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Repurposing the phenylthiazole scaffold with 1,3,4-oxadiazole for selective, potent and well-tolerated antifungal activity†

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Invasive fungal infections (IFIs) represent a critical health threat, particularly among immunocompromised individuals, with mortality rates reaching up to 50%. The growing resistance to existing antifungal therapies necessitates the development of novel agents. Here, we rationally designed phenylthiazole-based oxadiazole derivatives to enhance selectivity and potency against resistant fungal strains. Among the tested compounds, compound **35** (which emerged as a lead candidate) demonstrated potent activity against *Candida albicans* (MIC = 1–2 μg mL^{-1}), *Candida glabrata* (MIC = 0.5–1 μg mL^{-1}), and multidrug-resistant *Candida auris* (MIC = 2–4 μg mL^{-1}), outperforming fluconazole and matching amphotericin B. Additionally, compound **35** showed minimal cytotoxicity (88% cell viability at 16 μg mL^{-1}) and negligible hemolytic activity, indicating a superior safety profile.

1 Introduction

Invasive fungal infections (IFIs) are a significant global health burden, causing roughly 1.5 to 2 million deaths annually.[1,2] These life-threatening infections predominantly strike immunocompromised individuals, including organ transplant recipients, AIDS patients, and cancer patients undergoing immunosuppressive therapy. Additionally, patients hospitalized with severe underlying infections are also susceptible to IFIs.[3,4] *Candida*, *Aspergillus*, and *Cryptococcus* species are the most frequent fungal culprits responsible for IFIs.[5] *Candida*, particularly in intensive care unit (ICU) patients, is the leading cause of invasive fungal infections (invasive candidiasis). Notably, it ranks as the fourth most common bloodstream infection within the United States. Mortality rates associated with candidemia can be as high as 50%, while invasive aspergillosis carries a mortality rate ranging from 30–50%.[6–8]

For the treatment of IFIs, clinicians primarily rely on three drug classes: polyenes, echinocandins, and azoles.[9,10] Polyenes, such as amphotericin B and mycostatin, demonstrate potent antifungal activity against a wide range of pathogens. However, their use is often limited by the potential for moderate to severe nephrotoxicity and allergic contact dermatitis.[11,12] Echinocandins offer a favorable safety profile compared to other antifungals and exhibit fungicidal activity against *Candida* species. Nevertheless, their efficacy is diminished against *Aspergillus*, the most common fungal culprit in pneumonia.[13] Azoles (Fig. 1A), owing to their high therapeutic index, have emerged as the mainstay of antifungal therapy. Their broad-spectrum activity and generally well-tolerated nature make them the preferred choice for many IFIs.[14,15]

The widespread use of azoles, particularly fluconazole, for treating fungal infections has unfortunately led to the emergence of multidrug-resistant fungal strains. These strains exhibit resistance not only to fluconazole but also to other azole antifungals like itraconazole and voriconazole.[16] Notably, *Candida krusei* has been shown to possess innate resistance to fluconazole, meaning it is resistant without prior exposure to the drug.[17] Furthermore, a concerning number of *Candida* and *Aspergillus* species isolates, responsible for an estimated 1.4 million deaths annually, have developed significant resistance to fluconazole.[18]

The alarming rise of antifungal resistance, coupled with the inherent toxicity and adverse effects associated with existing antifungals, presents a significant unmet medical need. This challenge is further compounded by the difficulties in registering new antifungal compounds to combat resistant fungal strains. Consequently, the development of novel, potent antifungals is of paramount importance.

Azole antifungal drugs, such as fluconazole, voriconazole, isavuconazole, ketoconazole, and itraconazole, rely heavily on 5-membered nitrogenous heterocycles like triazoles, imidazoles, tetrazoles, and thiazoles for their antifungal activity (Fig. 1A). 1,3,4-Oxadiazoles share structural similarity with commonly used azole antifungals, such as triazoles and imidazoles, enabling them to target the same fungal enzymes. For example, replacing a portion

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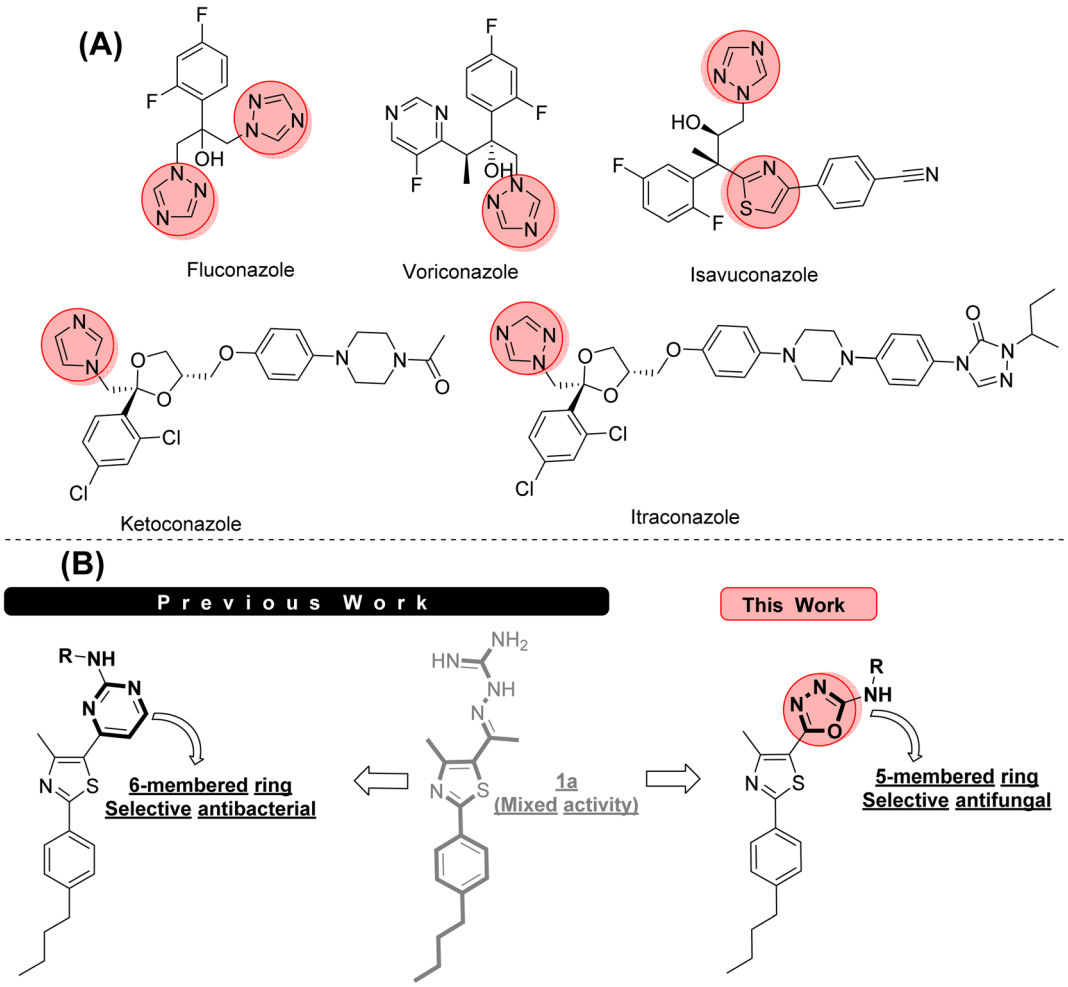

Fig. 1 (A) Structures of a typical azole antifungal drugs. (B) Rational design of this work.

of the fluconazole molecule with a 1,3,4-oxadiazole moiety has been shown to directly enhance its antifungal activity.[19] On the other hand, the phenylthiazole scaffold, well-established for its antimicrobial activity against multi-drug resistant pathogens, shows mixed activity (antibacterial and antifungal) in its lead compound **1a**. This compound possesses two key pharmacophoric features: an aminoguanidine head and an *n*-butyl tail. Notably, altering the *n*-butyl tail by replacing it with either a longer or shorter alkyl chain resulted in a decline in antimicrobial activity.[20,21] Optimizing **1a** by rigidifying the aminoguanidine head with a pyrimidine ring (a 6-membered ring) yielded selective antibacterial hits (Fig. 1B).[21,22] Herein, we aimed to design selective antifungal compounds. Therefore, we propose inserting a 1,3,4-oxadiazole ring into the phenylthiazole scaffold (Fig. 1B). We hypothesize that incorporating this ring may enhance selectivity and potency towards resistant fungal strains, leading to the development of novel and selective antifungal lead compounds (Fig. 1B).

2 Results and discussion

2.1. Chemistry

4-Butylbenzoyl chloride **2** was added portion-wise to an ammonia solution while keeping the reaction mixture stirred at 0–5 °C to produce 4-butylbenzamide **3**. The product was dried and dissolved in dry THF, and the flask was charged with Lawesson's reagent to obtain 4-butylbenzthioamide **4**. Ester **5** was prepared in high yield using thioamide **4** and ethyl α-chloroacetoacetate. The key intermediate acid hydrazide **6** was then obtained *via* treatment of the obtained ethyl ester **5** with hydrazine hydrate (Scheme 1). Adding a carbon disulfide to **6** in the presence of potassium hydroxide followed by methylation of the free thiol group afforded the 5-methylmercapto derivative **8**. The later was oxidized by *m*-chloroperbenzoic acid (*m*-CPBA) to yield methylsulfone analogue **9**, the key intermediate of this scheme, which was utilized to access nucleophilic substitution reaction. So that, treatment of methylsulfone **9** with thirty-five different nitrogenous nucleophiles include primary and secondary amines, hydrazine, diamines, aminoalcohols, guanidines and carboximidines afforded the final products **10–44** (Scheme 1).

2.2. Biological evaluation

2.2.1. Initial screening and structure activity relationship (SAR) study. The initial antimicrobial screening was performed using clinically-relevant pathogens (methicillin-resistant

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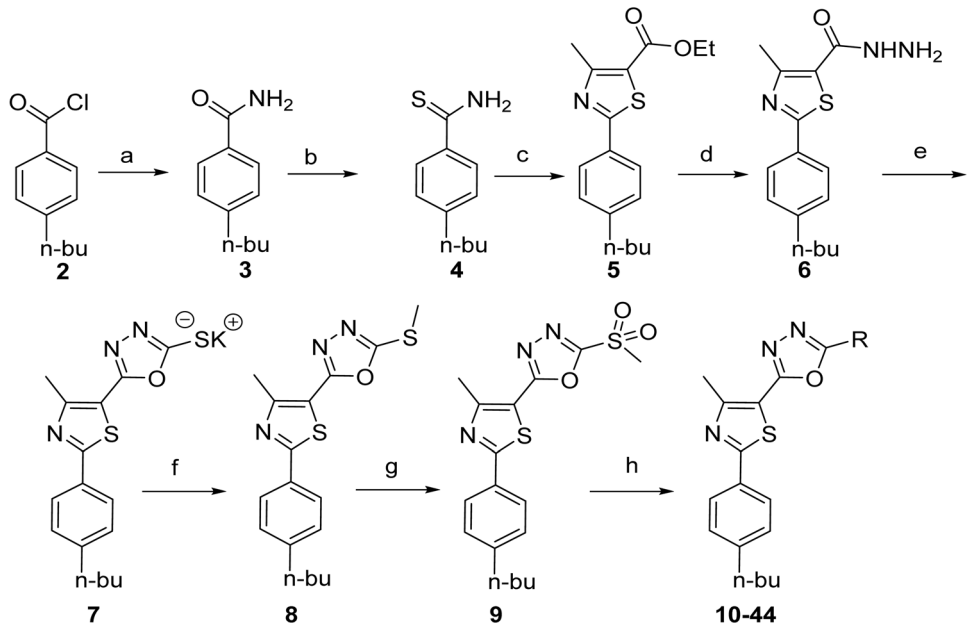

Reagents and conditions: (a) NH_4OH, 0-23 °C, 2-5 h; 98% (b) Lawesson's reagent, dry THF, 23 °C, 5-24 h; 88% (c) Ethyl 2-chloro-3-oxobutanoate, absolute EtOH, heat at reflux, 4 h, 92% (d) Absolute EtOH, $NH_2NH_2{\cdot}H_2O$, heat at reflux, 8 h; 98% (e) CS_2, KOH, EtOH, heat at reflux,12 h; 89% (f) dimethyl sulfate, KOH, H_2O, stirring at 23 °C, 2 h; 91% (g) MCPBA, dry DCM, 23 °C, 16 h; 93% (h) appropriate amine, hydrazine, guanidine or carboximidate; K_2CO_3, DMF, heat at 80 °C for 0.5 - 12 h, 49-98%.

