.49, 133.96, 133.23, 130.80, 130.52, 129.71, 129.43, 128.30, 127.21, 126.17, 122.54, 113.42 (aromatic ring C), 60.54 (-O-**C**H$_2$), 36.52 (-S-CH_2-), 25.74 (Ar-**C**H-$(CH_3)_2$), 23.32 (Ar-CH-(**C**H$_3$)**$_2$**), 21.43 (Ar-**C**H$_3$).

*2-hydroxy-5-(2-((5-(2-isopropyl-5-methylphenoxy)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetyl)benzamide (**4e**)*:

Analytical calculated for $C_{28}H_{28}N_4O_4S$ (%): C, 65.10; H, 5.46; N, 10.85; S, 6.21; Found (%): C, 65.43; H, 5.58; N, 10.53; S, 6.43; light yellow powder; m.p. 119 - 120ºC; yield 61%; IR(KBr) υ_{max} cm^{-1}: 3354 (N-H amide), 3074 (C-H arom), 1675 (C=O ketone), 1671 (C=O amide), 1632 (-N=CH-), 1383 (O-H), 1253 (C-O-C); MS (EI, 70 eV): m/z = 517.4 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 12.15 (s, 1H, -OH), 8.23 - 8.21 (d, 1H, ArH), 8.18 (d, 1H, ArH), 8.15 (s, 2H, -CO-NH_2), 7.87 (t, 2H, ArH), 7.60 (t, 1H, ArH), 7.54 (d, 2H, ArH), 7.51 (d, 1H, ArH), 7.07 (d, 1H, ArH), 6.82 (s, 1H, ArH), 6.78 (d, 1H, ArH), 5.00 (s, 2H, Ar-O-CH_2), 4.39 (s, 2H, -S-CH_2), 2.81 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.26 (s, 3H, Ar-CH_3), 1.00 - 0.97 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 192.40 (C=O), 167.62 (aromatic ring C), 167.45 (C=O amide), 155.52 (aromatic ring C), 154.49 (C_3-triazole ring), 152.18 (C_2-triazole ring), 136.39, 134.31, 133.99, 131.85, 130.74, 129.68, 129.24, 128.22, 127.95, 126.17, 122.54, 116.98, 115.21, 113.42 (aromatic ring C), 60.87 (-O-**C**H$_2$), 36.78 (-S-CH_2-), 25.61 (Ar-**C**H-$(CH_3)_2$), 23.27 (Ar-CH-(**C**H$_3$)**$_2$**), 21.24 (Ar-**C**H$_3$).

*2-((4-allyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazol-3-yl)thio)acetamide (**4f**)*:

Analytical calculated for $C_{18}H_{24}N_4O_2S$ (%): C, 59.87; H, 6.71; N, 15.54; S, 8.90; Found (%): C, 60.18; H, 6.59; N, 15.33; S, 9.08; white powder; m.p. 162 - 163ºC; yield 89%; IR(KBr) υ_{max} cm^{-1}: 3301 (N-H amide), 3154 (C-H arom), 3052 (=C-H), 1677 (C=O amide), 1612 (-N=CH-), 1254 (C-O-C); MS (EI, 70 eV): m/z = 361.2 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 7.31 (s, 2H, -CO-NH_2), 7.09 (d, 1H, ArH), 6.96 (s, 1H, ArH), 6.77 (d, 1H, ArH), 5.90 (m, 1H, C**H**=CH_2), 5.17 (s, 2H, Ar-O-CH_2), 4.94 - 4.93 and 4.91 (d, 1H each, =CH_2), 4.76 (d, 2H, -CH_2), 3.88 (s, 2H, -S-CH_2), 3.12 (m, 1H, Ar-C**H**-$(CH_3)_2$), 2.29 (s, 3H, Ar-CH_3), 1.09 - 1.08 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 169.05 (C=O), 154.22 (aromatic ring C), 152.35 (C_2-triazole

ring), 151.22 (C_3-triazole ring), 136.29, 132.77 (aromatic ring C), 131.19 (-CH=), 126.23, 122.49 (aromatic ring C), 118.17 (=CH_2), 113.35 (aromatic ring C), 60.46 (-O-**C**H_2), 46.39 (-CH_2-), 41.05 (-S-CH_2-), 25.97 (Ar-**C**H-(CH_3)$_2$), 23.41 (Ar-CH-(**C**H_3)$_2$), 21.40 (Ar-**C**H_3).

