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Anti-*Candida* activity and cytotoxicity of a large library of new *N*-substituted-1,3-thiazolidin-4-one derivatives

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Abstract

On the basis of the recent findings about the biological properties of thiazolidinones and taking into account the encouraging results about the antifungal activity of some (thiazol-2-yl)hydrazines, new *N*-substituted heterocyclic derivatives were designed combining the thiazolidinone nucleus with the hydrazonic portion. In details, 1,3-thiazolidin-4-ones bearing (cyclo)aliphatic or (hetero)aromatic moieties linked to the N1-hydrazine at C2 were synthesized and classified into three series according to the aromatic or bicyclic rings connected to the lactam nitrogen of the thiazolidinone. These molecules were assayed for their anti-*Candida* effects in reference to the biological activity

of the conventional topic (clotrimazole, miconazole, tioconazole) and systemic drugs (fluconazole, ketoconazole, amphotericin B). Finally, we investigated the selectivity against fungal cells by testing the compounds endowed with the best MICs on Hep2 cells in order to assess their cell toxicity (CC_{50}) and we noticed that two derivatives were less cytotoxic than the reference drug clotrimazole. Moreover, a preliminary molecular modelling approach has been performed against lanosterol 14-α demethylase (CYP51A1) to rationalize the activity of the tested compounds and to specify the target protein or enzyme.

Keywords: Antimycotic effect; *Candida* spp.; Cytotoxicity; *N*-substituted thiazolidinones.

1. Introduction

Opportunistic infections caused by common fungi such as *Candida* species are increasing in the last decades, affecting the lifestyle of the patients in a manner that is proportional to the degree of the infection, which in turn depends on the state of health of the patient [1]. In this regard, apart from the mucocutaneous mycosis with a functional epithelial damage, in patients suffering from serious diseases such as cancer, AIDS, or in those who received transplantation therapy or a broad spectrum antibiotic therapy, the immune system is compromised and this could lead to systemic mycosis [2]. Among fungal infections, recent data showed that there are many cases caused by non-*albicans* species [3-5] such as *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. sakè*. Particularly, candidemia caused by *C. glabrata* is rising [6], but some epidemiologic studies revealed that systemic candidiasis also provoked by *C. albicans*, *C. tropicalis* and *C. parapsilosis* is emerging, especially in South America [7-10].

With regard to the therapeutic strategy, the most used drugs belong to the class of azoles because of their good profile in terms of safety and bioavailability, but an increasing incidence of drug resistance occurs, due to the massive use of these azole-based agents [11]. Therefore, it is of primary importance to discover new candidates as antimycotic agents to cope with these alarming data and to manage systemic candidiasis especially in immunocompromised patients. Among novel compounds, thiazolidinones have proven to possess several biological properties, including the antifungal activity [12-16]. Furthermore, in our previous works we reported different scaffolds of [4-(4'-substituted-phenyl)thiazol-2-yl]hydrazine derivatives that have shown to be active as anti-*Candida* agents at very low micromolar concentrations, with low cell toxicity and a synergistic action with clotrimazole (Scheme 1). In details, compounds containing aliphatic and cycloaliphatic groups at the hydrazonic portion and substituted phenyl rings at C4 of the thiazole showed very low MIC values especially towards *C. albicans* and *C. glabrata*. Similarly, other derivatives with heterocyclic and aromatic substituents at C2 such as furan, thiophene, pyridine, benzodioxole and naphthalene were very active towards *C. albicans* [17-21].

On the basis of these findings about the (thiazol-2-yl)hydrazine scaffold and taking into account the therapeutic importance of thiazolidinone nucleus, especially with regard to its antimycotic activity [22], we developed a large library of new compounds belonging to three different series (**1A-26A**, **1B-26B**, **1C-26C**) as promising anti-*Candida* agents combining three chemically different, but pharmacologically compatible moieties such as a thiazolidinone, a hydrazonic portion and an aromatic or a bicyclic substituent linked at the lactamic nitrogen (**Scheme 1**).

According to the established guidelines of Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST), we evaluated the susceptibility of twenty-two clinical *Candida* spp. strains to our compounds by determining their minimum inhibitory concentration (MIC) [23]. Finally, some of the compounds endowed with the best MICs were tested to assess their cell toxicity (CC_{50}) on Hep2 cells in order to evaluate their selectivity against fungal cells and we found that two derivatives were less cytotoxic than the conventional drug clotrimazole.

2. Chemistry

We designed novel 1,3-thiazolidin-4-one derivatives in which the portion supported by N1-hydrazine moiety consisted of aliphatic chains (C_3-C_8, linear, branched, unsaturated), cyclic structures, aromatic, bicyclic and (hetero)aromatic rings (furan, thiophene, benzene, pyridine, benzodioxole, and naphthalene), whereas the lactam NH at the 4-thiazolidinone nucleus was functionalized with electrophiles endowed with different steric features obtaining three scaffolds, each of twenty-six compounds, by reaction with 4-nitrobenzyl bromide (**A series**), 1-(chloromethyl)naphthalene (**B series**) and *N*-(chloromethyl)phthalimide (**C series**).

The synthetic strategy is outlined in **Scheme 2**. Several carbonyl compounds reacted directly with thiosemicarbazide in ethanol using catalytic amounts of acetic acid. The resulting thiosemicarbazones were cyclized with ethyl-bromoacetate in methanol and sodium acetate to give the 1,3-thiazolidin-4-one derivatives [24,25]. Finally, by the reaction between the thiazolidinones and 4-nitrobenzyl bromide, 1-(chloromethyl)naphthalene and *N*-(chloromethyl)phthalimide in

anhydrous acetone and potassium carbonate we obtained the *N*-functionalized 4-thiazolidinone derivatives (**1A-26A**, **1B-26B** and **1C-26C**). All the synthesized compounds were purified by column chromatography before characterization by spectroscopic methods (Experimental section) and elemental analysis (Supporting information).

3. Antibacterial and anti-*Candida* activity

Derivatives **1A-26A**, **1B-26B** and **1C-26C**, dissolved in dimethylsulfoxide (DMSO), were evaluated for their antibacterial activity. Organisms from routine clinical Gram-positive (*Staphylococcus aureus*, *Staphylococcus warneri*, *Streptococcus faecalis*, *Streptococcus α-hemolyticus*) and Gram-negative isolates (*Escherichia coli*, *Proteus mirabilis*, *Enterobacter* spp., *Klebsiella oxytoca*) from the respiratory tract were collected from specimens of patients at the Hospital 'Azienda Policlinico Umberto I°' (Sapienza University of Rome). The isolates were subcultured on a qualified medium to ensure purity. The isolates were identified by conventional methodologies; all isolates were subcultured to ensure optimal growth. The *in vitro* antibacterial activities of the compounds were determined by the broth microdilution method, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [26] with Mueller-Hinton II broth (BBL Microbiology Systems, Cockeysville, MD).

Once verified the absence of antibacterial effects, the antifungal activity was evaluated against twenty-two clinical fungal isolates of *Candida* spp. (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis* and *C. sakè*) and compared with topical and systemic reference drugs clotrimazole, fluconazole, ketoconazole, miconazole, tioconazole and amphotericin B (see **Tables 1-3**). The used clinical isolates were recently collected from specimens of patients at the 'Azienda Policlinico Umberto I°' (Sapienza University of Rome) and were obtained from haematology/oncology and surgery departments, which also included an intensive care unit. In particular, the samples were isolated from the upper and lower respiratory tract, blood, and indwelling venous catheters; the isolates were identified by conventional methodologies. Prior to testing, each isolate was subcultured on a qualified medium to ensure purity and optimal growth.

All derivatives were dissolved in DMSO. The *in vitro* antifungal activities were determined by the broth microdilution method with Sabouraud dextrose broth (BBL Microbiology Systems, Cockeysville, MD) as recommended by the NCCLS [27].

4. Cell toxicity

Some of the compounds endowed with the strongest antifungal effect against *Candida* spp., namely **1C**, **6A**, **6B**, **6C**, **7A** and **10A**, were also assayed at five concentrations ranging from 0.05 to 100 μg/mL (Supporting information) to evaluate their cytotoxic activity against a cultured cell line derived from a human laryngeal epidermoid carcinoma (Hep2) with respect to the reference drug (clotrimazole), as reported in **Table 4**. Cell viability was analyzed using Trypan Blue exclusion test [28].

5. Results and discussion

Three new large series of *N*-substituted-1,3-thiazolidin-4-one derivatives were synthesized in high yields and tested *in vitro* for their antimicrobial effect against several bacterial and fungal clinical isolates. None of the products had shown antibacterial activity, therefore the products demonstrated to be selectively active towards mycotic pathogens. According to the *in vitro* biological data, we observed that most of the compounds bearing aliphatic and cycloaliphatic moieties showed the best antifungal activity (MICs values of 1-2 μg/mL), similar or better than the reference drugs; only few derivatives with (hetero)aromatic moieties were effective, while compounds with benzodioxole and naphthalene linked to the hydrazine moiety were not active. In details, with regard to **A series** provided with a 4-nitrobenzyl substituent at the lactam NH, compound **1A** was active against *C. albicans* and *glabrata* at 4 μg/mL; among the (cyclo)aliphatic compounds, also derivatives **2A**-**4A**, **8A**-**12A**, **14A** and **18A** possessed MIC values of 2 μg/mL, while only the heterocyclic derivatives **19A** and **21A** and the aromatic derivative **22A** were active at low concentrations against *C.* non-*albicans* spp. Three compounds of this series had MICs of 1 μg/mL, displaying their antifungal effect at lower concentrations than those of the reference drugs: compound **6A** against *C. albicans* and *glabrata*, compound **7A** towards *C. glabrata*, and compound **10A** against *C. tropicalis*. In **B**

series, bearing a naphthalene nucleus linked to the lactam nitrogen, compound **1B** was biologically active towards all the *Candida* spp. (MICs of 2-4 µg/mL), also derivatives **2B-15B** and **18B** had anti-*Candida* effect at 2 µg/mL but not against all the studied species. The branched aliphatic compound **6B** possessed a minimum inhibitory concentration of 1 µg/mL against *C. glabrata*. Similarly to **A series**, some (hetero)aromatic compounds were effective: the furan derivative **19B** was active against *C.* non-*albicans* spp. while the thiophene derivative **21B** and the benzene derivative **22B** were effective also towards *C. albicans*. Unlike **A series**, in this series the pyridine derivative **24B** was active with the MIC value of 2 µg/mL against all the species. Finally, in **C series** we observed the same trend of the previous **A** and **B** series: compounds **1C** and **6C** had MIC values of 1 µg/mL against *C.* non-*albicans* spp. and were effective also against *C. albicans* (MIC = 4 µg/mL and MIC = 2 µg/mL, respectively); most of the (cyclo)aliphatic derivatives and compounds **19C**, **21C**, **22C** and **24C** were good antifungal agents even if not against all *Candida* species. In consideration of these results, some of the most active derivatives against *Candida albicans* and non-*albicans* (**1C**, **6A**, **6B**, **6C**, **7A** and **10A**,) were tested for their cytotoxic effects (CC_{50}) by Trypan blue exclusion test at five different concentrations, and compared to clotrimazole (**Table 4**). While compound **1C** was cytotoxic at 22 µg/mL and compounds **6A** and **7A** showed CC_{50} values similar to the one of clotrimazole (32 µg/mL for **6A** and 35 µg/mL for **7A**), the less cytotoxic derivatives were compounds **6B** and **6C** with CC_{50} values of 95 µg/mL and 99 µg/mL, respectively. These two molecules demonstrated to be very selective against fungal species, especially against *C. glabrata*, since they displayed cytotoxicity towards human cells at much higher concentrations than those required for the pharmacological effect towards fungal cells (the selectivity index, defined as the ratio between CC_{50} and MIC value for *C. glabrata*, was 95 for **6B** and 99 for **6C**).

To conclude, we designed and synthesized three series of new *N*-substituted thiazolidinone derivatives and we found that many compounds have proven to be good and selective antifungal agents. Regarding two active compounds (**6B** and **6C**), we observed that they possessed a very low

toxicity against human cells with respect to clotrimazole. Therefore, these derivatives can be taken into account as new lead compounds for the development of promising antifungal drugs.