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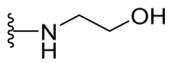
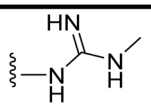
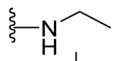
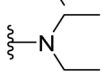
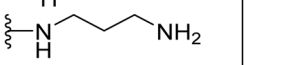
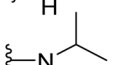
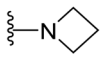
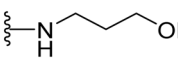
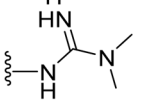
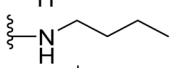
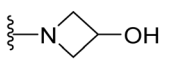
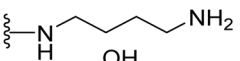
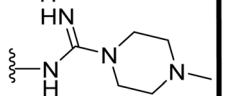
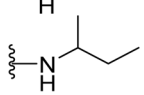
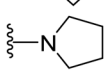
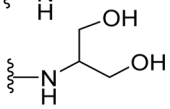
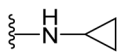
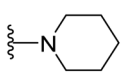
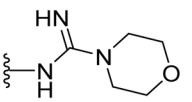
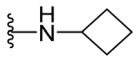
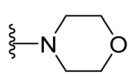
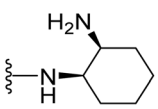
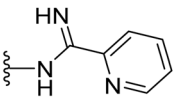
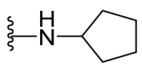
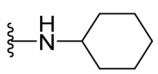
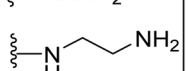
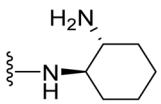
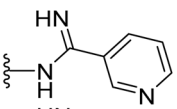
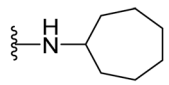
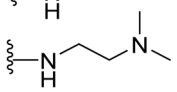
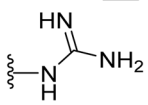
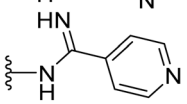
10		20		30		38	
11		21		31			
12		22		32		39	
13		23		33		40	
14		24		34			
15		25				41	
16		26		35		42	
17		27					
18		28		36		43	
19		29		37		44	

Scheme 1

Staphylococcus aureus (MRSA), *Escherichia coli*, *Clostridium difficile* and *Neisseria gonorrhea* strains) and (*Candida albicans*) strains.

The antimicrobial results that have been presented in (Table 1) indicated that oxadiazole derivatives with aliphatic side chains, primary or secondary amines (compounds **10–26**), are

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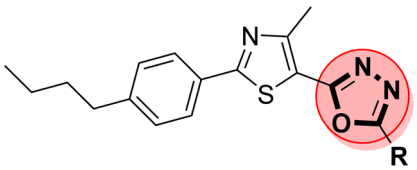
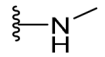
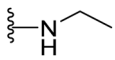
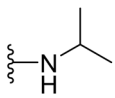
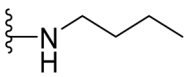
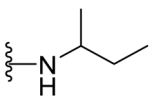
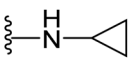
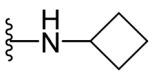
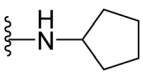
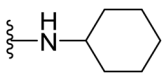
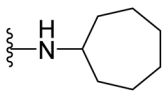
Table 1 The minimum inhibitory concentration (MIC in μg mL^{-1}) of new synthesized oxadiazole derivatives and control drugs (gentamicin, linezolid, vancomycin, cefixime, amphotericin B and fluconazole) initially screened against methicillin-resistant *Staphylococcus aureus*, *Clostridium difficile*, *Escherichia coli*, *Neisseria gonorrhoeae*, and *Candida albicans* isolates



Cpd	Side chain	MRSA NRS384 (MRSA USA300)	*C. difficile* ATCC BAA 1870	*E. coli* JW55031 (TolC Mutant)	*N. gonorrhoeae* 181	*C. albicans* SS5314 (wild-type)
10		>64	>64	>64	>64	>64
11		16	8	16	32	8
12		>64	>64	>64	>64	>64
13		>64	>64	>64	>64	>64
14		>64	64	>64	64	>64
15		>64	>64	>64	>64	>64
16		>64	>64	>64	>64	>64
17		>64	64	>64	64	>64
18		32	32	>64	>64	>64
19		>64	64	>64	64	>64
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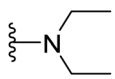
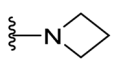
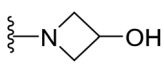
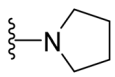
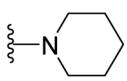
21		>64	>64	>64	64	>64
22		16	>64	32	64	8
23		>64	>64	>64	>64	>64
24		>64	>64	>64	>64	>64
25		>64	64	>64	>64	>64

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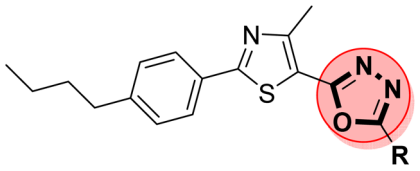
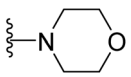


Cpd	Side chain	MRSA NRS384 (MRSA USA300)	*C. difficile* ATCC BAA 1870	*E. coli* JW55031 (TolC Mutant)	*N. gonorrhoeae* 181	*C. albicans* SS5314 (wild-type)
26		>64	32	>64	>64	>64
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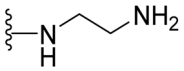
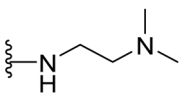
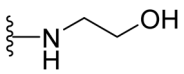
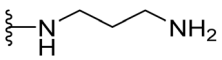
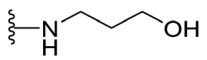
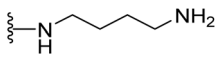
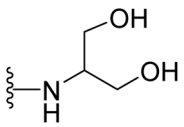
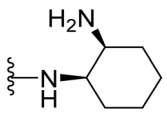
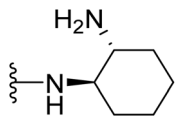
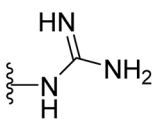
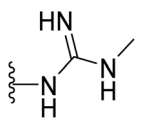
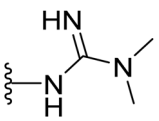
28		>64	32	>64	64	64
29		16	8	16	64	16
30		>64	>64	>64	>64	>64
31		16	8	32	64	16
32		>64	>64	>64	64	>64
33		32	16	32	64	64
34		>64	16	>64	32	32
35		>64	32	>64	32	4
36		64	32	64	>64	8
37		>64	>64	>64	32	>64
38		>64	>64	>64	64	>64
39		>64	64	>64	>64	>64

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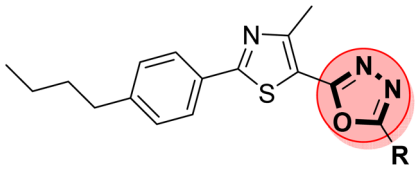
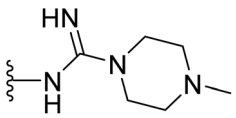
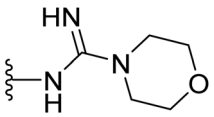
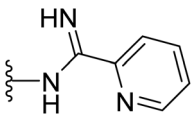
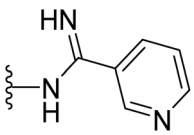
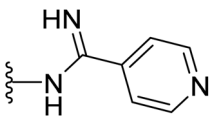
Table 1 (*Contd.*)



Cpd	Side chain	MRSA NRS384 (MRSA USA300)	*C. difficile* ATCC BAA 1870	*E. coli* JW55031 (TolC Mutant)	*N. gonorrhoeae* 181	*C. albicans* SS5314 (wild-type)
40		8	16	>64	64	8
41		>64	>64	>64	>64	>64
42		>64	>64	>64	>64	>64
43		>64	>64	>64	>64	>64
44		>64	>64	>64	> 64	>64
Linezolid		1	>64	8	NT	NT
Vancomycin		1	NT	NT	NT	NT
Gentamicin		NT	≤0.5	≤0.5	NT	NT
Cefixime		NT	NT	NT	1	NT
Amphotericin B		NT	NT	NT	NT	1
Fluconazole		NT[a]	NT	NT	NT	>64

[a] NT; not tested.

void from any antimicrobial activity, with the exceptions of ethylamine **11** and azetidine **22** derivatives. Compound **11** exhibited mild antimicrobial activity against all five tested microbes, with MIC values ranging from 8 to 32 μg mL^{-1}. In contrast, azetidine derivative **22** demonstrated moderate antibacterial activity against MRSA (MIC = 16 μg mL^{-1}) and promising antifungal activity against *C. albicans* (MIC = 8 μg mL^{-1}). These results collectively suggested that the presence of a hydrophobic region around the oxadiazole position-2 is unfavorable and highlights the crucial role of the terminal heteroatom as a hydrogen bond donor HBD and/or acceptor HBA.

Adding another polar heteroatom led to a new set of structures. The oxadiazole **27** bearing the hydrazine nitrogenous side chain was first synthesized and tested in this set. Compound **27** was limited moderate activity against *C. difficile* with MIC of 8 μg mL^{-1}. Unfortunately, **27** was inactive against all other tested microorganisms (Table 1).

Increasing the distance between the two heteroatoms resulted in the synthesis of diamines and amino alcohols (compounds **28–36**), which showed a slight improvement in anti-MRSA activity. Notably, the *N*,*N*-dimethyl ethylenediamine derivative **29** and the propylenediamine derivative **31** exhibited MIC values of 16 μg mL^{-1} against MRSA and 8 μg mL^{-1} against *C. difficile*. Additionally, both compounds demonstrated promising antifungal activity against *C. albicans* (MIC = 8 μg mL^{-1}). Interestingly, the 1,2-diaminocyclohexane derivatives (**35** and **36**) exhibited selective antifungal activity against *C. albicans* with MIC values of 4 and 8 μg mL^{-1}, respectively (Table 1).