*1-((4-allyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazol-3-yl)thio)propan-2-one (**4g**)*:

Analytical calculated for $C_{19}H_{25}N_3O_2S$ (%): C, 63.48; H, 7.01; N, 11.69; S, 8.92; Found (%): C, 63.26; H, 7.15; N, 11.46; S, 9,11; light brown powder; m.p. 57 - 58ºC; yield 61%; %; IR(KBr) υ_{max} cm^{-1}: 3049 (C-H arom), 3029 (=C-H), 1705 (C=O), 1613 (-N=CH-), 1256 (C-O-C); MS (EI, 70 eV): m/z = 360.2 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 7.03 (d, 1H, ArH), 6.95 (s, 1H, ArH), 6.74 (d,1H, ArH), 5.95 (m, 1H, C**H**=CH_2), 5.16 (s, 2H, Ar-O-CH_2), 4.93 - 4.90 (d, 1H each, =CH_2), 4.74 (d, 2H, -CH_2), 3.90 (s, 2H, -S-CH_2), 3.17 (m, 1H, Ar-C**H**-(CH_3)$_2$), 2.35 (s, 3H, CO-CH_3), 2.30 (s, 3H, Ar-CH_3), 1.08 - 1.07 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 199.05 (C=O), 154.42 (aromatic ring C), 152.45 (C_2-triazole ring), 151.19 (C_3-triazole ring), 136.21, 132.79 (aromatic ring C), 131.10 (-CH=), 126.34, 122.51 (aromatic ring C), 118.13 (=CH_2), 113.09 (aromatic ring C), 60.51 (-O-**C**H_2), 46.51 (-CH_2-), 41.12 (-S-CH_2-), 25.88 (Ar-**C**H-(CH_3)$_2$), 23.11 (Ar-CH-(**C**H_3)$_2$), 21.29 (Ar-**C**H_3);

*2-((4-allyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazol-3-yl)thio)-1-phenylethanone (**4h**)*:

Analytical calculated for $C_{24}H_{27}N_3O_2S$ (%): C, 68.38; H, 6.46; N, 9.97; S, 7.61; Found (%): C, 68.15; H, 6.71; N, 10.14; S, 7.38; white powder; m.p. 134 - 135ºC; yield 32%; IR(KBr) υ_{max} cm^{-1}: 3053 (C-H arom), 3027 (=C-H), 1687 (C=O), 1611 (-N=CH-), 1252 (C-O-C); MS (EI, 70 eV): m/z = 422.4 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 7.97 (d, 2H, ArH), 7.94 (d, 2H, ArH), 7.78 (t, 1H, ArH), 7.05 (d, 1H, ArH), 6.95 (s, 1H, ArH), 6.80 (d, 1H, ArH), 5.92 (m, 1H, C**H**=CH_2), 5.11 (s, 2H, Ar-O-CH_2), 4.95 - 4.93 (d, 1H each, =CH_2), 4.80 (d, 2H, -CH_2-), 3.92 (s, 2H, -S-CH_2), 3.13 (m, 1H, Ar-C**H**-(CH_3)$_2$), 2.28 (s, 3H, Ar-CH_3), 1.09 - 1.08 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 190.13 (C=O), 154.42 (aromatic ring C), 152.65 (C_2-triazole ring), 151.58 (C_3-triazole ring), 136.21, 132.89 (aromatic ring C), 131.27 (-CH=), 131.10, 129.38, 129.06, 127.53, 126.34, 122.51 (aromatic ring C), 118.29 (=CH_2), 113.09 (aromatic ring C), 60.39 (-O-**C**H_2), 46.33 (-CH_2-), 41.10 (-S-CH_2-), 25.95 (Ar-**C**H-(CH_3)$_2$), 23.42 (Ar-CH-(**C**H_3)$_2$), 21.19 (Ar-**C**H_3).

*2-((4-allyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazol-3-yl)thio)-1-(4-chlorophenyl)ethanone (**4i**)*:

Analytical calculated for $C_{24}H_{26}ClN_3O_2S$ (%): C, 63.21; H, 5.75; N, 9.21; S, 7.03; Found (%): C, 63.40; H, 5.49; N, 9.44; S, 6.89; light yellow powder; m.p. 144 - 145ºC; yield 39%; %; IR(KBr) υ_{max} cm^{-1}: 3085 (C-H arom), 3057 (=C-H), 1678 (C=O), 1612 (-N=CH-), 1253 (C-O-C), 728 (C-Cl); MS (EI, 70 eV): m/z = 456.5 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 8.01 (d, 2H, ArH), 7.99 (d, 2H, ArH), 7.06 (d, 1H, ArH), 6.99 (s, 1H, ArH), 6.78 (d, 1H, ArH), 5.96 (m, 1H, C**H**=CH_2), 5.13 (s, 2H, Ar-O-CH_2), 4.91 - 4.89 (d, 1H each, =CH_2), 4.81 (d, 2H, -CH_2-), 3.97 (s, 2H, -S-CH_2), 3.15 (m, 1H, Ar-C**H**-(CH_3)$_2$), 2.31 (s, 3H, Ar-CH_3), 1.10 - 1.09 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 190.13 (C=O), 154.39 (aromatic ring C), 152.59 (C_2-triazole ring), 151.44 (C_3-triazole ring), 136.29, 133.44, 132.87 (aromatic ring C), 131.30 (-CH=), 131.24, 129.51, 127.33, 126.14, 122.37 (aromatic ring C), 118.25 (=CH_2), 113.41 (aromatic ring C), 60.43 (-O-**C**H_2), 46.26 (-CH_2-), 41.17 (-S-CH_2-), 25.78 (Ar-**C**H-(CH_3)$_2$), 23.33 (Ar-CH-(**C**H_3)$_2$), 21.29 (Ar-**C**H_3).

*5-(2-((4-allyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazol-3-yl)thio)acetyl)-2-hydroxybenzamide (**4j**)*:

Analytical calculated for $C_{25}H_{28}N_4O_4S$ (%): C, 62.48; H, 5.87; N, 11.66; S, 6.67; Found (%): C, 62.71; H, 5.65; N, 11.45; S, 6.80; light yellow powder; m.p. 147 - 148ºC; yield 61%; IR(KBr) υ_{max} cm^{-1}: 3350 (N-H amide), 3098 (C-H arom), 3077 (=C–H), 1683 (C=O ketone), 1668 (C=O amide), 1612 (-N=CH-), 1253 (C-O-C); MS (EI, 70 eV): m/z = 481.4 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 12.02 (s, 1H, -OH), 8.20 (s, 2H, -CO-NH_2), 8.11 (s, 1H, ArH), 8.04 (d, 1H, ArH), 7.88 (s, 1H, ArH), 7.09 (d, 1H, ArH), 6.91 (s, 1H, ArH), 6.81 (d, 1H, ArH), 6.01 (m, 1H, C**H**=CH_2), 5.23 (s, 2H, Ar-O-CH_2), 5.02 - 4.98 (d, 1H each, =CH_2), 4.85 (d, 2H, -CH_2-), 3.99 (s, 2H, -S-CH_2), 3.20 (m, 1H, Ar-C**H**-(CH_3)$_2$), 2.32 (s, 3H, Ar-CH_3), 1.11 - 1.10 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 190.57 (C=O), 167.43 (aromatic ring C), 167.39 (C=O amide), 154.35 (aromatic ring C), 152.69 (C_2-triazole ring), 151.52 (C_3-triazole ring), 136.30, 134.12, 132.91 (aromatic ring C), 131.33 (-CH=), 128.95, 127.90, 126.19, 122.40 (aromatic ring C), 118.31 (=CH_2), 117.11, 115.05, 113.38 (aromatic ring C), 60.50 (-O-**C**H_2), 46.30 (-CH_2-), 41.55 (-S-CH_2-), 26.01 (Ar-**C**H-(CH_3)$_2$), 23.58 (Ar-CH-(**C**H_3)$_2$), 21.36 (Ar-**C**H_3).

*2-((4-ethyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazol-3-yl)thio)acetamide (**4k**)*:

Analytical calculated for $C_{17}H_{24}N_4O_2S$ (%): C, 58.59; H, 6.94; N, 16.08; S, 9.20; Found (%): C, 58.81; H, 6.82; N, 16.24; S, 9.07; white powder; m.p. 177 - 178ºC; yield 91%; IR(KBr) υ_{max} cm^{-1}: 3283 (N-H amide), 3050 (C-H arom), 1680 (C=O amide), 1613 (-N=CH-), 1254 (C-O-C); MS (EI, 70 eV): m/z = 349.3 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 7.73 (s, 2H, CO-NH_2), 7.13 (d, 1H, ArH), 6.99 (s, 1H, ArH), 6.82 (d, 1H, ArH), 5.22 (s, 2H, Ar-O-CH_2), 4.09 (m, 2H, -C**H$_2$**-), 4.03 (s, 2H, -S-CH_2), 3.18 (m, 1H, Ar-C**H**-(CH_3)$_2$), 2.31 (s, 3H, Ar-CH_3), 1.29 (t, 3H, -CH_2-C**H$_3$**), 1.10 - 1.09 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 169.15 (C=O), 155.04 (aromatic ring C), 148.82 (C_2-triazole ring), 147.03 (C_3-triazole ring), 136.48, 133.90, 126.40, 122.61,