6. Molecular modeling studies

Determining the molecular targets of the tested compounds either by biological assays or by molecular modelling studies is an extremely difficult task since organisms consists of thousands of proteins and other macromolecules (e.g., DNA and lipids) that can interact with the compounds under investigation. We focused on three enzymes that are important targets for well established antifungal drugs, *i.e.*, lanosterol 14-α demethylase (CYP51A1), 1,3-β-D-glucan synthase and squalene-epoxidase. The sequences of the three enzymes were obtained for *C. albicans* and a default BLAST search was performed to identify crystal structures of related enzymes in the Brookhaven Protein Databank (PDB server). No structures were obtained for the latter two enzymes, but a high resolution crystal structure of lanosterol 14-α demethylase was obtained for *Saccharomyces cerevisiae* in complex with lanosterol (pdb code: 4lxj; 1.90 Å; 65% sequence identity to *C. albicans* enzyme).

Afterwards, the sequences of lanosterol 14-α demethylase from *C. glabrata*, *C. parapsilosis* and *C. tropicalis* were obtained and the sequences alignments were created for all four *Candida* enzymes and *Saccharomyces cerevisiae* enzyme. All sequence identities are higher than 60% and the residue conservation in the binding pocket (defined as all residues within 4.5 Å of lanosterol) of the enzymes are high (Table 5). Therefore, *Saccharomyces cerevisiae* lanosterol 14-α demethylase was used as a model protein for docking studies of a representative set of compounds (*i.e.*, compounds **1A**, **1B**, **1C** and **20C**). The endogenous substrate of lanosterol 14-α demethylase is the hydrophobic lanosterol and the ligand-protein binding interactions are mostly hydrophobic in nature. Van-der-Waals interactions are formed with the side chains of Tyr126, Leu129, Phe134, Phe236, Pro238, Phe241, Leu380, Phe384 and Met509 (Figure 1A). Similarly, docking studies suggests that the synthesized compounds could also form mainly Van-der-Waals interactions with the hydrophobic binding pocket (Figure 1B) similarly to lanosterol.

7. Experimental section

The chemicals, solvents for synthesis, and spectral grade solvents were purchased from Aldrich (Italy) and used without further purification. Melting points (uncorrected) were determined automatically on an FP62 apparatus (Mettler-Toledo). ^{1}H NMR spectra were recorded at 400 MHz on a Bruker spectrometer using $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts are expressed as δ units (parts per millions) relative to the solvent peak. Coupling constants *J* are valued in Hertz (Hz). IR spectra (neat) were registered on a Perkin Elmer FT-IR Spectrometer Spectrum 1000. Elemental analyses for C, H, and N were recorded on a Perkin-Elmer 240 B microanalyzer and the analytical results were within ± 0.4% of the theoretical values for all compounds. All reactions were monitored by TLC performed on 0.2 mm thick silica gel plates (60 F_{254} Merck).

In general, the IR spectrum (neat) for derivatives **1A-26A**, **1B-26B**, and **1C-26C** showed a band at about 3027 cm^{-1} due to the stretching of C_{sp2}-H, at about 1693 due to the stretching of C=O bond, at about 1622 for the stretching of C=N, and at about 1582 and 1447 for C=C stretching.

*7.1. General procedure for the synthesis of compounds **1A-26A**, **1B-26B** and **1C-26C**.*

The initial carbonyl compound (50 mmol) was dissolved/suspended in ethanol (50 mL) and magnetically stirred with thiosemicarbazide (50 mmol) and catalytic amounts of acetic acid for 8-24 h at room temperature. The obtained thiosemicarbazone was filtered, washed with appropriate solvent (*n*-hexane, petroleum ether or diethyl ether) and dried under vacuum. The intermediate thiosemicarbazone (50 mmol) reacted with ethyl-bromoacetate (50 mmol), in methanol (50 mL) and sodium acetate (50 mmol) at room temperature under magnetic stirring for 24 h. The resulting 4-thiazolidinone was poured on ice, filtered or extracted with chloroform (3 x 100 mL) and purified by column chromatography (SiO_2, ethyl acetate/*n*-hexane). Then, the obtained thiazolidinone (50 mmol) was dissolved/suspended in 50 mL of anhydrous acetone in the presence of anhydrous potassium carbonate (50 mmol), and reacted with equimolar amounts of 4-nitrobenzyl bromide, 1-(chloromethyl)naphthalene and *N*-(chloromethyl)phthalimide for 24-48 h. The product was poured

on ice, filtered or extracted with chloroform (3 x 50 mL) and purified by column chromatography (SiO_2, ethyl acetate/*n*-hexane) in order to obtain the title compounds in high yields.

*7.1.1. 3-(4-Nitrobenzyl)-2-(2-(propan-2-ylidene)hydrazono)thiazolidin-4-one (**1A**).*

White powder, mp 157-163 °C, 79% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.00 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 3.82 (s, 2H, CH_2, thiazolidinone), 5.05 (s, 2H, $ArCH_2$), 7.60-7.62 (d, J = 8.4 Hz, 2H, Ar), 8.18-8.20 (d, J = 8.4 Hz, 2H, Ar).

*7.1.2. 3-(4-Nitrobenzyl)-2-(2-(butan-2-ylidene)hydrazono)thiazolidin-4-one (**2A**).*

Yellow powder, mp 108-112 °C, 75% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.01-1.05 (t, 3H, CH_3), 1.85 (s, 3H, CH_3), 2.23-2.29 (q, 2H, CH_2), 4.01 (s, 2H, CH_2, thiazolidinone), 4.98 (s, 2H, $ArCH_2$), 7.59-7.61 (d, J = 8.4 Hz, 2H, Ar), 8.20-8.22 (d, J = 8.4 Hz, 2H, Ar).

*7.1.3. 3-(4-Nitrobenzyl)-2-(2-(pentan-2-ylidene)hydrazono)thiazolidin-4-one (**3A**).*

Yellow powder, mp 103-105 °C, 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96-1.00 (t, 3H, CH_3), 1.59-1.68 (q, 2H, CH_2), 1.97 (s, 3H, CH_3), 2.30-2.34 (t, 2H, CH_2), 3.81 (s, 2H, CH_2, thiazolidinone), 5.05 (s, 2H, $ArCH_2$), 7.61-7.63 (d, J = 8.8 Hz, 2H, Ar), 8.19-8.21 (d, J = 8.8 Hz, 2H, Ar).

*7.1.4. 3-(4-Nitrobenzyl)-2-(2-(pentan-3-ylidene)hydrazono)thiazolidin-4-one (**4A**).*

Yellow powder, mp 95-96 °C, 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.98-1.02 (t, 3H, CH_3), 1.14-1.17 (t, 3H, CH_3), 2.35-2.45 (q, 4H, 2 x CH_2), 3.81 (s, 2H, CH_2, thiazolidinone), 5.05 (s, 2H, $ArCH_2$), 7.60-7.62 (d, J = 8.4 Hz, 2H, Ar), 8.18-8.20 (d, J = 8.4 Hz, 2H, Ar).

*7.1.5. 3-(4-Nitrobenzyl)-2-(2-(hex-5-en-2-ylidene)hydrazono)thiazolidin-4-one (**5A**).*

Grey powder, mp 110-111 °C, 83% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.85 (s, 3H, CH_3), 2.25-2.29 (m, 2H, CH_2), 2.33-2.36 (m, 2H, CH_2) 4.01 (s, 2H, CH_2, thiazolidinone), 4.94-4.98 (m, 1H, $=CH_2$), 4.99 (s, 2H, $ArCH_2$), 5.03-5.07 (d, J = 16.8 Hz, 1H, $=CH_2$) 5.79-5.89 (m, 1H, CH=), 7.59-7.61 (d, J = 8.4 Hz, 2H, Ar), 8.20-8.22 (d, J = 8.4 Hz, 2H, Ar).

*7.1.6. 3-(4-Nitrobenzyl)-2-(2-(4-methylpentan-2-ylidene)hydrazono)thiazolidin-4-one (**6A**).*

Yellow powder, mp 70-75 °C, 69% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95-0.97 (d, 6H, 2 x CH_3), 1.95 (s, 3H, CH_3), 2.01-2.04 (m, 1H, CH), 2.21-2.23 (d, 2H, CH_2), 3.81 (s, 2H, CH_2,

thiazolidinone), 5.05 (s, 2H, $ArCH_2$), 7.61-7.63 (d, *J* = 8.0 Hz, 2H, Ar), 8.19-8.21 (d, *J* = 8.0 Hz, 2H, Ar).

*7.1.7. 3-(4-Nitrobenzyl)-2-(2-(heptan-2-ylidene)hydrazono)thiazolidin-4-one (**7A**).*

Yellow powder, mp 64-69 °C, 72% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87-0.98 (m, 3H, CH_3), 1.27-1.42 (m, 4H, CH_2), 1.52-1.61 (m, 2H, CH_2), 1.97 (s, 3H, CH_3), 2.32-2.36 (t, 2H, CH_2), 3.82 (s, 2H, CH_2, thiazolidinone), 5.05 (s, 2H, $ArCH_2$), 7.61-7.63 (d, *J* = 8.0 Hz, 2H, Ar), 8.19-8.21 (d, *J* = 8.0 Hz, 2H, Ar).

*7.1.8. 3-(4-Nitrobenzyl)-2-(2-(heptan-3-ylidene)hydrazono)thiazolidin-4-one (**8A**).*

Yellow oil, 63% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86-0.89 (t, 3H, CH_3), 0.93-1.01 (m, 2H, CH_2), 1.13-1.18 (m, 1H, CH_2), 1.22-1.31 (m, 2H, CH_2), 1.35-1.42 (m, 1H, CH_2), 1.54-1.62 (m, 1H, CH_2), 2.31-2.43 (m, 1H, CH_2, 3H, CH_3), 3.81 (s, 2H, CH_2, thiazolidinone), 5.07 (s, 2H, $ArCH_2$), 7.56-7.61 (m, 2H, Ar), 8.18-8.20 (d, *J* = 8.8 Hz, 2H, Ar).

*7.1.9. 3-(4-Nitrobenzyl)-2-(2-(octan-2-ylidene)hydrazono)thiazolidin-4-one (**9A**).*

Yellow powder, mp 64-70 °C, 69% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91-0.92 (t, 3H, CH_3), 1.30-1.36 (m, 6H, CH_2), 1.55-1.60 (m, 2H, CH_2), 1.97 (s, 3H, CH_3), 2.31-2.35 (t, 2H, CH_2), 3.81 (s, 2H, CH_2, thiazolidinone), 5.05 (s, 2H, $ArCH_2$), 7.61-7.63 (d, *J* = 8.4 Hz, 2H, Ar), 8.19-8.21 (d, *J* = 8.4 Hz, 2H, Ar).

*7.1.10. 3-(4-Nitrobenzyl)-2-(2-cyclopentylidenehydrazono)thiazolidin-4-one (**10A**).*

Yellow powder, mp 173-175 °C, 87% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.69-1.71 (m, 4H, cyclopentane), 2.32-2.36 (m, 4H, cyclopentane), 4.02 (s, 2H, CH_2, thiazolidinone), 4.96 (s, 2H, $ArCH_2$), 7.59-7.61 (d, *J* = 8.8 Hz, 2H, Ar), 8.20-8.22 (d, *J* = 8.8 Hz, 2H, Ar).

*7.1.11. 3-(4-Nitrobenzyl)-2-(2-(2-methylcyclopentylidene)hydrazono)thiazolidin-4-one (**11A**).*

Yellow powder, mp 130-133 °C, 78% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37-1.39 (d, *J* = 7.6 Hz, 3H, CH_3), 1.35-1.39 (m, 1H, cyclopentane), 1.65-1.71 (m, 1H, cyclopentane), 1.88-1.92 (m, 1H, cyclopentane), 2.05-2.09 (m, 1H, cyclopentane), 2.38-2.43 (m, 1H, cyclopentane), 2.55-2.62 (m,

2H, cyclopentane), 3.81 (s, 2H, CH_2, thiazolidinone), 5.03 (s, 2H, $ArCH_2$), 7.62-7.64 (d, *J* = 8.4 Hz, 2H, Ar), 8.19-8.21 (d, *J* = 8.4 Hz, 2H, Ar).

