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1,2,4-Triazole derivatives as novel and potent antifungal agents: Design, synthesis and biological evaluation

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ABSTRACT

Fungal infections are still threatening human health due to resistance to existing drugs, therefore, the design and development of novel antifungal agents is to be necessary and also, is interesting topic for medicinal chemist. Azole derivatives are one of the promising antifungal agents, which exert their activities through inhibition of cytochrome P450 14α-demethylase (CYP51). In this regard, a new series of 1,2,4-triazole derivatives (**7a-i**) were designed, synthesized and confirmed with IR, ^{1}HNMR, ^{13}CNMR and Mass spectrum. The antifungal activity of these compounds were investigated against several yeasts (*candida* species), filamentous and clinical strains using the CLSI method. Furthermore, to measure the cytotoxic activity, MTT assay was also done against MRC-5 as normal human fibroblasts cell line. Our results represented that most of the compounds had appropriate activity ranging from 0.5-256 µg/mL, especially, compounds **7g** and **7h** were found to be more potent against yeast strains and clinical strain of Fluconazole-resistant and Fluconazole-sensitive with MIC values of 0.5 µg/mL compared to the Fluconazole as control drug. Subsequently, molecular docking studies were performed to find the binding energy and interaction mode of these compounds in the active site of 14α-demethylase enzyme as plausible target of azole compounds. According to *in vitro* antifungal assay and *in silico* ADME predictions, compounds **7g** and **7h** can be considered as potent candidates for further studies.

1. Introduction

Fungal infections are one of the common health problems, particular in immunocompromised patients (patients suffering from cancer, AIDS and transplant). The application of anticancer and immunosuppressant drugs has susceptible patients to the infection caused by opportunistic organisms especially fungi [1]. Currently, azole drugs including Fluconazole, Ketoconazole, Itraconazole, Voriconazole, etc. widely used in the clinic for the treatment of fungal infections, however, problems associated with these antifungal drugs such as resistance, undesirable side effects and toxicity, have emphasized the need for the development of new antifungal agents [2–4].

In the past decades, 1,2,4-triazole derivatives have received much attention in medicinal chemistry due to their broad-spectrum biological activities such as antiviral [5], antibacterial [6], antifungal [7,8], anti-tubercular [9–11], immunosuppressant [12], antihypertensive [13], anti-inflammatory [14,15], anticonvulsant [16,17], analgesic [18], hypoglycemic [19], antidepressant [20,21] and anticancer [22–24] activities. 1,2,4-Triazole derivatives are an important class of antifungal agents that widely use in the treatment of fungal infections [3]. They act through inhibition of cytochrome P450 14α-demethylase (CYP51) which is an essential enzyme in the sterol biosynthetic pathway [3,25]. Fluconazole and Voriconazole are the 1,2,4 triazole base antifungal drugs, which are widely used in the treatment of fungal infections due to their important features such as broad-spectrum activity and low toxicity [26,27], (Fig. 1).

Abbreviations: MIC, Minimum Inhibitory Concentration; ADME, Absorption, Distribution, Metabolism, and Excretion; CLSI, Clinical & Laboratory Standards Institute; MTT, 3-(4,5-di**methylthiazol**-2-yl)-2,5-diphenyl**tetrazolium**bromide.

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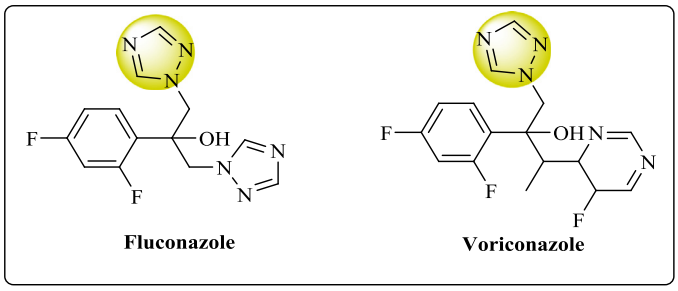


Fig. 1. Chemical structures of Fluconazole and Voriconazole.

In the present study, a novel series of 1,3-diphenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (**7a-e**) and 1,3,3-triphenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (**7f-i**) derivatives were designed, synthesized and evaluated as antifungal agents against several natural and clinical strains of fungi using the CLSI method. The cytotoxic activity of the synthesized compounds was investigated against MRC-5 as normal human fibroblasts cell line using MTT assay. In the following, molecular docking study was performed to elucidate the binding mode of these ligands in the active site of 14α-demethylase enzyme as plausible target of azole compounds.

