ORIGINAL ARTICLE

SYNTHESIS AND MOLECULAR DOCKING STUDY OF SOME NEW 1,4-PHENYLENE-BISTHIAZOLES AS FUNGAL LANOSTEROL 14α-DEMETHYLASE INHIBITORS

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Abstract

The present work reports the synthesis, physico-chemical, spectral characterization and molecular docking study of a novel series of 1,4-phenylene-bisthiazoles. The newly synthesized 1,4-phenylene-bisthiazole derivatives were obtained with good yields through a Hantzsch condensation reaction between the thioamide intermediate and various alfa-haloketones or alfa-haloesters. The proposed structure of the compounds was confirmed by quantitative elemental analysis and spectral data: mass spectrometry and proton nuclear magnetic resonance. A molecular docking study was performed in order to investigate the potential binding affinity of the synthesized compounds towards the fungal lanosterol 14α-demethylase. The results of the molecular docking study showed that these compounds have potential antifungal activity and could be considered for further *in vitro* biological evaluation.

Rezumat

Cercetarea de faţă cuprinde sinteza, caracterizarea fizico-chimică şi spectrală precum şi studiul de andocare moleculară a unei noi serii de 1,4-fenilen-bistiazoli. Sinteza noilor compuşi cu structură 1,4-fenilen-bistiazolică s-a realizat cu randamente bune, printr-o reacţie de condensare Hantzsch, între un intermediar cu structură tioamidică şi diverse alfa-halocetone sau alfa-haloesteri. Structura compușilor a fost confirmată prin analiză elementală cantitativă și metode spectrale: spectrometrie de masă și spectroscopie de rezonanță magnetică nucleară de proton. S-a efectuat un studiu de andocare moleculară pentru a urmări afinitatea de legare a noilor compuşi sintetizaţi de lanosterol 14α-demetilaza fungică. Rezultatele studiului de andocare moleculară au evidenţiat faptul că aceşti compuşi au potenţial antifungic şi pot face obiectul unor studii *in vitro* de evaluare a activităţii biologice.

Keywords: 1,4-phenylene-bisthiazoles, lanosterol 14α-demethylase, molecular docking, Hantzsch reaction, anti-*Candida*

Introduction

Candida genus includes about 200 different species, but only a few species can cause serious infection in human, particularly in immunocompromised patients (treatment with chemotherapy, corticosteroids, immunosuppressive agents, patients with AIDS) [20]. The spectrum of *Candida* infections is diverse, starting from superficial infections, such as cutaneous candidiasis, to invasive candidiasis, including candidemia [1]. The incidence of systemic opportunistic infections caused by fungi in immunocompromised patients is increasing, which has led to many treatment failures in patients receiving long-term antifungal therapy [6].

The azole class of antifungal compounds, which includes imidazoles and triazoles, is the most widely used, with more than fifteen molecules authorized for clinical practice. Epidemiological studies, conducted over the last decade, have shown that there is a significant increase in the frequency of fungal infections resistant to imidazole and triazole agents [13, 24].

A growing global concern related to the increased number of multidrug-resistant *Candida* infections and the need for discovering and developing innovative molecules with superior activity, pharmacokinetics and tolerability, explain researchers' effort in identifying compounds with a scaffold that is similar to the clinically approved azole antifungals' scaffold or with a totally new chemical profile.

Thiazole and its wide range of derivatives are an important class of substances in medicinal chemistry due to their various biological activities [4, 9, 12, 15, 16]. Many thiazoles were found to be associated with antimicrobial properties against a variety of clinically relevant fungal pathogens [5, 11, 19].

Computer-aided drug design represents a step forward in the rational design of new biologically active molecules. The modern *in silico* methods, can help new drugs' development and saving time and resources. Based on the knowledge of a specific biological target, we can nowadays create new agents with promising potential in key enzymes' or pathways' modulation [10].

The molecular docking studies can be used to elucidate the interactions between a small molecule and a protein at atomic level. These facts help us to characterize the behaviour of ligands in the binding site of target proteins, as well as to predict the binding affinity of drug candidates to their biological targets. Furthermore, *in silico* evaluation facilitates the understanding of the mechanism of action of newly synthesized compounds [14].