113.45 (aromatic ring C), 60.54 (-O-**C**H_2), 39.17 (-CH_2-), 37.21 (-S-CH_2-), 26.08 (Ar-**C**H-(CH_3)$_2$), 23.31 (Ar-CH-(**C**H_3)$_2$), 21.41 (Ar-**C**H_3), 13.92 (-CH_3**)**.

*1-((4-ethyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazol-3-yl)thio)propan-2-one (**4l**)*:

Analytical calculated for $C_{18}H_{25}N_3O_2S$ (%): C, 62.22; H, 7.25; N, 12.09; S, 9.23; Found (%): C, 62.43; H, 7.37; N, 12.33; S, 9.11; white powder; m.p. 73 - 74ºC; yield 67%; IR(KBr) υ_{max} cm^{-1}: 3034 (C-H arom), 1710 (C=O), 1611 (-N=CH-), 1254 (C-O-C); MS (EI, 70 eV): m/z = 348.2 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 7.14 (d, 1H, ArH), 6.98 (s, 1H, ArH), 6.83 (d, 1H, ArH), 5.25 (s, 2H, Ar-O-CH_2), 4.10 (m, 2H, -C**H$_2$**-), 4.05 (s, 2H, -S-CH_2), 3.19 (m, 1H, Ar-C**H**-(CH_3)$_2$), 2.37 (s, 3H, CO-CH_3), 2.34 (s, 3H, Ar-CH_3), 1.32 (t, 3H, -CH_2-C**H$_3$**), 1.09 - 1.08 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 199.18 (C=O), 155.11 (aromatic ring C), 148.53 (C_2-triazole ring), 147.99 (C_3-triazole ring), 136.51, 133.88, 126.32, 122.59, 113.33 (aromatic ring C), 60.58 (-O-**C**H_2), 39.21 (-CH_2-), 37.25 (-S-CH_2-), 26.01 (Ar-**C**H-(CH_3)$_2$), 23.43 (Ar-CH-(**C**H_3)$_2$), 21.39 (Ar-**C**H_3), 13.88 (-CH_3**)**.

*2-((4-ethyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazol-3-yl)thio)-1-phenylethanone: (**4m**)*:

Analytical calculated for $C_{23}H_{27}N_3O_2S$ (%): C, 67.45; H, 6.65; N, 10.26; S, 7.83; Found (%): C, 67.76; H, 6.49; N, 10.31; S, 7.75; light yellow powder; m.p. 105 - 106ºC; yield 40%; IR(KBr) υ_{max} cm^{-1}: 3045 (C-H arom), 1649 (C=O), 1614 (-N=CH-), 1238 (C-O-C); MS (EI, 70 eV): m/z = 410.2 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 8.02 (d, 2H, ArH), 7.72 - 7.69 (t, 1H, ArH), 7.60 (d, 2H, ArH), 7.17 (d, 1H, ArH), 7.01 (s, 1H, ArH), 6.92 (d, 1H, ArH), 5.24 (s, 2H, Ar-O-CH_2), 4.12 (m, 2H, -C**H$_2$**-), 4.08 (s, 2H, -S-CH_2), 3.20 (m, 1H, Ar-C**H**-(CH_3)$_2$), 2.36 (s, 3H, Ar-CH_3), 1.35 (t, 3H, -CH_2-C**H$_3$**), 1.09 - 1.08 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 192.38 (C=O), 154.93 (aromatic ring C), 148.72 (C_2-triazole ring), 148.21 (C_3-triazole ring), 136.52, 133.79, 130.94, 129.57, 129.14, 127.15, 126.29, 122.51, 113.46 (aromatic ring C), 60.72 (-O-**C**H_2), 39.16 (-CH_2-), 37.17 (-S-CH_2-), 26.03 (Ar-**C**H-(CH_3)$_2$), 23.27 (Ar-CH-(**C**H_3)$_2$), 21.44 (Ar-**C**H_3), 14.03 (-CH_3**)**.

