

THIAZOLYL-OXADIAZOLE DERIVATIVES TARGETING LANOSTEROL 14 α -DEMETHYLASE AS POTENTIAL ANTIFUNGAL AGENTS: DESIGN, SYNTHESIS AND MOLECULAR DOCKING STUDIES

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Abstract

A series of novel thiazolyl-oxadiazole derivatives were synthesized by oxidative cyclization or dehydrative cyclization. The compounds were characterised using quantitative elemental analysis, ¹H NMR, ¹³C NMR and mass spectrometry. Since lanosterol 14 α -demethylase is an attractive target in the design of antifungal agents, we used this enzyme in molecular docking trials with all synthesized compounds. By this means, we explored the interactions and identified structural elements that could increase their activity in order to develop new antifungal agents that specifically target this enzyme.

Rezumat

A fost sintetizată o nouă serie de derivați de tiazolil-1,3,4-oxadiazol prin ciclizare oxidativă sau ciclizare dehidrativă. Compușii obținuți au fost caracterizați cu ajutorul analizei elementale cantitative, ¹H RMN, ¹³C RMN și spectrometrie de masă. Toți compușii sintetizați au fost incluși într-un studiu de andocare moleculară, pe diferite modele tridimensionale ale lanosterol 14 α -demetilazei. Aceasta reprezintă o țintă atractivă în designul de compuși cu activitate antifungică. Prin intermediul acestui studiu am explorat interacțiunile și am identificat elementele structurale care ar putea influența activitatea antifungică, în scopul de a dezvolta noi compuși care să aibă ca țintă această enzimă.

Keywords: thiazolyl-oxadiazole, oxidative cyclization, dehydrative cyclization, *Candida albicans*, molecular docking

Introduction

Candida albicans is the most important opportunistic fungal pathogen. It usually resides as a commensal fungi, but it can cause infections when the host becomes debilitated or immuno-compromised [11, 24, 25]. Fungal infections represent an important complication and a major cause of mortality and morbidity in immuno-compromised patients suffering from infectious diseases, cancer and in organ transplant cases [12]. Over the past years azoles, such as fluconazole, miconazole and voriconazole were extensively used in the treatment of fungal infections [24]. These compounds act by inhibiting CYP51 in the process of ergosterol biosynthesis through a mechanism in which the heterocyclic nitrogen atom binds to the heme iron atom [23]. Although azoles have a pivotal role in the antifungal treatment, their increasing use led to an increase in drug resistance issues [17]. These health concerns boost the development of chemical compounds with high efficiency and broad activity spectrum.

As an important class of heterocyclic compounds, 1,3,4-oxadiazoles are associated with a broad biological activity spectrum including antibacterial, antifungal, analgesic, anti-inflammatory, antiviral and anticancer effects [5, 26, 27]. Moreover, the 1,3,4-oxadiazole heterocycle is a bioisostere of amides and esters, which enables it to participate in hydrogen bonding interactions with the targets, leading to an increase of the biological activity. On the other hand, the heterocyclic compounds bearing the thiazole moiety exhibit antitumor, antibacterial, antifungal, antitubercular and anti-inflammatory activities [1, 18, 20].

The association of this two structural motifs (thiazole and 1,3,4-oxadiazole) in the same unit could be an interesting approach to discover new drugs, with a possible synergistic effect.

In order to obtain compounds with intensified biological activity, we synthesized and characterized a new series of compounds that contain both heterocycles in one molecular framework. In this paper, we also included a molecular docking study, in order to estimate the interaction potential of the thiazolyl-1,3,4-oxadiazoles

with lanosterol 14 α -demethylase. The results obtained suggested that the molecules could be exploited for the development of antifungal agents.

Materials and Methods

Chemistry

Solvents and reagents used for synthesis and purification were purchased from Alfa Aesar (Karlsruhe, Germany). All chemicals were of analytical grade purity. The purity of the synthesized compounds was verified by thin layer chromatography that was carried out on precoated Silica Gel 60F254 sheets using heptane – ethyl-acetate 1:1 as developant and UV absorption for visualization. The melting points were measured using an Electrothermal melting point meter and are uncorrected. Elemental analyses were performed using a Vario El CHNS instrument. All synthesized compounds yielded spectral data consistent with the proposed structure and microanalysis within 0.4% of the theoretical values. LC-MS analyses were performed on an Agilent 1100 series and an Agilent Ion Trap SL, mass spectrometer. ¹H-NMR and ¹³C NMR were performed on a Bruker Avance NMR spectrometer operating at 500 MHz, in dimethyl sulfoxide (DMSO)-d₆ as solvent. Chemical shift values were reported relative to tetramethylsilane (TMS) as internal standard and were expressed in ppm. The synthesis of compounds **1** and **2** was previously reported [8, 21].

General procedure for the synthesis of diacylhydrazines (1a-h) [2]

1 mL phosphorous oxychloride was added to an equimolar (20 mmol) mixture of thiazolylhydrazide (**1**) and aromatic carboxylic acid in tetrahydrofuran (15 mL). This mixture was stirred at room temperature. After 1 - 2 h a precipitate appeared. This was filtered and washed with tetrahydrofuran.

General procedure for the synthesis of the N-acylhydrazones (2i-u) [7]

An equimolar (20 mmol) mixture of hydrazide (**1**, **2**) and aldehyde was refluxed in ethanol (10 mL) for 4 h in the presence of a catalytic amount of glacial acetic acid. The mixture was cooled and the solid was separated by filtration and recrystallized from ethanol.

The structures of the intermediate compounds (**1a-h**, **2i-u**) were confirmed by the results obtained from quantitative elemental analysis (CHNS). These were concordant with the calculated values. The purity of the compounds was confirmed by TLC.

