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Research Article

In Vivo Antifungal Activity and Computational Studies of Some Azole Derivatives against *Candida Albicans*

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Resistance of *Candida* species is a major problem in the management of *Candida* infection. This study investigated in vivo antifungal activities of several new imidazole and triazole derivatives in a *C. albicans* systemic infection. The efficacy of derivatives was determined against systemic infection by *C. albicans* in mice with cyclophosphamide-induced immunosuppression, and the antifungal activities of the synthesized compounds were evaluated in comparison with fluconazole. Compounds **3** and **8** had the highest efficacy with minimum inhibitory concentration (MIC) values of 0.5–1 μM against the *C. albicans* pathogen. In vivo activities in immunosuppressed mice were also greater than fluconazole. Furthermore, docking analysis was carried out to know the binding mode of imidazole and triazole derivatives to the CYP51 active site of *C. albicans* and dihydrofolate reductase as a valid antifungal target. The docking study found that the antifungal results are well correlated with docking results. ADMET and in silico physicochemical parameters were also performed. This study demonstrates that compounds **3** and **8** are potential antifungal candidates against the *C. albicans* pathogen.

1. Introduction

Fungal infections have dramatically increased due to immunosuppressive agents in different clinical cases. Among fungal infections, candidiasis is a serious cause of morbidity and mortality in immunocompromised patients. There are more than 17 different *Candida* species that are known as etiological agents of human infection. Among *Candida* species, *C. albicans* is the most frequent fungal pathogen that causes the most invasive infections [1–4]. *C. albicans* is a commensal fungus of the oral cavity and gastrointestinal tract, which causes life-threatening mucosal and systematic infection [5]. It has been reported that *Candida* is the fourth cause of healthcare-associated bloodstream infections in the United States [6]. The intrinsic resistance to antifungal agents and also, acquired drug resistance during treatment is becoming a major problem in the management of *Candida* infection. Many antifungal drugs used to treat fungal infections include polyenes, azoles, echinocandins, allylamines, and chitin synthesis inhibitors. Azoles are the largest family of antifungal drugs [2, 6, 7]. The azole antifungal agents block the synthesis of CYP450 14α-demethylase to prevent ergosterol biosynthesis from lanosterol in yeast and fungi [5, 8]. Azoles are five-membered heterocyclic rings and the most successful antifungal agents (clotrimazole, ketoconazole, fluconazole, voriconazole, posaconazole, and itraconazole) are the most common ones (Figure 1). Currently, many newer compounds have been designed and synthesized to mitigate many disadvantages of antifungal drugs such as resistance to fungal infection therapy, side effects, efficacy, and toxicity. Our group has synthesized small molecules as antimicrobial agents containing imidazole, benzimidazole, triazole, benzotriazole, nitrotriazole, and thiazole in recent years. Several of those synthesized compounds have been investigated as antifungal agents in in vitro and were shown that many of them