*1-(4-chlorophenyl)-2-((4-ethyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazol-3-yl)thio)ethanone (**4n**)*:

Analytical calculated for $C_{23}H_{26}ClN_3O_2S$ (%): C, 62.22; H, 5.90; N, 9.46; S, 7.22; Found (%): C, 62.46; H, 5.69; N, 9.28; S, 7.34; yellow powder; m.p. 142 - 143ºC; yield 39%; IR(KBr) υ_{max} cm^{-1}: 3055 (C-H arom), 1659 (C=O), 1614 (-N=CH-), 1238 (C-O-C), 708 (C-Cl); MS (EI, 70 eV): m/z = 444.1 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 8.10 (d, 2H, ArH), 7.63 (d, 2H, ArH), 7.16 (d, 1H, ArH), 6.98 (s, 1H, ArH), 6.86 (d,1H, ArH), 5.27 (s, 2H, Ar-O-CH_2), 4.12 (m, 2H, -C**H$_2$**-), 4.09 (s, 2H, -S-CH_2), 3.23 (m, 1H, Ar-C**H**-(CH_3)$_2$), 2.39 (s, 3H, Ar-CH_3), 1.38 (t, 3H, -CH_2-C**H$_3$**), 1.10 - 1.09 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 192.25 (C=O), 154.96 (aromatic ring C), 148.91 (C_2-triazole ring), 147.18 (C_3-triazole ring), 136.55, 134.12, 133.91, 131.02, 129.63, 127.18, 126.35, 122.54, 113.49 (aromatic ring C), 60.69 (-O-**C**H_2), 39.20 (-CH_2-), 37.30 (-S-CH_2-), 26.05 (Ar-**C**H-(CH_3)$_2$), 23.29 (Ar-CH-(**C**H_3)$_2$), 21.46 (Ar-**C**H_3), 14.10 (-CH_3**)**.

*5-(2-((4-ethyl-5-(2-isopropyl-5-methylphenoxy)-4H-1,2,4-triazol-3-yl)thio)acetyl)-2-hydroxybenzamide (**4o**)*:

Analytical calculated for $C_{24}H_{28}N_4O_4S$ (%): C, 61.52; H, 6.02; N, 11.96; S, 6.84; Found (%): C, 61.75; H, 5.88; N, 12.29; S, 6.62; white powder; m.p. 133 - 134ºC; yield 84%; IR(KBr) υ_{max} cm^{-1}: 3355 (N-H amide), 3110 (C-H arom), 1684 (C=O ketone), 1660 (C=O amide), 1629 (-N=CH-), 1260 (C-O-C); MS (EI, 70 eV): m/z = 469.5 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ/ppm): 12.19 (s, 1H, -OH), 8.22 (d, 1H, ArH), 8.19 (s, 2H, -CO-NH_2), 8.15 (s, 1H, ArH), 7.72 (d, 1H, ArH), 7.19 (d, 1H, ArH), 7.02 (s, 1H, ArH), 6.89 (d, 1H, ArH), 5.30 (s, 2H, Ar-O-CH_2), 4.12 (s, 2H, -S-CH_2), 4.06 (m, 2H, -C**H$_2$**-), 3.29 (m, 1H, Ar-C**H**-(CH_3)$_2$), 2.38 (s, 3H, Ar-CH_3), 1.37 (t, 3H, -CH_2-C**H$_3$**), 1.09 - 1.08 (d, 6H, Ar-CH-(C**H$_3$**)$_2$); ^{13}C-NMR (125 MHz, DMSO-d_6, δ/ppm): 192.68 (C=O), 167.73 (aromatic ring C), 167.59 (C=O amide), 154.91 (aromatic ring C), 148.56 (C_2-triazole ring), 148.11 (C_3-triazole ring), 136.59, 133.74, 132.04, 130.94, 129.57, 129.14, 128.99, 127.30, 127.15, 126.39, 122.52, 118.83, 114.97, 113.50 (aromatic ring C), 60.74 (-O-**C**H_2), 39.07 (-CH_2-), 37.32 (-S-CH_2-), 26.10 (Ar-**C**H-(CH_3)$_2$), 23.28 (Ar-CH-(**C**H_3)$_2$), 21.50 (Ar-**C**H_3), 14.05 (-CH_3**)**.