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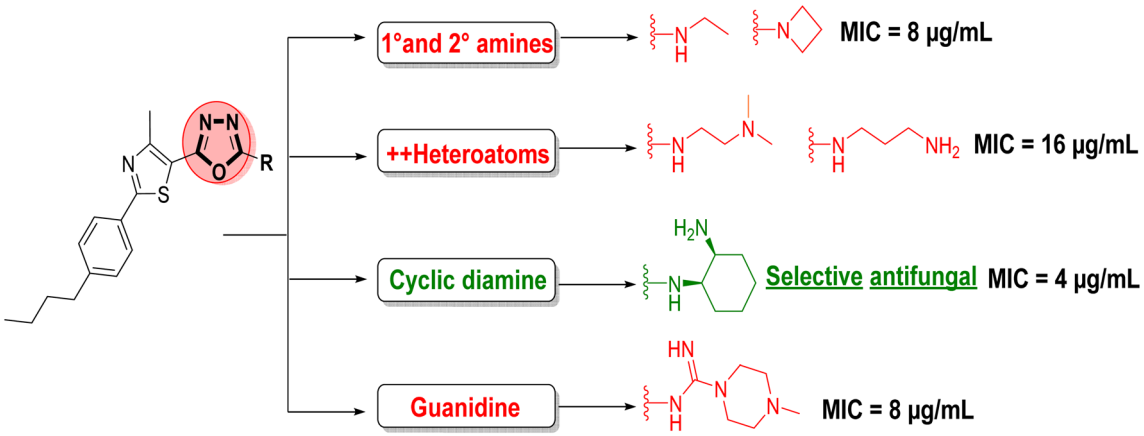

Fig. 2 SAR study of antifungal activity.

Further expansion of the nitrogenous side chain provided the guanidine compounds **37–39**, which were void from any antimicrobial activity. The antibacterial activity of guanidine-containing compound **37** was not improved even by adding one or two methyl groups to the terminal amino group (compounds **38** and **39**). However, further increasing the number of carbon units around the terminal amine (compounds **40**) notably enhanced both anti-MRSA and anti-fungal activity, with MIC value improved from above 64 to 8 μg mL^{-1}. Finally, replacing the *n*-methyl piperazine side chain with a morpholine ring (compound **41**) or aromatic pyridines (compounds **42–44**) had a negative impact on the antibacterial activity of the tested compounds, as these compounds were void of any antimicrobial activity (Table 1).

The SAR analysis for antifungal activity indicates that oxadiazole derivatives with hydrophobic regions and simple aliphatic side chains, such as compounds **10–26**, lack significant activity, except for ethylamine **11** and azetidine **22**, which exhibit mild antifungal effects (MIC = 8 μg mL^{-1}). Introducing additional heteroatoms, as in compound **27**, or extending the distance between heteroatoms, as in compounds **28–36**, improved antifungal activity, with *N*,*N*-dimethyl ethylenediamine **29** and propylenediamine **31** active against *C. albicans* (MIC = 8 μg mL^{-1}). Notably, selective antifungal activity was observed only in the 1,2-diaminocyclohexane derivatives **35** and

Table 2 The minimum inhibitory concentrations (MICs) of compound **35** against different yeast strains

		MICs (μg mL^{-1})			
No.	Fungal strain	**35**	Amphotericin	Fluconazole	Itraconazole
1	*C. albicans* NR-29448	2	1	>128	NT[a]
2	*C. albicans* ATCC 10231	1	0.5	0.25	NT
3	*C. albicans* ATCC 64124	1	2	128	NT
4	*C. glabrata* ATCC MYA-2950	0.5	0.5	16	NT
5	*C. glabrata* ATCC 15126	1	1	4	NT
6	*C. glabrata* ATCC 66032	0.5	1	8	NT
7	*C. glabrata* ATCC 2001	1	1	8	NT
8	*C. glabrata* CAB 524041	1	1	8	NT
9	*C. krusei* ATCC 14243	1	1	16	NT
10	*C. krusei* ATCC 34135	2	2	16	NT
11	*C. krusei* CAB 396420	1	1	16	NT
12	*C. auris* AR0385	2	2	>128	NT
13	*C. auris* AR0389	4	2	>128	NT
14	*C. auris* AR0390	2	2	>128	NT
15	*Cryptococcus gattii* NR-43208	1	1	2	NT
16	*Cryptococcus gattii* NR-43210	1	1	2	NT
17	*Cryptococcus gattii* NR-43209	0.5	1	4	NT
18	*Cryptococcus neoformans* NR-41298	1	1	2	NT
19	*Cryptococcus neoformans* NR-41300	2	1	2	NT
20	*Cryptococcus neoformans* NR-48770	1	1	2	NT
21	*Aspergillus fumigatus* 303	>16	1	NT	0.5
22	*Aspergillus fumigatus* 304	>16	1	NT	0.5

[a] NT; not tested.

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36, which exhibited MIC values of 4 and 8 μg mL^{-1}, respectively, with no significant antibacterial activity, underscoring the importance of a rigid cyclic diamine structure. Guanidine-containing compounds **37–39** were inactive, while increasing carbon units in compound **40** restored antifungal activity (MIC = 8 μg mL^{-1}). Substitutions with morpholine **41** or pyridines **42–44** eliminated activity, highlighting the importance of specific side chains for selectivity (Fig. 2).

2.2.2. Evaluation of compound 35 antifungal activity against a wider panel of fungal isolates. To explore the spectrum of antifungal activity of compound **35**, its minimum inhibitory concentrations (MICs) were assessed against a comprehensive panel of fungal pathogens, including *Candida* species, *Cryptococcus* species, and *Aspergillus fumigatus* (Table 2). The results were compared with established antifungal agents, including amphotericin B, fluconazole, and itraconazole. Compound **35** exhibited potent activity against *Candida albicans*, with MICs of 1–2 μg mL^{-1}, matching amphotericin B and outperforming fluconazole in strains resistant to the latter. For example, in the case of *C. albicans* NR-29448, compound **35** maintained an MIC of 2 μg mL^{-1}, whereas fluconazole showed MICs >128 μg mL^{-1}. Similarly, compound **35** demonstrated consistent activity against *Candida glabrata*, with MICs ranging from 0.5–1 μg mL^{-1}, comparable to amphotericin B and superior to fluconazole, which displayed variability across strains.

Notably, compound **35** showed efficacy against *Candida auris*, a multidrug-resistant pathogen of critical clinical concern. It achieved MICs of 2–4 μg mL^{-1}, whereas fluconazole was ineffective (MICs >128 μg mL^{-1}). The compound also displayed potent activity against *Cryptococcus gattii* and *Cryptococcus neoformans*, with MICs of 0.5–2 μg mL^{-1}, aligning closely with amphotericin B and outperforming fluconazole, which exhibited reduced activity in some strains. However, the efficacy of compound **35** was less pronounced against *Aspergillus fumigatus*, where MICs exceeded 16 μg mL^{-1}.

2.2.3. Safety profile of compound 35. The safety profile of compound **35** was evaluated in comparison to amphotericin B and fluconazole through cytotoxicity assays on monkey kidney epithelial (Vero) cells and hemolysis assays on sheep red blood cells. The results consistently highlight the superior safety margin of compound **35**.

In the cytotoxicity assay (Fig. 3), compound **35** demonstrated minimal impact on cell viability across all tested concentrations. At 8 μg mL^{-1} and 16 μg mL^{-1}, compound **35** maintained a high cell viability of 88%, in stark contrast to amphotericin B, which exhibited significant cytotoxicity, with cell viabilities of 40% and 18%, respectively. This steep decline in cell viability for amphotericin B underscores its well-documented narrow therapeutic window and high off-target toxicity. Compound **35**'s gentle dose–response curve highlights its potential as a safer antifungal agent for systemic use.

In the hemolysis assay (Fig. 4), amphotericin B exhibited a pronounced dose-dependent hemolytic activity, causing substantial hemolysis at higher concentrations, with levels exceeding 100% at 128 μg mL^{-1}. In contrast, compound **35** showed negligible hemolytic activity, comparable to that of fluconazole, across all tested concentrations. The absence of significant red blood cell lysis even at elevated concentrations reinforces the favorable safety profile of compound **35** and

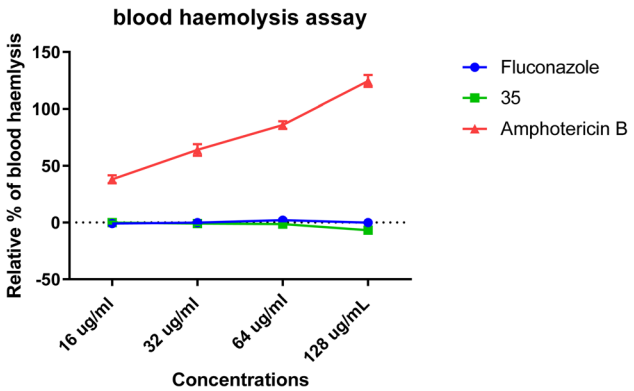

Fig. 4 RBCs hemolysis assay of different concentrations of **35** and antifungal drugs.

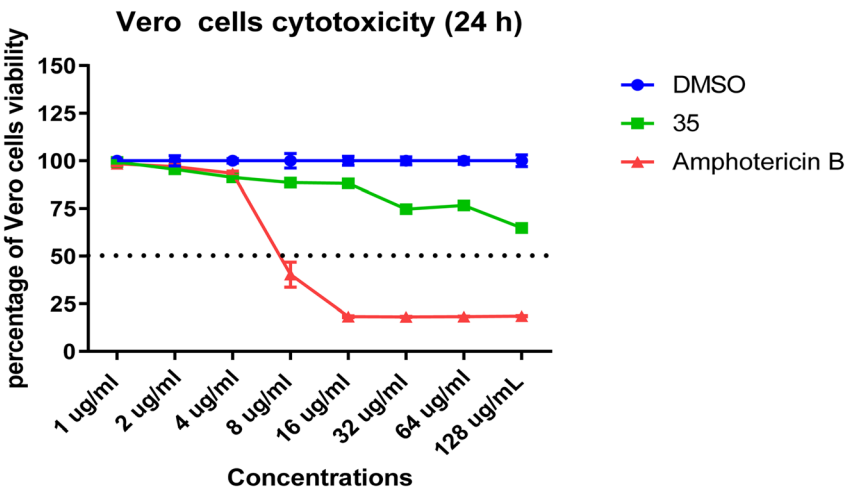

Fig. 3 *In vitro* cytotoxicity assay of different concentrations of **35** and amphotericin-B against monkey kidney epithelial (Vero) cells.