*7.1.12. 3-(4-Nitrobenzyl)-2-(2-(3-methylcyclopentylidene)hydrazono)thiazolidin-4-one (**12A**).*

Yellow powder, mp 206-210 °C, 84% yield; ^1H NMR (400 MHz, $CDCl_3$): δ 1.09-1.11 (d, 3H, CH_3), 1.36-1.41 (m, 1H, cyclopentane), 1.93-2.01 (m, 1H, cyclopentane), 2.07-2.20 (m, 2H, cyclopentane), 2.33-2.43 (m, 1H, cyclopentane), 2.61-2.72 (m, 2H, cyclopentane), 3.83 (s, 2H, CH_2, thiazolidinone), 5.03 (s, 2H, $ArCH_2$), 7.61-7.63 (d, *J* = 8 Hz, 2H, Ar), 8.19-8.21 (d, *J* = 8 Hz, 2H, Ar).

*7.1.13. 3-(4-Nitrobenzyl)-2-(2-cyclohexylidenehydrazono)thiazolidin-4-one (**13A**).*

Grey powder, mp 147-150 °C, 81% yield; ^1H NMR (400 MHz, $CDCl_3$): δ 1.66 (bs, 4H, cyclohexane), 1.76 (bs, 2H, cyclohexane), 2.37-2.40 (m, 2H, cyclohexane), 2.59 (bs, 2H, cyclohexane), 3.82 (s, 2H, CH_2, thiazolidinone), 5.05 (s, 2H, $ArCH_2$), 7.59-7.61 (d, *J* = 8.4 Hz, 2H, Ar), 8.18-8.20 (d, *J* = 8.4 Hz, 2H, Ar).

*7.1.14. 3-(4-Nitrobenzyl)-2-(2-(2-methylcyclohexylidene)hydrazono)thiazolidin-4-one (**14A**).*

Yellow powder, mp 136-138 °C, 80% yield; ^1H NMR (400 MHz, $CDCl_3$): δ 1.16-1.18 (d, *J* = 6.4 Hz, 3H, CH_3), 1.35-1.47 (m, 3H, cyclohexane), 1.78-1.82 (m, 2H, cyclohexane), 1.95-2.00 (m, 2H, cyclohexane), 2.41-2.44 (m, 1H, cyclohexane), 3.13-3.17 (m, 1H, cyclohexane), 3.80 (s, 2H, CH_2, thiazolidinone), 5.05 (s, 2H, $ArCH_2$), 7.60-7.62 (d, *J* = 8.4 Hz, 2H, Ar), 8.18-8.20 (d, *J* = 8.4 Hz, 2H, Ar).

*7.1.15. 3-(4-Nitrobenzyl)-2-(2-(4-methylcyclohexylidene)hydrazono)thiazolidin-4-one (**15A**).*

White powder, mp 123-128 °C, 78% yield; ^1H NMR (400 MHz, $CDCl_3$): δ 0.97-0.99 (d, *J* = 6.4 Hz, 3H, CH_3), 1.08-1.18 (m, 1H, cyclohexane), 1.21-1.31 (m, 1H, cyclohexane), 1.69-1.76 (m, 1H, cyclohexane), 1.85-1.98 (m, 3H, cyclohexane), 2.24-2.32 (m, 1H, cyclohexane), 2.53-2.56 (m, 1H, cyclohexane), 3.24-3.28 (m, 1H, cyclohexane), 3.81 (s, 2H, CH_2, thiazolidinone), 5.05 (s, 2H, $ArCH_2$), 7.59-7.61 (d, *J* = 8.8 Hz, 2H, Ar), 8.18-8.20 (d, *J* = 8.8 Hz, 2H, Ar).

*7.1.16. 3-(4-Nitrobenzyl)-2-(2-cycloheptylidenehydrazono)thiazolidin-4-one (**16A**).*

Yellow powder, mp 106-110 °C, 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.58-1.71 (m, 8H, cycloheptane), 2.54-2.62 (m, 4H, cycloheptane), 3.81 (s, 2H, CH_2, thiazolidinone), 5.04 (s, 2H, $ArCH_2$), 7.61-7.63 (d, J = 8.8 Hz, 2H, Ar), 8.18-8.20 (d, J = 8.8 Hz, 2H, Ar).

*7.1.17. 3-(4-Nitrobenzyl)-2-(2-cyclooctylidenehydrazono)thiazolidin-4-one (**17A**).*

Yellow powder, mp 124-126 °C, 87% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37-1.44 (m, 4H, cyclooctane), 1.55-1.64 (m, 4H, cyclooctane), 1.83-1.84 (m, 2 H, cyclooctane), 2.44-2.50 (m, 4H, cyclooctane), 3.83 (s, 2H, CH_2, thiazolidinone), 5.05 (s, 2H, $ArCH_2$), 7.60-7.62 (d, J = 8 Hz, 2H, Ar), 8.18-8.20 (d, J = 8 Hz, 2H, Ar).

*7.1.18. 3-(4-Nitrobenzyl)-2-(2-(1-cyclohexylethylidene)hydrazono)thiazolidin-4-one (**18A**).*

Yellow powder, mp 130-133 °C, 85% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25-1.35 (m, 5H, cyclohexane), 1.57-1.73 (m, 8H, cyclohexane), 1.82-1.89 (m, 1H, cyclohexane), 4.02 (s, 2H, CH_2, thiazolidinone), 4.98 (s, 2H, $ArCH_2$), 7.58-7.60 (d, J = 8 Hz, 2H, Ar), 8.20-8.22 (d, J = 8 Hz, 2H, Ar).

*7.1.19. 3-(4-Nitrobenzyl)-2-(2-(furan-2-ylmethylene)hydrazono)thiazolidin-4-one (**19A**).*

Yellow powder, mp 150-155 °C, 76% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.09 (s, 2H, CH_2, thiazolidinone), 5.03 (s, 2H, $ArCH_2$), 6.65-6.67 (m, 1H, furan), 6.98-6.99 (d, J = 3.2 Hz, 1H, furan), 7.61-7.63 (d, J = 8.8 Hz, 2H, Ar), 7.90 (s, 1H, furan), 8.21-8.23 (d, J = 8.8 Hz, 2H, Ar), 8.26 (s, 1H, CH=).

*7.1.20. 3-(4-Nitrobenzyl)-2-(2-(1-(furan-2-yl)ethylidene)hydrazono)thiazolidin-4-one (**20A**).*

Yellow powder, mp 182-187 °C, 82% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.33 (s, 3H, CH_3), 3.87 (s, 2H, CH_2, thiazolidinone), 5.12 (s, 2H, $ArCH_2$), 6.51 (bs, 1H, furan), 6.90-6.91 (m, 1H, furan), 7.56-7.61 (m, 1H, furan), 7.63-7.65 (d, J = 8 Hz, 2H, Ar), 8.20-8.22 (d, J = 8 Hz, 2H, Ar).

*7.1.21. 3-(4-Nitrobenzyl)-2-(2-(1-(thiophen-2-yl)ethylidene)hydrazono)thiazolidin-4-one (**21A**).*

Yellow powder, mp 160-164 °C, 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.42 (s, 3H, CH_3), 3.86 (s, 2H, CH_2, thiazolidinone), 5.11 (s, 2H, $ArCH_2$), 7.08 (bs, 1H, thiophene), 7.40 (m, 2H, thiophene), 7.63-7.66 (m, 2H, Ar), 8.20-8.22 (m, 2H, Ar).

*7.1.22. 3-(4-Nitrobenzyl)-2-(2-(1-phenylethylidene)hydrazono)thiazolidin-4-one (**22A**).*

Yellow powder, mp 152-155 °C, 85% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.43 (s, 3H, CH_3), 3.86 (s, 2H, CH_2, thiazolidinone), 5.14 (s, 2H, $ArCH_2$), 7.42-7.44 (m, 3H, Ar), 7.66-7.68 (d, J = 8.8 Hz, 2H, Ar), 7.87-7.89 (m, 2H, Ar), 8.21-8.23 (d, J = 8.8 Hz, 2H, Ar).

*7.1.23. 3-(4-Nitrobenzyl)-2-(2-(pyridin-3-ylmethylene)hydrazono)thiazolidin-4-one (**23A**).*

Red powder, mp 231-233 °C, 83% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.15 (s, 2H, CH_2, thiazolidinone), 5.06 (s, 2H, $ArCH_2$), 7.47-7.54 (m, 1H, Ar), 7.62-7.64 (d, J = 8.0 Hz, 2H, Ar), 8.14-8.18 (m, 1H, Ar), 8.22-8.24 (d, J = 8.0 Hz, 2H, Ar), 8.51 (s, 1H, CH=), 8.62-8.70 (m, 1H, Ar), 8.91 (s, 1H, Ar).

*7.1.24. 3-(4-Nitrobenzyl)-2-(2-(1-(pyridin-2-yl)ethylidene)hydrazono)thiazolidin-4-one (**24A**).*

Yellow powder, mp 227-230 °C, 86% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.40 (s, 3H, CH_3), 4.12 (s, 2H, CH_2, thiazolidinone), 5.09 (s, 2H, $ArCH_2$), 7.44-7.47 (m, 1H, Ar), 7.65-7.67 (d, J = 8.4 Hz, 2H, Ar), 7.86-7.90 (m, 1H, Ar), 8.05-8.07 (d, J = 8.0 Hz, 1H, Ar), 8.23-8.25 (d, J = 8.4 Hz, 2H, Ar), 8.63-8.64 (m, 1H, Ar).

*7.1.25. 3-(4-Nitrobenzyl)-2-(2-(benzo[d][1,3]dioxol-5-ylmethylene)hydrazono)thiazolidin-4-one (**25A**).*

Grey powder, mp 185-187 °C, 79% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.09 (s, 2H, CH_2, thiazolidinone), 5.03 (s, 2H, $ArCH_2$), 6.10 (s, 2H, CH_2 benzodioxole), 7.00-7.02 (d, J = 7.6 Hz, 2H, Ar), 7.25-7.30 (m, 2H, Ar), 7.60-7.62 (d, J = 8.8 Hz, 2H, Ar), 8.21-8.23 (d, J = 8.0 Hz, 1H, Ar), 8.34 (s, 1H, CH=).

*7.1.26. 3-(4-Nitrobenzyl)-2-(2-(naphthalen-1-ylmethylene)hydrazono)thiazolidin-4-one (**26A**).*

Yellow powder, mp 185-191 °C, 84% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.92 (s, 2H, CH_2, thiazolidinone), 5.17 (s, 2H, $ArCH_2$), 7.52-7.58 (m, 2H, Ar), 7.60-7.66 (m, 1H, Ar), 7.68-7.70 (d, J = 8.0 Hz, 2H, Ar), 7.92-7.98 (m, 3H, Ar), 8.20-8.22 (d, J = 8.0 Hz, 2H, Ar), 8.93-8.95 (d, J = 8.0 Hz, 1H, Ar), 9.05 (s, 1H, CH=).

*7.1.27. 3-(Naphthalen-1-ylmethyl)-2-(2-(propan-2-ylidene)hydrazono)thiazolidin-4-one (**1B**).*

Orange oil, 67% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.67 (s, 3H, CH_3), 1.81 (s, 3H, CH_3), 3.60 (s, 2H, CH_2, thiazolidinone), 5.23 (s, 2H, $ArCH_2$), 7.17-7.21 (t, 1H, Ar), 7.23-7.36 (m, 3H, Ar), 7.56-7.58 (d, *J* = 8.0 Hz, 2H, Ar), 8.05-8.07 (d, *J* = 8.0 Hz, 1H, Ar).

*7.1.28. 2-(2-(Butan-2-ylidene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**2B**).*

Orange oil, 63% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12-1.20 (m, 3H, CH_3), 1.89 (s, 3H, CH_3), 2.30-2.38 (m, 2H, CH_2), 3.83 (s, 2H, CH_2, thiazolidinone), 5.48 (s, 2H, $ArCH_2$), 7.41-7.45 (m, 1H, Ar), 7.50-7.59 (m, 3H, Ar), 7.80-7.82 (d, *J* = 8.0 Hz, 1H, Ar), 7.88-7.90 (d, *J* = 8.0 Hz, 1H, Ar), 8.29-8.32 (m, 1H, Ar).