2. Results and discussion

2. 1. Design approach

The new set of 1,2,4-triazole derivatives as antifungal agents were designed based on the chemical scaffolds of Isavuconazole (**a**), Voriconazole (**b**) and Fluconazole (**c**) drugs which are currently used in the treatment of fungal infections. These drugs containing 1,2,4-triazole ring, difluorophenyl group, and a tertiary alcohol group in their structures. These moieties are critical for their antifungal activities. The mechanism of action of these drugs, like other azoles, are inhibition of cytochrome P450 14α-demethylase (CYP51). According to the previous reports, the nitrogen atom of triazole ring bind to the iron atom of the heme moiety of CYP-450 and diholophenyl moiety have key interactions in the active site of enzyme [3]. Therefore, we applied 1,2,4-triazole ring, a dihalophenyl moiety and a carbonyl group for design of new compounds (Fig. 2).

2.2. Chemistry

The target derivatives (**7a-i)** were synthesized according to the general method depicted in Scheme 1. In the first step, Friedel Crafts acylation was occur between mono or di-substituted benzene (**1a-e**) and chloroacetyl chloride (**2**) in the presence of aluminum trichloride ($AlCl_3$) as a strong Lewis acid catalyst to obtain intermediates **3a-e**, in high yields [28,29]. Afterwards, the reaction of intermediates **3a-e** with 1,2,4-triazole (**4**) in the presence of sodium bicarbonate ($NaHCO_3$) and under reflux condition led to the successful synthesis of the corresponding intermediates **5a-e**. Finally, the expect products (**7a-i)** were obtained from the reaction of benzyl bromide (**6a**) or benzhydryl bromide (**6b**) with intermediates **5a-e** in the presence of sodium hydride (NaH) as a strong base and in acetonitrile (CH_3CN) as solvent, in high yields.

The structures of the target compounds (**7a-i)** were characterized using IR, ^{1}H NMR, ^{13}C NMR, and Mass spectroscopic techniques (Table 1).

2.3. Antifungal assay

The antifungal activities of the synthesized compounds (**7a-i**) were evaluated against several types of yeasts (*C. albicans, C. dubliniensis, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, C. neoformans*), filamentous fungi (*A. flavus, A. fumigatus, A. clavatus, P. boydii, E. dermatitidis*) and clinical strains using CLSI method. Fluconazole was used as a positive control drug. The results of the biological evaluation are shown in Tables 2 and 3.