General procedure for the synthesis of thiazolyl-oxadiazoles (3a-h) [4, 16]

10 mmols diacyl-hydrazine (**1a-h**) were refluxed in 3 mL phosphorous oxychloride, for 8 h. The reaction was monitored by TLC. After the completion of the reaction, the mixture was poured over crushed ice

and allowed to precipitate. The precipitate was filtered and recrystallized from ethanol.

General procedure for the synthesis of thiazolyl-oxadiazoles (4i-u) [6, 10]

10 mmols N-acylhydrazone (**2i-u**) were dissolved in 5 mL DMSO, followed by addition of potassium carbonate (3 mmols) and iodine (2 mmols). The reaction mixture was stirred at room temperature (r.t.) (2 - 4 h) until the reaction was complete (monitored by TLC). The solid was filtered, washed with water and recrystallized from ethanol.

Molecular docking study

The molecular docking study was carried out using AutoDock (AD) 4.2 [15] and AutoDock Vina (ADV) 1.1.2 [3].

The dataset of the 3D thiazolyl-oxadiazoles (**3a-h**, **4i-u**) was designed with HyperChem 6, using the geometry optimization function and the minimal energy conformation [13]. All input docking files of the compounds were prepared using AutoDock Tools 1.5.6 [15] (ADT). Ligand preparation included addition of Gasteiger charges and the removal of non-polar hydrogen atoms (all hydrogen atoms in our compounds). The maximum number of rotatable bonds was set to 6 for each ligand.

The study was performed on 3 subtypes of lanosterol 14 α -demethylase (or CYP51) from three fungal strains: 4UYL – *Aspergillus fumigatus* (resolution 2.8 Å), 5EQB – *Saccharomyces cerevisiae* (resolution 2.19 Å) and a model from *Candida albicans* built by homology modelling. Since for the moment, no crystallographic data of lanosterol 14 α -demethylase from *Candida albicans* is available, we obtained the FASTA format amino acid sequence of the enzyme from Universal Protein Resource (<http://www.uniprot.org/uniprot/P10613>). This was submitted to Protein Data Bank and Swiss Model [9, 14, 19, 22] as queries for the protein BLAST analysis. Based on the BLAST search results we have chosen 5EQB for homology modelling which provided a sequence identity of 65% with the P10613 sequence. Using Swiss-Model [9, 14, 19, 22] we built the three dimensional homology model of CYP51 of *Candida albicans* based on the template of the crystal structure of 5EQB, further called CACYP51. Crystal structures of macro-molecular targets with co-crystallized ligands were obtained from Protein Data Bank. All structures were obtained by X-ray diffraction. In order to check the molecular docking settings and the accuracy of the docking method, we performed an initial re-docking of the former co-crystallized ligands (itraconazole from the 5EQB complex and voriconazole from the 4UYL complex). Root mean square deviation (RMSD) values between the top ranked predicted conformation of every re-docked ligand and the observed X-ray position in the crystal structure was within the range 0.796 to 1.707 Å. Re-docking predictions of the co-

crystallized ligands were made with a very good accuracy. RMSD being less than 2 Å, the docking protocol was validated. The grid size (x, y, z) was set to 75x75x75 points with a 0.375 Å grid spacing for AD and 28x28x28 grid points for ADV. These were centred on the torsion root of the co-crystallized ligands. All water molecules were removed, polar hydrogen atoms were added, non-polar hydrogen atoms were merged, rotatable bonds were defined, carboxylic moieties were deprotonated and Gasteiger partial charges were assigned.

The best conformers were established by AD, based on the Lamarckian genetic algorithm. Amide bonds were set non-rotatable. For each compound AD searched for 100 conformers. The maximum number of energy evaluations was set to 9×10^4 with a rate of mutation of 0.02, a crossover rate of 0.8, a step size for translations of 2 Å and a cluster tolerance of 2 Å.

For ADV the global search exhaustiveness was set to 50 and the maximum number of conformers was set to 20. The difference allowed between the best and the worst binding affinity of the conformers was set to maximum 4 kcal/mol. The conformation, with the best ranked affinity for the protein, was chosen by the ADV empirical scoring function.

To evaluate the accuracy of the docking results, we tested as positive controls the co-crystallized ligands: itraconazole (IC), voriconazole (VC) and supplementary, fluconazole (FC), miconazole (MC) and ketoconazole (KC).

Docking studies were performed on a single machine with a CPU Intel Core 2 Duo E6550, 3 GB RAM, running Microsoft Windows XP SP2 as operating system. Visualization and analysis of the docking results was performed using ADT and PyMOL1.1. Amino acids in the foreground were removed for clarity.