The most important and widely studied mechanisms of resistance to azole antifungals is the modification of the binding affinity of these drugs to target lanosterol 14α-demethylase, which is a key enzyme in the biosynthesis of sterols, very important components of the fungal cell. This enzyme has been extensively studied in the last years for the discovery of novel pharmaceutical active agents, which are capable to inhibit it, in order to avoid resistance acquired by fungi to classical azoles [18].

In the present study we report the synthesis, physico-chemical and spectral characterization and a molecular docking study of a new series of 1,4-phenylene-bisthiazole as potential inhibitors of lanosterol 14α-demethylase.

Materials and Methods

Chemistry

All reagents and solvents were used as purchased from Sigma-Aldrich (Buchs, Switzerland) and Alfa Aesar (Karlsruhe, Germany). Analytical thin layer chromatography (TLC) was used to verify the purity of the synthesized compounds and was performed on precoated Silica Gel $60F_{254}$ sheets, using ethyl-acetate - heptane 7:3 as mobile phase and UV absorption as visualization. Melting points (m.p.) were recorded on an Electrothermal capillary melting point meter and are uncorrected. Elemental analysis was performed on a Vario El CHNS apparatus and the results are within ± 0.4% of the theoretical values. The ^{1}H NMR spectra were recorded at room temperature, in deuterated dimethylsulfoxide (DMSO-d_6), on a Bruker Avance NMR spectrometer (Bruker, Karlsruhe, Germany) operating at 500 MHz. Chemical shifts (δ values) are given in parts per million relative to tetramethylsilane (TMS) as internal standard. Multiplicities are given as s (singlet), d (doublet), m (multiplet), dd (double doublet). LC-MS analyses were recorded on an Agilent 1100 series and an Agilent Ion Trap SL mass spectrometer. ESI was carried out in the positive ion mode.

*Synthesis of 4-(2-methylthiazol-4-yl)benzonitrile (**3**)*

A mixture of thioacetamide **1** (3.75 g, 50 mmol) and 4-(2-bromoacetyl)benzonitrile **2** (11.15 g, 50 mmol) was refluxed for 3 hours in the minimum required volume of 96% ethanol. After cooling, the reaction mixture was poured into ice-cold water and the resulting precipitate was separated by filtration. The product obtained was recrystallized from ethanol.

*Synthesis of 4-(2-methylthiazol-4-yl)benzothioamide (**4**)*

4-(2-methylthiazol-4-yl)benzonitrile **3** (8.00 g, 40 mmol) was added to a solution of triethylamine (TEA, 8 mL) in 96% ethanol (40 mL). The resulting suspension was saturated with hydrogen sulfide, which was produced from iron (II) sulfide and hydrochloric acid in a Kipp apparatus, and stirred at room temperature. The progress of conversion was monitored by TLC. After the reaction was complete, the crystalline product was filtered, washed with water, dried and purified by recrystallization from ethanol.

*General procedure for the synthesis of 1,4-phenylene-bisthiazoles (**5a-j**)*

To a suspension of 4-(2-methylthiazol-4-yl)benzothio-amide in 96% ethanol, an equimolar quantity of the appropriate alfa-haloketone or alfa-haloester was added. The resulting mixture was brought to reflux for 2 - 3 h. The completion of the reaction was monitored by TLC. The precipitate was isolated by filtration, washed with water and dried. Pure compounds were obtained after recrystallization using methanol.

Molecular docking study

Because resistant strains of *Candida albicans* are responsible for life threatening infections across the globe, we evaluated the potential binding affinity of the synthesized compounds, 1,4-phenylene-bisthiazoles **5a-j**, to lanosterol 14α-demethylase. Fluconazole, itraconazole, ketoconazole, miconazole and voriconazole were used as reference compounds.

FASTA sequence of targeted enzyme from *Candida albicans* was taken from Universal Protein Resource (http://www.uniprot.org/uniprot/P10613). A BLAST analysis, based on P10613 sequence, indicated that structure 5EQB from Protein Data Bank has the closest similarity (65%) and was further used for homology modelling using SWISS-MODEL [2, 3, 7, 8].