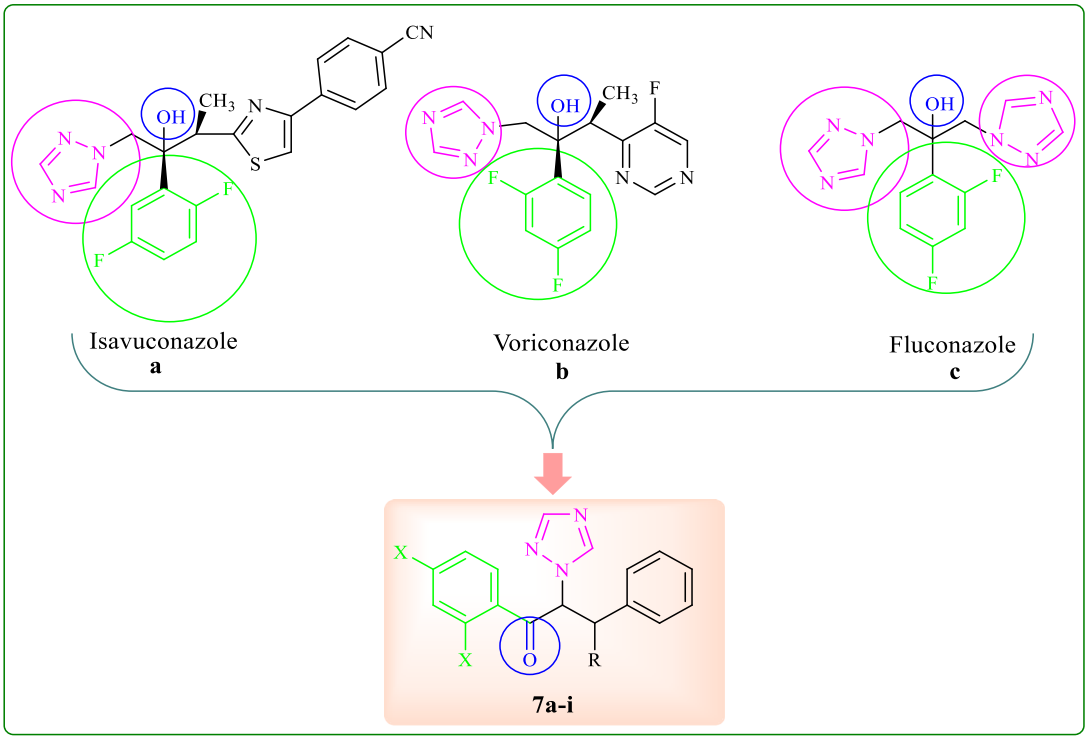
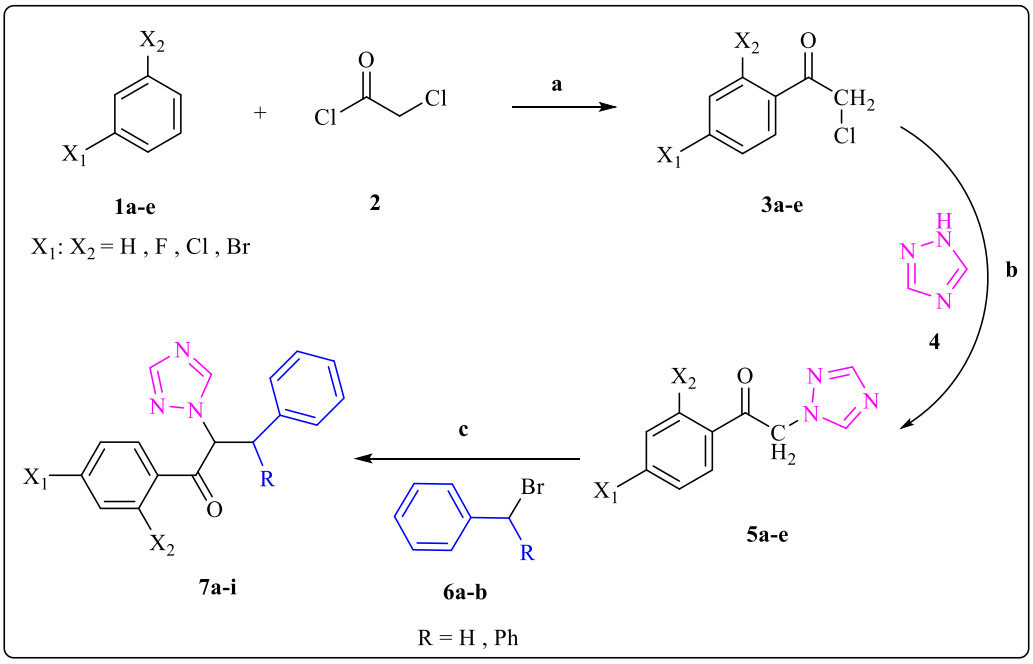


Fig. 2. Design of new 1,2,4-triazole derivatives **7a-i**.



Scheme 1. Synthesis of compounds **7a-i**. Reagents and conditions: a) $AlCl_3$, CH_2Cl_2, r.t., 24 h, b) $NaHCO_3$, toluene, reflux, 20 h, c) NaH, CH_3CN, reflux, 24 h.

Table 1
Chemical structures and physical properties of the synthesized compounds **7a-i.**