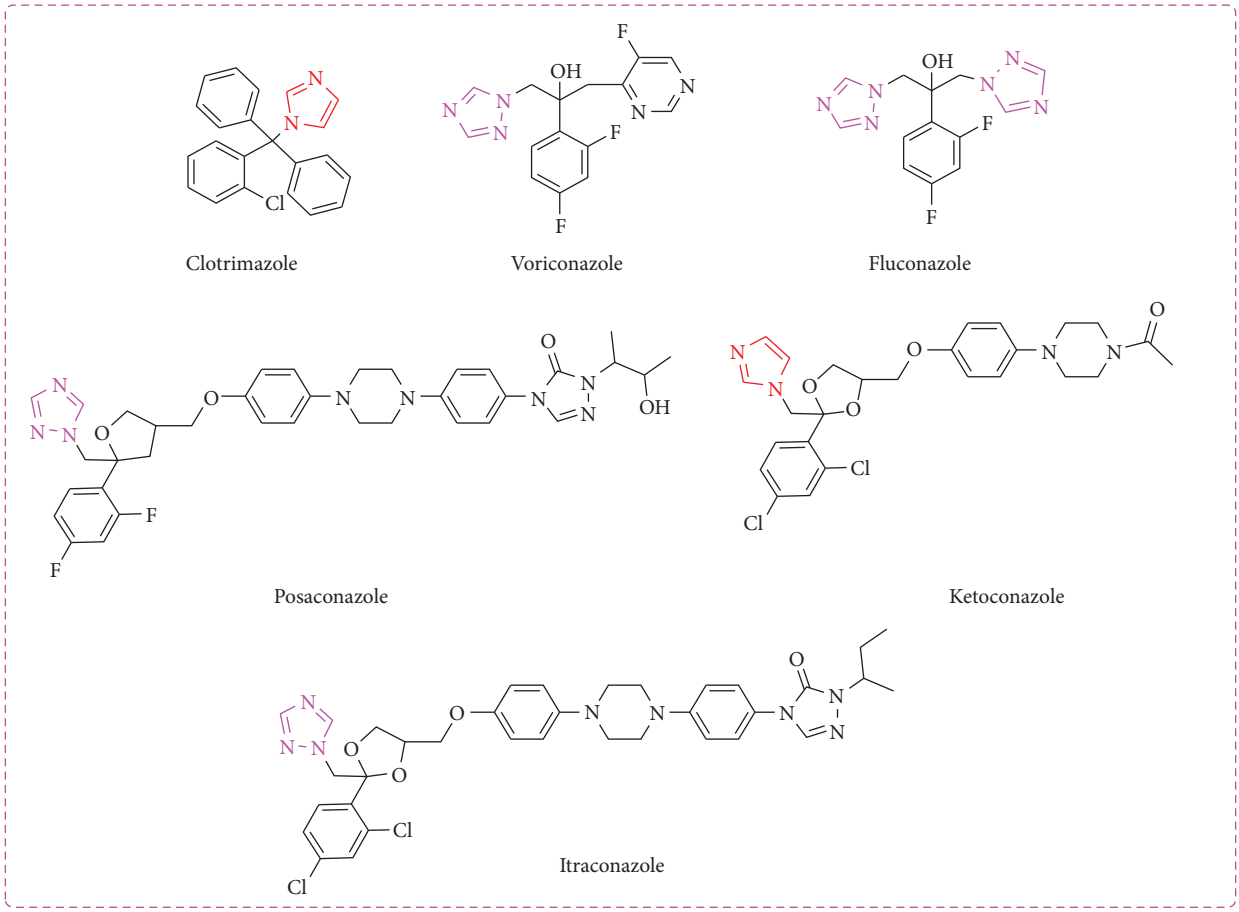


Figure 1: Some antifungal agents with triazole and imidazole rings.

had anticandidal activities against standard and clinical spices of *Candida* [9–15]. The present work describes the antifungal activity of some compounds with different in vitro antifungal effects against *C. albicans* [13, 16, 17]. The immunosuppressed mice model with C. *albicans* was used to evaluate the effectiveness of treatments with several benzimidazole and triazole derivatives in vivo in systemic infection. As it was mentioned, cytochrome P_{450} 14α-demethylase (CYP51) is a key enzyme in sterol biosynthesis for the fungal cell membrane. On the other hand, another possible mechanism for azole derivatives is dihydrofolate reductase (DHFR) inhibitors, especially in *C. albicans* pathogen [18]. Bacteria and fungi need DHFR coenzymes to grow and proliferate; thus selective inhibition of DHFR can be considered a suitable target in fungal diseases. In this regard, we performed molecular docking studies to find out their binding interaction and energy towards CYP450 14α-demethylase (CYP51) and DHFR as the main target for this purpose. Absorption, distribution, metabolism, excretion, toxicity (ADMET) calculations, and physicochemical parameters were also included.

2. Materials and Methods

All solvents and reagents were purchased from Merck or Fluka. *C. albicans* was extracted from consulted patients at the Shiraz University of Medical Sciences and was identified by the Department of Mycology. All tested compounds were synthesized in the Department of Medicinal Chemistry, Shiraz University of Medical Sciences [12, 13]. The mouse breeding facility provided the animals at Shiraz animal laboratory, Shiraz, Iran. The ethical guidelines for using experimental animals were followed in all tests under ethical committee acts of Iran University of Medical Sciences and the European Communities Council Directive 24 November 1986 (86/609/EEC).

2.1. Antifungal Assay

2.1.1. Preparation of Fungal Suspension. Before testing, *C. albicans* were purified and subculture on Sabouraud dextrose agar (SDA) media. Stock inoculums were prepared by suspending three colonies of the tested yeasts in 5 mL of sterile 0.9% NaCl and adjusting the turbidity of the inoculums to 0.5 at a 530 nm wavelength to yield a stock suspension of 1×10^5 to 1×10^6 cell/ml.

2.1.2. In Vitro Study. The anticandidal activity of all compounds was evaluated against clinical species of *C. albicans*. The compounds were dissolved in DMSO at the 200 mg/ml concentration, then the serial dilutions for all compounds (625 to 0.5 μg/mL) were prepared in 96-well plates using

Table 1: Chemical structures of investigated compounds and in vitro MIC (μg/ml) against clinical *C. albicans* (12, 13).

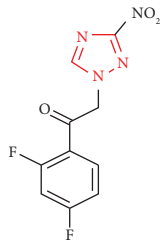
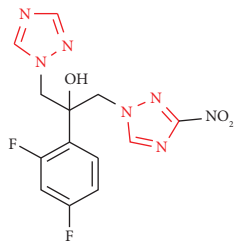
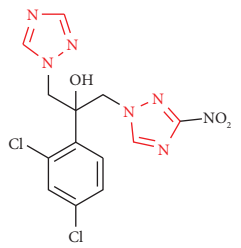
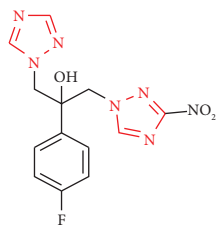
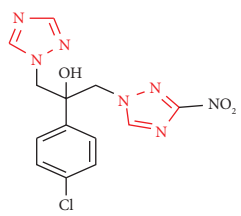
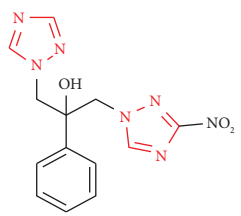
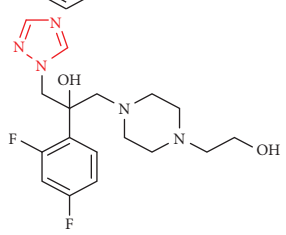
Entry	Chemical structure	MIC (12, 13)	MIC (in this study)
1		NT	256
2		4 (12)	1
3		0.5 (12)	0.5
4		128 (12)	1
5		16 (12)	1
6		256 (12)	256
7		64 (12)	1