The molecular docking study was carried out using AutoDock 4.2 [17] and AutoDock Vina 1.1.2 [23]. The synthesized 1,4-phenylene-bisthiazoles derivatives and the reference compounds were docked into the active site of the enzyme, in the cubic space whose centre was defined by coordinates x = 23.134, y = 13.943, z = 19.959. The grid size (x, y, z) was set to 75 x 75 x 75 points with a 0.375 Å grid spacing for AutoDock and 28 x 28 x 28 grid points for Auto Dock Vina. AutoDock searched for 25 conformers

with 2 Å cluster tolerance. Exhaustiveness was set to 10 for AutoDock Vina. Using two software solutions with different algorithms, in the molecular docking study, would help to filter the results and eliminate the potential false positive results from this virtual screening. In other words, we set a threshold value of binding affinity (ΔG) at -10 kcal/mol. Inhibition constant was calculated as:

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

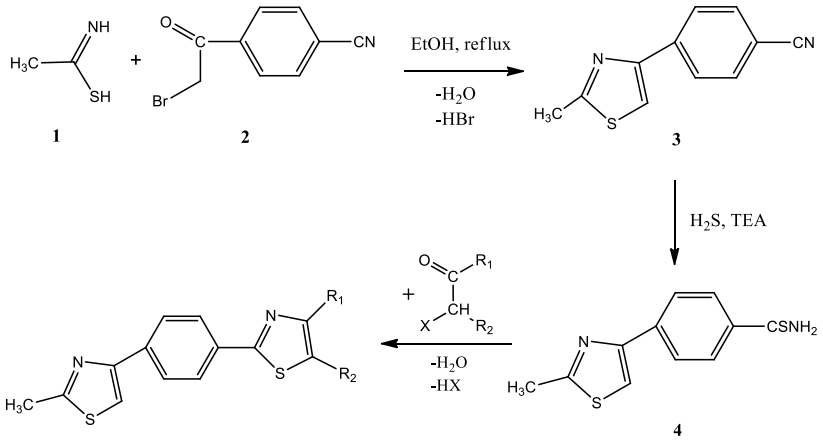
where R = 1.98719 cal/(mol x K) (Regnault constant) and T = 298.15 K = 25°C. Compounds that did not meet this criteria on both software in our screening were considered inactive.

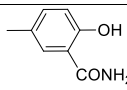
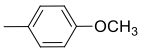
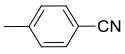
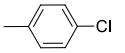
Both in our molecules and in the target macromolecule, polar hydrogen atoms were added, rotatable bonds were defined, carboxylic moieties were deprotonated and Gasteiger partial charges were assigned. Amide bonds were set non-rotatable. Dataset files containing the ligands and the macromolecule were prepared using the previous reported protocol [22]. The other settings were left as default.

Results and Discussion

Chemistry

The chemical development of the 1,4-phenylene-bisthiazoles followed the reaction sequences outlined in Figure 1.



Compound	R_1	R_2
5a		H
5b		H
5c		H
5d		H
5e		H
5f		H
5g	CH_3	$COCH_3$
5h	CH_3	$COOC_2H_5$
5i	CH_2-$COOC_2H_5$	H
5j	CH_2Cl	H

TEA = triethylamine

Figure 1.
Synthesis of 1,4-phenylene-bisthiazoles **5a-j**

The target 1,4-phenylene-bisthiazoles **5a-j** were synthesized, with good yields, through a Hantzsch condensation reaction, between the thioamide key intermediate **4** and various alfa-haloketones or alfa-haloesters. The thioamide key intermediate **4**, having a thiazole moiety in its structure, was obtained in a two-steps synthesis. In the first step, 4-(2-methyl-thiazol-4-yl)benzonitrile **3** was synthesized through the reaction between thioacetamide **1** and 4-(2-bromoacetyl)benzonitrile **2**, in 96% ethanol at reflux. The next step was the conversion of 4-(2-methyl-thiazol-4-yl)benzonitrile **3** to 4-(2-methylthiazol-4-yl)benzothioamide **4** using hydrogen sulfide gas.

The progress of the reactions was monitored by thin layer chromatography. All the newly synthesized compounds were purified by recrystallization and were characterized by melting point, elemental analysis and spectroscopic data (MS and ^{1}H NMR). The structures of the 1,4-phenylene-bisthiazole derivatives **5a-j** were correlated with the data obtained from quantitative elemental analysis, mass spectrometry and ^{1}H NMR.