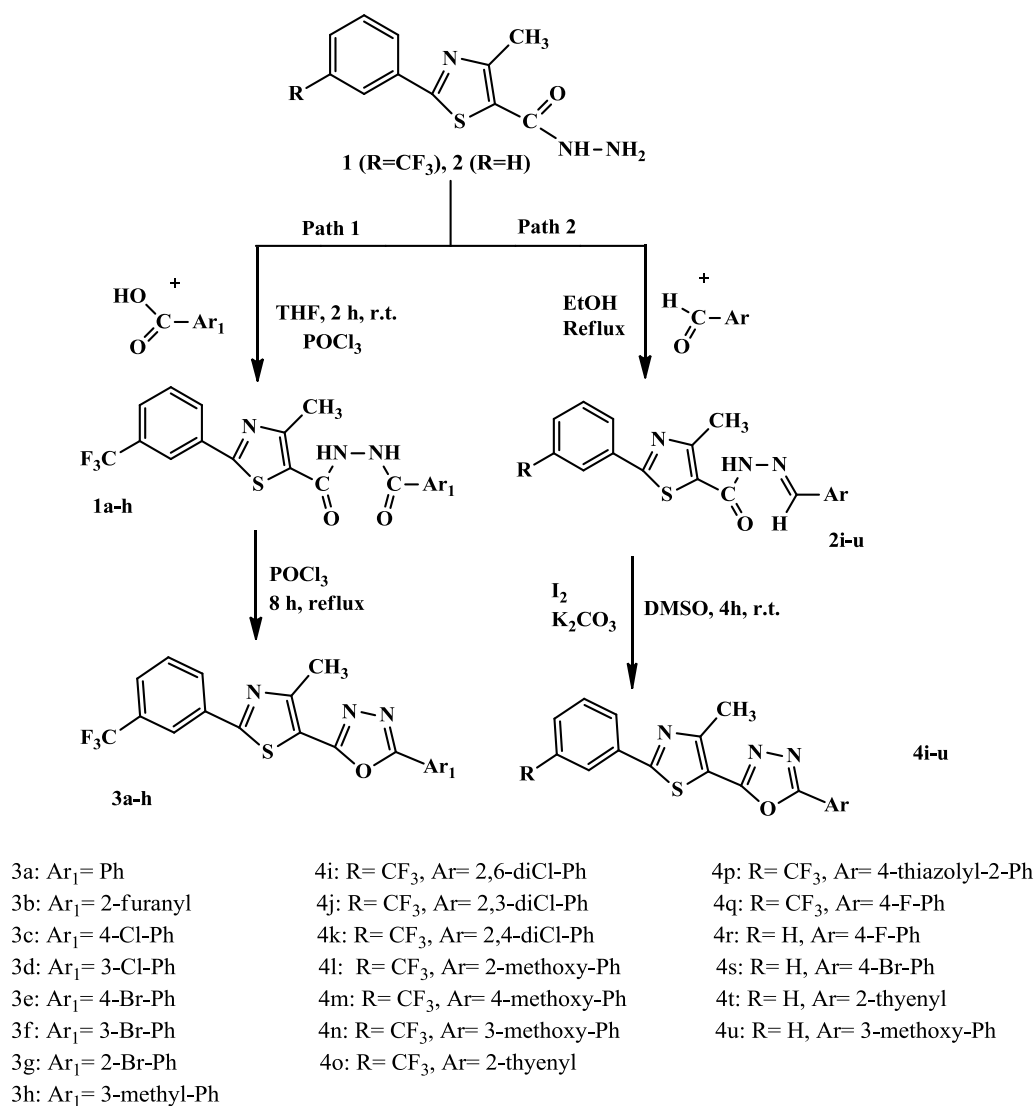


Figure 1.

The procedures for the synthesis of thiazolyl-1,3,4-oxadiazoles
(EtOH = Ethylic alcohol; POCl₃ = phosphoryl chloride; THF = tetrahydrofuran)

Results and Discussion

Chemistry

We used two methods for the synthesis of thiazolyl-1,3,4-oxadiazoles (Figure 1): a dehydrative cyclization method using phosphorous oxychloride (PCl_5) (Path 1) and an oxidative cyclization method using $\text{K}_2\text{CO}_3/\text{I}_2$ (Path 2). This allowed us to increase the structural variety of the synthesized compounds. Mild reaction conditions, operational simplicity, environmental friendly catalyst and higher yields make the oxidative cyclization method superior to the dehydrative cyclization method. The completion of each reaction was monitored by thin layer chromatography (TLC) and the structures were confirmed by quantitative elemental analysis and spectroscopic analysis: mass spectrometry, ^1H NMR and ^{13}C NMR.

The structures of the newly synthesized compounds (**3a-h**, **4i-u**) were correlated with the data obtained from quantitative elemental analysis, ^1H NMR, ^{13}C NMR and mass spectrometry, given below.

2-(2-(3-(Trifluoromethyl)phenyl)4-methylthiazol-5-yl)-5-phenyl-1,3,4-oxadiazole (**3a**)

$\text{C}_{19}\text{H}_{12}\text{F}_3\text{N}_3\text{OS}$ (387.07). Yellow powder. Yield 50%. M.p.: 210°C. ^1H NMR δ , 8.35 (d, 1H, Phe- CF_3), 8.33 (s, 1H, Phe- CF_3), 8.1 (dd, 2H, Phe), 7.96 (d, 1H, Phe- CF_3), 7.82 (t, 1H, Phe- CF_3), 7.67 (m, 3H, Phe), 2.88 (s, 3H, CH_3 thiazole). ^{13}C NMR δ , 166.20 (C), 155.96 (C), 151.36 (C), 149.70 (C), 135.24 (C), 135.0 (C), 132.25 (CH), 130.50 (C), 129.85 (CH), 129.42 (CH), 129.18 (2CH), 127.17 (CH), 127.04 (2CH), 124.461 (CH), 121.97 (CF₃), 113.60 (C), 16.14 (CH₃). MS: m/z 388.5 ($\text{M}+\text{H}^+$)

2-(Furan-2-yl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (**3b**)

$\text{C}_{17}\text{H}_{10}\text{F}_3\text{N}_3\text{O}_2\text{S}$ (377.04). White powder. Yield 50%. M.p.: 198.7°C. ^1H NMR δ , 8.35 (d, 1H, Phe- CF_3), 8.32 (s, 1H, Phe- CF_3), 8.12 (d, 1H, furan), 7.95 (d, 1H, Phe- CF_3), 7.82 (t, 1H, Phe- CF_3), 7.47 (d, 1H, furan), 6.85 (m, 1H, furan), 2.85 (s, 3H, CH_3 thiazole). ^{13}C NMR δ , 166.23 (C), 155.96 (C), 152.45 (C), 149.70 (C), 147.56 (C), 145.49 (CH), 135.31 (C), 135.05 (C), 132.28 (CH), 129.46 (CH), 127.08 (CH), 124.31 (CH), 121.99 (CF₃), 112.32 (C), 116 (CH), 110.68 (CH), 16.13 (CH₃). MS: m/z 378.3 ($\text{M}+\text{H}^+$)