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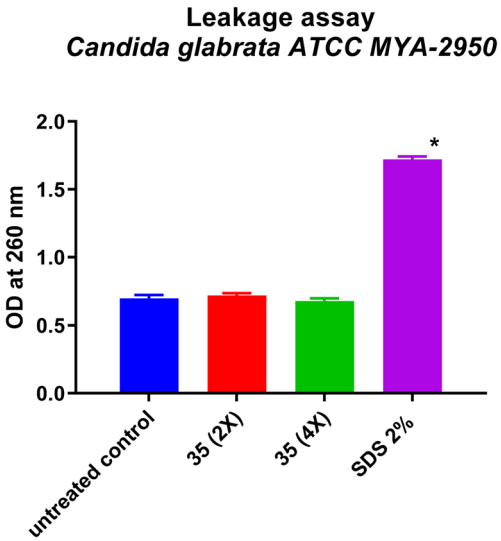

Fig. 5 Leakage assay of *Candida glabrata* ATCC MYA-2950 after 4 h incubation with 2× and 4× MIC of **35** compound and 2% SDS.

indicates its reduced risk of causing hemolytic toxicity during systemic administration.

2.2.4. Effect of compound 35 on fungal cell membrane. The integrity of the fungal cell membrane is crucial for maintaining cellular homeostasis, and agents that disrupt this membrane typically lead to rapid fungicidal effects. However, such disruption can also result in off-target toxicity in host cells due to non-selective interactions. In this study, we assessed the effect of compound **35** on the membrane integrity and permeability of *Candida glabrata* ATCC MYA-2950 by measuring the release of intracellular components at 260 nm after treatment (Fig. 5). The results showed that compound **35**, even at 2× and 4× MIC concentrations, did not induce significant leakage of intracellular materials, suggesting that it does not disrupt the fungal cell membrane. This finding implies that compound **35** does not directly target the membrane, differentiating its mechanism of action from membrane-active agents like amphotericin B and SDS. Compound **35** may exert its antifungal effects by targeting intracellular pathways or inhibiting key enzymes, warranting further investigation into its specific mechanism (Fig. 6).

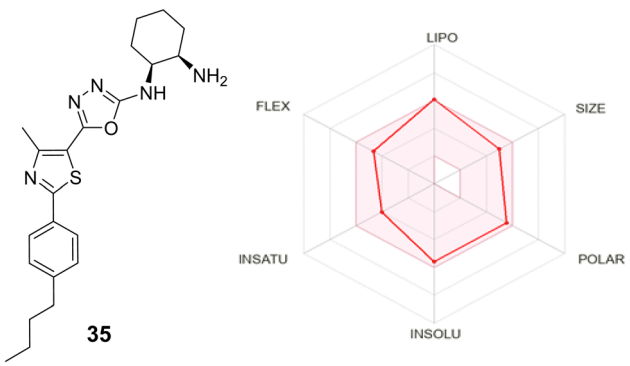

Fig. 6 *In silico* ADME profile of **35**.

2.3. *In silico* ADME profile

The ADME (absorption, distribution, metabolism, and excretion) properties of the most active compound **35** were evaluated using the SwissADME online tool. Based on Lipinski's rule of five, a compound is considered to have acceptable drug-like ADME characteristics if it meets no more than one of the following criteria: molecular weight below 500 daltons, fewer than 5 hydrogen bond donors (*n*-OHNH), fewer than 10 hydrogen bond acceptors (*n*-ON), an octanol–water partition coefficient (MLogP) below 4.5, and a total polar surface area (TPSA) of 140 or less. As summarized in (Table 3), the analysis revealed that compound **35** complies with all these criteria, indicating it fully adheres to Lipinski's rule. Furthermore, the predicted solubility class suggests that compound **35** is moderately soluble. These results highlight that compound **35** possesses favorable ADME properties, supporting its potential as a therapeutic candidate.

3 Conclusion

The rational design of phenylthiazole-based oxadiazole derivatives led to the identification of compound **35**, a promising antifungal agent with potent activity against a wide spectrum of fungal species, including resistant strains. Its excellent safety profile, as evidenced by low cytotoxicity and hemolytic activity, and its non-disruptive mechanism highlight its therapeutic potential. These results demonstrate the value of legand-based drug design in addressing the limitations of current antifungal treatments and support further development of compound **35** as a novel antifungal therapy.

4 Experimental section

4.1. Chemistry

4.1.1. General. ^{1}H NMR spectra were run at 400 MHz and 13C spectra were determined at 100 MHz in deuterated

Table 3 *In silico* ADME profile of compound **35**

Compound	*M* log *P*[a]	TPSA	*n*-ON	*n*-OHNH	NRB	L.Vio.	Log *S* (ESOL)	Solubility class
35	0.21	118.10 Å^2	6	2	7	0	−5.57	Moderately soluble

[a] The logarithm of partition coefficient between *n*-octanol and water (*M* log *P*); topological polar surface area (TPSA); number of hydrogen bond acceptors (*n*-ON); number of hydrogen bond donors (*n*-OHNH); number of rotatable bonds (NRB); Lipinski's violation (L.Vio.); the logarithm of solubility (log *S*) = log(solubility measured in mol L^{-1}).

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dimethyl sulfoxide (DMSO-d_6) on a Varian Mercury VX-400 NMR spectrometer. Chemical shifts are given in parts per million (ppm) on the delta (d) scale. Chemical shifts were calibrated relative to those of the solvents. The progress of reactions was monitored through thin-layer chromatography (TLC) using Merck silica gel IB2-F plates (0.25 mm thickness), where the spots were visualized through Vilber VL-6.LC UV lamp, with wavelength 365/254 nm. Mass spectra were recorded on a compact Mass Spectrometer (TLC-MS, Advion, USA) using APCI and ESI modes. Melting points were determined and were uncorrected, using capillary tubes with a Stuart SMP30 apparatus. All yields reported refer to isolated yields.

*4.1.1.1. Synthesis of 2-(4-butylphenyl)-4-methylthiazole-5-carbohydrazide (**6**).* To a solution of ester derivative **5** (1.29 g, 4 mmol) in ethanol (15 mL), hydrazine hydrate (99%, 1 mL, 20 mmol) was added dropwise. The reaction mixture was heated at reflux for 6 h then allowed to cool down to room temperature. The formed solid was separated by filtration and crystallized from ethanol to provide the desired product as white crystals (1.1 g, 93%); mp = 133–135 °C; IR (KBr) cm^{-1}: 3289, 3185 (NH_2), 3160 (NH), 3026 (C–H aromatic), 2987, 2943 (C–H aliphatic), 1668 (C=O amide); ^{1}H NMR (DMSO-d_6) δ: 9.66 (brs, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 4.53 (brs, 2H), 2.78 (s, 3H), 2.68 : 2.65 (m, 2H), 1.58 : 1.56 (m, 2H), 1.38 : 1.30 (m, 2H), 0.93–0.89 (m, 3H); MS (*m*/*z*) 389 (M^+, 100%).

4.1.1.2. Synthesis of 5-(2-(4-butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazole-2-thiolate (7). Potassium hydroxide (0.4 g, 10 mmol) was added to a solution of **6** (3 g, 10 mmol) in ethanol (15 mL), followed by drop-wise addition of carbon disulphide (3 mL, 110 mmol) over 0.5 h. The reaction mixture was stirred at room temperature for an additional 15 min and then heated to reflux until the evolution of hydrogen sulfide gas ceased. After completion of the reaction, as monitored by TLC, the obtained product was poured on cold water (50 mL), washed with water, dried and crystallized from ethanol to provide thiol derivative as yellow crystals (3.3 g, 89%); mp > 300 °C.

*4.1.1.3. Synthesis of 2-(2-(4-butylphenyl)-4-methylthiazol-5-yl)-5-(methylthio)-1,3,4-oxadiazole (**8**).* The obtained yellow solid **7** (0.25 g, 4.2 mmol) was dissolved in water (15 mL). Then, dimethyl sulfate (0.5 mL, 4 mmol) was added dropwise with vigorous stirring. After 2 h, the formed solid was filtered and washed with copious amounts of water to yield the titled compound as a yellowish white solid (0.68 g, 91%); mp = 154 °C; ^{1}H NMR (DMSO-d_6) δ: 7.90 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 2.77 (s, 3H), 2.71 (s, 3H), 2.64 : 2.62 (m, 2H), 1.62 : 1.56 (m, 2H), 1.37–1.31 (m, 2H), 0.93 : 0.89 (m, 3H); MS (*m*/*z*) 345 (M^+, 14%).

*4.1.1.4. Synthesis of 2-(2-(4-butylphenyl)-4-methylthiazol-5-yl)-5-(methylsulfonyl)-1,3,4 oxadiazole (**9**).* To a solution of *S*-methyl derivative **8** (0.5 g, 1.3 mmol) in dry DCM (5 mL), *m*-CPBA (0.514 g, 2.9 mmol) diluted with DCM (5 mL) was added portion-wise with continuous stirring. Afterward, the reaction mixture was kept at room temperature for 4 h, additional DCM (10 mL) was added and the reaction mixture was washed with 25 mL of 5% aqueous solution of sodium metabisulfite, and 25 mL of 5% aqueous sodium carbonate. The organic layer was separated, dried and concentrated under reduced pressure to give the desired product as yellow crystals (0.5 g, 93%) mp = 126 °C; ^{1}H NMR (DMSO-d_6) δ: 7.92 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 3.83 (s, 3H), 2.78 (s, 3H), 2.74 : 2.65 (m, 2H), 1.58 : 1.57 (m, 2H), 1.38 : 1.36 (m, 2H), 0.93 : 0.89 (m, 3H); MS (*m*/*z*) 377 (M^+, 100%).

4.1.2. Compounds 10–44

*4.1.2.1. General procedure **A**.* To a solution of **9** (0.1 g, 0.25 mmol) in dry DMF (5 mL), appropriate amine, diamine, aminoalcohol, hydrazine, guanidine, guanidine analogue or carboximidate (0.4 mmol) was added. The reaction mixture was heated at 80 °C for 0.5–12 h, and then poured over ice water (50 mL). The formed solid was extracted with ethyl acetate (10 mL). The organic layer was evaporated under reduced pressure. The obtained crude material was then purified by crystallization or column chromatography. Physical properties and spectral analysis of isolated products are listed below.

*4.1.2.2. 5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-N-methyl-1,3,4-oxadiazol-2-amine (**10**).* Following the general procedure (**A**), and using methylamine (13 μL, 0.4 mmol), compound **10** was obtained as yellowish white solid (0.078 g, 91%); mp = 170 °C; ^{1}H NMR (DMSO-d_6) δ: 7.89 (d, J = 8.4 Hz, 2H), 7.77 (brs, 1H), 7.36 (d, J = 8.4 Hz, 2H), 2.87 (s, 3H), 2.65 (s, 3H), 2.63 : 2.56 (m, 2H), 1.61 : 1.55 (m, 2H), 1.37 : 1.32 (m, 2H), 0.92 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 166.6, 164.2, 153.8, 153, 146.3, 130.2, 129.7, 126.7, 114.9, 35.1, 33.2, 29.5, 22.2, 17.4, 14.2; MS (*m*/*z*) 328 (M^+, 100%); anal. calc. for: ($C_{17}H_{20}N_4OS$, Mwt = 328): C, 62.17; H, 6.14; N, 17.06%; found: C, 62.22; H, 6.19; N, 17.09%.