Data obtained from the quantitative elemental analysis of the synthesized compounds was within ±0.4% of the theoretical values. The structures of the intermediates *N*-substituted-acyl-thiosemicarbazides **2a-c** were confirmed by their IR spectra through the presence of the absorption peaks characteristic for N-H (3316 - 3268 cm^{-1}), C=O (1681 - 1659 cm^{-1}) and C=S (1274 - 1266 cm^{-1}) stretching vibrations and by NMR spectroscopy. The ^{1}H-NMR spectra of compounds **2a-c** displayed specific signals for NH protons as singlet at 7.14 - 7.90 ppm, and for the protons from aryl-alkyl-ether linkage as singlet at 4.98 - 5.23 ppm. The ^{13}C-NMR spectra of these compounds showed specific signals for the C=S and C=O carbons at 180.09 - 182.75 ppm and 165.34 - 167.45 ppm, respectively. Cyclization reactions to the corresponding 3-mercapto-1,2,4-triazole compounds **3a-c** were confirmed by the IR spectra through the disappearance of the absorption band characteristic for the C=O stretching. There are two possible tautomeric isoforms corresponding for the 1,2,4-triazole-3-yl-mercapto compounds, due to the mobility of the H atom, which can be attached either to the nitrogen (the thione form) or to the sulphur atom (the thiol form) [24]. The

absence of the absorption band characteristic for the N-H stretching vibration in the IR spectra of the 1,2,4-triazole compounds, as well as the presence of the absorption band corresponding to the S-H stretching vibration, indicates that they exist predominantly as thiol isoform, in solid state. This fact was also confirmed by the ^{1}H-NMR spectra of the above-mentioned compounds, which displayed characteristic signal for the deshielded SH proton as singlet at 13.84 - 14.05 ppm, and by the ^{13}C-NMR spectra, through the absence of the C=S characteristic signal. ^{1}H-NMR spectra of the target 1,2,4-triazole-3-yl-mercapto derivatives confirmed their structures, based on the disappearance of the signal characteristic for the SH proton and the presence of the additional signals corresponding to the α-halocarbonyl moiety.

Molecular docking study

The target 1,2,4-triazole-3yl-mercapto derivatives and fluconazole, as control inhibitor, were docked into the catalytic site of both fungal and human lanosterol 14α-demethylase. The predicted binding affinity for each compound, in terms of binding energy (ΔG), as well as the calculated inhibition constants (K_i) for the best docking poses are presented in Table I.

Table I

Predicted binding energies (ΔG) and inhibition constants (K_i) of the tested compounds towards the fungal and human lanosterol 14α-demethylase

Compound	ΔG (kcal/mol)		K_i (nM)	
	5EQBCA	3JUS	5EQBCA	3JUS
3a	-11.05	-9.32	7.95	147.35
3b	-9.28	-8.84	157.64	331.27
3c	-9.04	-8.26	236.37	881.73
4a	-11.42	-10.66	4.26	15.35
4b	-12.17	-10.17	1.20	35.10
4c	-14.02	-11.20	0.05	6.17
4d	-14.20	-11.56	0.04	3.36
4e	-13.58	-12.34	0.11	0.90
4f	-9.90	-9.19	55.36	183.50
4g	-10.39	-9.12	24.21	206.51
4h	-11.36	-10.48	4.71	20.80
4i	-12.66	-10.54	0.52	18.80
4j	-12.00	-10.90	1.60	10.24
4k	-10.22	-9.08	32.26	220.94
4l	-9.73	-8.89	73.76	304.47
4m	-11.81	-10.44	2.20	22.25
4n	-11.73	-10.18	2.52	34.51
4o	-12.80	-11.85	0.41	2.06
fluconazole	-6.92	-6.74	8463.86	11468.60

The results of the molecular docking run demonstrated a good binding affinity towards *Candida albicans* lanosterol 14α-demethylase for all the tested compounds. The synthesized 3-*S*-substituted-1,2,4-triazole derivatives possess binding energy values ranging from -8.13 to -14.20 kcal/mol, significantly lower than that of fluconazole (-6.92 kcal/mol). The calculated K_i values showed that the tested compounds are weaker binders of the human CYP51 than of the fungal homologous, so they might be associated with a reduced mammalian toxicity.

All the synthesized compounds interact with the target enzyme through the formation of two hydrogen bonds between the phenol moiety of Tyr118 residues and the nitrogen atoms N1 and N2 of the 1,2,4-triazole heterocycle. Moreover, the ketone group of the 3-mercapto-substituent forms a polar bond with the His377-Ser378 peptide bridge. Figure 2 shows the representation of the binding patterns into the catalytic site of fungal CYP51 for the compound **4d** (carbon atoms in pink), which showed the highest inhibitory activity against 5EQBCA. The non-interacting amino acid residues and tertiary structures in the foreground were removed for clarity.