2-(4-Chlorophenyl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (**3c**)

$\text{C}_{19}\text{H}_{11}\text{ClF}_3\text{N}_3\text{OS}$ (421.03). Yellow powder. Yield 40%. M.p.: 236.5°C. ^1H NMR δ , 8.35 (d, 1H, Phe- CF_3), 8.33 (s, 1H, Phe- CF_3), 8.18 (d, 1H, Phe-Cl), 8.16 (d, 1H, Phe-Cl), 7.96 (d, 1H, Phe- CF_3), 7.82 (t, 1H, Phe- CF_3), 7.74 (d, 1H, Phe-Cl), 7.73 (d, 1H, Phe-Cl), 2.88 (s, 3H, CH_3 thiazole). ^{13}C NMR δ , 167.23 (C), 154.91 (C), 151.47 (C), 149.84 (C), 137.5 (C), 135.25 (C), 135.05 (C), 132.35 (CH), 129.85 (C), 129.53 (2CH), 128.36 (CH), 127.40 (2CH), 127.07 (CH), 122.71 (CH), 122.18 (CF₃), 113.37 (C), 16.12 (CH₃). MS: m/z 422.6 ($\text{M}+\text{H}^+$)

2-(3-Chlorophenyl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (**3d**)

$\text{C}_{19}\text{H}_{11}\text{ClF}_3\text{N}_3\text{OS}$ (421.03). White powder. Yield 45%. M.p.: 229°C. ^1H NMR δ , 8.35 (d, 1H, Phe- CF_3), 8.33 (s, 1H, Phe- CF_3), 8.21 (s, 1H, Phe-Cl), 8.13 (d, 1H, Phe-Cl), 7.96 (d, 1H, Phe- CF_3), 7.90 (d, 1H, Phe-Cl), 7.82 (t, 1H, Phe- CF_3), 7.65 (t, 1H, Phe-Cl), 2.87 (s, 3H, CH_3 thiazole). ^{13}C NMR δ , 166.63 (C), 153.95 (C), 152.37 (C), 150.74 (C), 136.2 (C), 135.75 (C), 135.15 (C), 131.22 (CH), 129.97 (C), 129.78 (CH), 129.51 (CH), 128.41 (CH), 127.35 (CH), 126.22 (CH), 125.09 (CH), 124.41 (CH), 121.08 (CF₃), 113.22 (C), 16.11 (CH₃). MS: m/z 422.2 ($\text{M}+\text{H}^+$)

2-(4-Bromophenyl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (**3e**)

$\text{C}_{19}\text{H}_{11}\text{BrF}_3\text{N}_3\text{OS}$ (464.98). White powder. Yield 30%. M.p.: 230°C. ^1H NMR δ , 8.36 (d, 1H, Phe- CF_3), 8.33 (s, 1H, Phe- CF_3), 8.17 (d, 1H, Phe-Br), 8.15 (d, 1H, Phe-Br), 7.95 (d, 1H, Phe- CF_3), 7.83 (t, 1H, Phe- CF_3), 7.73 (d, 1H, Phe-Br), 7.72 (d, 1H, Phe-Br), 2.88 (s, 3H, CH_3 thiazole). ^{13}C NMR δ , 166.63 (C), 154.96 (C), 153.37 (C), 149.84 (C), 135.85 (C), 136.5 (C), 134.26 (C), 132.42 (CH), 131.99 (2CH), 129.97 (C), 129.68 (CH), 128.09 (CH), 124.85 (2CH), 123.31 (CH), 121.67 (CF₃), 113.56 (C), 16.10 (CH₃). MS: m/z 466.1 ($\text{M}+\text{H}^+$)

2-(3-Bromophenyl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (**3f**)

$\text{C}_{19}\text{H}_{11}\text{BrF}_3\text{N}_3\text{OS}$ (464.98). Yellow powder. Yield 55%. M.p.: 231.5°C. ^1H NMR δ , 8.35 (d, 1H, Phe- CF_3), 8.33 (s, 1H, Phe- CF_3), 8.20 (s, 1H, Phe-Br), 8.10 (d, 1H, Phe-Br), 7.95 (d, 1H, Phe- CF_3), 7.9 (d, 1H, Phe-Br), 7.82 (t, 1H, Phe- CF_3), 7.6 (t, 1H, Phe-Br), 2.88 (t, 3H, CH_3 thiazole). ^{13}C NMR δ , 169.93 (C), 155.37 (C), 151.96 (C), 149.77 (C), 135.26 (C), 134.99 (C), 132.29 (CH), 131.92 (CH), 131.27 (CH), 129.88 (CH), 129.77 (C), 128.49 (CH), 127.19 (CH), 125.48 (CH), 124.31 (CH), 124.2 (C), 121.90 (CF₃), 114.32 (C), 16.14 (CH₃). MS: m/z 466.5 ($\text{M}+\text{H}^+$)

2-(2-Bromophenyl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (**3g**)