*4-(2-methylthiazol-4-yl)benzonitrile (**3**)* Pale yellow solid. Yield 78%. M.p. 159°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.14 (dd, 2H, phenyl), 8.11 (dd, 2H, phenyl), 7.97 (s, 1H, thiazole-C_5H), 2.76 (s, 3H, -CH_3). MS (ESI, 70 eV): *m/z* 201.04 (M+H$^+$). *Anal*. Calcd. for $C_{11}H_8N_2S$ (%): C, 65.97; H, 4.03; N, 13.99; S, 16.01. Found (%): C, 65.78; H, 4.25; N, 14.03; S, 15.89.

*4-(2-methylthiazol-4-yl)benzothioamide (**4**)* Yellow solid. Yield 75%. M.p. 205°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.37 (s, 2H, -NH_2), 8.12 (s, 1H, thiazole-C_5H), 7.95 (dd, 2H, phenyl), 7.81 (dd, 2H, phenyl), 2.73 (s, 3H, -CH_3). MS (ESI, 70 eV): *m/z* 235.03 (M+H$^+$). *Anal*. Calcd. for $C_{11}H_{10}N_2S_2$ (%): C, 56.38; H, 4.30; N, 11.95; S, 27.37. Found (%): C, 56.69; H, 4.22; N, 11.67; S, 27.52.

*2-hydroxy-5-(2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-4-yl)benzamide (**5a**)* Yellow solid. Yield 84%. M.p. 246-7°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 11.8 (s, 1H, OH), 8.57 (s, 1H, thiazole-C_5H), 8.24 (d, 1H, phenyl-R), 8.17 (s, 2H, -NH_2), 8.14 (s, 1H, thiazole-C_5H), 8.07 (s, 1H, phenyl-R), 7.96 (dd, 2H, phenyl), 7.81 (dd, 2H, phenyl) 7.12 (d, 1H, phenyl-R), 2.71 (s, 3H, -CH_3). MS (ESI, 70 eV): *m/z* 394.5 (M+H$^+$). *Anal*. Calcd. for $C_{20}H_{15}N_3O_2S_2$ (%): C, 61.05; H, 3.84; N, 10.68; S, 16.30. Found (%): C, 61.18; H, 3.92; N, 10.83; S, 16.21.

*4-(4-methoxyphenyl)-2-(4-(2-methylthiazol-4-yl)-phenyl)thiazole (**5b**)* Yellow solid. Yield 91%. M.p. 196°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.59 (s, 1H, thiazole-C_5H), 8.05 (dd, 2H, phenyl-OCH_3), 7.88 (dd, 2H, phenyl), 7.49 (dd, 2H, phenyl), 7.37 (s, 1H, thiazole-C_5H), 7.06 (dd, 2H, phenyl- OCH_3), 3.96 (s, 3H, -CH_3), 2.75 (s, 3H, -CH_3). MS (ESI, 70 eV): *m/z* 365.5 (M+H$^+$). *Anal*. Calcd. for $C_{20}H_{16}N_2OS_2$ (%): C, 65.91; H, 4.42; N, 7.69; S, 17.59. Found (%): C, 65.79; H, 4.50; N, 7.57; S, 17.71.

*4-(2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-4-yl)-benzonitrile (**5c**)* Yellow solid. Yield 89%. M.p. 223°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.49 (s, 1H, thiazole-C_5H), 8.29 (dd, 2H, phenyl), 8.14 (dd, 2H, phenyl-CN), 8.12 (dd, 2H, phenyl-CN), 8.11 (s, 1H, thiazole-C_5H), 7.98 (dd, 2H, phenyl), 2.75 (s, 3H, -CH_3). MS (ESI, 70 eV): *m/z* 360.6 (M+H$^+$). *Anal*. Calcd. for $C_{20}H_{13}N_3S_2$ (%): C, 66.82; H, 3.65; N, 11.69; S, 17.84. Found (%): C, 66.89; H, 3.49; N, 11.77; S, 17.91.