*4.1.2.3. 5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-N-ethyl-1,3,4-oxadiazol-2-amine (**11**).* Following the general procedure (**A**), and using ethylamine (18 μL, 0.4 mmol), compound **11** was obtained as white solid (0.085 g, 95%); mp = 115 °C; ^{1}H NMR (DMSO-d_6) δ: 7.90 (d, J = 8.4 Hz, 2H), 7.77 (brs, 1H), 7.35 (d, J = 8.4 Hz, 2H), 3.26 : 3.24 (m, 2H), 2.67 (s, 3H), 2.63 : 2.60 (m, 2H), 1.62 : 1.55 (m, 2H), 1.26 : 1.22 (m, 2H), 1.18 : 1.16 (m, 3H), 0.93 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 166.6, 164.7, 153.7, 152.9, 146.1, 130.2, 129.6, 126.9, 114.9, 37.9, 35.1, 33.2, 22.2, 17.4, 14.4, 13.7; MS (*m*/*z*) 342 (M^+, 100%); anal. calc. for: ($C_{18}H_{22}N_4OS$, Mwt = 342): C, 63.13; H, 6.48; N, 16.36%; found: C, 63.19; H, 6.52; N, 16.40%.

*4.1.2.4. 5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-N-isopropyl-1,3,4-oxadiazol-2-amine (**12**).* Following the general procedure (**A**), and using isopropylamine (23 μL, 0.4 mmol), compound **12** was obtained as white solid (0.085 g, 92%); mp = 135 °C; ^{1}H NMR (DMSO-d_6) δ: 8.05 (brs, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 3.78 : 3.71 (m, 1H), 2.84 (s, 3H), 2.68 : 2.54 (m, 2H), 1.58 : 1.57 (m, 2H), 1.33 : 1.28 (m, 2H), 1.23 : 1.21 (m, 6H), 0.93 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 166.6, 162.9, 153.7, 152.8, 146.1, 130.2, 129.6, 126.9, 115, 45.5, 35.1, 33.2, 22.7, 22, 17.4, 14; MS (*m*/*z*) 356 (M^+, 68%); anal. calc. for: ($C_{19}H_{24}N_4OS$, Mwt = 356): C, 64.02; H, 6.79; N, 15.72%; found: C, 64.07; H, 6.84; N, 15.77%.

*4.1.2.5. N-Butyl-5-(2-(4-butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-amine (**13**).* Following the general procedure (**A**), and using butylamine (25 μL, 0.4 mmol), compound **13** was obtained as buff solid (0.09 g, 93%); mp = 175 °C; ^{1}H NMR (DMSO-d_6) δ: 7.90 (d, J = 8.4 Hz, 2H), 7.77 (brs, 1H), 7.38 (d, J = 8.4 Hz, 2H), 3.26 : 3.21 (m, 2H), 2.68 (s, 3H), 2.62 : 2.51 (m, 2H),

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1.62 : 1.58 (m, 4H), 1.39 : 1.31 (m, 4H), 0.98 : 0.91 (m, 6H); ^{13}C NMR (DMSO-d_6) δ: 166.6, 163.7, 153.7, 152.8, 146.2, 130.2, 129.7, 126.6, 115, 42.7, 35.1, 33.2, 31.3, 22.1, 19.8, 17.3, 14.2, 14; MS (*m*/*z*) 370 (M^+, 100%); anal. calc. for: ($C_{20}H_{26}N_4OS$, Mwt = 370): C, 64.83; H, 7.07; N, 15.12%; found: C, 64.88; H, 7.11; N, 15.15%.

*4.1.2.6. N-(sec-Butyl)-5-(2-(4-butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-amine (**14**).* Following the general procedure (**A**), and using *sec*-butylamine (28 μL, 0.4 mmol), compound **14** was obtained as yellow solid (0.089 g, 92%); mp = 120 °C; ^{1}H NMR (DMSO-d_6) δ: 7.93 (d, J = 8.4 Hz, 2H), 7.81 (brs, 1H), 7.33 (d, J = 8.4 Hz, 2H), 3.58 : 3.55 (m, 1H), 2.78 (s, 3H), 2.68 : 2.51 (m, 4H), 1.60 : 1.57 (m, 2H), 1.37 : 1.33 (m, 2H), 1.22 : 1.20 (m, 3H), 0.94 : 0.89 (m, 6H); ^{13}C NMR (DMSO-d_6) δ: 166.5, 163.2, 153.6, 152.7, 146.1, 130.2, 129.6, 126.6, 115, 51, 35.1, 33.2, 29.1, 22.1, 20.3, 17.4, 14.2, 10.8; MS (*m*/*z*) 370 (M^+, 48%); anal. calc. for: ($C_{20}H_{26}N_4OS$, Mwt = 370): C, 64.83; H, 7.07; N, 15.12%; found: C, 64.87; H, 7.11; N, 15.17%.

*4.1.2.7. 5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-N-cyclopropyl-1,3,4-oxadiazol-2-amine (**15**).* Following the general procedure (**A**), and using cyclopropylamine (18 μL, 0.4 mmol), compound **15** was obtained as yellow solid (0.06 g, 65%); mp = 165 °C; ^{1}H NMR (DMSO-d_6) δ: 8.20 (brs, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 3.09 : 3.07 (m, 1H), 2.68 (s, 3H), 2.64 : 2.62 (m, 2H), 1.58 : 1.56 (m, 2H), 1.37 : 1.31 (m, 2H), 0.93 : 0.89 (m, 3H), 0.75 : 0.72 (m, 2H), 0.59 : 0.53 (m, 2H); ^{13}C NMR (DMSO-d_6) δ: 166.7, 164.1, 153.8, 153.3, 146.3, 130.2, 129.7, 126.7, 114.9, 35.1, 33.2, 24.6, 22.1, 17.3, 14.2, 6.8; MS (*m*/*z*) 354.3 (M^+, 100%); anal. calc. for: ($C_{19}H_{22}N_4OS$, Mwt = 354): C, 64.38; H, 6.26; N, 15.81%; found: C, 64.44; H, 6.29; N, 15.85%.

*4.1.2.8. 5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-N-cyclobutyl-1,3,4-oxadiazol-2-amine (**16**).* Following the general procedure (**A**), and using cyclobutylamine (20 μL, 0.4 mmol), compound **16** was obtained as white solid (0.091 g, 95%); mp = 155 °C; ^{1}H NMR (DMSO-d_6) δ: 8.19 (brs, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 4.07 : 4.03 (m, 1H), 2.78 (s, 3H), 2.70 : 2.64 (m, 2H), 2.31 : 2.26 (m, 2H), 2.08 : 2.03 (m, 2H), 1.73 : 1.70 (m, 2H), 1.58 : 1.56 (m, 2H), 1.33 : 1.28 (m, 2H), 0.93 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 166.7, 162.5, 153.8, 152.9, 146.3, 130.2, 129.6, 127.2, 114.9, 48.2, 35.1, 33.2, 30.4, 22.1, 17, 14.8, 14.2; MS (*m*/*z*) 368 (M^+, 100%); anal. calc. for: ($C_{20}H_{24}N_4OS$, Mwt = 368): C, 65.19; H, 6.57; N, 15.20%; found: C, 65.26; H, 6.62; N, 15.26%.

*4.1.2.9. 5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-N-cyclopentyl-1,3,4-oxadiazol-2-amine (**17**).* Following the general procedure (**A**), and using cyclopentylamine (34 μL, 0.4 mmol), compound **17** was obtained as brown solid (0.091 g, 91%); mp = 165 °C; ^{1}H NMR (DMSO-d_6) δ: 8.16 (brs, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 3.93 : 3.88 (m, 1H), 2.68 (s, 3H), 2.57 : 2.53 (m, 2H), 1.92 : 1.90 (m, 2H), 1.72 : 1.70 (m, 2H), 1.58 : 1.52 (m, 4H), 1.33 : 1.23 (m, 4H), 0.93 : 0.87 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 166.6, 163.2, 153.7, 152.9, 146.2, 130.2, 129.7, 126.3, 115, 54.8, 35.1, 33.2, 32.6, 23.7, 22.1, 17.3, 14.2; MS (*m*/*z*) 382 (M^+, 100%); anal. calc. for: ($C_{21}H_{26}N_4OS$, Mwt = 382): C, 65.94; H, 6.85; N, 14.65%; found: C, 65.98; H, 6.89; N, 14.69%.

*4.1.2.10. 5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-N-cyclohexyl-1,3,4-oxadiazol-2-amine (**18**).* Following the general procedure (**A**), and using cyclohexylamine (40 μL, 0.4 mmol), compound **18** was obtained as brown solid (0.08 g, 78%); mp = 176 °C; ^{1}H NMR (DMSO-d_6) δ: 8.02 (brs, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 3.88 : 3.82 (m, 1H), 2.81 (s, 3H), 2.68 : 2.56 (m, 4H), 2.01 : 1.96 (m, 2H), 1.73 : 1.67 (m, 2H), 1.56 : 1.52 (m, 4H), 1.33 : 1.30 (m, 4H), 0.95 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 166.5, 162.9, 153.6, 152.7, 146.2, 130.2, 129.3, 126.3, 115, 52.3, 35.1, 33.3, 32.6, 25.3, 24.8, 22.2, 17, 14.2; MS (*m*/*z*) 396 (M^+, 100%); anal. calc. for: ($C_{22}H_{28}N_4OS$, Mwt = 396): C, 66.63; H, 7.12; N, 14.13%; found: C, 66.66; H, 7.15; N, 14.17%.

*4.1.2.11. 5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-N-cycloheptyl-1,3,4-oxadiazol-2-amine (**19**).* Following the general procedure (**A**), and using cycloheptylamine (50 μL, 0.4 mmol), compound **19** was obtained as orange solid (0.091 g, 85%); mp = 188 °C; ^{1}H NMR (DMSO-d_6) δ: 8.13 (brs, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 3.65 : 3.63 (m, 1H), 2.78 (s, 3H), 2.73 : 2.68 (m, 2H), 1.99 : 1.95 (m, 2H), 1.59 : 1.26 (m, 14H), 0.93 : 0.91 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 166.6, 162.8, 153.6, 152.8, 146.1, 130.9, 129.6, 127.2, 115, 54.5, 35.1, 34.4, 33.2, 29.3, 23.9, 22.1, 17.4, 14.2; MS (*m*/*z*) 410 (M^+, 100%); anal. calc. for: ($C_{23}H_{30}N_4OS$, Mwt = 410): C, 67.28; H, 7.37; N, 13.65%; found: C, 67.34; H, 7.41; N, 13.67%.

*4.1.2.12. 5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-N,N-dimethyl-1,3,4-oxadiazol-2-amine (**20**).* Following the general procedure (**A**), and using dimethylamine (18 μL, 0.4 mmol), compound **20** was obtained as brown solid (0.07 g, 79%); mp = 110 °C; ^{1}H NMR (DMSO-d_6) δ: 7.89 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 3.07 (s, 6H), 2.68 (s, 3H), 2.64 : 2.62 (m, 2H), 1.62 : 1.55 (m, 2H), 1.33 : 1.28 (m, 2H), 0.93 : 0.91 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 166.7, 164.6, 153.8, 153.5, 146.3, 130.2, 129.7, 126.7, 114.8, 38.1, 35.1, 33.2, 22.2, 17.3, 14.2; MS (*m*/*z*) 342 (M^+, 100%); anal. calc. for: ($C_{18}H_{22}N_4OS$, Mwt = 342): C, 63.13; H, 6.48; N, 16.36%; found: C, 63.15; H, 6.51; N, 16.39%.