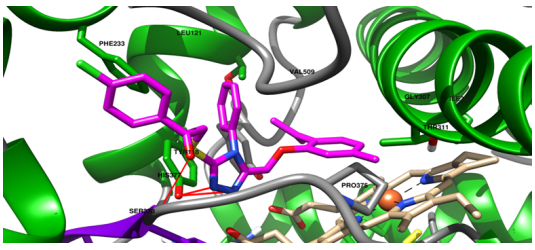


Figure 2.

The top binding conformation of compound **4d** (carbon atoms in pink) to the active site of the fungal lanosterol 14α-demethylase. Enzyme loops are depicted in grey and the heme carbon atoms in fawn. Protein α helices are depicted in green and β sheets in purple

The docking simulation showed that all the synthesized compounds tend to interact with the amino acids from the access channel to the enzyme's catalytic site. The "T" shape of the compounds enables the insertion of the N4-substituent of the 1,2,4-triazole

heterocycle (phenyl, allyl or ethyl) through the amino acids from the access channel (Val509, Tyr132, Phe233). Since the accessibility to the catalytic site of CYP51 is mediated by hydrophobic interactions between the ligand and the hydrophobic amino acids residues, compounds having a large aromatic N4-substituent are better inhibitors than those having a smaller substituent. The above mentioned are supported by the smaller K_i values of compounds N4-phenyl-substituted (**4a-e**), compared with those N4-allyl-substituted (**4f-j)** or N4-ethyl-substituted (**4k-o**). The orientation of the phenyl substituents (represented in Figure 3 and Figure 4 for the compound **4d**) enable π-π type interactions with the aromatic rings of Tyr132 and Phe228. In addition, there might be π-cation interactions between the aromatic ring of thymol and the heme Fe^{2+}.

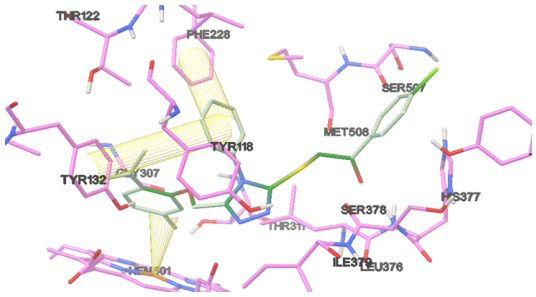


Figure 3.

Interaction of the compound **4d** with the active site of lanosterol 14α-demethylase; π-π interactions are depicted with yellow cylinders and π-cation interactions are depicted with yellow cone

The fungal CYP51 inhibition induced by the screened compounds was mediated mainly by non-covalent interactions with the amino acid residues from the access channel to the active site. As a result, mammalian toxicity raised from the cross-over inhibition of the human CYP isoforms, through the covalent-coordination of the heme Fe^{2+}, is avoided. Based on the above observations, the synthesized compounds might be exploited for the development of novel non-competitive CYP51 inhibitors, this mechanism of action being associated with a reduction in both mammalian toxicity and fungal resistance to antifungal therapy [5].

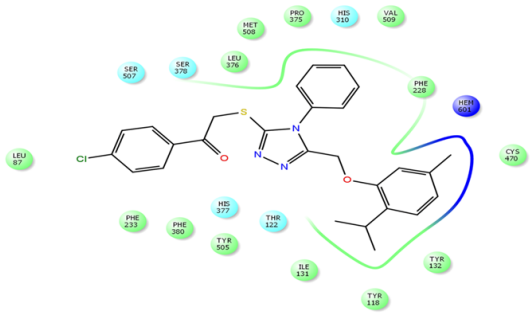


Figure 4.

A general view of the compound **4d** inserted in the catalytic site of the fungal lanosterol 14α-demethylase

Antifungal activity assay

The antifungal properties of the synthesized compounds were evaluated *in vitro*, against two *Candida albicans* strains (*C. albicans* ATCC 10231 and *C. albicans* ATCC 18804) and one non-albicans *Candida* strain (*C. krusei* ATCC 6258). The broth microdilution method was used for the determination of MIC and MFC values. Stock solutions (1 mg/mL) were prepared by dissolving the tested compounds and the reference antifungal, fluconazole, in sterile DMSO. The results obtained are presented in Table II.