*4-(4-chlorophenyl)-2-(4-(2-methylthiazol-4-yl)phenyl)-thiazole (**5d**)* Yield 94%. M.p. 209°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.51 (s, 1H, thiazole-C_5H), 8.08 (dd, 2H, phenyl), 7.84 (dd, 2H, phenyl-Cl), 7.81 (dd, 2H, phenyl), 7.46 (s, 1H, thiazole-C_5H), 7.35 (dd, 2H, phenyl-Cl), 2.74 (s, 3H, -CH_3). MS (ESI, 70 eV): *m/z* 369.5 (M+H$^+$). *Anal*. Calcd. for $C_{19}H_{13}ClN_2S_2$ (%): C, 61.86; H, 3.55; Cl, 9.61; N, 7.59; S, 17.38. Found (%): C, 61.93; H, 3.47; N, 7.67; S, 17.43.

*2-methyl-4-(4-(4-(naphthalen-1-yl)thiazol-2-yl)phenyl)-thiazole (**5e**)* Light orange solid. Yield 89%. M.p. 179°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.66 (d, 1H, naph), 8.35 (s, 1H, thiazole-C_5H), 8.22 (d, 1H, naph), 8.20-8.14 (m, 4H, naph), 8.11 (m, 1H, naph) 8.06 (dd, 2H, phenyl), 7.97 (s, 1H, thiazole-C_5H), 7.56 (dd, 2H, phenyl), 2.76 (s, 3H, -CH_3). MS (ESI, 70 eV): *m/z* 385.6 (M+H$^+$). *Anal*. Calcd. for $C_{23}H_{16}N_2S_2$ (%): C, 71.84; H, 4.19; N, 7.29; S, 16.68 Found (%): C, 71.92; H, 4.28; N, 7.41; S, 16.59.

*2-methyl-4-(4-(4-phenylthiazol-2-yl)phenyl)thiazole (**5f**)* Yellow solid. Yield 97%. M.p. 172-3°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.21 (s, 1H, thiazole-C_5H), 8.09-7.58 (m, 10H, phenyl), 7.36 (s, 1H, thiazole-C_5H) 2.75 (s, 3H, CH_3). MS (ESI, 70 eV): *m/z* 335.5 (M+H$^+$). *Anal*. Calcd. for $C_{19}H_{14}N_2S_2$ (%): C, 68.23; H, 4.22; N, 8.38; S, 19.17. Found (%): C, 68.14; H, 4.41; N, 8.29; S, 19.36.

*1-(4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-5-yl)ethanone (**5g**)* Yellow solid. Yield 86%. M.p. 168°C. ^{1}H NMR (DMSO- d_6, 500 MHz) δ ppm: 8.13 (s, 1H, thiazole-C_5H), 8.11 (dd, 2H, phenyl), 8.08 (dd, 2H, phenyl), 2.75 (s, 3H, -CH_3), 2.73 (s, 3H, -CH_3), 2.59 (s, 3H, -CH_3). MS (ESI, 70 eV): *m/z* 315.4 (M+H$^+$). *Anal*. Calcd. for $C_{16}H_{14}N_2OS_2$ (%): C, 61.12; H, 4.49; N, 8.81, S, 20.40. Found (%): C, 61.07; H, 4.58; N, 8.73; S, 20.51.

*Ethyl 4-methyl-2-(4-(2-methylthiazol-4-yl)phenyl)-thiazole-5-carboxylate (**5h**)* Yellow solid. Yield 92%. M.p. 105°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.13 (s, 1H, thiazole-C_5H), 8.11 (dd, 2H, phenyl), 8.07 (dd, 2H, phenyl), 4.30 (m, 2H, -CH_2-), 2.74 (s, 3H, -CH_3), 2.71 (s, 3H, -CH_3), 1.31 (m, 3H, -CH_3). MS (ESI, 70 eV): *m/z* 345.4 (M+H$^+$). *Anal*. Calcd. for $C_{17}H_{16}N_2O_2S_2$ (%): C, 59.28; H,

4.68; N, 8.13; S, 18.62. Found (%): C, 59.37; H, 4.58; N, 8.24; S, 18.48.

*Ethyl 2-(2-(4-(2-methylthiazol-4-yl)phenyl)thiazol-4-yl)acetate (**5i**)* Yellow solid. Yield 81%. M.p. 66°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.08 (s, 1H, thiazole-C_5H), 8.07 (dd, 2H, phenyl), 8.06 (dd, 2H, phenyl), 7.56 (s, 1H, thiazole-C_5H), 4.16 (m, 2H, -CH_2-), 4.12 (s, 2H, -CH_2-), 2.74 (s, 3H, -CH_3), 1.23 (m, 3H, -CH_3). MS (ESI, 70 eV): *m/z* 345.5 ($M+H^+$). *Anal*. Calcd. for $C_{17}H_{16}N_2O_2S_2$ (%): C, 59.28; H, 4.68; N, 8.13; S, 18.62. Found (%): C, 59.37; H, 4.81; N, 8.02; S, 18.83.