$\text{C}_{19}\text{H}_{11}\text{BrF}_3\text{N}_3\text{OS}$ (464.98). White powder. Yield 43%. M.p.: 167°C. ^1H NMR δ , 8.35 (d, 1H, Phe- CF_3), 8.33 (s, 1H, Phe- CF_3), 8.00 (d, 1H, Phe-Br), 7.96 (d, 1H, Phe- CF_3), 7.91 (d, 1H, Phe-Br), 7.81 (t, 1H, Phe- CF_3), 7.78 (t, 1H, Phe-Br), 7.66 (t, 1H, Phe-Br), 2.89 (s, 3H, CH_3 thiazole). ^{13}C NMR δ , 167.13 (C), 156.37 (C), 155.96 (C), 149.64 (C), 135.29 (C), 134.05 (C), 132.85 (CH), 132.62 (C), 131.22 (CH), 129.68 (CH), 129.35 (CH), 127.64 (CH), 127.49 (CH), 126.82 (CH), 124.71 (CH), 121.88 (CF₃), 118.99 (C), 113.42 (C), 16.13 (CH₃). MS: m/z 466.4 ($\text{M}+\text{H}^+$)

2-(2-(3-(Trifluoromethyl)phenyl)4-methylthiazol-5-yl)-5-m-tolyl-1,3,4-oxadiazole (**3h**)

$\text{C}_{20}\text{H}_{14}\text{F}_3\text{N}_3\text{OS}$ (401.08). Yellow powder. Yield 40%. M.p.: 207°C. ^1H NMR δ , 8.34 (d, 1H, Phe- CF_3), 8.32 (s, 1H, Phe- CF_3), 8.02 (d, 1H, tolyl),

7.98 (s, 1H, tolyl), 7.95 (d, 1H, Phe-CF₃), 7.83 (t, 1H, Phe-CF₃), 7.45 (d, 1H, tolyl), 7.42 (t, 1H, tolyl), 2.87 (s, 3H, CH₃ thiazole), 2.43 (s, 3H, CH₃ tolyl). ¹³C NMR δ, 166.53 (C), 155.37 (C), 151.96 (C), 149.74 (C), 139.81 (C), 135.62 (C), 135.2 (C), 135.15 (C), 132.34 (CH), 130.41 (CH), 129.58 (CH), 128.96 (CH), 128.22 (CH), 127.99 (CH), 127.07 (CH), 124.11 (CH), 121.68 (CF₃), 112.32 (C), 20.87 (CH₃), 16.10 (CH₃). MS: *m/z* 402.3 (M+H⁺)

2-(2,6-Dichlorophenyl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (4i)
C₁₉H₁₀Cl₂F₃N₃OS (454.99). White powder. Yield 80%. M.p.: 158°C. ¹H NMR δ, 8.35 (d, 1H, Phe-CF₃), 8.33 (s, 1H, Phe-CF₃), 7.96 (d, 1H, Phe-CF₃), 7.82 (t, 1H, Phe-CF₃), 7.72 (t, 1H, Phe-Cl), 7.51 (d, 1H, Phe-Cl), 7.42 (d, 1H, Phe-Cl), 2.87 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 165.83 (C), 156.37 (C), 152.96 (C), 149.88 (C), 135.45 (C), 135.26 (C), 132.72 (CH), 132.2 (2C), 130.85 (CH), 129.88 (CH), 129.74 (2CH), 129.37 (C), 127.99 (CH), 124.81 (CH), 121.88 (CF₃), 112.98 (C), 16.09 (CH₃). MS: *m/z* 456.9 (M+H⁺)

2-(2,3-Dichlorophenyl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (4j)
C₁₉H₁₀Cl₂F₃N₃OS (454.99). White powder. Yield 85%. M.p.: 176°C. ¹H NMR δ, 8.35 (d, 1H, Phe-CF₃), 8.33 (s, 1H, Phe-CF₃), 7.96 (d, 1H, Phe-CF₃), 7.82 (t, 1H, Phe-CF₃), 7.74 (t, 1H, Phe-Cl), 7.62 (d, 1H, Phe-Cl), 7.61 (d, 1H, Phe-Cl), 2.88 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 169.13 (C), 155.37 (C), 153.96 (C), 150.74 (C), 134.8 (C), 133.2 (C), 132.88 (C), 132.22 (CH), 131.08 (CH), 129.38 (CH), 129.11 (CH), 128.91 (C), 128.26 (C), 127.83 (CH), 127.09 (CH), 124.41 (CH), 121.98 (CF₃), 113.32 (C), 16.14 (CH₃). MS: *m/z* 456.4 (M+H⁺)

2-(2,4-Dichlorophenyl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (4k)
C₁₉H₁₀Cl₂F₃N₃OS (454.99). Yellow powder. Yield 85%. M.p.: 191°C. ¹H NMR δ, 8.35 (d, 1H, Phe-CF₃), 8.33 (s, 1H, Phe-CF₃), 8.14 (d, 1H, Phe), 7.97 (d, 1H, Phe), 7.95 (d, 1H, Phe-CF₃), 7.81 (t, 1H, Phe-CF₃), 7.73 (dd, 1H, Phe), 2.87 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 165.43 (C), 155.37 (C), 152.46 (C), 149.84 (C), 136.90 (C), 134.2 (C), 133.69 (C), 133.05 (C), 132.42 (CH), 131.52 (CH), 130.12 (CH), 129.78 (CH), 129.51 (C), 128.09 (CH), 126.92 (CH), 122.41 (CH), 121.78 (CF₃), 112.42 (C), 16.10 (CH₃). MS: *m/z* 456.7 (M+H⁺)