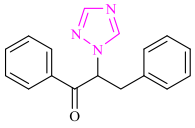
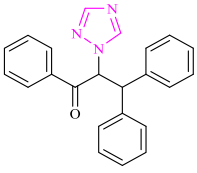
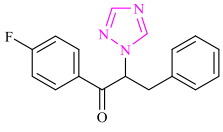
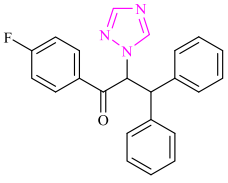
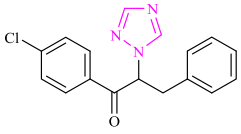
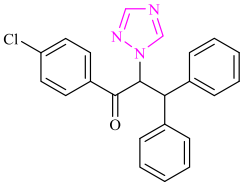
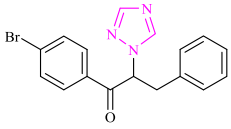
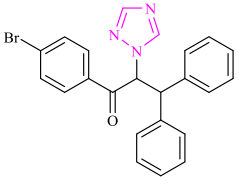
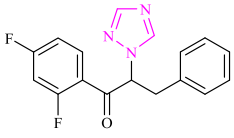
Entry	Chemical structure	M.P. (°C)	Yield (%)	Entry	Chemical structure	M.P. (°C)	Yield (%)
7a		145-148	75	**7f**		161-164	73
7b		65-68	61	**7g**		149-152	58
7c		116-119	72	**7h**		140-142	67
7d		128-131	69	**7i**		110-113	63
7e		78-80	56				

Table 2
Antifungal activities of the target compounds against Yeasts and filamentous fungi (MICs, μg/mL).

Fungi Entry	*C. albicans*	*C. dubliniensis*	*C. glabrata*	*C. krusei*	*C. parapsilosis*	*C. tropicalis*	*C. neoformans*	*A. flavus*	*A. fumigatus*	*A. clavatus*	*P. boydii*	*E. dermatitidis*
7a	G	G	G	G	G	G	4	G	G	G	G	G
7b	1	28	64	G	64	32	1	G	G	G	G	G
7c	1	16	32	128	32	8	0.5	G	G	G	G	G
7d	1	8	G	G	8	G	0.5	G	G	G	G	G
7e	16	32	32	256	32	16	4	32	G	128	128	G
7f	0.5	2	16	G	8	2	0.5	0.5	G	G	G	G
7g	0.5	0.5	0.5	64	0.5	0.5	0.5	8	G	G	G	G
7h	0.5	0.5	0.5	0.5	0.5	0.5	0.5	4	G	G	G	G
7i	0.5	4	32	G	32	2	0.5	G	G	G	G	G
Fluconazole	4	1	4	16	2	1	4	4	1	4	16	16

Table 3
Antifungal activities of the target compounds against clinical strains (MICs, μg/mL).

Tested Fungi Compounds	*Sensitive 629*	*Sensitive 638*	*Sensitive 639*	*Resistant 8808*	*Resistant 625*	*Resistant 2303*
7a	G	G	G	G	G	G
7b	G	32	G.	64	32	32
7c	32	16	64	32	1	16
7d	G	8	G	G	1	32
7e	32	32	64	64	64	G
7f	16	0.5	8	G	G	G
7g	4	0.5	1	64	G	G
7h	8	0.5	0.5	G	G	G
7i	32	0.5	0.5	G	G	G
Fluconazole	4	1	4	16	32	16

All the synthesized compounds can be classified into two series, 1,3-diphenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (**7a-e**) and 1,3,3-triphenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (**7f-i**) derivatives. In the first series (**7a-e**), compounds 7**b-d** with an electronegative substitution on the benzoyl moiety at C1 propane-1-one position, demonstrated a promising activity toward *C. albicans* and *C. neoformans* with MIC value of 0.5-1 μg/mL compared to Fluconazole with MIC value of 4 μg/mL (Table 2). In addition, it was observed that the presence of two fluorine atoms at the benzoyl ring (compound **7e**) led to a decrease in the activity against all the tested candida microorganism. Compound **7a** showed the least effect against all the tested yeast and filamentous strains except *C. neoformans* strain. The potency of compound **7a** against *C. neoformans* is comparable to Fluconazole (MIC = 4 μg/mL).