*4-(chloromethyl)-2-(4-(2-methylthiazol-4-yl)phenyl)-thiazole (**5j**)* Yellow solid. Yield 73%. M.p. 124°C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.1 (s, 1H, thiazole C_5-H), 8.01 (dd, 2H, phenyl), 7.59 (s, 1H, thiazole-C_5H), 7.53 (dd, 2H, phenyl), 4.89 (s, 2H, CH2), 2.72 (s, 3H, -CH_3). MS (ESI, 70 eV): *m/z* 307.7 ($M+H^+$). *Anal*. Calcd. for $C_{14}H_{11}ClN_2S_2$ (%): C, 54.80; H, 3.61; N, 9.13; S, 20.90. Found (%): C, 54.91; H, 3.47; N, 9.31; S, 20.77.

Molecular docking study

The results obtained from the molecular docking study of the tested compounds 1,4-phenylene-bis-thiazoles **5a-j** (binding affinity and computed inhibition constant) are presented in Table I. Cluster analysis of conformations resulted from AutoDock is presented in Table II.

Table I

Binding energies (kcal/mol) and inhibition constants (nM) for the synthesized 1,4-phenylene-bisthiazoles **5a-j**

Compound	AutoDock Vina		AutoDock	
	ΔG[a]min (kcal/mol)	Ki[b] (nM)	ΔG[a]min (kcal/mol)	Ki[b] (nM)
5a	**-10.00**	**46.76**	**-11.66**	**2.84**
5b	-9.70	77.59	**-10.24**	**31.19**
5c	**-10.20**	**33.37**	**-10.91**	**10.07**
5d	-9.90	55.36	**-10.72**	**13.87**
5e	**-11.90**	**1.89**	**-11.91**	**1.86**
5f	-9.50	108.74	-9.90	55.36
5g	-8.70	419.58	-8.72	405.65
5h	-8.50	588.05	-8.98	261.56
5i	-8.40	696.17	-9.03	240.39
5j	-7.90	1618.89	-8.34	770.35
Fluconazole	-8.20	975.69	-6.97	7778.89
Itraconazole	-11.00	8.64	-15.93	0.002
Ketoconazole	-11.60	3.14	-13.27	0.08
Miconazole	-9.70	77.58	-10.20	33.36
Voriconazole	-11.40	4.40	-12.28	0.99

[a]ΔG = Variation of Gibbs free energy; [b]Ki = Inhibition constant

Table II

Cluster analysis of conformations for the synthesized 1,4-phenylene-bisthiazoles **5a-j**

Compound	Conformations in the 2 Å cluster of the best binding conformation		The cluster with the most conformations, if it does not contain the best binding conformation		
	NoC[a]	Mean ΔG[b] in cluster (kcal/mol)	NoC[a]	Mean ΔG[b] in cluster (kcal/mol)	ΔG[b]min (kcal/mol)
5a	4	-11.21	7	-10.53	-10.60
5b	4	-10.18	14	-10.01	-10.11
5c	13	-10.85	N/A	N/A	N/A
5d	5	-10.67	16	-10.34	-10.44
5e	17	-11.72	N/A	N/A	N/A
5f	13	-9.66	N/A	N/A	N/A
5g	7	-8.50	N/A	N/A	N/A
5h	5	-8.81	11	-8.86	-8.70
5i	1	-9.03	10	-8.62	-8.90
5j	5	-8.28	N/A	N/A	N/A
Fluconazole	6	-6.49	N/A	N/A	N/A
Itraconazole	3	-14.99	7	-12.47	-13.37
Ketoconazole	7	-12.42	N/A	N/A	N/A
Miconazole	1	-10.20	4	-8.25	-8.48
Voriconazole	7	-12.06	N/A	N/A	N/A

[a]NoC = Number of conformations; [b]ΔG = Variation of Gibbs free energy; N/A = Not applicable

Taking binding affinity equal or less than -10 kcal/mol as threshold value, compounds **5a**, **5c** and **5e** are predicted to be the best inhibitors of lanosterol 14α-demethylase in our series. Compounds **5b** and **5d** seemed to have potential affinity of lanosterol 14α-demethylase on AutoDock, but they did not meet these criteria on AutoDock Vina. Top compounds **5a**, **5c** and **5e** are predicted to be better inhibitors than fluconazole.