*7.1.29. 3-(Naphthalen-1-ylmethyl)-2-(2-(pentan-2-ylidene)hydrazono)thiazolidin-4-one (**3B**).*

Orange oil, 65% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95-0.98 (t, 3H, CH_3), 1.28-1.36 (m, 2H, CH_2), 1.87 (s, 3H, CH_3), 2.27-2.31 (t, 2H, CH_2), 3.83 (s, 2H, CH_2, thiazolidinone), 5.47 (s, 2H, $ArCH_2$), 7.41-7.63 (m, 4H, Ar), 7.80-7.93 (m, 2H, Ar), 8.27-8.32 (m, 1H, Ar).

*7.1.30. 3-(Naphthalen-1-ylmethyl)-2-(2-(pentan-3-ylidene)hydrazono)thiazolidin-4-one (**4B**).*

Orange oil, 67% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87-0.91 (t, 3H, CH_3), 1.16-1.20 (t, 3H, CH_3), 2.31-2.39 (q, 4H, 2 x CH_2), 3.79 (s, 2H, CH_2, thiazolidinone), 5.48 (s, 2H, $ArCH_2$), 7.43-7.47 (t, 1H, Ar), 7.51-7.61 (m, 3H, Ar), 7.81-7.83 (d, *J* = 8.4 Hz, 1H, Ar), 7.89-7.91 (d, *J* = 8.0 Hz, 1H, Ar), 8.33-8.35 (d, *J* = 8.0 Hz, 1H, Ar).

*7.1.31. 2-(2-(Hex-5-en-2-ylidene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**5B**).*

Orange oil, 63% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.89 (s, 3H, CH_3), 2.35-2.43 (m, 4H, CH_2), 3.83 (s, 2H, CH_2, thiazolidinone), 5.01-5.10 (m, 2H, $=CH_2$), 5.47 (s, 2H, $ArCH_2$), 5.83-5.93 (m, 1H, CH=), 7.41-7.45 (t, 1H, Ar), 7.50-7.58 (m, 3H, Ar), 7.80-7.82 (d, *J* = 8.4 Hz, 1H, Ar), 7.88-7.90 (d, *J* = 8.4 Hz, 1H, Ar), 8.30-8.32 (d, *J* = 8.4 Hz, 1H, Ar).

*7.1.32. 2-(2-(4-Methylpentan-2-ylidene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**6B**).*

Orange oil, 61% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.64-0.66 (d, 6H, 2xCH_3), 1.74-1.81 (m, 1H, CH), 2.01 (s, 3H, CH_3), 2.19-2.21 (d, 2H, CH_2), 3.83 (s, 2H, CH_2, thiazolidinone), 5.46 (s, 2H, ArCH_2), 7.41-7.49 (m, 1H, Ar), 7.50-7.60 (m, 3H, Ar), 7.80-7.92 (m, 1H, Ar), 8.15-8.17 (d, *J* = 8.0 Hz, 1H, Ar), 8.25-8.27 (d, *J* = 8.0 Hz, 1H, Ar).

*7.1.33. 2-(2-(Heptan-2-ylidene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**7B**).*

White powder, mp 148-153, 77% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.58-1.59 (m, 2H CH_2, 6H CH_3), 1.72-1.74 (m, 2H, CH_2), 2.35-2.38 (t, 2H, CH_2), 2.48-2.51 (t, 2H, CH_2), 3.84 (s, 2H, CH_2, thiazolidinone), 5.47 (s, 2H, ArCH_2), 7.40-7.45 (t, 1H, Ar), 7.50-7.58 (m, 3H, Ar), 7.80-7.82 (d, *J* = 8.0 Hz, 1H, Ar), 7.88-7.90 (d, *J* = 8.0 Hz, 1H, Ar), 8.30-8.32 (d, *J* = 8.0 Hz, 1H, Ar).

*7.1.34. 2-(2-(Heptan-3-ylidene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**8B**).*

Orange oil, 65% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83-0.87 (t, 3H, CH_3), 0.92-0.96 (t, 3H, CH_3), 1.23-1.37 (m, 5H, CH_2), 2.26-2.34 (m, 3H, CH_2), 3.84 (s, 2H, CH_2, thiazolidinone), 5.46 (s, 2H, ArCH_2), 7.40-7.58 (m, 4H, Ar), 7.80-7.92 (m, 2H, Ar), 8.21-8.27 (m, 1H, Ar).

*7.1.35. 3-(Naphthalen-1-ylmethyl)-2-(2-(octan-2-ylidene)hydrazono)thiazolidin-4-one (**9B**).*

Pink powder, mp 64-68 °C, 82% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90-0.94 (t, 3H, CH_3), 1.03-1.08 (m, 1H, CH_2), 1.24-1.32 (m, 5H, CH_2), 1.53-1.58 (m, 1H, CH_2), 1.88 (s, 3H, CH_3), 2.02 (bs, 1H, CH_2), 2.28-2.33 (m, 2H, CH_2), 3.83 (s, 2H, CH_2, thiazolidinone), 5.47 (s, 2H, ArCH_2), 7.41-7.58 (m, 4H, Ar), 7.80-7.82 (d, *J* = 8.0 Hz, 1H, Ar), 7.88-7.90 (d, *J* = 8.0 Hz, 1H, Ar), 8.30-8.33 (d, *J* = 8.0 Hz, 1H, Ar).

*7.1.36. 2-(2-Cyclopentylidenehydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**10B**).*

Orange powder, mp 60-65 °C, 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27-1.35 (m, 1H, cyclopentane), 1.73-1.79 (m, 3H, cyclopentane), 2.36-2.40 (m, 2H, cyclopentane), 2.47-2.50 (m, 2H, cyclopentane), 3.84 (s, 2H, CH_2, thiazolidinone), 5.45 (s, 2H, ArCH_2), 7.41-7.45 (t, 1H, Ar), 7.48-7.59 (m, 3H, Ar), 7.80-7.82 (d, *J* = 8.0 Hz, 1H, Ar), 7.86-7.92 (m, 1H, Ar), 8.32-8.34 (d, *J* = 8.0 Hz, 1H, Ar).

*7.1.37. 2-(2-(2-Methylcyclopentylidene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**11B**).*

Yellow powder, mp 95-96 °C, 94% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20-1.21 (d, J = 6.4 Hz, 3H, CH_3), 1.28-1.36 (m, 1H, cyclopentane), 1.59-1.64 (m, 1H, cyclopentane), 1.79-1.85 (m, 1H, cyclopentane), 2.03-2.07 (m, 1H, cyclopentane), 2.26-2.36 (m, 1H, cyclopentane), 2.47-2.60 (m, 2H, cyclopentane), 3.82 (s, 2H, CH_2, thiazolidinone), 5.45 (s, 2H, $ArCH_2$), 7.41-7.45 (t, 1H, Ar), 7.50-7.61 (m, 3H, Ar), 7.80-7.82 (d, *J* = 8.4 Hz, 1H, Ar), 7.87-7.89 (d, *J* = 8.0 Hz, 1H, Ar), 8.33-8.35 (d, *J* = 8.4 Hz, 1H, Ar).

*7.1.38. 2-(2-(3-Methylcyclopentylidene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**12B**).*

Red oil, 64% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.05-1.08 (d, 3H, CH_3), 1.27-1.35 (m, 4H, cyclopentane), 2.25-2.34 (m, 1H, cyclopentane), 2.46-2.66 (m, 2H, cyclopentane), 3.84 (s, 2H, CH_2, thiazolidinone), 5.45 (s, 2H, $ArCH_2$), 7.41-7.45 (t, 1H, Ar), 7.48-7.57 (m, 3H, Ar), 7.81-7.83 (d, *J* = 8.0 Hz, 1H, Ar), 7.88-7.90 (d, *J* = 8.0 Hz, 1H, Ar), 8.28-8.34 (m, 1H, Ar).

*7.1.39. 2-(2-Cyclohexylidenehydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**13B**).*

Yellow powder, mp 148-150 °C, 91% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.57-1.62 (m, 3H, cyclohexane), 1.71-1.74 (m, 2H, cyclohexane), 2.20 (s, 1H, cyclohexane), 2.35-2.38 (t, 2H, cyclohexane), 2.48-2.51 (t, 2H, cyclohexane), 3.83 (s, 2H, CH_2, thiazolidinone), 5.47 (s, 2H, $ArCH_2$), 7.40-7.44 (t, 1H, Ar), 7.50-7.58 (m, 3H, Ar), 7.80-7.82 (d, *J* = 8.0 Hz, 1H, Ar), 7.88-7.90 (d, *J* = 7.6 Hz, 1H, Ar), 8.30-8.32 (d, *J* = 8.0 Hz, 1H, Ar).

*7.1.40. 2-(2-(2-Methylcyclohexylidene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**14B**).*

Yellow powder, mp 132-133 °C, 96% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22-1.23 (d, *J* = 6.4 Hz, 3H, CH_3), 1.28-1.44 (m, 2H, cyclohexane), 1.47-1.53 (m, 1H, cyclohexane), 1.74-1.76 (m, 2H, cyclohexane), 1.87-1.95 (m, 2H, cyclohexane), 2.37-2.42 (m, 1H, cyclohexane), 3.05-3.08 (m, 1H, cyclohexane), 3.81 (s, 2H, CH_2, thiazolidinone), 5.47 (s, 2H, $ArCH_2$), 7.40-7.44 (t, 1H, Ar), 7.49-

7.57 (m, 3H, Ar), 7.79-7.81 (d, *J* = 8.0 Hz, 1H, Ar), 7.87-7.89 (d, *J* = 7.6 Hz, 1H, Ar), 8.31-8.33 (d, *J* = 7.6 Hz, 1H, Ar).

*7.1.41. 2-(2-(4-Methylcyclohexylidene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**15B**).*

Brown oil, 60% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22-1.23 (d, 3H, CH_3), 1.28-1.39 (m, 1H, cyclohexane), 1.46-1.58 (m, 1H, cyclohexane), 1.86-2.20 (m, 4H, cyclohexane), 2.48-2.56 (m, 1H, cyclohexane), 2.79-2.82 (m, 1H, cyclohexane), 3.44-3.48 (m, 1H, cyclohexane), 4.11 (s, 2H, CH_2, thiazolidinone), 5.74 (s, 2H, $ArCH_2$), 7.68-7.72 (t, 1H, Ar), 7.77-7.85 (m, 3H, Ar), 8.07-8.09 (d, *J* = 8.4 Hz, 1H, Ar), 8.15-8.17 (d, *J* = 7.6 Hz, 1H, Ar), 8.58-8.60 (d, *J* = 8.4 Hz, 1H, Ar).

*7.1.42. 2-(2-Cycloheptylidenehydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**16B**).*

Brown oil, 65% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89-0.98 (m, 3H, cycloheptane), 1.22-1.33 (m, 1H, cycloheptane), 1.34-1.37 (m, 3H, cycloheptane), 1.62-1.76 (m, 4H, cycloheptane), 2.45-2.48 (m, 1H, cycloheptane), 3.95 (s, 2H, CH_2, thiazolidinone), 5.62 (s, 2H, $ArCH_2$), 7.41-7.63 (m, 4H, Ar), 7.86-7.90 (m, 1H, Ar), 8.11-8.13 (m, 1H, Ar), 8.39-8.41 (m, 1H, Ar).

*7.1.43. 2-(2-Cyclooctylidenehydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**17B**).*

Orange oil, 67% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.16-1.46 (m, 5H, cyclooctane), 1.53-1.61 (m, 3H, cyclooctane), 1.69-1.80 (m, 2H, cyclooctane), 2.27-2.39 (m, 2H, cyclooctane), 2.41-2.48 (m, 2H, cyclooctane), 3.83 (s, 2H, CH_2, thiazolidinone), 5.45 (s, 2H, $ArCH_2$), 7.40-7.44 (t, 1H, Ar), 7.50-7.60 (m, 3H, Ar), 7.80-7.82 (d, *J* = 8.0 Hz, 1H, Ar), 7.88-7.90 (d, *J* = 8.0 Hz, 1H, Ar), 8.29-8.31 (d, *J* = 8.0 Hz, 1H, Ar).