*4.1.2.13. 5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-N,N-diethyl-1,3,4-oxadiazol-2-amine (**21**).* Following the general procedure (**A**), and using diethylamine (25 μL, 0.4 mmol), compound **21** was obtained as yellow solid (0.068 g, 70%); mp = 158 °C; ^{1}H NMR (DMSO-d_6) δ: 7.87 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 3.49 : 3.44 (m, 4H), 2.67 (s, 3H), 2.62 : 2.51 (m, 2H), 1.59 : 1.57 (m, 2H), 1.30 : 1.29 (m, 2H), 1.18 : 1.12 (m, 6H), 0.92 : 0.90 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 166.6, 163.4, 153.6, 153.2, 146.2, 130.2, 129.6, 126.7, 114.9, 43.5, 35.1, 33.2, 22.2, 17.3, 14.2, 13.4; MS (*m*/*z*) 370 (M^+, 100%); anal. calc. for: ($C_{20}H_{26}N_4OS$, Mwt = 370): C, 64.83; H, 7.07; N, 15.12%; found: C, 64.87; H, 7.11; N, 15.14%.

*4.1.2.14. 2-(Azetidin-1-yl)-5-(2-(4-butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazole (**22**).* Following the general procedure (**A**), and using azetidine (40 mg, 0.4 mmol), compound **22** was obtained as yellow solid (0.07 g, 76%); mp = 230 °C; ^{1}H NMR (DMSO-d_6) δ: 7.90 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 4.18 : 4.13 (m, 4H), 2.68 (s, 3H), 2.63 : 2.61 (m, 2H), 2.45 : 2.42 (m, 2H), 1.61 : 1.59 (m, 2H), 1.31 : 1.28 (m, 2H), 0.93 : 0.91 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 167.1, 164.7, 154.3, 153.4, 146.4, 130.2, 129.7, 126.8, 114.6, 52.5, 35.3, 33.2, 22.1, 17.8, 17.4, 14.2; MS (*m*/*z*) 354 (M^+, 100%); anal. calc. for: ($C_{19}H_{22}N_4OS$, Mwt = 354): C, 64.38; H, 6.26; N, 15.81%; found: C, 64.43; H, 6.29; N, 15.85%.

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*4.1.2.15. 1-(5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)azetidin-3-ol (**23**).* Following the general procedure (**A**), and using 3-hydroxyazetidine (25 mg, 0.4 mmol), compound **23** was obtained as white solid (0.09 g, 93%); mp = 190 °C; ^{1}H NMR (DMSO-d$_6$) δ: 7.88 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 5.85 (brs, 1H), 4.67 : 4.65 (m, 1H), 4.32 (dd, J = 4.6 Hz, J = 9.2 Hz, 2H), 3.96 (dd, J = 4.8 Hz, J = 9.2 Hz, 2H), 2.68 (s, 3H), 2.63 : 2.59 (m, 2H), 1.61 : 1.59 (m, 2H), 1.37 : 1.33 (m, 2H), 0.93 : 0.91 (m, 3H); ^{13}C NMR (DMSO-d$_6$) δ: 167.1, 164.8, 154.5, 154.4, 146.4, 130.2, 129.7, 126.8, 114.5, 62.4, 62.2, 35.1, 33.2, 22.1, 17.4, 14.2; MS (*m*/*z*) 370 (M$^+$, 97%); anal. calc. for: ($C_{19}H_{22}N_4O_2S$, Mwt = 370): C, 61.60; H, 5.99; N, 15.12%; found: C, 61.66; H, 6.04; N, 15.16%.

*4.1.2.16. 2-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-5-(pyrrolidin-1-yl)-1,3,4-oxadiazole (**24**).* Following the general procedure (**A**), and using pyrrolidine (28 μL, 0.4 mmol), compound **24** was obtained as yellow solid (0.086 g, 90%); mp = 115 °C; ^{1}H NMR (DMSO-d$_6$) δ: 7.89 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 3.50 : 3.48 (m, 4H), 2.68 (s, 3H), 2.64 : 2.61 (m, 2H), 2.00 : 1.98 (m, 4H), 1.59 : 1.57 (m, 2H), 1.37 : 1.35 (m, 2H), 0.93 : 0.91 (m, 3H); ^{13}C NMR (DMSO-d$_6$) δ: 166.6, 162.4, 153.7, 153.4, 146.2, 130.2, 129.7, 126.6, 114.9, 48, 35.1, 33.2, 25.5, 22.2, 17.3, 14.2; MS (*m*/*z*) 368 (M$^+$, 100%); anal. calc. for: ($C_{20}H_{24}N_4OS$, Mwt = 368): C, 65.19; H, 6.57; N, 15.20%; found: C, 65.22; H, 6.59; N, 15.23%.

*4.1.2.17. 2-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-5-(piperidin-1-yl)-1,3,4-oxadiazole (**25**).* Following the general procedure (**A**), and using piperidine (34 μL, 0.4 mmol), compound **25** was obtained as yellow solid (0.092 g, 93%); mp = 123 °C; ^{1}H NMR (DMSO-d$_6$) δ: 7.93 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 3.48 : 3.44 (m, 4H), 2.69 (s, 3H), 2.65 : 2.62 (m, 2H), 1.62 : 1.53 (m, 6H), 1.34 : 1.28 (m, 2H), 0.91 : 0.85 (m, 5H); ^{13}C NMR (DMSO-d$_6$) δ: 166.5, 163.5, 153.7, 152.9, 146.5, 130.4, 130.3, 126.6, 113.3, 64.6, 36.9, 35.1, 33.5., 32.9, 21.9, 18.9, 13.9; MS (*m*/*z*) 382 (M$^+$, 100%); anal. calc. for: ($C_{21}H_{26}N_4OS$, Mwt = 382): C, 65.94; H, 6.85; N, 14.65%; Found: C, 65.97; H, 6.85; N, 14.66%.

*4.1.2.18. 4-(5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)morpholine (**26**).* Following the general procedure (**A**), and using morpholine (35 μL, 0.4 mmol), compound **26** was obtained as greenish yellow solid (0.048 g, 49%); mp = 143 °C; ^{1}H NMR (DMSO-d$_6$) δ: 7.89 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 3.75 : 3.73 (m, 4H), 3.48 : 3.46 (m, 4H), 2.69 (s, 3H), 2.68 : 2.65 (m, 2H), 1.62 : 1.59 (m, 2H), 1.32 : 1.26 (m, 2H), 0.91 : 0.86 (m, 3H); ^{13}C NMR (DMSO-d$_6$) δ: 166.6, 165.9, 154.3, 153, 146.5, 130, 126.8, 126.5, 115.3, 54.5, 44.3, 35.2, 33.1, 22.3, 17.5, 14.5; MS (*m*/*z*) 384 (M$^+$, 100%); anal. calc. for: ($C_{20}H_{24}N_4O_2S$, Mwt = 384): C, 62.48; H, 6.29; N, 14.57%; found: C, 62.52; H, 6.33; N, 14.60%.

*4.1.2.19. 2-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-5-hydrazinyl-1,3,4-oxadiazole (**27**).* Following the general procedure (**A**), and using and using hydrazine hydrate (5 mL), compound **27** was obtained as grayish black solid (0.072 g, 85%); mp = 170 °C; ^{1}H NMR (DMSO-d$_6$) δ: 8.74 (brs, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 4.56 (brs, 2H), 2.77 (s, 3H), 2.69 : 2.58 (m, 2H), 1.61 : 1.55 (m, 2H), 1.37 : 1.32 (m, 2H), 0.91 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d$_6$) δ: 166.5, 163.5, 153.7, 152.9, 146.5, 130, 126.8, 126.6, 115, 35.9, 33.6, 22.5, 17.4, 14.9; MS (*m*/*z*) 329 (M$^+$, 100%); anal. calc. for: ($C_{16}H_{19}N_5OS$, Mwt = 329): C, 58.34; H, 5.81; N, 21.26%; found: C, 58.38; H, 5.85; N, 21.28%.

*4.1.2.20. N-(5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)ethane-1,2-diamine (**28**).* Following the general procedure (**A**), and using ethane-1,2-diamine (20 μL, 0.4 mmol), compound **28** was obtained as yellowish brown solid (0.085 g, 92%); mp = 230 °C; ^{1}H NMR (DMSO-d$_6$) δ: 8.35 (brs, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 3.74 : 3.72 (m, 2H), 2.69 (s, 3H), 2.61 : 2.57 (m, 2H), 2.45 : 2.43 (m, 2H), 1.75 (brs, 2H), 1.59 : 1.57 (m, 2H), 1.34 : 1.31 (m, 2H), 0.93 : 0.90 (m, 3H); ^{13}C NMR (DMSO-d$_6$) δ: 166.7, 162.4, 153.7, 153.4, 146.2, 130.6, 126.5, 126.4, 115, 55.5, 36.9, 35.1, 31.3, 22.7, 18.5, 13.9; MS (*m*/*z*) 357 (M$^+$, 46%); anal. calc. for: ($C_{18}H_{23}N_5OS$, Mwt = 357): C, 60.48; H, 6.49; N, 19.59%; found: C, 60.52; H, 6.53; N, 19.59%.

*4.1.2.21. N-(5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)-N,N-dimethylethane-1,2-diamine (**29**).* Following the general procedure (**A**), and using *N*,*N*-Dimethylethylenediamine (30 μL, 0.4 mmol), compound **29** was obtained as yellow solid (0.095 g, 95%); mp = 179 °C; ^{1}H NMR (DMSO-d$_6$) δ: 7.93 (brs, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 3.84 : 3.82 (m, 2H), 2.77 (s, 3H), 2.66 : 2.62 (m, 2H), 2.45 : 2.42 (m, 2H), 2.18 (s, 6H), 1.59 : 1.57 (m, 2H), 1.34 : 1.32 (m, 2H), 0.90 : 0.88 (m, 3H); ^{13}C NMR (DMSO-d$_6$) δ: 166.6, 164.7, 153.7, 152.9, 146.2, 130.2, 129.3, 126.2, 114, 58.2, 45.6, 35.1, 33, 22.2, 17.4, 14.7, 14.2; MS (*m*/*z*) 385 (M$^+$, 71%); anal. calc. for: ($C_{20}H_{27}N_5OS$, Mwt = 385): C, 62.31; H, 7.06; N, 18.17%; found: C, 62.34; H, 7.08; N, 18.20%.