Table 1: Continued.

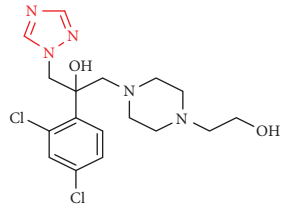
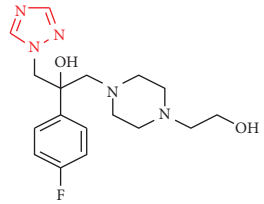
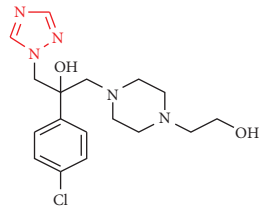
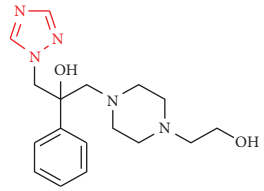
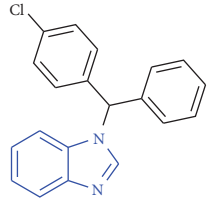
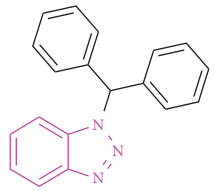
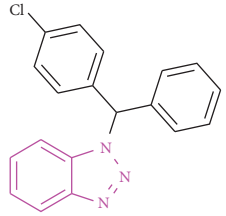
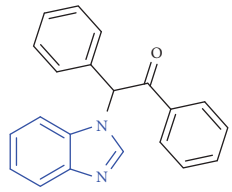
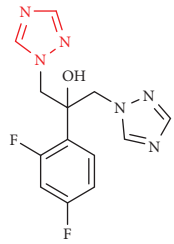
Entry	Chemical structure	MIC (12, 13)	MIC (in this study)
8		2 (12)	1
9		256 (12)	256
10		256 (12)	128
11		256 (12)	256
12		64 (11)	4
13		>256 (11)	G
14		>256 (11)	G

Table 1: Continued.

Entry	Chemical structure	MIC (12, 13)	MIC (in this study)
15		64 (11)	16
Fluconazole		1 (12) 256 (11)	1

NT: not tested, G: growth.

RPMI 1640 media [19, 20]. The broth microdilution method determined minimal inhibitory concentrations (MIC) for the compounds, as explained in our previous study [13]. The chemical structures of all studied compounds and the *in-vitro* MIC (μg/ml) value toward clinical *C. albicans* are shown in Table 1.

2.1.3. In vivo Antifungal Study. In vivo anticandidal study was conducted according to a previously described method with some modifications [21]. 100 females specific-pathogen-free female CD1 mice at six weeks of age (23–30 g body weight) were provided by the mouse breeding facility at Shiraz animal laboratory, Shiraz, Iran. The mice were kept in an environment with a controlled temperature of 22°C and relative humidity of 30%. Light conditions as 12 hours of light and 12 hours of darkness were held. All the procedures agreed with the Committee for Research Ethics of the Shiraz University of Medical Sciences (No. 98-01-36-19478). The mice were rendered neutropenic before infection with *Candida albicans* by intraperitoneal injection of cyclophosphamide for 4 days with 150 mg/kg dose and 5th day with 100 mg/kg dose. On the 6th day, the neutropenic mice were inoculated via the lateral tail vein with 0.1 ml of the provided *C. albicans* suspension. Drug treatment was performed 2 h after infection in different doses, 5, 10, and 20 mg/kg/i.p (Table 2). Antifungal evaluation for investigated compounds was performed compared to fluconazole (5, 10, and 20 mg/kg/oral) as a positive control. After 24 h, the mice were killed with ether, and both kidneys were extracted as the initial end-organ of the study. The kidneys were homogenized under aseptic conditions and then cultured in an incubator at 35°C for 24 h on SDA media. The kidneys were processed for colony-forming unit (CFU) enumeration compared to the control group [22, 23]. The CFU was obtained for all groups. The ratio of CFU for the tested compounds to CFU for fluconazole (positive control) as well as not treatment group (negative control) were considered for antifungal effects evaluation (Table 2).

2.2. Statistical Analysis. The statistical tests were carried out using the SPSS, 22. The mean CFU in all groups was analyzed using a *T*-test ($N = 5$). The ratio of CFU of the tested compounds to fluconazole and control was obtained and then analyzed using a *T*-test. One-way analysis of variance (ANOVA) was used to test the significance among different doses of applied compounds. $p < 0.05$ was considered statistically significant ($N = 5$).