*7.1.44. 2-(2-(1-Cyclohexylethylidene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**18B**).*

White powder, mp 116-121 °C, 85% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20-1.40 (m, 6H, cyclohexane), 1.69-1.72 (m, 1H, cyclohexane), 1.81 (bs, 3H, cyclohexane), 1.85 (s, 3H, CH_3), 2.20-2.26 (m, 1H, cyclohexane), 3.82 (s, 2H, CH_2, thiazolidinone), 5.47 (s, 2H, $ArCH_2$), 7.41-7.45 (t, 1H,

Ar), 7.50-7.57 (m, 3H, Ar), 7.80-7.82 (d, *J* = 8.0 Hz, 1H, Ar), 7.88-7.90 (d, *J* = 8.0 Hz, 1H, Ar), 8.30-8.32 (d, *J* = 8.0 Hz, 1H, Ar).

*7.1.45. 2-(2-(Furan-2-ylmethylene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**19B**).*

Orange powder, mp 145-150 °C, 84% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.91 (s, 2H, CH_2, thiazolidinone), 5.53 (s, 2H, $ArCH_2$), 6.51-6.55 (m, 1H, furan), 6.82-6.83 (d, *J* = 3.2 Hz, 1H, furan), 7.42-7.49 (m, 1H Ar, 1H furan), 7.52-7.61 (m, 3H Ar), 7.81-7.83 (d, *J* = 8.0 Hz, 1H, Ar), 7.89-7.91 (d, *J* = 7.6 Hz, 1H, Ar), 8.21 (s, 1H, CH=), 8.29-8.31 (d, *J* = 8.4 Hz, 1H, Ar).

*7.1.46. 2-(2-(1-(Furan-2-yl)ethylidene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one (**20B**).*

Orange powder, mp 169-171 °C, 89% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.23 (s, 3H, CH_3), 3.88 (s, 2H, CH_2, thiazolidinone), 5.53 (s, 2H, $ArCH_2$), 6.48 (bs, 1H, furan), 6.85-6.86 (d, *J* = 3.6 Hz, 1H, furan), 7.40-7.46 (t, 1H, Ar), 7.51-7.60 (m, 3H Ar, 1H furan), 7.81-7.83 (d, *J* = 7.6 Hz, 1H, Ar), 7.88-7.90 (d, *J* = 8.4 Hz, 1H, Ar), 8.32-8.34 (d, *J* = 8.4 Hz, 1H, Ar).

*7.1.47. 3-(Naphthalen-1-ylmethyl)-2-(2-(1-(thiophen-2-yl)ethylidene)hydrazono)thiazolidin-4-one (**21B**).*

Orange powder, mp 137-143 °C, 83% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.32 (s, 3H, CH_3), 3.87 (s, 2H, CH_2, thiazolidinone), 5.51 (s, 2H, $ArCH_2$), 7.04-7.07 (m, 1H, thiophene), 7.36-7.38 (m, 2H, thiophene), 7.42-7.46 (t, 1H, Ar), 7.51-7.58 (m, 3H, Ar), 7.81-7.83 (d, *J* = 8.0 Hz, 1H, Ar), 7.89-7.91 (d, *J* = 8.0 Hz, 1H, Ar), 8.29-8.35 (m, 1H, Ar).

*7.1.48. 3-(Naphthalen-1-ylmethyl)-2-(2-(1-phenylethylidene)hydrazono)thiazolidin-4-one (**22B**).*

Orange powder, mp 155-156 °C, 87% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.34 (s, 3H, CH_3), 3.88 (s, 2H, CH_2, thiazolidinone), 5.56 (s, 2H, $ArCH_2$), 7.40-7.47 (m, 4H, Ar), 7.52-7.61 (m, 3H, Ar), 7.82-7.91 (m, 4H, Ar), 8.34-8.36 (d, *J* = 8.4 Hz, 1H, Ar).

*7.1.49. 3-(Naphthalen-1-ylmethyl)-2-(2-(pyridin-3-ylmethylene)hydrazono)thiazolidin-4-one (**23B**).*

Orange powder, mp 71-73 °C, 90% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.93 (s, 2H, CH_2, thiazolidinone), 5.55 (s, 2H, $ArCH_2$), 7.31-7.61 (m, 5H, Ar), 7.82-7.92 (m, 2H, Ar), 8.14-8.16 (m, 1H, Ar), 8.20-8.25 (m, 1H, Ar), 8.39-8.40 (m, 1H, Ar), 8.65-8.67 (m, 1H, Ar), 8.89 (bs, 1H, CH=).

7.1.50. 3-(Naphthalen-1-ylmethyl)-2-(2-(1-(pyridin-2-yl)ethylidene)hydrazono)thiazolidin-4-one ***(24B)****.*

Yellow powder, mp 125-126 °C, 92% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.47 (s, 3H, CH_3), 3.88 (s, 2H, CH_2, thiazolidinone), 5.57 (s, 2H, $ArCH_2$), 7.44-7.66 (m, 5H, Ar), 7.70-7.75 (m, 1H, Ar), 7.83-7.91 (m, 2H, Ar), 8.18-8.21 (m, 1H, Ar), 8.33-8.40 (m, 1H, Ar), 8.63 (bs, 1H, Ar).

7.1.51. 2-(2-(Benzo[d][1,3]dioxol-5-ylmethylene)hydrazono)-3-(naphthalen-1-ylmethyl)thiazolidin-4-one ***(25B)****.*

Yellow powder, mp 128-130 °C, 85% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.18 (s, 2H, CH_2, thiazolidinone), 5.41 (s, 2H, $ArCH_2$), 6.09 (s, 2H, CH_2, benzodioxole), 6.98-7.00 (d, J = 8.0 Hz, 1H, Ar), 7.20-7.22 (m, 1H, Ar), 7.26-7.29 (m, 2H, Ar), 7.42-7.49 (m, 1H, Ar), 7.59-7.64 (m, 2H, Ar), 7.87-7.88 (m, 1H, Ar), 7.98-8.00 (m, 1H, Ar), 8.21-8.30 (m, 2H: Ar + CH=).

7.1.52. 3-(Naphthalen-1-ylmethyl)-2-(2-(naphthalen-1-ylmethylene)hydrazono)thiazolidin-4-one ***(26B)****.*

Orange powder, mp 185-190 °C, 93% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.96 (s, 2H, CH_2, thiazolidinone), 5.61 (s, 2H, $ArCH_2$), 7.45-7.47 (m, 1H, Ar), 7.549-7.58 (m, 4H, Ar), 7.61-7.65 (m, 2H, Ar), 7.83-7.85 (m, 1H, Ar), 7.91-7.99 (m, 4H, Ar), 8.30-8.36 (m, 1H, Ar), 8.85-8.92 (m, 1H, Ar), 9.00 (s, 1H, CH=).

7.1.53. 2-((2-(2-(Propan-2-ylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione ***(1C)****.*

Yellow powder, mp 119-124 °C, 99% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.79 (s, 3H, CH_3), 1.88 (s, 3H, CH_3), 3.95 (s, 2H, CH_2, thiazolidinone), 5.52 (s, 2H, $ArCH_2$), 7.73-8.31 (m, 4H, Ar).

7.1.54. 2-((2-(2-(Butan-2-ylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione ***(2C)****.*

White powder, mp 135-142 °C, 87% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08-1.12 (t, 3H, CH_3), 2.20 (s, 3H, CH_3), 2.27-2.31 (q, 2H, CH_2), 3.83 (s, 2H, CH_2, thiazolidinone), 5.73 (s, 2H, $ArCH_2$), 7.76 (bs, 2H, Ar), 7.86-7.87 (m, 2H, Ar).

*7.1.55. 2-((2-(2-(Pentan-2-ylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**3C**).*

White powder, mp 125-126 °C, 84% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92-0.95 (t, 3H, CH_3), 1.59-1.61 (m, 2H, CH_2), 1.93 (s, 3H, CH_3), 2.23-2.27 (t, 2H, CH_2), 3.83 (s, 2H, CH_2, thiazolidinone), 5.73 (s, 2H, $ArCH_2$), 7.75-7.76 (m, 2H, Ar), 7.86-7.87 (m, 2H, Ar).

*7.1.56. 2-((2-(2-(Pentan-3-ylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**4C**).*

White powder, mp 155-156 °C, 89% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87-0.91 (t, 3H, CH_3), 1.09-1.12 (t, 3H, CH_3), 2.28-2.34 (q, 2H, CH_2), 2.38-2.44 (q, 2H, CH_2), 3.83 (s, 2H, CH_2, thiazolidinone), 5.73 (s, 2H, $ArCH_2$), 7.74-7.76 (m, 2H, Ar), 7.86-7.88 (m, 2H, Ar).

*7.1.57. 2-((2-(2-(Hex-5-en-2-ylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**5C**).*

White powder, mp 133-135 °C, 80% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.94 (s, 3H, CH_3), 2.33-2.39 (m, 4H, 2xCH_2), 3.83 (s, 2H, CH_2, thiazolidinone), 4.96-4.98 (m, 1H, $=CH_2$), 5.02-5.07 (m, 1H, $=CH_2$), 5.73 (s, 2H, $ArCH_2$), 5.81-5.87 (m, 1H, CH=), 7.74-7.76 (m, 2H, Ar), 7.86-7.88 (m, 2H, Ar).

*7.1.58. 2-((2-(2-(4-Methylpentan-2-ylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**6C**).*

Yellow powder, mp 66-67 °C, 75% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.91-0.92 (d, J = 6.8 Hz, 6H, 2 x CH_3), 1.91 (s, 3H, CH_3), 1.94-2.01 (m, 1H, CH), 2.14-2.16 (d, J = 7.2 Hz, 2H, CH_2), 3.82 (s, 2H, CH_2, thiazolidinone), 5.73 (s, 2H, $ArCH_2$), 7.74-7.80 (m, 2H, Ar), 7.86-7.88 (m, 2H, Ar).

*7.1.59. 2-((2-(2-(Heptan-2-ylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**7C**).*

Grey powder, mp 84-85 °C, 85% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.19-1.29 (m, 8H, CH_2), 1.92 (s, 3H, CH_3), 2.20-2.28 (t, 3H, CH_3), 3.78 (s, 2H, CH_2, thiazolidinone), 5.53 (s, 2H, $ArCH_2$), 7.88-7.89 (m, 4H, Ar).

*7.1.60. 2-((2-(2-(Heptan-3-ylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**8C**).*

White powder, mp 70-74 °C, 86% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82-0.93 (m, 2H, CH_2, 3H, CH_3), 1.08-1.12 (m, 1H, CH_2), 1.30-1.37 (m, 3H, CH_3), 1.51-1.55 (m, 1H, CH_2), 2.24-2.32 (m, 2H, CH_2), 2.36-2.44 (m, 2H, CH_2), 3.82 (s, 2H, CH_2, thiazolidinone), 5.71 (s, 2H, $ArCH_2$), 7.73-75 (m, 2H, Ar), 7.85-7.86 (m, 2H, Ar).

*7.1.61. 2-((2-(2-(Octan-2-ylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**9C**).*

Brown powder, mp 65-70 °C, 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89-0.91 (m, 4H, CH_2, 3H, CH_3), 1.53-1.62 (m, 3H, CH_2), 1.93 (s, 3H, CH_3), 2.24-2.35 (m, 2H, CH_2), 2.44-2.47 (m, 1H, CH_2), 3.83 (s, 2H, CH_2, thiazolidinone), 5.72 (s, 2H, $ArCH_2$), 7.70-76 (m, 2H, Ar), 7.86-7.88 (m, 2H, Ar).

*7.1.62. 2-((2-(2-Cyclopentylidenehydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**10C**).*

Pink powder, mp 169-175 °C, 85% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.62 (bs, 4H, cyclopentane), 2.15 (bs, 2H, cyclopentane), 2.29 (bs, 2H, cyclopentane), 3.98 (s, 2H, CH_2, thiazolidinone), 5.51 (s, 2H, $ArCH_2$), 7.84-7.93 (m, 4H, Ar).

*7.1.63. 2-((2-(2-(2-Methylcyclopentylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**11C**).*

White powder, mp 158-162 °C, 83% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14-1.17 (d, J = 10 Hz, 3H, CH_3), 1.28-1.31 (m, 2H, cyclopentane), 1.83 (bs, 1H, cyclopentane), 1.98-2.02 (m, 1H,

cyclopentane), 2.25-2.30 (m, 1H, cyclopentane), 2.48-2.54 (m, 2H, cyclopentane), 3.83 (s, 2H, CH_2, thiazolidinone), 5.71 (s, 2H, $ArCH_2$), 7.76-7.77 (m, 2H, Ar), 7.86-7.88 (m, 2H, Ar).