In the second series (**7f-i**), compounds **7g** and **7h** with F and Cl substitutions at the para position of benzoyl ring, indicated the highest antifungal activity against all the tested yeast strains with MIC values of 0.5μg/mL which are more potent than Fluconazole as standard drug (Table 2). Compound **7i** with Br substitution at benzoyl ring showed less activity than similar compounds in this series (**7g** and **7h**). It could be due to its large size and consequently its reduced ability to penetrate into the cells. Structure activity relationship (SAR) studies indicated that the presence of one or two halogen atoms (F, Cl, Br) at the para position of benzoyl ring at the C-1 of propane -1-one, as well as the presence of one or two phenyl ring at C-3 position were required to the antifungal activity. Generally, the tested compounds with the F, Cl and Br substitution had the most efficacy in order to Cl>F>Br. It is probably due to their increased lipophilicity and consequently better penetration into the cells. Compounds with Br substitution likely due to their large size and consequently their reduced ability to penetrate into the cells, were less effective than similar compounds containing F and Cl substitution. Over-all, the second series (i.e.: 1,3,3 triphenyl propane 1-one compounds) represented better effects than the first series (i.e.: 1,3 diphenyl propane 1-one compounds).

Today, because of widespread use of antifungal agents, drug resistance is one of the most important problems in human health. Based on the biological results, all of the synthesized compounds were further evaluated toward Fluconazole-resistant strains (resistance 8800, 625 and 2303) and Fluconazole-sensitive (sensitive 629, 638 and 639) strains. As depicted in Table 3, compounds **7g** and **7h** exhibit significant antifungal activity against Fluconazole-sensitive strains. Also, compound **7c** and **7d** showed moderate inhibitory effect on Fluconazole-resistant strains with MIC values in the range of 1-32 μg/mL

2.4. Cytotoxicity activity

The cytotoxicity activity of the tested compounds (**7a-i**) was evaluated against normal human fibroblasts cell line (MRC-5) according to the previously reported methods [30–32]. The IC_{50} and therapeutic index (TI) values of these compounds are illustrated in Table 4. The cytotoxicity data revealed that the most potent compounds **7g** and **7h** showed considerably low toxicity against normal human fibroblasts cell line (MRC-5) with IC_{50} values of >180 μM. Subsequently, the therapeutic index of compounds **7a-i** were calculated by dividing IC_{50} value to MIC value [33]. The therapeutic index of compounds **7g** and **7h** was in the range of 360->600 for all tested yeasts, which indicated that these compounds had no toxic effect on normal cell line at therapeutic doses required for inhibition of fungal growth and they can be more profoundly investigated to achieve strong antifungal agents in the future.

2.5. Molecular docking study

Since the cytochrome P450 lanosterol 14α-demethylase enzyme (CYP51) is a vital enzyme for sterol biosynthesis of fungal cell membrane, the molecular docking studies were applied to understand the pattern of the interaction between ligands and CYP51 in the active site of enzyme. All the docking procedures were done

Table 4
IC_{50} values of all compounds against MRC-5 and their therapeutic indexes toward studied fungi.

		Therapeutic index										
Entry	IC_{50} (μM)	*C. albicans*	*C. dubliniensis*	*C. glabrata*	*C. krusei*	*C. parapsilosis*	*C. tropicalis*	*C. neoformans*	*A. flavus*	*A. fumigatus*	*A. clavatus*	*P. boydii*	*E. dermatitidis*
7a	>300	–	–	–	–	–	–	>75	–	–	–	–	–
7b	>300	>300	>10.7	>4.7	–	>4.7	>9.4	>300	–	–	–	–	–
7c	294	294	18.37	9.18	2.3	9.18	36.7	588	–	–	–	–	–
7d	54.9	54.9	6.86	–	–	6.86	–	109.8	–	–	–	–	–
7e	285	17.8	8.9	8.9	1.11	8.9	17.8	71.2	8.9	–	2.2	2.2	–
7f	267.3	534.6	133.6	16.7	–	33.4	133.6	534.6	534.6	–	–	–	–
7g	>300	>600	>600	>600	>4.7	>600	>600	>600	>37.5	–	–	–	–
7h	180	360	360	360	360	360	360	360	45	–	–	–	–
7i	29.4	58.8	7.35	0.92	–	0.92	14.7	58.8	–	–	–	–	–

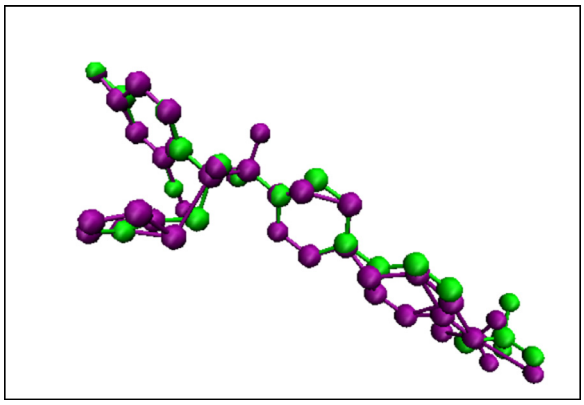


Fig. 3. Two conformations of co-crystal ligand (VT1) in the cytochrome P_{450} 14α-demethylase (CYP51) active site: The green model represented the crystal orientation and a purple color showed the redocked model.