2.3. Docking Study Procedure. The 3D structures of CYP51 (PDB ID: 5FSA) and DHFR (PDB ID: 4HOF) were downloaded from Protein Data Bank (https://www.rcsb.org). The structures of azole derivatives were drawn, optimized, and converted to PDBQT format. For the preparation of receptors, the cocrystal ligand and water molecules of CYP51 and DHFR were removed. Missing hydrogen atoms and nonpolar hydrogens were corrected to obtain the PDBQT format of receptors by AutoDock Tools package (1.5.6) [24]. The size of grid box $60 \times 60 \times 60$ points in *x*, *y*, and *z* directions was selected for 5FSA and 4HOF targets. The number of points in *x*, *y*, and *z* was chosen 188.27, 25.12, and 74.88 for 5FSA and 3.34, −0.59,

Table 2: Anticandidal activity of compounds **1**–**15** in comparison with fluconazole and control using obtained CFU/kidney.

Compound	Dose (mg/kg)	No of colony (compound/fluconazole)	No of colony (compound/control)	CFU (±SD)
	10	8.23*	1.59^{NS}	799.29 (85.25)
1	20	11.2*	0.76*	382.92 (54.10)
	50	9*	0.27**	137.97 (21.12)
	10	2.5*	0.48*	242.80 (15.23)
2	20	1.05^{NS}	0.07**	35.89 (5.21)
	50	3.50^{NS}	0.1**	53.65 (8.94)
	10	4.15*	1.38*	403.04 (71.25)
3	20	1.72^{NS}	0.11**	58.80 (15.44)
	50	1.00^{NS}	0.03**	15.33 (2.31)
	10	4.41*	0.85*	428.29 (44.36)
4	20	5*	0.34*	170.95 (21.87)
	50	3*	0.09**	45.99 (8.91)
	10	8.75*	1.69*	849.80 (56.98)
5	20	18.4*	1.25**	629.09 (55.21)
	50	17*	0.52**	260.61 (25.25)
	10	9.75*	1.89*	946.92 (101.43)
6	20	16*	1.09**	547.04 (32.65)
	50	20*	0.61**	306.60 (10.12)
	10	2.27*	0.44**	220.46 (11.85)
7	20	5.73*	0.31**	159.90 (16.37)
	50	11.04*	0.33**	169.24 (29.56)
	10	1.8^{NS}	0.35**	174.81 (27.38)
8	20	3.21^{NS}	0.22**	109.74 (28.46)
	50	2.1^{NS}	0.06**	32.19 (8.95)
	10	2.74*	0.53**	266.8 (43.27)
9	20	4.72*	0.32**	161.37 (50.66)
	50	12.33*	0.37**	189.16 (27.44)
	10	3.80*	0.70**	369.05 (20)
10	20	3.98*	0.27**	136.07 (7.76)
	50	11.87*	0.36**	181.96 (7.21)
	10	3.88*	0.75**	376.82 (22.17)
11	20	6.31*	0.43**	215.73 (19.34)
	50	12.92*	0.39**	198.06 (8.37)
	10	6.25*	1.21*	607.00 (65.59)
12	20	4.5*	0.30**	153.85 (11.85)
	50	G	0.05**	—
	10	105.2**	1.58^{NS}	G
13	20	59.25**	0.36^{NS}	G
	50	G	—	G
	10	G	—	G
14	20	G	—	G
	50	G	—	G
	10	4.16*	0.80**	404.01 (61.44)
15	20	1.25^{NS}	0.08**	42.73 (6.87)
	50	1.25^{NS}	0.03**	19.16 (3.27)
	10	1	0.19**	97.12 (5.66)
Fluconazole	20	1	0.06**	34.19 (3.36)
	50	1	0.03**	15.33 (1.86)
Control				500 (5)

CFU = colony-forming unit, SD = standard deviation, * $p < (0.05)$, ** $p < (0.01)$, NS = not significant, and G = growth.

and 27.53 for 4HOF by AutoDock Vina (1.1.2). An in-house batch script (DOCKFACE) was used to run the docking protocol. Discovery Studio Visualizer v20.1.0.19295 was used to visualize the binding interactions of compounds [25].

2.4. In silico ADMET Calculations. SwissADME and the pre-ADMET online server (https://preadmet.bmdrc.org/) were used to obtain the drug-likeness and physicochemical properties of all structures.

3. Results and Discussion

3.1. Antifungal Assay and Structure Activity Relationship (SAR). Fifteen derivatives of triazoles, benzimidazole, and benzotriazole were evaluated against clinical species of *C. albicans* using the CLS method. As shown in Table 1, all compounds except compounds **13** and **14** had anti-Candida activity in the range of 0.5–265 μ/mL, and these results are the same as our previous in vitro studies [11, 12]. The tested compounds can be classified into three categories. The first category is compounds **2**–**6** which possessed 4-nitrotriazole moiety at C_1 of propane 2-ol. Among these compounds, compound **3** bearing 2, 4 dichloro at phenyl ring (MIC = 0.5 μg/ml) showed excellent antifungal effect compared to fluconazole and compounds **2**, **4,** and **5** had MIC values of 1 μg/ml as the same as fluconazole. However, compound **6** as an unsubstituted derivative displayed the least effect.