2-(2-Methoxyphenyl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (4l)
C₂₀H₁₄F₃N₃O₂S (417.08). White powder. Yield 90%. M.p.: 201°C. ¹H NMR δ, 8.35 (d, 1H, Phe-CF₃), 8.33 (s, 1H, Phe-CF₃), 7.96 (d, 1H, Phe-CF₃), 7.88 (d, 1H, Phe-OCH₃), 1H, 7.81 (t, 1H, Phe-CF₃), 7.53 (d, 1H, Phe-OCH₃), 7.3 (m, 1H, Phe-OCH₃), 7.25 (m, 1H, Phe-OCH₃), 3.82 (s, 3H, OCH₃), 2.84 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 166.83 (C), 155.84 (C), 155.47 (C), 154.06 (C), 149.27 (C), 135.77 (C), 135.05

(C), 133.24 (CH), 132.45 (CH), 129.68 (CH), 127.29 (CH), 126.84 (CH), 124.81 (CH), 123.29 (C), 121.68 (CF₃), 120.82 (CH), 115.53 (CH), 113.42 (C), 55.20 (OCH₃), 16.12 (CH₃). MS: *m/z* 418.4 (M+H⁺)

2-(4-Methoxyphenyl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (4m)
C₂₀H₁₄F₃N₃O₂S (417.08). White powder. Yield 85%. M.p.: 220°C. ¹H NMR δ, 8.35 (d, 1H, Phe-CF₃), 8.33 (s, 1H, Phe-CF₃), 8.2 (d, 1H, Phe-OCH₃), 8.18 (d, 1H, Phe-OCH₃), 7.95 (d, 1H, Phe-CF₃), 7.83 (t, 1H, Phe-CF₃), 7.70 (d, 1H, Phe OCH₃), 7.68 (d, 1H, Phe-OCH₃), 3.87 (s, 3H, OCH₃), 2.89 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 165.13 (C), 155.37 (C), 151.16 (C), 149.27 (C), 135.29 (C), 137.05 (C), 132.12 (CH), 129.08 (CH), 127.19 (CH), 124.92 (C), 124.31 (CH), 121.08 (CF₃), 120.87 (C), 116.60 (2CH), 115.82 (2CH) 113.62 (C), 55.40 (OCH₃), 16.09 (CH₃). MS: *m/z* 418.3 (M+H⁺)

2-(3-Methoxyphenyl)-5-(2-(3-(trifluoromethyl)phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (4n)
C₂₀H₁₄F₃N₃O₂S (417.08). White powder. Yield 87%. M.p.: 198°C. ¹H NMR δ, 8.32 (d, 1H, Phe-CF₃), 8.30 (s, 1H, Phe-CF₃), 7.93 (d, 1H, Phe-CF₃), 7.80 (t, 1H, Phe-CF₃), 7.65 (d, 1H, Phe-OCH₃), 7.57 (d, 1H, Phe-OCH₃), 7.55 (s, 1H, Phe-OCH₃), 7.24 (m, 1H, Phe OCH₃), 3.88 (s, 3H, OCH₃), 2.86 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 166.43 (C), 160.76 (C), 155.37 (C), 152.86 (C), 149.14 (C), 135.28 (C), 135.07 (C), 132.22 (CH), 130.76 (CH), 130.62 (C), 129.78 (CH), 127.89 (CH), 125.54 (CH), 124.51 (CH), 121.18 (CF₃), 117.96 (CH), 113.62 (C), 108.61 (CH), 55.76 (OCH₃), 16.24 (CH₃). MS: *m/z* 418.2 (M+H⁺)

2-(2-(3-(Trifluoromethyl)phenyl)4-methylthiazol-5-yl)-5-(thiophen-2-yl)-1,3,4-oxadiazole (4o)
C₁₇H₁₀F₃N₃OS₂ (393.02). Yellow powder. Yield 95%. M.p.: 235°C. ¹H NMR δ, 8.35 (d, 1H, Phe), 8.33 (s, 1H, Phe), 8.02 (d, 1H, Phe), 7.96 (s, 1H, thiophene), 7.94 (d, 1H, thiophene), 7.82 (t, 1 H, Phe), 7.35 (t, 1 H, thiophene), 2.86 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 166.13 (C), 155.96 (C), 151.37 (C), 149.74 (C), 135.2 (C), 135.05 (C), 133.44 (CH), 132.98 (C), 132.22 (CH), 131.07 (CH), 129.38 (CH), 127.09 (CH), 124.41 (CH), 122.06 (CH), 121.98 (CF₃), 113.32 (C), 16.14 (CH₃). MS: 394.5 *m/z* (M+H⁺)

2-(2-(3-(Trifluoromethyl)phenyl)4-methylthiazol-5-yl)-5-(2-phenylthiazol-4-yl)-1,3,4-oxadiazole (4p)
C₂₂H₁₃F₃N₄OS₂ (470.05). White powder. Yield 93%. M.p.: 235°C. ¹H NMR δ, 8.74 (s, 1H, thiazole), 8.36 (d, 1H, Phe-CF₃), 8.33 (s, 1H, Phe-CF₃), 8.08 (d, 1 H, Phe-CF₃), 8.07 (d, 1H, Phe), 7.95 (d, 1H, Phe), 7.83 (t, 1H, Phe-CF₃), 7.59 (m, 3H, Phe), 2.9 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 171.8 (C), 166.13 (C), 155.37 (C), 152.50 (C), 151.98 (C), 149.14 (C), 135.25 (C), 135.09 (C), 133.34 (C), 132.27 (CH), 131.98 (CH), 129.38 (CH), 128.88 (2CH), 127.49 (CH), 127.27 (2CH), 124.71 (CH), 122.98 (CF₃), 118.80 (CH), 113.52 (C), 16.04 (CH₃). MS: *m/z* 471.5 (M+H⁺)

2-(4-Fluorophenyl)-5-(2-(3-(trifluoromethyl)-phenyl)4-methylthiazol-5-yl)-1,3,4-oxadiazole (4q)