*7.1.64. 2-((2-(2-(3-Methylcyclopentylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**12C**).*

White powder, mp 174-175 °C, 77% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31-1.32 (d, 3H, CH_3), 1.55-1.58 (m, 1H, cyclopentane), 2.20 (bs, 1H, cyclopentane), 2.29-2.35 (m, 3H, cyclopentane), 2.84-2.87 (m, 2H, cyclopentane), 4.12 (s, 2H, CH_2, thiazolidinone), 5.97 (s, 2H, $ArCH_2$), 8.02-8.04 (m, 2H, Ar), 8.13-8.15 (m, 2H, Ar).

*7.1.65. 2-((2-(2-Cyclohexylidenehydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**13C**).*

Yellow powder, mp 184-189 °C, 80% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.52 (bs, 4H, cyclohexane), 1.68-1.69 (m, 2H, cyclohexane), 2.29-2.32 (t, 2H, cyclohexane), 2.55-2.58 (t, 2H, cyclohexane), 3.84 (s, 2H, CH_2, thiazolidinone), 5.72 (s, 2H, $ArCH_2$), 7.74-7.76 (m, 2H, Ar), 7.85-7.87 (m, 2H, Ar).

*7.1.66. 2-((2-(2-(3-Methylcyclohexylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**14C**).*

Yellow powder, mp 183-184 °C, 86% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10-1.11 (d, J = 6.4 Hz, 3H, CH_3), 1.26-1.32 (m, 2H, cyclohexane), 1.49-1.54 (m, 1H, cyclohexane), 1.71-1.77 (m, 2H, cyclohexane), 1.88-1.95 (m, 2H, cyclohexane), 2.32-2.37 (m, 1H, cyclohexane), 3.18-3.22 (m, 1H, cyclohexane), 3.82 (s, 2H, CH_2, thiazolidinone), 5.73 (s, 2H, $ArCH_2$), 7.73-7.75 (m, 2H, Ar), 7.85-7.87 (m, 2H, Ar).

*7.1.67. 2-((2-(2-(4-Methylcyclohexylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**15C**).*

Orange oil, 67% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87-0.88 (m, 3H, CH_3), 1.26-1.30 (t, 1H, cyclohexane), 1.37-1.39 (m, 2H, cyclohexane), 1.79-1.92 (m, 3H, cyclohexane), 2.16-2.24 (m, 1H,

cyclohexane), 2.45-2.48 (m, 1H, cyclohexane), 3.31-3.35 (m, 1H, cyclohexane), 3.84 (s, 2H, CH_2, thiazolidinone), 5.72 (s, 2H, $ArCH_2$), 7.74-7.76 (m, 2H, Ar), 7.85-7.87 (m, 2H, Ar).

*7.1.68. 2-((2-(2-Cycloheptylidenehydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**16C**).*

Yellow powder, mp 118-119 °C, 81% yield; ^1H NMR (400 MHz, $CDCl_3$): δ 1.38-1.54 (m, 8H, cycloheptane), 1.65 (bs, 1H, cycloheptane), 2.46-2.53 (m, 3H, cycloheptane), 3.83 (s, 2H, CH_2, thiazolidinone), 5.72 (s, 2H, $ArCH_2$), 7.74-7.76 (m, 2H, Ar), 7.86-7.88 (m, 2H, Ar).

*7.1.69. 2-((2-(2-Cyclooctylidenehydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**17C**).*

Red oil, 65% yield; ^1H NMR (400 MHz, $CDCl_3$): δ 1.33-1.49 (m, 8H, cyclooctane), 2.37-2.42 (m, 3H, cyclooctane), 2.44-2.53 (m, 3H, cyclooctane), 3.84 (s, 2H, CH_2, thiazolidinone), 5.73 (s, 2H, $ArCH_2$), 7.74-7.76 (m, 2H, Ar), 7.86-7.88 (m, 2H, Ar).

*7.1.70. 2-((2-(2-(1-Cyclohexylethylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**18C**).*

Yellow powder, mp 153-154 °C, 80% yield; ^1H NMR (400 MHz, $CDCl_3$): δ 1.23-1.40 (m, 3H cyclohexane), 1.59-1.67 (m, 1H, cyclohexane), 1.70-1.87 (m, 4H, cyclohexane), 1.91 (s, 3H, CH_3), 2.16-2.21 (m, 1H, cyclohexane), 2.44-2.52 (m, 2H, cyclohexane), 4.13 (s, 2H, CH_2, thiazolidinone), 5.72 (s, 2H, $ArCH_2$), 7.75-7.77 (m, 2H, Ar), 7.85-7.87 (m, 2H, Ar).

*7.1.71. 2-((2-(2-((Furan-2-yl)methylene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**19C**).*

Yellow powder, mp 154-155°C, 91% yield; ^1H NMR (400 MHz, $CDCl_3$): δ 3.90 (s, 2H, CH_2, thiazolidinone), 5.75 (s, 2H, $ArCH_2$), 6.50 (bs, 1H, furan), 6.82-6.83 (m, 1H, furan), 7.56 (bs, 1H, furan), 7.75-7.77 (m, 2H, Ar), 7.88-7.90 (m, 2H, Ar), 8.16-8.17 (m, 1H, CH=).

*7.1.72. 2-((2-(2-(1-(Furan-2-yl)ethylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**20C**).*

Yellow powder, mp 208-211 °C, 82% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.18 (s, 3H, CH_3), 3.73 (s, 2H, CH_2, thiazolidinone), 5.58 (s, 2H, $ArCH_2$), 6.58-6.60 (m, 1H, furan), 6.96-6.98 (m, 1H, furan), 7.81 (bs, 1H, furan), 7.83-7.90 (m, 4H, Ar).

*7.1.73. 2-((2-(2-(1-(Thiophen-2-yl)ethylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**21C**).*

Orange powder, mp 165-170 °C, 84% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H, CH_3), 3.87 (s, 2H, CH_2, thiazolidinone), 5.78 (s, 2H, $ArCH_2$), 7.02-7.04 (m, 1H, thiophene), 7.34-7.37 (m, 2H, thiophene), 7.74-7.76 (m, 2H, Ar), 7.87-7.89 (m, 2H, Ar).

*7.1.74. 2-((2-(2-(1-Phenylethylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**22C**).*

White powder, mp 185-186 °C, 89% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.39 (s, 3H, CH_3), 3.89 (s, 2H, CH_2, thiazolidinone), 5.80 (s, 2H, $ArCH_2$), 7.39 (bs, 3H, Ar), 7.75-7.77 (m, 2H, Ar), 7.82-7.89 (m, 4H, Ar).

*7.1.75. 2-((2-(2-((Pyridin-3-yl)methylene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**23C**).*

Yellow powder, mp 189-190 °C, 82% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.87 (s, 2H, CH_2, thiazolidinone), 5.71 (s, 2H, $ArCH_2$), 7.27-7.30 (m, 1H, Ar), 7.69-7.72 (m, 2H, Ar), 7.82-7.84 (m, 2H, Ar), 8.03-8.08 (m, 1H, Ar), 8.28-8.29 (m, 1H, Ar), 8.57-8.58 (m, 1H, Ar), 8.81 (s, 1H, CH=).

*7.1.76. 2-((2-(2-(1-(Pyridin-2-yl)ethylidene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**24C**).*

White powder, mp 181-184 °C, 94% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H, CH_3), 3.90 (s, 2H, CH_2, thiazolidinone), 5.82 (s, 2H, $ArCH_2$), 7.70-7.75 (m, 3H, Ar), 7.89-7.90 (m, 2H, Ar), 8.12-8.17 (m, 2H, Ar), 8.61-8.62 (m, 1H, Ar).

*7.1.77. 2-((2-(2-((Benzo[d][1,3]dioxol-6-yl)methylene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**25C**).*

Yellow powder, mp 125-130 °C, 85% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 2H, CH_2, thiazolidinone), 5.75 (s, 2H, $ArCH_2$), 5.99 (s, CH_2, benzodioxole), 6.81-6.83 (m, 1H, Ar), 7.10-7.13 (m, 1H, Ar), 7.37-7.38 (m, 1H, Ar), 7.74-7.77 (m, 2H, Ar), 7.89-7.91 (m, 2H, Ar), 8.22-8.23 (m, 1H, CH=).

*7.1.78. 2-((2-(2-((Naphthalen-1-yl)methylene)hydrazono)-4-oxothiazolidin-3-yl)methyl)isoindoline-1,3-dione (**26C**).*

Yellow powder, mp 146-147 °C, 90% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.94 (s, 2H, CH_2, thiazolidinone), 5.82 (s, 2H, $ArCH_2$), 7.51-7.57 (m, 2H, Ar), 7.72-7.82 (m, 3H, Ar + CH=), 7.85-7.98 (m, 5H, Ar), 8.85-8.96 (m, 2H, Ar).

7.2. Antibacterial activity

Microtiter plates containing serial dilutions of each compound ranging from 256 to 0.5 μg/mL were inoculated with each organism to yield the appropriate density (10^5/mL) in a 100 μL final volume; each plate included positive controls (bacteria without the compound), and a negative control (medium only). The plates were incubated for 18 to 22 h at 35 °C. The minimum inhibitory concentration (MIC) for all isolates was defined as the lowest concentration of antibacterial agent that completely inhibited the growth of the organism, as detected by the unaided eye.

7.3. Antifungal activity

Microtiter plates containing serial dilutions of each compound were inoculated with each organism to yield the appropriate density (10^3/mL) in a 100 μL final volume; each plate included positive controls (fungi without a compound) and a negative control (medium only). The plates were incubated for 24 h at 37 °C. The MIC for all isolates was defined as the lowest concentration of antifungal agents that completely inhibited the growth of the organism, as detected by unaided eye.

7.4. Cytotoxicity assay

The Hep2 cell line, delivered from a human epidermoid carcinoma of the larynx and purchased from a Korean Cell Line Bank (KCLB No. 10023), was a kind gift by Prof. R. Misasi (Department of Experimental Medicine-Sapienza University of Rome). Cells were cultured in Dulbecco's

modified eagle media (DMEM), supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% streptomycin-penicillin. Cell lines were maintained as adherent type cultures under humidified atmosphere in 5% CO_2 at 37 °C in Dulbecco's modified Eagle's culture medium. Experiments were performed on cells grown to 60-70% confluency. The stock solutions of the investigated compounds were prepared in sterile DMSO and successive dilutions were made in culture medium; the percentage of DMSO present in culture medium never exceeded 0.5%. Hep2 cells in the exponential phase of growth (1 x 10^5/mL) were seeded into 24-well microplate and incubated for 24 h with five different concentrations of the compounds (0.05-100 μg/mL). Some plates containing cells alone or cells and DMSO represented the negative controls, whereas cells incubated with 1 mM natrium nitroprusside represented the positive one. After incubation time, cells were mechanically scraped off from the plates, resuspended in fresh medium and incubated for 30 min with gentle shaking at 37 °C in atmosphere of 5% CO_2 to recover from the eventual stress. An aliquot was then diluted (1:1) with a solution 0.4% Trypan blue stain. After few minutes at room temperature, cells were counted under an optical microscope in a Thoma hemocytometer chamber by two different operators. On the basis that Trypan blue is a vital dye [28] and can enter and interact with the cells unless the plasmatic membrane is damaged, blue stained cells were considered as having died. Values are expressed as % of viable cells. Cell viability in control samples was always 97-98%.

7.5. Molecular modelling studies

Sequences of *C. albicans* lanosterol 14-α demethylase (XP716761), 1,3-β-D-glucan synthase (BAA21540) and squalene-epoxidase (AAC49715) were obtained from the National Center for Biotechnology Information (NCBI). A BLAST search with default settings was performed to obtain crystal structures of proteins with high sequence similarity form the Brookhaven Protein Databank (PDB server). A high resolution crystal structure of *Saccharomyces cerevisiae* lanosterol 14-α demethylase in complex with lanosterol was obtained (pdb code: 4lxj; 1.90 Å). This enzyme shows the highest overall sequence identity to the *C. albicans* enzyme (65%).

Subsequently, the sequences of lanosterol 14-α demethylase from *C. glabrata* (AAX39317), *C. parapsilosis* (CCE41385) and *C. tropicalis* (AAX39316) were obtained from NCBI and the sequences of all four *Candida* enzymes were compared to *Saccharomyces cerevisiae* enzyme.