In the second category, bearing piperazine ethanol moieties (**7**–**11**) compounds **7** and **8** containing di-fluorophenyl or di-chlorophenyl ring displayed better antifungal effect (MIC = 1 μg/ml). Reduction or elimination of the electronegative group resulted in analog **9**–**11** led to diminishing the activity. Finally, in the third category, clotrimazole base structure (**12**–**15**) compounds, **12** and **15** showed moderate activity with MIC values of 4 and 12 μg/ml, respectively. The structure-activity relationship (SAR) illustrated that the incorporation of electronegative substituents such as Cl and F at the phenyl ring in series 1 is necessary for antifungal effects. Furthermore, in the case of series 2 (a fluconazole-like compound with piperazine ethanol moieties), compounds bearing two electronegative substitutions had the best activity. It seems that increasing the electronegative substitution at phenyl moiety may improve the potency. The SAR for the third category (**12**–**15**) is the same as other groups. Generally, the first family (fluconazole-like compounds bearing 4-nitrotriazole) showed better effects than the other evaluated compounds.

The in vivo anticandidal activity was investigated against *C. albicans* using immunosuppressed mice. All synthesized compounds were applied in 10, 20, and 50 mg/kg/i.p and results showed that all tested compounds had antifungal activities. Figure 2 presents the mean of CFU for control, fluconazole, and active compounds in doses 10, 20, and 50 mg/kg. The obtained CFU after 24 hr was less than the control for the most investigated compounds (Figure 2). Therefore, as shown in Figure 2, all compounds had antifungal activity in 3 doses compared to the control group, except compounds **1**, **5**, and **6**. One-way analysis of variance test (ANOVA) results confirmed that the antifungal activities were dose-dependent with $p < 0.05$. The ratio of obtained CFU for the tested compounds to the CFU of the control showed that most compounds had significant antifungal activity compared with the control (Table 2). The ratio of CFU from the compounds to fluconazole was determined (Table 2). Results showed that the antifungal effects of compound **8** in all doses and compounds **3** and **15** (in doses of 20 and 50 mg/kg) were comparable with fluconazole. Antifungal activities of these compounds were not statically different from fluconazole (Table 2). Although other compounds had significantly antifungal effects less than fluconazole (Table 2), all tested compounds had significant antifungal activities compared to negative control except compounds **1** (10 mg/kg), **13**, and **14**. These results were consistent with in vitro antifungal evaluation in this study and our previous studies against other species of fungi (Table 1). In vitro antifungal evaluation and docking studies into the active site of 14α-demethylase had shown that compounds **3** and **8** with two chlorine groups had the best antifungal activity compared with fluconazole [12]. Accordingly, compounds **3** and **8** could be candidates for more evaluations in the future.

3.2. Docking Analysis Results. The molecular Docking studies were performed to investigate the binding mode and interaction of azole derivatives toward cytochrome P_{450} lanosterol 14α-demethylase and DHFR enzymes of *C. albicans*. All the docking protocols were performed with RMSD values below 2 Å. The docking scores of all molecules (**1**–**15**) with two plausible targets are shown in Table 3. From our results, compounds **3** and **8** with high antifungal activity, represented stronger score dockings in the two studied enzymes compared to other compounds.

The interactions of two azole derivatives (**1** and **3**) the least and most active compounds with CYP51 and DHFR were presented in Figures 3 and 4. In the case of **3**, Ile 131 and Ile 304 are involved in pi-alkyl interaction with 1, 2, 4 triazole ring and pi-pi interaction between Tyr 118 and 4-nitrotriazole moiety. The 2, 4 dichloro moiety of compound **3** interacted with His 310 and Leu 376 by pi-alkyl interactions. The groups of Phe 126, Thr 122, Gly 307, Thr 311, Phe 228, Leu 121, Ser 378, Ile 379, Phe 233, Tyr 132, and Gly 303 were involved in creating a pocket around compound **3** by van der Waals forces. The triazole moiety pointed to the HEM group in CYP51 active site with pi-sigma interaction.

As shown in Figure 3, the most important residues in the binding of compound **1** were van der Waals interaction with Leu 376, Leu 121, Tyr 132, Ile 131, Ile 304, Gly 303, Gly 307, Tyr 311, and Phe 228. However, the 2, 4 di-fluoro moieties were involved in pi-interaction with HEM 580.