Compound **5e** was predicted to have good binding affinity of the enzyme on both AutoDock and Auto Dock Vina, better than fluconazole and miconazole. However, it did not have the potency of itraconazole, ketoconazole or voriconazole. Its binding manner was homogenous, 17 out of 25 conformations predicted are in the same 2 Å cluster.

Interaction between lanosterol 14α-demethylase and the compound **5e**, with the highest potential as inhibitor of the target enzyme, is depicted in Figure 2. The aminoacids in the background were faded.

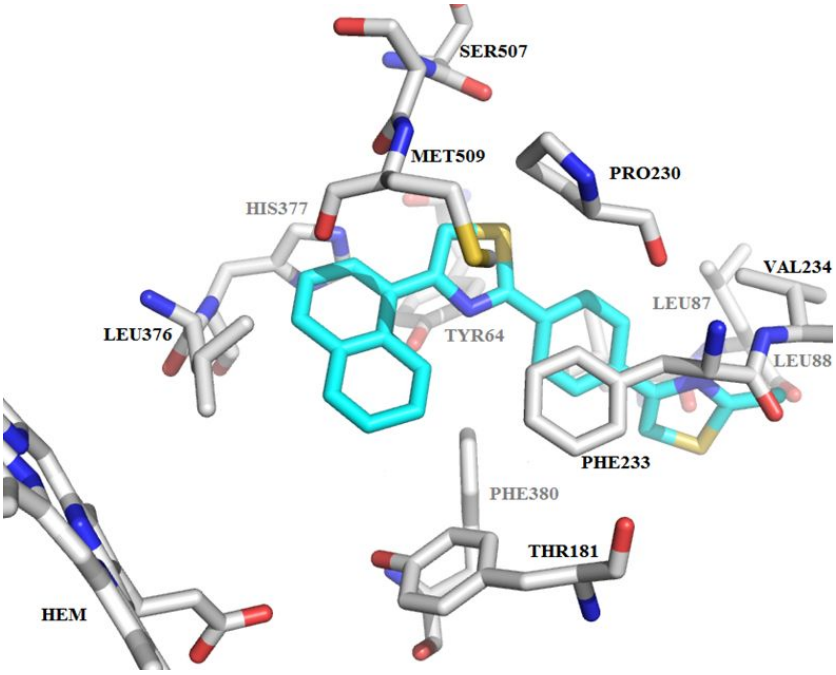


Figure 2.
Lanosterol 14α-demethylase molecular docking and binding mode of compound **5e**

Compounds **5a-j** were predicted to interact with lanosterol 14α-demethylase more in a hydrophobic manner. None of the compounds could have polar contacts with the active site or with the amino acid residues from the active site of the enzyme. All interactions predicted, which took place, are with amino acids from the access channel to the active site of lanosterol 14α-demethylase, such as LEU376, HIS377, MET509, SER507, TYR64, PRO230, LEU87, VAL234, LEU88, PHE 233, PHE380 and THR181. The new synthesized compounds were predicted to be non-competitive inhibitors of the enzyme. This proposed mechanism of action has been reported in the literature as potential avoidance of fungal resistance to classical azoles [21].

Conclusions

In conclusion, a series of 1,4-phenylene-bisthiazole derivatives were successfully synthesized by Hantzsch condensation reaction. The structure of the new molecules was confirmed by elemental analysis, mass spectrometry and ^{1}H NMR. A molecular docking study on lanosterol 14α-demethylase was performed in order to elucidate the mechanism of action of the synthesized compounds. The obtained *in silico* results suggested that the new 1,4-phenylene-bisthiazoles may be considered for further *in vitro* studies as potential anti-*Candida* agents and for various chemical pharmacomodulation in order to optimize the hydrophilic-lipophilic balance and increase the anti-fungal activity.

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