Table II

Minimum Inhibitory Concentration (μg/mL) and Minimum Fungicidal Concentration (μg/mL) of the tested compounds

Compound	*C. albicans* (ATCC 10231)		*C. albicans* (ATCC 18804)		*C. krusei* (ATCC 6258)	
	MIC	MFC	MIC	MFC	MIC	MFC
3a	62.5	125	62.5	125	62.5	125
3b	**15.62**	**31.25**	**15.62**	**31.25**	31.25	62.5
3c	62.5	125	62.5	125	31.25	62.5
4a	62.5	125	62.5	125	62.5	125
4b	62.5	125	62.5	125	62.5	125
4c	62.5	125	62.5	125	62.5	125
4d	31.25	62.5	31.25	62.5	31.25	62.5
4e	62.5	125	62.5	125	31.25	62.5
4f	62.5	125	62.5	125	62.5	125
4g	31.25	62.5	31.25	62.5	62.5	125
4h	62.5	125	62.5	125	62.5	125
4i	62.5	125	62.5	125	31.25	62.5
4j	62.5	125	62.5	125	62.5	125
4k	62.5	125	62.5	125	62.5	125
4l	31.25	62.5	31.25	62.5	62.5	125
4m	31.25	62.5	31.25	62.5	62.5	125
4n	62.5	125	62.5	125	31.25	62.5
4o	62.5	125	62.5	125	62.5	125
fluconazole	**15.62**	**31.25**	**15.62**	**31.25**	**15.62**	**31.25**

MIC values registered for all the tested compounds were equal or inferior to that of the reference antifungal drug, fluconazole. The compound **3b,** having an allyl substituent attached to the N4 nitrogen of the 1,2,4-triazole nucleus, proved to be the most promising molecule, being as active as fluconazole against the *Candida albicans* ATCC 10231 and *Candida albicans* ATCC 18804 strains.

The presence of a halogenated aryl substituent attached to the 3-mercapto group proved to be favourable for the antifungal activity. The 1,2,4-triazole-3-yl-mercapto derivatives possessing a 4-Cl-phenyl moiety exhibited the stronger antifungal effect against *Candida krusei* ATCC 6258 strain, as compounds **4d**, **4i** and **4n** had smaller MIC values compared with the others 1,2,4-triazolyl-thioethers.

The presence of the 4-Cl-phenyl substituent also led to an improved activity against *Candida albicans* strains in the case of the 3-*S*-substituted-4-phenyl-1,2,4-triazole derivatives (**4a-e**), compound **4d** exhibiting the smallest MIC value in this series. Moreover, its high antifungal effect is supported by the results of the docking simulation, compound **4d** possessing the lowest binding energy value towards the target enzyme 5EQBCA.

The substitution of the 3-mercapto group with a propan-2-one moiety seems to improve the activity of the 1,2,4-triazole-3-yl-mercapto derivatives having an N4-allyl (**4f-j**) or ethyl (**4k-o**) substituent against *C. albicans* strains, as shown by the MIC values of the compounds **4g** and **4l**.

The determination of MFC confirmed the obtained MIC values. The MFC/MIC ratio values for all the tested compounds were equal to 2, this fact suggesting a fungicidal effect [8] of the newly synthesized 1,2,4-triazole derivatives.

Conclusions

A new series of 1,2,4-triazole-3-yl-mercapto derivatives were obtained in good yields, through a stepwise reaction protocol, and were investigated for their antifungal properties. The data obtained from the *in silico* simulation showed that the synthesized compounds are potent inhibitors of fungal lanosterol 14α-demethylase, their affinity for the human target enzyme being significantly weaker. CYP51 inhibitory activity was not mediated by the covalent-coordination of heme Fe^{2+}, so the synthesized compounds may have both a reduced mammalian toxicity and a lower risk that the fungal strains might develop resistance, compared to the azole class. According to the registered MIC and MFC values, the compound **3b** proved to be the most promising molecule, being as active as the reference antifungal drug against *Candida albicans* strains. The target compounds (**4a-o**) exhibited a moderate to good activity against both *Candida* strains, their antifungal properties being inferior to fluconazole. Substitution of the 3-mercapto group of the 1,2,4-triazole nucleus with a 4-Cl-phenyl moiety was associated with an improved antifungal activity, as supported by the MIC values of the compounds **4d**, **4i** and **4n**.

The results obtained encourage us to optimize further the synthesized compounds, in order to develop novel non-competitive fungal lanosterol 14α-demethylase inhibitors.

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