*4.1.2.22. 2-((5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)amino)ethan-1-ol (**30**).* Following the general procedure (**A**), and using ethanolamine (20 μL, 0.4 mmol), compound **30** was obtained as white solid (0.092 g, 98%); mp = 184 °C; ^{1}H NMR (DMSO-d$_6$) δ: 7.93 (brs, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 4.81 (brs, 1H), 4.62 : 4.60 (m, 2H), 3.32 : 3.26 (m, 2H), 2.76 (s, 3H), 2.68 : 2.65 (m, 2H), 1.59 : 1.57 (m, 2H), 1.34 : 1.32 (m, 2H), 0.92 : 0.90 (m, 3H); ^{13}C NMR (DMSO-d$_6$) δ: 168.6, 167, 155.2, 154, 146.9, 130.9, 126.9, 126.1, 115.5, 65.6, 64.6, 35.3, 33.1, 22.3, 16.5, 13.9; MS (*m*/*z*) 358 (M$^+$, 100%); anal. calc. for: ($C_{18}H_{22}N_4O_2S$, Mwt = 358): C, 60.31; H, 6.19; N, 15.63%; found: C, 60.35; H, 6.22; N, 15.66%.

*4.1.2.23. N^1-(5-(2-(4-butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)propane-1,3-diamine (**31**).* Following the general procedure (**A**), and using propane-1,3-diamine (28 μL, 0.4 mmol), compound **31** was obtained as yellow solid (0.08 g, 83%); mp = 252 °C; ^{1}H NMR (DMSO-d$_6$) δ: 7.89 (d, J = 8.4 Hz, 2H), 7.76 (brs, 1H), 7.35 (d, J = 8.4 Hz, 2H), 3.45 : 3.42 (m, 2H), 2.68 (s, 3H), 2.62 : 2.59 (m, 2H), 2.43 : 2.41 (m, 2H), 1.79 (brs, 2H), 1.58 : 1.56 (m, 2H), 1.33 : 1.29 (m, 2H), 1.09 : 1.06 (m, 2H), 0.92 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d$_6$) δ: 166.8, 163.6, 153.8, 152.9, 146.3, 130.2, 126.7, 126, 114.9, 46.6, 37, 35.1, 33.3, 33.2, 22.1, 17.4, 14.2; MS (*m*/*z*) 371 (M$^+$, 75%); anal. calc. for: ($C_{19}H_{25}N_5OS$, Mwt = 371): C, 61.43; H, 6.78; N, 18.85%; found: C, 61.45; H, 6.81; N, 18.89%.

*4.1.2.24. 3-((5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)amino)propan-1-ol (**32**).* Following the general

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procedure (**A**), and using 3-aminopropan-1-ol (28 μL, 0.4 mmol), compound **32** was obtained as yellow solid (0.091 g, 94%); mp = 249 °C; ^{1}H NMR (DMSO-d$_6$) *δ*: 7.93 (brs, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 4.57 (brs, 1H), 3.52 : 3.50 (m, 2H), 3.31 : 3.29 (m, 2H), 2.77 (s, 3H), 2.63 : 2.60 (m, 2H), 1.76 : 1.74 (m, 2H), 1.61 : 1.56 (m, 2H), 1.31 : 1.26 (m, 2H), 0.92 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d$_6$) *δ*: 166.9, 161.5, 154.3, 153.8, 146.4, 130.9, 126.3, 124.1, 115.9, 64.6, 38.3, 37.9, 35.1, 34.1, 22.3, 17.5, 14.9; MS (*m*/*z*) 372 (M$^+$, 100%); anal. calc. for: ($C_{19}H_{24}N_4O_2S$, Mwt = 372): C, 61.27; H, 6.49; N, 15.04%; found: C, 61.33; H, 6.53; N, 15.07%.

*4.1.2.25. N-(5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)butane-1,4-diamine (**33**).* Following the general procedure (**A**), and using butane-1,4-diamine (34 μL, 0.4 mmol), compound **33** was obtained as yellow solid (0.096 g, 96%); mp = 209 °C; 1H NMR (DMSO-d6) *δ*: 7.92 (brs, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 3.87 : 3.82 (m, 2H), 3.26 : 3.23 (m, 2H), 2.78 : 2.72 (m, 2H), 2.68 (s, 3H), 2.64 : 2.62 (m, 2H), 1.60 : 1.58 (m, 2H), 1.48 : 1.46 (m, 2H), 1.33 : 1.31 (m, 2H), 0.93 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d$_6$) *δ*: 166.5, 164.9, 154, 152.8, 145.4, 130.6, 126.9, 126.3, 115.4, 64.6, 37.9, 35.1, 34.6, 31.3, 26.5, 26.4, 17.5, 14.9; MS (*m*/*z*) 385 (M$^+$, 68%); anal. calc. for: ($C_{20}H_{27}N_5OS$, Mwt = 385): C, 62.31; H, 7.06; N, 18.17%; found: C, 62.33; H, 7.08; N, 18.19%.

*4.1.2.26. 2-((5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)amino)propane-1,3-diol (**34**).* Following the general procedure (**A**), and using 2-aminopropane-1,3-diol (30 μL, 0.4 mmol), compound **34** was obtained as red solid (0.093 g, 93%); mp = 126 °C; ^{1}H NMR (DMSO-d$_6$) *δ*: 7.90 (d, *J* = 8.4 Hz, 2H), 7.76 (brs, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 4.79 (brs, 2H), 3.84 : 3.79 (m, 4H), 3.59 : 3.57 (m, 1H), 2.78 (s, 3H), 2.70 : 2.64 (m, 2H), 1.58 : 1.55 (m, 2H), 1.33 : 1.31 (m, 2H), 0.93 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d$_6$) *δ*: 168.6, 164.7, 160.6, 156.3, 146.7, 130.2, 129.9, 126.2, 113.7, 60.5, 58, 35.1, 33.2, 22.1, 17.4, 14.2; MS (*m*/*z*) 388 (M$^+$, 100%); anal. calc. for: ($C_{19}H_{24}N_4O_3S$, Mwt = 388): C, 58.74; H, 6.26; N, 14.42%; found: C, 58.77; H, 6.29; N, 14.45%.

*4.1.2.27. N-{5-[2-(4-Butylphenyl)-4-methylthiazol-5-yl]-1,3,4-oxadiazol-2-yl}cyclohexane-cis-1,2-diamine (**35**).* Following the general procedure (**A**), and using *cis*-1,2-Diaminocyclohexane (0.045 g, 0.4 mmol), compound **35** was obtained as brown solid (0.09 g, 84%); mp = 83 °C; ^{1}H NMR (DMSO-d$_6$) *δ*: 7.85 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.11 (brs, 1H), 3.90 : 3.88 (m, 1H), 3.54 : 3.51 (m, 1H), 3.14 : 3.08 (m, 2H), 2.70 (s, 3H), 2.61 : 2.59 (m, 2H), 1.58 : 1.52 (m, 2H), 1.30 : 1.20 (m, 8H), 1.05 (brs, 2H), 0.85 : 0.82 (m, 3H); ^{13}C NMR (DMSO-d$_6$) *δ*: 166.7, 161.4, 157.6, 153.4, 145.9, 130.8, 126.9, 126.5, 115.5, 49.3, 43.9, 39.9, 36.9, 35, 33.2, 26.7, 22.7, 22.1, 18.6, 14.2; MS (*m*/*z*) 411 (M$^+$, 60%); anal. calc. for: ($C_{22}H_{29}N_5OS$, Mwt = 411): C, 64.20; H, 7.10; N, 17.02%; found: C, 64.26; H, 7.15; N, 17.07%.

*4.1.2.28. N-{5-[2-(4-Butylphenyl)-4-methylthiazol-5-yl]-1,3,4-oxadiazol-2-yl}cyclohexane-trans-1,2-diamine (**36**).* Following the general procedure (**A**), and using *trans*-1,2-diaminocyclohexane (0.045 g, 0.4 mmol), compound **36** was obtained as brown solid (0.088 g, 81%); mp = 99 °C; ^{1}H NMR (DMSO-d$_6$) *δ*: 7.88 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.05 (brs, 1H), 3.86 : 3.78 (m, 2H), 2.86 : 2.80 (m, 2H), 2.68 (s, 3H), 2.62 : 2.57 (m, 2H), 1.58 : 1.53 (m, 2H), 1.30 : 1.11 (m, 8H), 1.03 (brs, 2H), 0.93 : 0.87 (m, 3H); ^{13}C NMR (DMSO-d$_6$) *δ*: 166.3, 161.5, 157.3, 153.5, 145.7, 130.9, 127.1, 126.6, 115.6, 40.5, 39.9, 39.4, 36.8, 35.1, 33.3, 25.4, 22.5, 22.2, 18.4, 14.2; MS (*m*/*z*) 411 (M$^+$, 62%); anal. calc. for: ($C_{24}H_{31}N_5S$, Mwt = 411): C, 64.20; H, 7.10; N, 17.02%; found: C, 64.27; H, 7.16; N, 17.05%.

*4.1.2.29. 1-(5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)guanidine (**37**).* Following the general procedure (**A**), and using guanidine hydrochloride (0.05 g, 0.5 mmol) and potassium carbonate anhydrous (0.1 g, 0.7 mmol), compound **37** was obtained as yellow solid (0.07 g, 76%); mp = 140 °C; ^{1}H NMR (DMSO-d$_6$) *δ*: 8.19 (brs, 2H), 7.93 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 8.4 Hz, 2H), 6.92 (brs, 2H), 2.73 (s, 3H), 2.65 : 2.63 (m, 2H), 1.59 : 1.57 (m, 2H), 1.34 : 1.32 (m, 2H), 0.93 : 0.91 (m, 3H); ^{13}C NMR (DMSO-d$_6$) *δ*: 166.5, 163.5, 154.3, 153.7, 146.9, 145, 130, 126.8, 126.6, 115, 35.1, 31.3, 22.4, 17.4, 14.9; MS (*m*/*z*) 356 (M$^+$, 30%); anal. calc. for: ($C_{17}H_{20}N_6OS$, Mwt = 356): C, 57.28; H, 5.66; N, 23.58%; found: C, 57.34; H, 5.70; N, 23.62%.

*4.1.2.30. 1-(5-(2-(4-butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)-3-methylguanidine (**38**).* Following the general procedure (**A**), and using methylguanidine hydrochloride (0.06 g, 0.5 mmol) and potassium carbonate anhydrous (0.1 g, 0.7 mmol), compound **38** was obtained as brown solid (0.09 g, 93%); mp = 160 °C; ^{1}H NMR (DMSO-d$_6$) *δ*: 7.88 (d, *J* = 8.4 Hz, 2H), 7.77 (brs, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 2.87 (brs, 1H), 2.78 (s, 3H), 2.67 (s, 3H), 2.61 : 2.59 (m, 2H), 1.61 : 1.59 (m, 2H), 1.35 : 1.29 (m, 2H), 0.91 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d$_6$) *δ*: 166.4, 164.2, 153.7, 153, 146.2, 146, 130.2, 129.9, 126.3, 114.7, 58, 35.1, 33.2, 22.2, 17, 14.2; MS (*m*/*z*) 370 (M$^+$, 41%); anal. calc. for: ($C_{18}H_{22}N_6OS$, Mwt = 370): C, 58.36; H, 5.99; N, 22.68%; found: C, 58.38; H, 6.01; N, 22.69%.