Ligands **1A**, **1B**, **1C** and **20C** were constructed using the MOE software package (v2014.09, Chemical Computing Group, Inc, Montreal). Docking studies were performed into the binding pocket of *Saccharomyces cerevisiae* lanosterol 14-α demethylase using the ChemScore scoring function and p450 settings (GOLD Suite v5.2, CCDC, Cambridge, UK). The binding pocket was defined to include all residues within 10 Å of the C10 atom of lanosterol.

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Conflict of interest

The authors state no conflict of interest and that they have received no payment in preparation of this manuscript.

References

[1] J. Kim, P. Sudbery, J. Microbiol. 49 (2011) 171-177.

[2] P. Fournier, C. Schwebel, D. Maubon, A. Vesin, B. Lebeau, L. Foroni, R. Hamidfar-Roy, M. Cornet, J.F. Timsit, H. Pelloux, J. Antimicrob. Chemother. 66 (2011) 2880-2886.

[3] M. Nucci, F. Queiroz-Telles, A.M. Tobon, A. Restrepo, A.L. Colombo, Clin. Infect. Dis. 51 (2010) 561-570.

[4] T. Mori, Y. Nakamura, J. Kato, K. Sugita, M. Murata, K. Kamei, S. Okamoto, Transpl. Infect. Dis. 14 (2012) 91-94.

[5] S. Silva, M. Negri, M. Henriques, R. Oliveira, D.W. Williams, J. Azeredo, FEMS Microbiol. Rev. 36 (2012) 288-305.

[6] D. Diekema, S. Arbefeville, L. Boyken, J. Kroeger, M. Pfaller, Diagn. Microbiol. Infect. Dis. 73 (2012) 45-48.

[7] A.L. Colombo, M. Nucci, B.J. Park, S.A. Nouer, B. Arthington-Skaggs, D.A. da Matta, D. Warnock, J. Morgan, J. Clin. Microbiol. 44 (2006) 2816-2823.

[8] S. Tobudic, C. Kratzer, E. Presterl, Mycoses 55 (2012) 24-32.

[9] M. Nucci, K.A. Marr, Clin. Infect. Dis. 41 (2005) 521-526.

[10] M.A. Pfaller, Am. J. Med. 125 (2012) S3-S13.

[11] A. Kunzler, P.D. Neuenfeldt, A.M. das Neves, C.M.P. Pereira, G.H. Marques, P.S. Nascente, M.H.V. Fernandes, S.O. Hübner, W. Cunico, Eur. J. Med. Chem. 64 (2013) 74-80.

[12] A. Verma, S.K. Saraf, Eur. J. Med. Chem. 43 (2008) 897-905.

[13] A. Dandia, R. Singh, S. Khaturia, C. Merienne, G. Morgant, A. Loupy, Bioorg. Med. Chem. 14 (2006) 2409-2417.

[14] D. Patel, P. Kumari, N. Patel, Eur. J. Med. Chem. 48 (2012) 354-362.

[15] B.M. Mistry, S. Jauhari, Med. Chem. Res. 22 (2013) 647-658.

[16] K. Omar, A. Geronikaki, P. Zoumpoulakis, C. Camoutsis, M. Soković, A. Ćirić, J. Glamočlija, Bioorg. Med. Chem. 18 (2010) 426-432.

[17] F. Chimenti, B. Bizzarri, E. Maccioni, D. Secci, A. Bolasco, R. Fioravanti, P. Chimenti, A. Granese, S. Carradori, D. Rivanera, D. Lilli, A. Zicari, S. Distinto, Bioorg. Med. Chem. Lett. 17 (2007) 4635-4640.

[18] F. Chimenti, S. Carradori, D. Secci, A. Bolasco, P. Chimenti, A. Granese, B. Bizzarri, J. Heterocyclic Chem. 46 (2009) 575-578.

[19] F. Chimenti, B. Bizzarri, A. Bolasco, D. Secci, P. Chimenti, A. Granese, S. Carradori, M. D'Ascenzio, D. Lilli, D. Rivanera, Eur. J. Med. Chem. 46 (2011) 378-382.

[20] D. Secci, B. Bizzarri, A. Bolasco, S. Carradori, M. D'Ascenzio, D. Rivanera, E. Mari, L. Polletta, A. Zicari, Eur. J. Med. Chem. 53 (2012) 246-253.

[21] S. Carradori, D. Secci, A. Bolasco, D. Rivanera, E. Mari, A. Zicari, L.V. Lotti, B. Bizzarri, Eur. J. Med. Chem. 65 (2013) 102-111.

[22] A.K. Jain, A. Vaidya, V. Ravichandran, S.K. Kashaw, R.K. Agrawal, Bioorg. Med. Chem. 20 (2012) 3378-3395.

[23] National Committee for Clinical Laboratory Standards 2009. Performance standards for antimicrobial susceptibility testing. Supplement M100-S19.Vol.29 n 3 National Committee for Clinical Laboratory Standards, Wayne, PA.

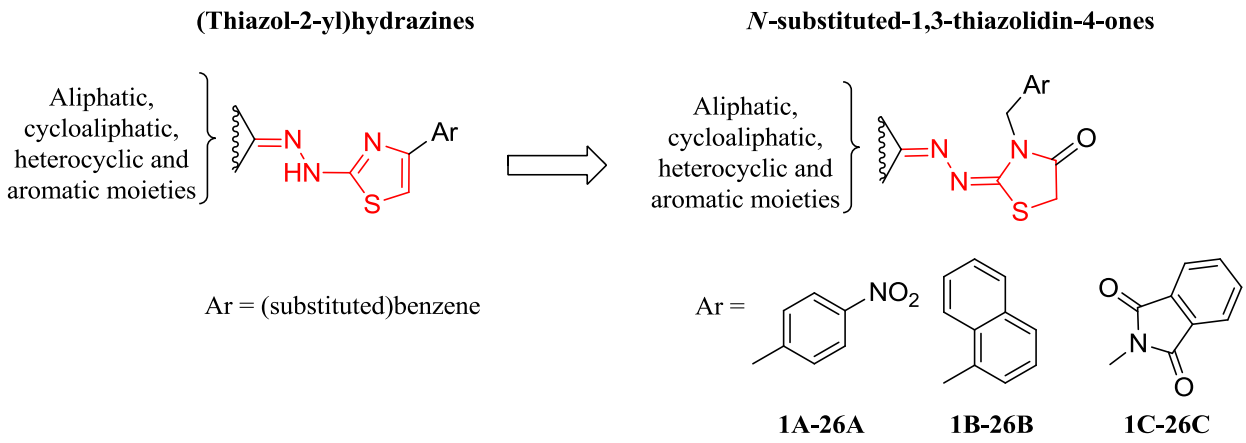
[24] M. D'Ascenzio, B. Bizzarri, C. De Monte, S. Carradori, A. Bolasco, D. Secci, D. Rivanera, N. Faulhaber, C. Bordón, L. Jones-Brando, Eur. J. Med. Chem. 86 (2014) 17-30.

[25] C. De Monte, B. Bizzarri, M.C. Gidaro, S. Carradori, A. Mollica, G. Luisi, A. Granese, S. Alcaro, G. Costa, N. Basilico, S. Parapini, M.M. Scaltrito, C. Masia, F. Sisto, J. Enzyme Inhib. Med. Chem. (2015) doi:10.3109/14756366.2014.1001755.

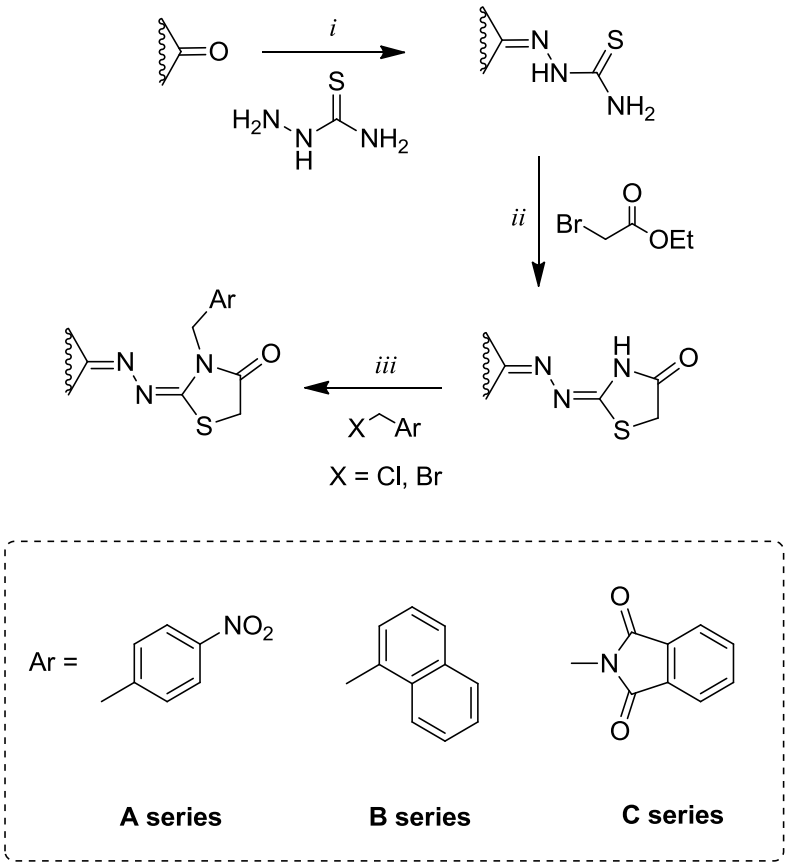
[26] National Committee for Clinical Laboratory Standards 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 8th Edition Approved standard M7-A8 vol 29 n.2. National Committee for Clinical Laboratory Standards, Wayne, PA.

[27] National Committee for Clinical Laboratory Standard. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard 3rd edition. DM27-A3 vol 28 n 14 National Committee for Clinical Laboratory Standard, Wayne, Pa.

[28] R.I. Freshney, Wiley-Liss: New York, (1987) 245-256.

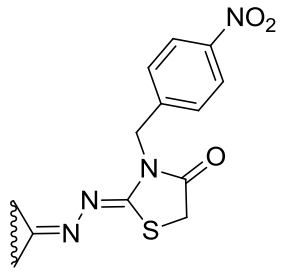
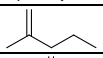
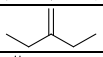
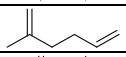
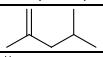
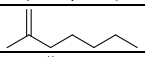
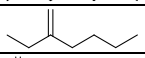
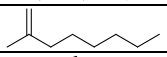
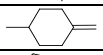


Scheme 1. Evolution from (thiazol-2-yl)hydrazine to *N*-substituted-1,3-thiazolidin-4-one scaffold.



Scheme 2. Synthesis of the derivatives **1A-26A**, **1B-26B** and **1C-26C**. Reagents and conditions: (*i*) EtOH, acetic acid (cat.), rt; (*ii*) MeOH, CH_3COONa, rt; (*iii*) anhydrous acetone, anhydrous K_2CO_3, reflux.

Table 1. Minimal inhibitory concentration (MIC) expressed as geometric mean of derivatives **1A-26A** and reference compounds against twenty-two clinical strains of *Candida* species.