C₁₉H₁₁F₄N₃OS (405.06). Yellow powder. Yield 97%. M.p.: 223°C. ¹H NMR δ, 8.34 (d, 1H, Phe-CF₃), 8.32 (s, 1H, Phe-CF₃), 8.19 (d, 1H, Phe-F), 8.17 (d, 1H, Phe-F), 7.75 (d, 1H, Phe-F), 7.74 (d, 1H Phe-F), 7.96 (d, 1H, Phe-CF₃), 7.82 (t, 1H, Phe-CF₃), 2.86 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 166.13 (C), 155.67 (C), 151.16 (C), 149.94 (C), 135.85 (C), 135.45 (C), 132.27 (CH), 131.56 (C), 129.18 (CH), 127.29 (CH), 126.57 (2CH), 124.51 (CH), 121.68 (CF₃), 118.80 (2CH), 163.99 (C), 113.02 (C), 16.10 (CH₃). MS: *m/z* 406.6 (M+H⁺)

2-(4-Fluorophenyl)-5-(4-methyl-2-phenylthiazol-5-yl)-1,3,4-oxadiazole (4r)

C₁₈H₁₂FN₃OS (337.07). Yellow powder. Yield 87%. M.p.: 183°C. ¹H NMR δ, 8.16 (d, 1H, Phe-F), 8.14 (d, 1H, Phe-F), 8.05 (m, 2H, Phe), 7.70 (d, 1H, Phe-F), 7.69 (d, 1H, Phe-F), 7.55 (m, 3H, Phe), 2.82 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 169.98 (C), 165.25 (C), 163.99 (C), 156.39 (C), 151.31 (C), 133.52 (C), 128.55 (2CH), 128.49 (C), 127.23 (2CH), 125.56 (2CH), 118.8 (CH), 115.79 (2CH), 112.88 (C), 16.13 (CH₃). MS: *m/z* 338.3 (M+H⁺)

2-(4-Bromophenyl)-5-(4-methyl-2-phenylthiazol-5-yl)-1,3,4-oxadiazole (4s)

C₁₈H₁₂BrN₃OS (396.99). Yellow powder. Yield 90%. M.p.: 216°C. ¹H NMR δ, 8.20 (d, 1H, Phe Br), 8.18 (d, 1H, Phe Br), 8.10 (m, 2H, Phe), 7.75 (d, 1H, Phe Br), 7.73 (d, 1H, Phe Br), 7.50 (m, 3H, Phe), 2.83 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 169.08 (C), 162.25 (C), 156.19 (C), 151.41 (C), 133.52 (C), 131.99 (2CH), 128.95 (2CH), 128.89 (C), 127.83 (2CH), 125.07 (C), 124.85 (2CH), 118.67 (CH), 112.98 (C), 16.17 (CH₃). MS: *m/z* 398.5 (M+H⁺)

2-(4-Methyl-2-phenylthiazol-5-yl)-5-(thiophen-2-yl)-1,3,4-oxadiazole (4t)

C₁₆H₁₁N₃OS₂ (325.03). Yellow powder. Yield 95%. M.p.: 196°C. ¹H NMR δ, 8.05 (m, 2H, Phe), 8.01

(dd, 1H, thiophene), 7.93 (dd, 1H, thiophene), 7.57 (m, 3H, Phe), 7.34 (m, 1H, thiophene), 2.83 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 169.08 (C), 162.85 (C), 155.39 (C), 152.39 (C), 133.52 (C), 131.27 (CH), 130.46 (CH), 128.55 (2CH), 127.99 (C), 127.23 (2CH), 123.16 (CH), 118.8 (CH), 113.98 (C), 16.15 (CH₃). MS: *m/z* 326.5 (M+H⁺)

2-(3-Methoxyphenyl)-5-(4-methyl-2-phenylthiazol-5-yl)-1,3,4-oxadiazole (4u)

C₁₉H₁₅N₃O₂S (349.09). Yellow powder. Yield 91%. M.p.: 145°C. ¹H NMR δ, 8.08 (m, 2H, Phe), 7.64 (d, 1H, Phe-OCH₃), 7.55 (d, 1H, Phe-OCH₃), 7.53 (s, 1H, Phe-OCH₃), 7.50 (m, 3H, Phe), 7.24 (m, 1H, Phe-OCH₃), 3.90 (s, 3H, OCH₃), 2.84 (s, 3H, CH₃ thiazole). ¹³C NMR δ, 169.98 (C), 163.25 (C), 160.76 (C), 157.39 (C), 152.01 (C), 134.56 (CH), 134.52 (C), 130.76 (CH), 130.41 (CH), 129.87 (C), 128.05 (2CH), 127.23 (2CH), 118.8 (CH), 117.96 (CH), 112.88 (C), 55.76 (OCH₃), 16.13 (CH₃). MS: *m/z* 350.6 (M+H⁺)

Molecular docking study

The binding energies corresponding to the new synthesized molecules were predicted with two programs (AD, ADV) and are presented in Table I.

The thiazolyl-oxadiazoles that contain the CF₃ group (3a-h, 4i-q) showed higher binding affinities than compounds 4r-u which lack this substituent.

Generally, compounds substituted in the ortho and meta position of the phenyl group, directly bound to the oxadiazole, have higher binding affinities.

Seven lead compounds were chosen by setting the maximum limit of the inhibition constant to 5 nM, which means all compounds with binding energies lower than -11.32 kcal/mol (calculated by each program AD and ADV). The values that met these criteria (bold) are showed in Table I.