on validated structures, with RMSD values below 2Å. The binding energies and interaction details of all the synthesized compounds (**7a-i**) were presented in Fig. 4, Fig. 5 and Table 5. Overall, compounds with highest antifungal effects (**7g** and **7h**) showed stronger binding energies in comparison to the other compounds. The superimposing of co-crystal ligand (VTI) before and after the docking procedure was represented in Fig. 3 [34,35].

Representation of docking interactions of **7a-e** were shown in Fig. 4. Compounds **7b**-**e** which had electronegative substitution on benzoyl moiety showed similar interactions including pi-pi and pi-alkyl interactions between benzoyl and Tyr 118, Leu 121 residues, pi-alkyl and pi-sigma bond of phenyl moiety with Met 508 and Leu 376. In addition, another pi-pi interaction was evident between HEM group and 1,2,4 triazole group, too. Compound **7a** with no substitution represent different interactions such as, pi-pi. pi-alkyl and carbon hydrogen bond with Tyr 118, Tyr 132, Leu 376, Ile 131 and Gly 307. Also, 1,2,4 triazole involved in pi-sigma interaction with HEM 601, too. In the second series (**7f-g**), di phenyl moiety and carbonyl group were involved in pi-pi, pi-sigma and carbon hydrogen interactions with Phe 233, Leu 88 and His 377. As depicted in Fig. 5, **7g**, showed two extra interactions such as, halogen interaction between 4-F substitution on benzoyl moiety and Met 508 and also, hydrogen bond with 1,2,4 triazole group and Ser 506. These extra and common interactions with cytochrome P_{450} 14α-demethylase (CYP51) created the **7g** as a promising inhibitor of CYP51 compared to other studied compounds. The most important residues in binding of **7h-i** were pi-alkyl and pi-pi interactions of diphenyl and benzoyl moiety with Leu 376, Tyr 118, Ile 304, Leu 121 and Ile 131. The benzoyl moiety of **7h-i** pointed to HEM group in CYP51 active site with pi-pi and pi-sigma interaction, respectively. These interactions stabilized **7h** and **7i** in the proper orientation of CYP51 enzyme.

2.6. In silico *ADME modeling study*

In silico ADME study was carried out to predict the drug-likeness and pharmacokinetic properties of the synthesized compounds **7a-i**. SwissADME online software was applied to obtain various ADME parameters of these derivatives, which are shown in Table 6. The ADME screening results indicated that all the synthesized derivatives **7a-i** obeyed the Lipinski's rule of 5 (MW < 500, cLogP < 5, HB donor ≤ 5, HB acceptor ≤ 10). In addition, the topological polar surface areas (TPSA) of the synthesized compounds were in favorable range (≤ 140) and so, admitted the Veber rule. Therefore, these derivatives show appropriate potential for oral bioavailability and can be considered as potent candidates for the discovery of promising antifungal agents [34–36].

Table 5
The bonding energies (kcal/mol) and the detailed interactions of the all synthesized compounds on CYP51 using AutoDock Vina.