		**Tested fungi (MIC µg/mL)**					
Compound	**N1-hydrazine moiety**	***C. albicans* (8)**	***C. glabrata* (4)**	***C. tropicalis* (3)**	***C. krusei* (3)**	***C. parapsilosis* (2)**	***C. sake'* (2)**
1A		4	4	2	2	2	2
2A		2	2	128	2	2	8
3A		2	2	64	16	16	8
4A		2	8	8	32	128	2
5A		4	8	8	32	32	8
6A		1	1	32	2	2	4
7A		4	1	64	2	2	2
8A		2	8	2	16	16	2
9A		4	2	128	128	256	256
10A		2	16	1	8	16	16
11A		4	16	2	2	8	2
12A		2	16	128	256	64	256
13A		8	32	8	16	8	16
14A		16	32	4	2	16	4
15A		32	16	32	8	8	4
16A		128	32	16	4	8	4
17A		4	16	8	16	16	16
18A		4	16	32	2	32	32
19A		4	8	2	16	16	2
20A		32	32	16	4	8	8
21A		8	2	4	2	2	8
22A		4	32	2	8	4	2

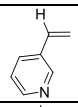
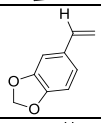
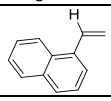
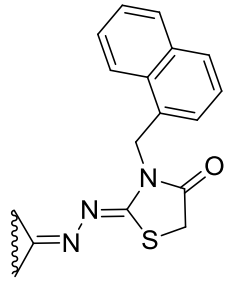
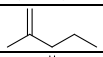
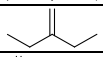
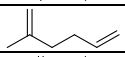
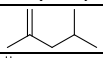
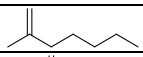
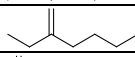
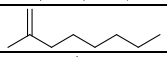
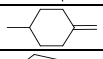
23A		256	32	256	128	32	32
24A		4	32	4	32	4	4
25A		256	32	16	8	16	16
26A		256	64	16	8	8	8
Fluconazole		2	2	2	2	2	2
Ketoconazole		2	2	2	4	2	4
Clotrimazole		2	2	2	2	2	2
Miconazole		2	4	2	4	4	2
Tioconazole		8	4	8	8	8	4
Amphotericin B		4	4	2	4	2	2

Table 2. Minimal inhibitory concentration (MIC) expressed as geometric mean of derivatives **1B-26B** against twenty-two clinical strains of *Candida* species.

		**Tested fungi (MIC µg/mL)**					
Compound	**N1-hydrazine moiety**	***C. albicans* (8)**	***C. glabrata* (4)**	***C. tropicalis* (3)**	***C. krusei* (3)**	***C. parapsilosis* (2)**	***C. sake'* (2)**
1B		4	4	2	2	2	2
2B		2	2	128	2	2	2
3B		2	2	64	2	2	16
4B		2	16	2	16	16	2
5B		2	2	8	2	2	2
6B		4	1	64	2	2	4
7B		4	8	64	2	2	2
8B		2	8	2	16	16	2
9B		2	2	8	8	16	16
10B		2	16	8	16	128	256
11B		4	32	2	2	8	2
12B		2	16	128	256	64	256
13B		2	32	8	16	8	32
14B		4	8	4	16	16	2
15B		2	16	128	8	8	4
16B		8	16	8	4	4	4
17B		4	32	8	4	4	4
18B		8	8	256	2	16	16
19B		16	8	8	16	16	2
20B		32	32	128	4	4	4
21B		2	8	2	16	16	2
22B		2	8	2	32	32	8

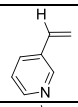
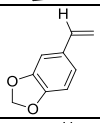
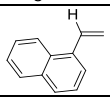
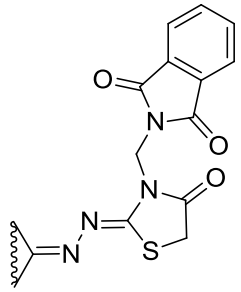
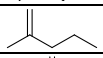
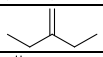
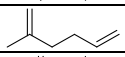
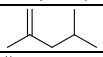
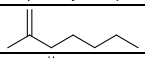
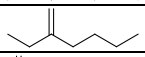
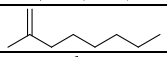
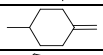
23B		4	8	8	32	32	32
24B		2	2	2	2	2	2
25B		8	4	16	8	16	8
26B		256	64	32	8	8	8

Table 3. Minimal inhibitory concentration (MIC) expressed as geometric mean of derivatives **1C**-**26C** against twenty-two clinical strains of *Candida* species.

		**Tested fungi (MIC µg/mL)**					
Compound	**N1-hydrazine moiety**	***C. albicans* (8)**	***C. glabrata* (4)**	***C. tropicalis* (3)**	***C. krusei* (3)**	***C. parapsilosis* (2)**	***C. sake'* (2)**
1C		4	1	1	2	2	2
2C		2	2	64	8	8	2
3C		2	2	4	2	2	4
4C		2	8	8	8	8	8
5C		4	32	4	32	128	8
6C		2	1	64	2	2	2
7C		4	8	128	2	2	2
8C		4	16	2	32	8	2
9C		2	16	16	8	16	16
10C		2	16	8	16	64	256
11C		16	32	4	2	8	8
12C		2	32	8	16	8	16
13C		8	16	8	16	8	32
14C		4	8	2	16	16	2
15C		2	32	8	8	8	4
16C		4	16	32	16	4	16
17C		4	32	8	4	4	4
18C		256	16	256	16	16	16
19C		2	8	8	4	2	2
20C		256	16	128	256	256	256
21C		4	32	2	4	4	8
22C		2	8	8	8	8	8

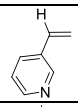
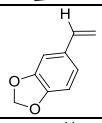
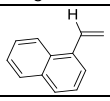
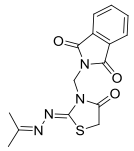
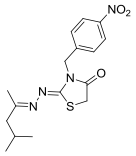
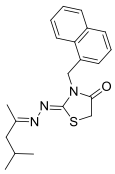
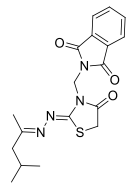
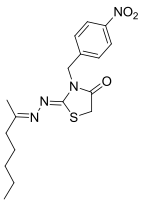
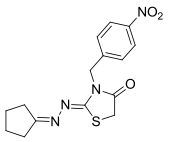
23C		256	8	16	8	16	32
24C		2	32	2	32	4	4
25C		8	8	8	32	128	128
26C		256	16	128	128	128	128

Table 4. Cytotoxic effect (CC_{50}) of the most active compounds and clotrimazole tested on Hep2 cells after 24 h of incubation at 37 °C using Trypan blue exclusion test expressed as cell survival fraction (%).

Compound		**Trypan Blue exclusion test[a] CC_{50} (µg/mL)**
1C		22
6A		32
6B		95
6C		99
7A		35
10A		28
Clotrimazole		38

[a]Cells incubated with culture medium alone represented the controls. Viability with 2 µL of DMSO= 94%, viability with 20 µL of DMSO= 87%. Data represent arithmetic means of at least three independent experiments.

Table 5. The sequence identity of the binding pocket residues of selected CYP51A1 (lanosterol 14-α demethylase) enzymes.

4LXJ	***C. albicans***	***C. glabrata***	***C. parapsilosis***	***C. tropicalis***
Tyr72	Tyr	Tyr	Tyr	Tyr
Gly73	Gly	Gly	Gly	Gly
Leu95	Leu	Leu	**Met**	Leu
Tyr126	Tyr	Tyr	Tyr	Tyr
His128	His	His	His	His
Leu129	Leu	Leu	Leu	Leu
Thr130	Thr	Thr	Thr	Thr
Phe134	Phe	Phe	Phe	Phe
Ile139	Ile	Ile	Ile	Ile
Tyr140	Tyr	Tyr	**Phe**	Tyr
Leu147	Leu	Leu	Leu	Leu
Lys151	Lys	Lys	Lys	Lys
Val154	Ala	**Val**	Ala	Ala
Phe236	Phe	Phe	Phe	Phe
Pro238	Pro	Pro	Pro	Pro
Phe241	Phe	Phe	Phe	Phe
Val242	Val	Val	Val	Val
Leu306	Leu	Leu	Leu	Leu
Gly310	Gly	Gly	Gly	Gly
Val311	**Ile**	Val	Val	Val
Met313	Met	Met	Met	Met
Gly314	Gly	Gly	Gly	Gly
Gly315	Gly	Gly	Gly	Gly
Thr318	Thr	Thr	Thr	Thr
Leu380	Leu	Leu	Leu	Leu
His381	His	His	His	His
Ser382	Ser	Ser	Ser	Ser
Leu383	Ile	**Leu**	Ile	Ile
Phe384	Phe	Phe	Phe	Phe
Cys470	Cys	Cys	Cys	Cys
Phe506	Tyr	**Phe**	Tyr	Tyr
Thr507	**Ser**	Thr	Thr	**Gln**
Ser508	Ser	Ser	Ser	Ser
Met509	Met	Met	Met	Met
Val510	Val	Val	Val	Val
Thr511	**Val**	Thr	Thr	Thr

The binding pocket is defined as all amino acids within 4.5 Å of lanosterol.

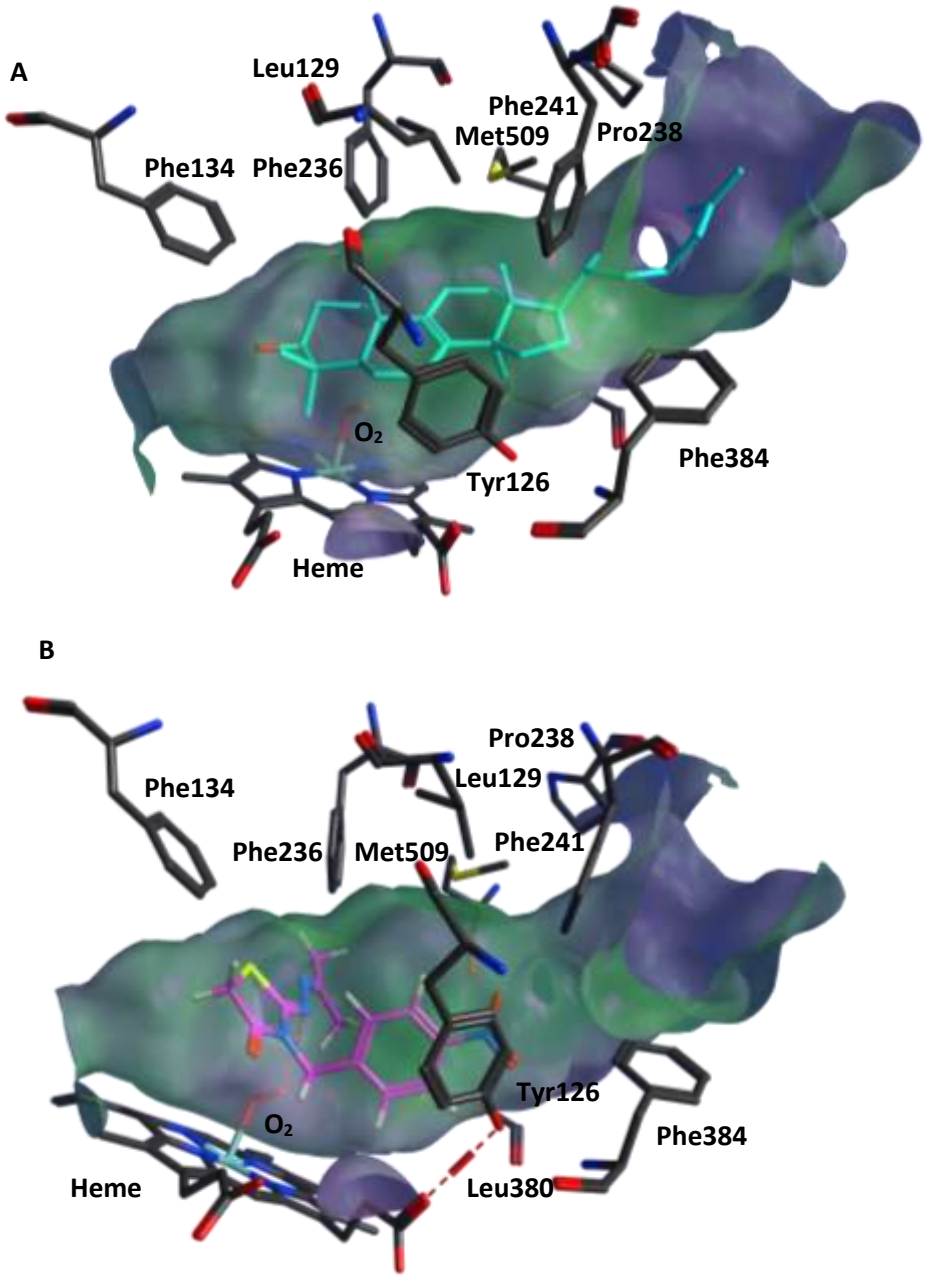


Figure 1. A) The binding interactions of lanosterol with its endogenous hydrophobic binding pocket in lanosterol 14-α demethylase (pdb: 4lxj). **B)** A representative docked pose of compound **1A** in the binding pocket of lanosterol 14-α demethylase. The binding pocket cavity is indicated in green (apolar residues) and purple (polar residues).