There also existed some pi interaction between the 2, 4 dichloro moiety of compound **3** and Phe 30, Ile 112, Ile 33, NDP 201, and Met 25 residue in the active site of the DHFR enzyme (Figure 4). Hydrogen bond and pi-alkyl interactions

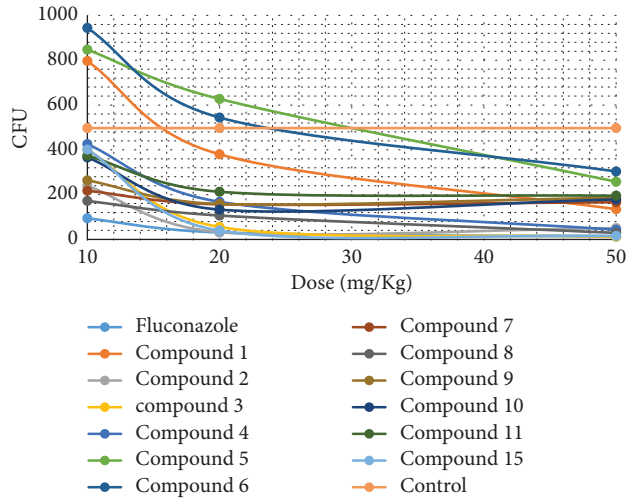


Figure 2: The CFU/kidney for tested compounds after 24 h infection in different doses.

Table 3: The bonding energies (kcal/mol) of the tested compounds on CYP51 and DHFR target using AutoDock Vina.

Entry	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Flu
5FSA	−8.2	−8.9	−9.5	−9.0	−8.8	−8.7	−8.4	−9.3	−8.6	−8.1	−8.3	−8.6	−7.8	−8.9	−8.2	−8.5
4HOF	−7.0	−7.5	−8.6	−7.2	−7.3	−7.4	−7.0	−8.1	−7.0	−7.0	−6.8	−7.6	−7.3	−7.6	−7.5	−6.8

Flu: fluconazole.

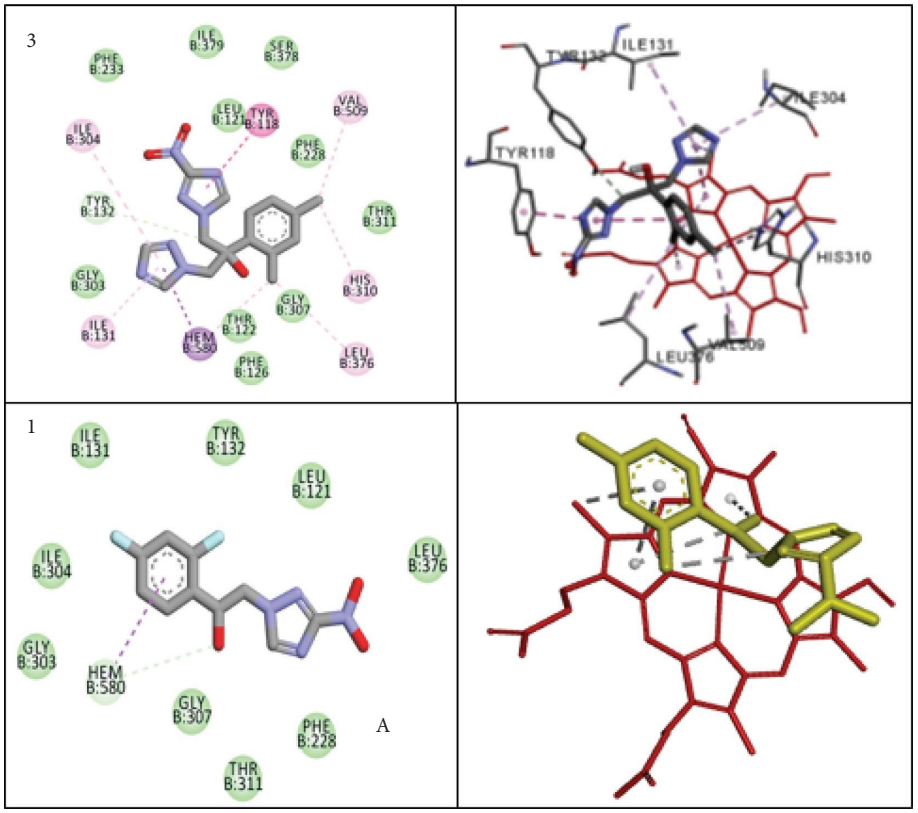


Figure 3: Interactions of 1 and 3 with the residues in the binding site of the CYP51 active (5FSA) target (pink: pi-alkyl, dark pink: pi-pi, green: van der Waals, purple: pi-sigma).