Entry	Amino Acid	Ligand involved moiety	Type of interaction	B.E (kcal/mol)	Entry	Amino Acid	Ligand involved moiety	Type of interaction	B.E (kcal/mol)
7a	Tyr 118, Tyr132	phenyl	pi-pi	-9.0	**7f**	Ala 61, Pro 230, Leu 87	di-phenyl & benzoyl & 1,2,4-triazole	pi-alkyl	-8.6
	Leu 376, Ile 131	1,2,4-triazole & benzoyl	pi-alkyl			Phe 233	di-phenyl	pi-pi	
	HEM 601	1,2,4-triazole	pi-sigma			Leu 88	di-phenyl	pi-sigma	
	Gly 307	C=O	Carbon hydrogen bond			His 377	benzoyl & C=O	pi-cation & carbon hydrogen bond	
	Gly 303, Ile 304, Phe 126, Phe 228, Met 508, Val 509, Thr 311, Thr 122, Leu 121	––	Vander waals			Val 234, Tyr 64, Lys 90, Ser 506, Phe 380, Tyr 505, Gly 65, Ser 507, Met 508	––	Vander waals	
7b	Met 508, Leu 121, Leu 376	phenyl & benzoyl	pi-alkyl	-9.3	**7g**	Phe 233, His 377	di-phenyl & benzoyl	pi-pi	-9.8
	HEM 601	1,2,4-triazole	pi-pi			Ser 506, His 377	1,2,4-triazole & C=O	Carbon hydrogen bond	
	Tyr 118	benzoyl	pi-pi			Met 508	4-F	Halogen interaction	
	Leu 376	phenyl	pi-sigma			Pro 230, Ala 61, Leu 87	1,2,4-triazole & benzoyl & di-phenyl	pi-alkyl	
						Leu 87, Leu 88	di-phenyl	pi-sigma	
	Thr 311, Thr 122, Phe 126, Phe 380, Phe 228, Phe 233, Gly 307, Ile 131, Tyr 132, His 310, Val 509	––	Vander waals			Leu 376, Phe 380, Tyr 505, Lys 90, Gly 65, Tyr 64, Ser 507	––	Vander waals	
7c	HEM 601	1,2,4-triazole	pi-pi	-9.4	**7h**	Leu 376, Val 509, Met 508	di-phenyl & 1,2,4-triazole	pi-alkyl	-10.1
	Tyr 118	benzoyl	pi-pi			Tyr 118, Phe 228	di-phenyl	pi-pi	
	Leu 376	phenyl	pi-sigma			HEM 601, Ile 131	benzoyl	pi-sigma	
	Met 508, Phe 233, Leu 121, Phe 380, Leu 376	benzoyl & phenyl	pi-alkyl			Ile 304, Leu 300, Leu 121, Hem 601, Ile 131	di-phenyl & benzoyl	alkyl	
	Tyr 132, Ile 131, Thr 122, His 310, Phe 228, Phe 126, Gly 307, Thr 311, Ser 378, Val 509	––	Vander waals			Leu 139, Phe 126, Tyr 132, Thr 122, His 310, Gly 307, Thr 311, Gly 303	––	Vander waals	
7d	Met 508, Phe 233, Phe 380, Leu 121, Leu 376	Benzoyl & phenyl	pi-alkyl	-9.7	**7i**	Leu 376	1,2,4-triazole & di-phenyl	pi-alkyl	-8.6
	Tyr 118	benzoyl	pi-pi			HEM 601, Tyr 118, Phe 126	benzoyl & di-phenyl	pi-pi	
	HEM 601	1,2,4-triazole	pi-pi			Leu 121, Ile 304, Ile 131, Phe 126, HEM 601	benzoyl & di-phenyl	alkyl	
	Leu 376	phenyl	pi-sigma						
	Ser 378, Gly 307, Thr 311, His 310, Val 509, Phe 228, Phe 126, Thr 122, Ile 131, Tyr 132	––	Vander waals			Gly 303, Gly 307, Phe 228, Thr 311, Tyr 132, His 310, Val 509, Ile 379, Ser 378, Phe 380, Phe 233, Thr 122, Met 508	––	Vander waals	
7e	Leu 376, Met 508, Leu 121	phenyl & benzoyl	pi-alkyl	-9.6	Flu.	Leu 121	phenyl	pi-alkyl	-8.4
	HEM 601	1,2,4-triazole	pi-pi			HEM 601	1,2,4-triazole	pi-alkyl & pi-cation	
	Tyr 118	benzoyl	pi-pi			Gly 303, Tyr 132	1,2,4-triazole & CH_2	Carbon hydrogen bond	
	Leu 376	phenyl	pi-sigma			Ile 131	1,2,4 triazole	pi-alkyl	
	Ser 378, Phe 380, Phe 233, Thr 122, Phe 228, Val 509, His 310, Gly 307, Thr 311, Ile 131, Phe 126, Tyr 132	––	Vander waals			Phe 233, Tyr 118, Thr 122, Thr 311, Gly 307, Ile 304, Phe 126, Leu 376, Phe 228, Met 508	––	Vander waals	