*4.1.2.31. 3-(5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)-1,1-dimethylguanidine (**39**).* Following the general procedure (**A**), and using 1,1-dimethylguanidine hydrochloride (0.06 g, 0.5 mmol) and potassium carbonate anhydrous (0.1 g, 0.7 mmol), compound **39** was obtained as yellow solid (0.06 g, 60%); mp = 175 °C; ^{1}H NMR (DMSO-d$_6$) *δ*: 7.90 (d, *J* = 8.4 Hz, 2H), 7.69 (brs, 2H), 7.36 (d, *J* = 8.4 Hz, 2H), 3.03 (s, 6H), 2.71 (s, 3H), 2.63 : 2.60 (m, 2H), 1.59 : 1.57 (m, 2H), 1.34 : 1.32 (m, 2H), 0.91 : 0.87 (m, 3H); ^{13}C NMR (DMSO-d$_6$) *δ*: 166.5, 163.7, 154.3, 153.7, 152.9, 146.4, 130, 126.8, 126.6, 115, 63.6, 35.1, 34.6, 22.3, 17.5, 14.9; MS (*m*/*z*) 384 (M$^+$, 37%); anal. calc. for: ($C_{19}H_{24}N_6OS$, Mwt = 384): C, 59.35; H, 6.29; N, 21.86%; found: C, 59.39; H, 6.32; N, 21.89%.

*4.1.2.32. N-(5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)-4-methylpiperazine-1-carboximidamide (**40**).* Following the general procedure (**A**), and and using 4-methylpiperazine-1-carboximidamide hydroiodide (0.11 g, 0.4 mmol) and potassium carbonate anhydrous (0.1 g, 0.7 mmol), compound **40** was obtained as orange solid (0.110 g, 92%); mp = 155 °C; ^{1}H NMR (DMSO-d$_6$) *δ*: 7.96 (brs, 2H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 3.62 : 3.57 (m, 4H), 2.70 (s, 3H), 2.62 : 2.59 (m, 2H), 2.34 : 2.32 (m, 4H), 2.21 (s, 3H), 1.62 : 1.55 (m, 2H), 1.33 : 1.28 (m, 2H), 0.93 : 0.87 (m, 3H); ^{13}C NMR (DMSO-d$_6$) *δ*: 166.9, 166.8, 156.9, 154, 153, 146.2, 130.3, 129.7, 126.5, 115.4, 54.6, 46, 44.4, 35.1, 33.2, 22.2, 17.5, 14.2; MS (*m*/*z*) 459 (M$^+$, 42%); anal. calc. for: ($C_{22}H_{29}N_7O_2S$, Mwt = 439): C, 60.11; H, 6.65; N, 22.31%; found: C, 60.15; H, 6.68; N, 22.34%.

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*4.1.2.33. N-(5-(2-(4-butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)morpholine-4-carboximidamide (**41**).* Following the general procedure (**A**), and using morpholine-4-carboximidamide hydroiodide (0.1 g, 0.4 mmol) and potassium carbonate anhydrous (0.1 g, 0.7 mmol), compound **41** was obtained as buff solid (0.07 g, 63%); mp = 200 °C; ^{1}H NMR (DMSO-d_6) δ: 7.97 (brs, 2H), 7.87 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 3.64 : 3.62 (m, 4H), 3.55 : 3.51 (m, 4H), 2.70 (s, 3H), 2.62 : 2.60 (m, 2H), 1.60 : 1.58 (m, 2H), 1.33 : 1.31 (m, 2H), 0.93 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 169.3, 166.6, 164.2, 153.8, 153, 146.3, 130.3, 129.7, 126.7, 114, 66.1, 44.8, 35.1, 33.2, 22.1, 17.5, 14.2; MS (*m*/*z*) 426 (M^+, 32%); anal. calc. for: ($C_{21}H_{26}N_6O_2S$, Mwt = 426): C, 59.13; H, 6.14; N, 19.70%; found: C, 59.15; H, 6.16; N, 19.73%.

*4.1.2.34. N-(5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)picolinimidamide (**42**).* Following the general procedure (**A**), and using picolinmidamide hydrochloride (0.06 g, 0.4 mmol) and potassium carbonate anhydrous (0.1 g, 0.7 mmol), compound **42** was obtained as yellow solid (0.1 g, 92%); mp = 188 °C; ^{1}H NMR (DMSO-d_6) δ: 9.47 (brs, 1H), 8.91 (brs, 1H), 8.78 : 8.72 (m, 1H), 8.41 : 8.38 (m, 1H), 8.07 : 8.05 (m, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.69 : 7.66 (m, 1H), 7.36 (d, J = 8.4 Hz, 2H), 2.78 (s, 3H), 2.68 : 2.66 (m, 2H), 1.59 : 1.53 (m, 2H), 1.33 : 1.31 (m, 2H), 0.92 : 0.90 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 167.8, 161.9, 159.2, 158.7, 158, 157.9, 155.1, 154.3, 154, 153.5, 146.5, 130.5, 130.3, 126.6, 113.3, 35.1, 31.3, 22.2, 18.9, 13.9; MS (*m*/*z*) 418 (M^+, 100%); anal. calc. for: ($C_{22}H_{22}N_6OS$, Mwt = 418): C, 63.14; H, 5.30; N, 20.08%; found: C, 63.18; H, 5.33; N, 20.11%.

*4.1.2.35. N-(5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)nicotinimidamide (**43**).* Following the general procedure (**A**), and using nicotinimidamide hydrochloride (0.06 g, 0.4 mmol) and potassium carbonate anhydrous (0.1 g, 0.7 mmol), compound **43** was obtained as yellow solid (0.1 g, 92%); mp = 139 °C; ^{1}H NMR (DMSO-d_6) δ: 9.61 (brs, 1H), 9.22 (brs, 1H), 9.06 (s, 1H), 8.82 : 8.79 (m, 1H), 8.44 : 8.41 (m, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.60 : 7.58 (m, 1H), 7.33 (d, J = 8.4 Hz, 2H), 2.78 (s, 3H), 2.68 : 2.63 (m, 2H), 1.61 : 1.60 (m, 2H), 1.36 : 1.34 (m, 2H), 0.93 : 0.90 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 166.4, 163.8, 160.4, 159.1, 157.8, 156.1, 154.5, 153.4, 152.8, 146.8, 135.9, 129.7, 126.6, 126.3, 115, 34.9, 33.2, 22.1, 17.4, 14.2; MS (*m*/*z*) 418 (M^+, 100%); anal. calc. for: ($C_{22}H_{22}N_6OS$, Mwt = 418): C, 63.14; H, 5.30; N, 20.08%; found: C, 63.18; H, 5.33; N, 20.09%.

*4.1.2.36. N-(5-(2-(4-Butylphenyl)-4-methylthiazol-5-yl)-1,3,4-oxadiazol-2-yl)isonicotinimidamide (**44**).* Following the general procedure (**A**), and using isonicotinimidamide hydrochloride (0.06 g, 0.4 mmol) and potassium carbonate anhydrous (0.1 g, 0.7 mmol), compound **44** was obtained as yellow solid (0.07 g, 64%); mp = 127 °C; ^{1}H NMR (DMSO-d_6) δ: 9.67 (brs, 1H), 9.07 (brs, 1H), 8.97 (d, J = 6 Hz, 2H), 8.00 (d, J = 6 Hz, 2H), 7.90 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 2.77 (s, 3H), 2.65 : 2.63 (m, 2H), 1.59 : 1.57 (m, 2H), 1.34 : 1.29 (m, 2H), 0.90 : 0.89 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 167.9, 166.3, 160.3, 155.5, 155.3, 150.8, 146.5, 141.6, 130.1, 129.6, 126.8, 121.9, 114.7, 35.1, 33.1, 22.2, 17.6, 14.2; MS (*m*/*z*) 418 (M^+, 100%); anal. calc. for: ($C_{22}H_{22}N_6OS$, Mwt = 418): C, 63.14; H, 5.30; N, 20.08%; found: C, 63.17; H, 5.34; N, 20.13%.

4.2 Biology

4.2.1. Fungal strains and growth condition. Fungal isolates were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and BEI Resources (Manassas, VA, USA). RPMI 1640, was obtained from Thermo Fisher Scientific (Waltham, MA). 3-(*N*-Morpholino) propanesulfonic acid (MOPS) (Sigma Aldrich, St. Louis, MO) and YPD broth and agar medium (Becton, Dickinson and Company, Franklin Lakes, NJ) were purchased from commercial vendors.

4.2.2. Determination of the minimum inhibitory concentration (MIC). The antifungal activities of the tested compound were determined by broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI 2017) for yeasts (M27). Minimum inhibitory concentrations (MICs) were determined as the lowest concentration of the compound that inhibit the fungal growth by 50% after incubation at 35 °C for 24 h for *Candida* and *Aspergillus* and 72 h for *Cryptococcus* species.[23]

4.2.3. Cytotoxicity of 35 against vero cells. The toxicity of **35** against monkey kidney epithelial cells (vero) was evaluated at different concentrations from 1 to 128 μg mL^{-1}. Briefly, vero cells were cultivated in MEM supplemented with 10% FBS, 1 mM sodium pyruvate, and penicillin–streptomycin at 37 °C with CO2 (5%). Control cells were treated with DMSO at a concentration equal to that in the compound-treated samples. The cells were incubated with the compounds and amphotericin (in triplicate) in a 96-well plate at 37 °C with CO_2 (5%) for 24 hours. The assay reagent MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2tetrazolium (Promega, Madison, WI, USA) was subsequently added and the plate was incubated for three hours. Absorbance readings (at OD_{490}) were taken using a kinetic microplate reader (Spectra MAX IX3, Molecular Device, CA, USA). The quantity of viable cells after treatment with each compound was expressed as a percentage of the viability of DMSO-treated control cells (average of triplicate wells ± standard deviation).[24–26]

4.2.4. RBCs hemolysis of 35. The toxicity of **35** was evaluated by the red blood cell (RBC) lysis assay. The freshly obtained 1 mL RBCs of sheep blood were diluted with 4 mL PBS and added to different concentrations of the compounds. Amphotericin-b and SDS 2% were used as positive controls whereas 1% DMSO and PBS were used as negative controls. After 45 min incubation period the 96-well plates were centrifuged at 2000 rpm for 10 min and the absorbance of supernatant were read at 540 nm taken using a kinetic microplate reader (Spectra MAX IX3, Molecular Device, CA, USA).[27,28]

4.2.5. Leakage assay of 35. We measured the effect of **35** on the permeability and integrity of fungal cell membrane after 4 hours treatment with 2× and 4× MIC compared to amphotericin and SDS 2%. We cultivated the fungal yeast *Candida glabrata* ATCC MYA-2950 in YPD broth overnight. Then the cells were centrifuged at 3000 rpm for 5 min and washed three times with sterile PBS. The cells then resuspended in PBS and treated with 2× and 4× MICs of **35** and the controls for 4 h after that, the cells were centrifuged at 10 000 rpm for 10 min and

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spectrophotometric measurements of intracellular components at 260 nm were determined in the cell supernatant.[29,30]

4.3 ADME prediction

ADME parameters were predicted using the Swiss ADME open-access web tool (**http://www.swissadme.ch**).

Ethical approval

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Al-Azhar University and Experiments were approved by the Animal Ethics Committee of Animal Care and Use of Faculty of Medicine Al-Azhar University.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

All the authors declare that they have no conflict of interest.

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