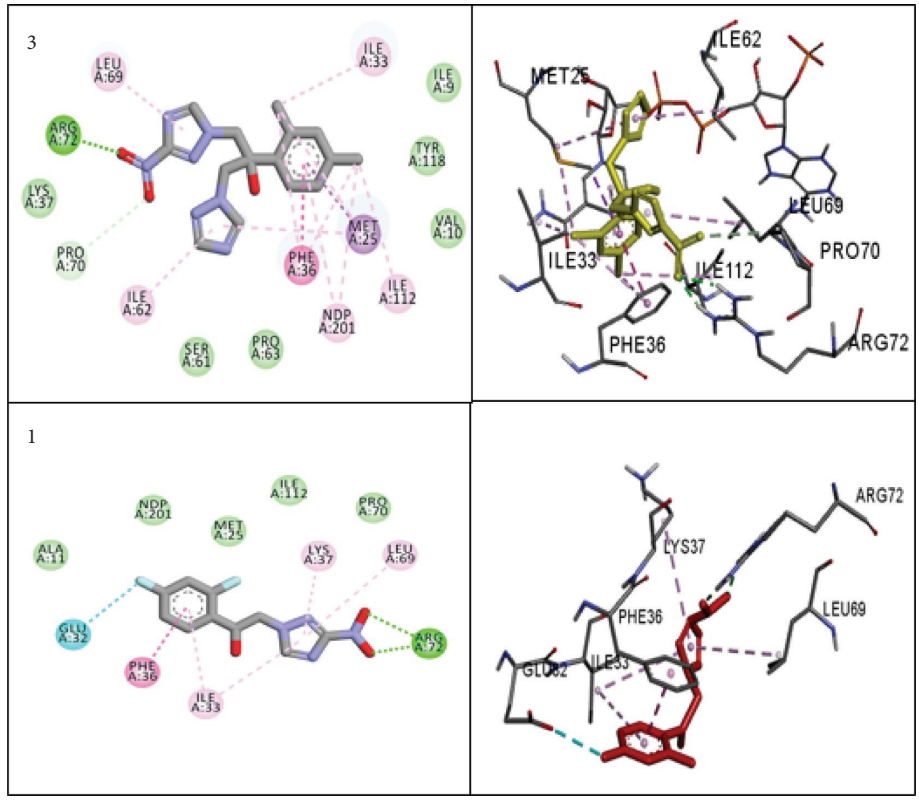


Figure 4: Interactions of 1 and 3 with the residues in the binding site of the DHFR active (4HOF) target (pink: pi-alkyl, dark pink: pi-pi, green: van der Waals, purple: pi-sigma, blue: halogen bond).

Table 4: Physiochemical properties of all azole derivatives (**1**–**15**).

Entry	MW[a]	LogP[b]	HBD[c]	HBA[d]	TPSA (Å)[e]	RB[f]	Lipinski/Veber violation
1	268.18	2.25	0	7	93.60	4	0
2	351.27	1.43	1	9	127.47	6	0
3	384.18	1.69	1	7	127.47	6	0
4	333.28	1.03	1	8	127.47	6	0
5	349.73	1.16	1	7	127.47	6	0
6	315.29	0.63	1	7	127.47	6	0
7	367.39	1.04	2	8	77.65	7	0
8	400.3	1.27	2	6	77.65	7	0
9	349.40	0.65	2	7	77.65	7	0
10	365.86	0.79	2	6	77.65	7	0
11	331.2	0.26	2	6	77.65	7	0
12	318.8	4.52	0	1	17.82	3	0
13	285.35	4.14	0	2	30.71	3	0
14	319.79	4.64	0	2	30.71	3	0
15	312.37	3.30	0	2	34.89	4	0
Fluconazole	306.28	1.47	1	7	81.65	5	0
Lipinski/Veber's rules	≤500	≤5	≤5	≤10	≤140	≤10	≤1

[a]Molecular weight (MW). [b]Logarithm of partition coefficient between n-octanol and water (LogP). [c]Number of hydrogen bond donors (HBD). [d]Number of hydrogen bond acceptors (HBA). [e]Topological polar surface area (TPSA). [f]Number of rotatable bonds (RB).

existed between the 4-nitrotriazole moiety and Arg 72 and Leu 62. The side chains consisting of amino acid residues Ser 61, Pro 63, Val 10, Tyr 118, Ile 9, Lys 37, and Pro 70 create van der Waals contacts with compound **3**. In the case of compound **1**, the fluoro atom of the phenyl ring established halogen interaction with Glu 32 and 2, 4 difluoro rings formed pi-pi and pi-alkyl interaction with Phe 36 and Ile 33, respectively. The 1,2, 4 triazole ring is involved in pi-alkyl interaction with Ile 33, Lys 37, and Leu 69. The nitro moiety formed a hydrogen bond interaction with Arg 72; we can also see some hydrophobic interaction with Ala 11, Met 25, Ile 112, Pro 70, and NDP 201 (Figure 4).

Table 5: Absorption and distribution profile of all azole derivatives (**1**–15).