Table I
Binding energies (kcal/mol) for the synthesized thiazolyl-1,3,4-oxadiazoles calculated with both programs (AD and ADV)

Enzyme Comp.	4UYL		5EQB		CACYP51	
	ADV	AD	ADV	AD	ADV	AD
3a	-11.20	-11.48	-11.40	-10.58	-10.40	-10.37
3b	-10.50	-10.47	-10.50	-9.56	-10.40	-9.31
3c	-11.20	-11.98	-11.40	-11.13	-11.20	-10.88
3d	-11.60	-11.46	-11.60	-10.90	-11.50	-10.73
3e	-10.80	-10.30	-11.20	-11.25	-10.70	-10.95
3f	-10.90	-11.98	-11.40	-11.17	-10.60	-10.93
3g	-11.60	-12.14	-11.80	-11.60	-11.60	-11.42
3h	-11.50	-11.54	-11.60	-10.93	-11.50	-10.73
4i	-11.20	-11.64	-11.40	-10.46	-11.30	-10.29
4j	-11.60	-11.68	-11.20	-11.42	-11.30	-11.28
4k	-11.30	-12.29	-11.60	-11.80	-11.50	-11.53
4l	-11.50	-12.09	-11.50	-10.96	-11.30	-10.87
4m	-11.00	-10.93	-10.80	-10.87	-10.50	-10.63

Enzyme Comp.	4UYL		5EQB		CACYP51	
	ADV	AD	ADV	AD	ADV	AD
4n	-11.10	-11.04	-10.90	-10.86	-10.90	-10.70
4o	-10.80	-10.80	-10.70	-10.13	-10.50	-9.90
4p	-11.40	-12.84	-11.90	-12.86	-11.30	-12.45
4q	-11.30	-10.32	-11.70	-10.50	-11.50	-10.35
4r	-10.10	-10.96	-10.50	-10.24	-10.40	-10.10
4s	-10.40	-11.02	-10.20	-11.01	-10.10	-10.72
4t	-9.00	-10.56	-9.40	-9.86	-9.40	-9.63
4u	-8.70	-11.84	-10.20	-10.81	-10.30	-10.64
FC	-7.90	-6.53	-8.10	-7.03	-8.20	-6.54
IC	-11.00	-14.38	-12.50	-14.09	-11.00	-15.52
KC	-10.60	-12.51	-12.00	-13.45	-11.60	-12.74
MC	-9.20	-9.90	-9.80	-10.32	-9.70	-8.21
VC	-12.20	-12.46	-11.80	-12.68	-11.40	-11.92

For the corresponding thiazolyl-1,3,4-oxadiazoles we calculated the inhibition constants (Table II) using the formula:

$$K_i = e^{\frac{\Delta G \times 1000}{R \times T}}$$

ΔG = binding energy

R = Regnault constant for ideal gases (1.98719 cal / mol * K)

T = absolute temperature (298.15 K)

Table II

Inhibition constants (nM) for the lead compounds

Enzyme Comp.	4UYL	5EQB	CACYP51
3d	3.97	-	-
3g	1.25	3.13	4.29
3h	3.49	-	-
4j	2.77	-	-
4k	0.98	2.25	3.53
4l	1.38	-	-
4p	0.39	0.37	-
IC	0.03	0.05	0.004
KC	0.67	0.14	0.45
MC	55.55	27.26	956.74
VC	0.73	0.50	1.84

The values are exponentially decreasing with the increasing affinity of the enzyme inhibitor.

Low values of K_i suggest that the compound is a good inhibitor. The inhibition constants were calculated by AutoDock 4.2.

Compound **4k** was considered the most performant agent since it showed a good inhibition on all the three enzymes, which have varying degrees of similarity.

It showed, mostly, hydrophobic interactions with all three enzymes. In most cases, these occur with the amino acids that form the access channel to the enzyme active site and not directly with the active centre of the enzyme as for itraconazole (Figure 2). The predominant amino acids located in the access channel to the enzyme active site in all three enzymes are alanine, phenylalanine, valine, leucine and isoleucine (about 70 % of the interactions were

formed between our compounds and this amino acids). We noticed potential hydrogen bonds with the nitrogen atoms from the oxadiazole system *via* tyrosine residues 136, 140 respectively.

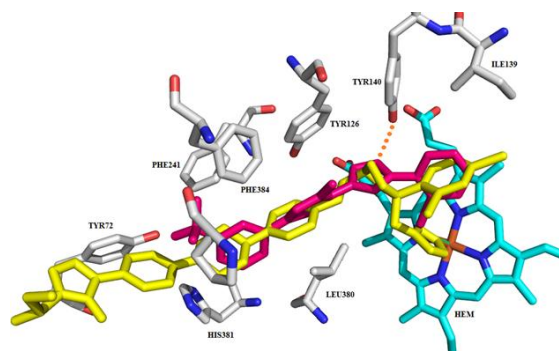


Figure 2.

5EQB molecular docking and binding mode of itraconazole (yellow) and **4k** (pink). The hydrogen bond is shown in orange and the covalent bond is shown in green.

The compounds are acting as non-competitive inhibitors of the lanosterol 14 α -demethylase, which means they are not competing with the substrate for the active site of the enzyme. This new potential mechanism of action of the thiazolyl-1,3,4-oxadiazoles could be harnessed by *in vitro* testing on strains that are resistant to azoles.

Conclusions

In conclusion, a new class of thiazolyl-1,3,4-oxadiazole derivatives was synthesized using two methods with different reaction mechanisms. The structures of the obtained compounds were confirmed by: elemental quantitative analysis, ^1H NMR, ^{13}C NMR and MS. These were subjected to an *in silico* study on lanosterol 14 α -demethylase from three different fungal strains. The obtained results were promising and will guide us for further *in vitro* studies.

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