Entry	HIA	Caco-2 permeability	BBB	P-glycoprotein	Plasma protein binding (PPB)	Skin
1	84.40	0.58	0.01	NS/I	82.16	−4.02
2	75.36	1.2	0.05	NS/I	76.23	−4.04
3	92.91	6.91	0.07	NS/I	88.69	−3.58
4	89.23	6.89	0.05	NS/I	83.50	−3.87
5	86.45	7.47	0.06	NS/I	81.10	−3.67
6	74.93	3.36	0.08	NS/NI	69.23	−3.63
7	93.50	26.69	0.03	NS/NI	27.79	−4.2
8	94.75	26.33	0.05	NS/NI	43.43	−3.86
9	93.48	20.57	0.06	NS/NI	23.41	−4.15
10	94.15	23.50	0.05	NS/NI	33.55	−3.95
11	93.47	22.79	0.03	NS/NI	24.78	−3.93
12	100.0	57.02	1.85	NS/NI	96.14	−2.23
13	100.0	53.04	4.41	NS/I	94.37	−2.32
14	100.0	44.32	1.90	NS/I	100.0	−2.37
15	99.04	56.38	5.33	NS/I	98.69	−2.38
Fluconazole	95.58	17.23	0.24	NS/NI	55.15	−4.18

NS = nonsubstrate; NI = noninhibitor; S = substrate; I = Inhibitor.

Table 6: Metabolism and toxicity profile of all compounds.

Entry	CYP2D6	CYP3A4	Carcinogenicity	hERG inhibition
1	NS/NI	S/NI	NC	Weak
2	NS/NI	S/NI	NC	Weak
3	NS/NI	S/NI	NC	Weak
4	NS/NI	S/NI	NC	Weak
5	NS/NI	S/NI	NC	Weak
6	NS/NI	S/NI	NC	Weak
7	S/I	S/I	NC	Weak
8	S/I	S/I	NC	Weak
9	S/I	S/I	NC	Weak
10	S/I	S/I	NC	Weak
11	S/I	S/I	NC	Weak
12	NS/NI	S/I	NC	Weak
13	NS/NI	S/NI	NC	Weak
14	NS/NI	S/I	NC	Weak
15	NS/NI	S/I	NC	Weak
Fluconazole	NS/NI	S/NI	NC	Weak

[a]Human ether-*a*-go-go related gene, NS = nonsubstrate; NI = noninhibitor; S = substrate; I = inhibitor; NC = noncarcinogenic; C = carcinogenic.

3.3. ADMET Results. The ADME properties and toxicity are two factors that had a significant role in the acceptance or rejection of compounds as drug candidates. The Swiss ADME software was utilized to predict the physiochemical properties of all azole derivatives [1–15, 26]. All compounds were in the acceptance range (268–400 g/mol) according to Lipinski's rules. Other properties including hydrogen bond donors and acceptors, rotatable bond number, and total polar surface area (TPSA) were convenient (Table 4). Moreover, all of the compounds except compound **14**, had reasonable lipophilicity (log P) values. According to these results, compounds **1** and **7**–**15** could be administered orally.

The absorption-distribution properties of all azole derivatives are shown in Table 5. The human intestinal absorptions (% HIA) were above 70, which represented well intestinal absorption in the transport of oral drugs to their biological targets [27]. The Caco-2 permeability of compounds (**3**–**5**) and (**7**–**15**) demonstrated that they had middle permeability across intestinal membranes. In the case of blood-brain barrier property, all of the compounds except **12**–**15** have low blood-brain barrier percentages and thus do not show a neurocytotoxicity effect. None of the compounds had a substrate of P- glycoprotein efflux transporters; also compounds **1**–**5** and **13**–**15** showed effects as an inhibitor on P-glycoprotein. All of the compounds except **12**–**15** had weak PPB with values less than 90% which could have an effect on their bioavailability and distribution across the cell membrane [24].

Table 6 illustrates the metabolism and toxicity profiles of tested compounds. Both CYP2D6 and CYP3A4 enzymes have an important role in the metabolism of drugs in the liver. In this regard, compounds **1**-**6** and **12**-**15** were not substrates or inhibitors of CYP2D6. In the case of CYP3A4 enzymes, compounds **7**–**12** and **14**–**15** were substrates/inhibitors of CYP3A4. All of the compounds were determined to be noncarcinogenic. All of the compounds can act as weak hERG inhibitors, which means that they cannot affect QT interval prolongation. Hence, they may not create violent cardiac side effects. Taken together, these results indicate that these compounds had proper physicochemical and pharmacokinetic properties.

4. Conclusion

Here, we studied in vivo and in vitro antifungal activities of several new azole derivatives toward the *C. albicans* pathogen. Fluconazole-like compounds (**2**–**11**) indicated better antifungal activity in a range of 0.5–256 (μg/ml). Compounds **3** and **8**, containing a 2,4-dichloro moiety on the phenyl ring at position 2 of the propane 2-ol, were the most potent derivatives in in vitro and in vivo studies. According to the molecular docking assessment, compound **3** indicated the best affinity and high docking score

compared to other studied compounds against lanosterol 14α-demethylase (CYP51) and DHFR targets through hydrogen bonding and some extent of hydrophobic interactions. All of the tested compounds fulfilled the Lipinski and Veber rules. In vivo and in vitro results were supported by in silico studies.

Data Availability

The data sets used and analyzed during the current study are available from the corresponding author upon reasonable request. We have presented all data in the form of Tables and Figures. The PDB codes (4HOF) and 5FSA were retrieved from the protein data bank (http://www.rcsb.org). https://www.rcsb.org/structure/4HOF and https://www.rcsb.org/structure/5FSA.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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