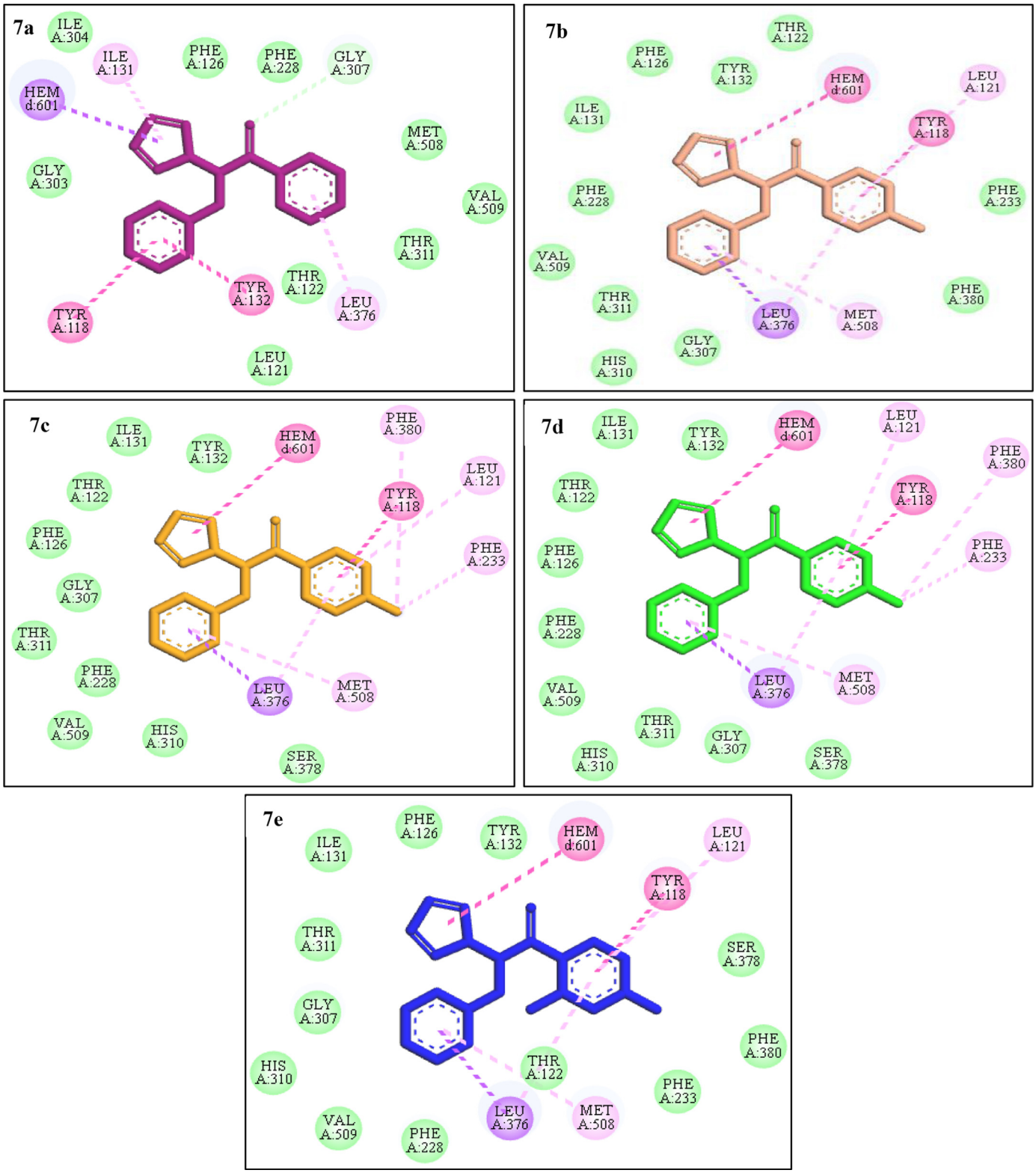


Fig. 4. 2D interactions of **7a-e** and Fluconazole in cytochrome P_{450} 14α-demethylase (CYP51) active site (green: Vander waals, light green: carbon hydrogen bond, dark pink: pi-pi, light pink: pi-alkyl, purple: pi-sigma, orange: pi-cation, blue: halogen bond).

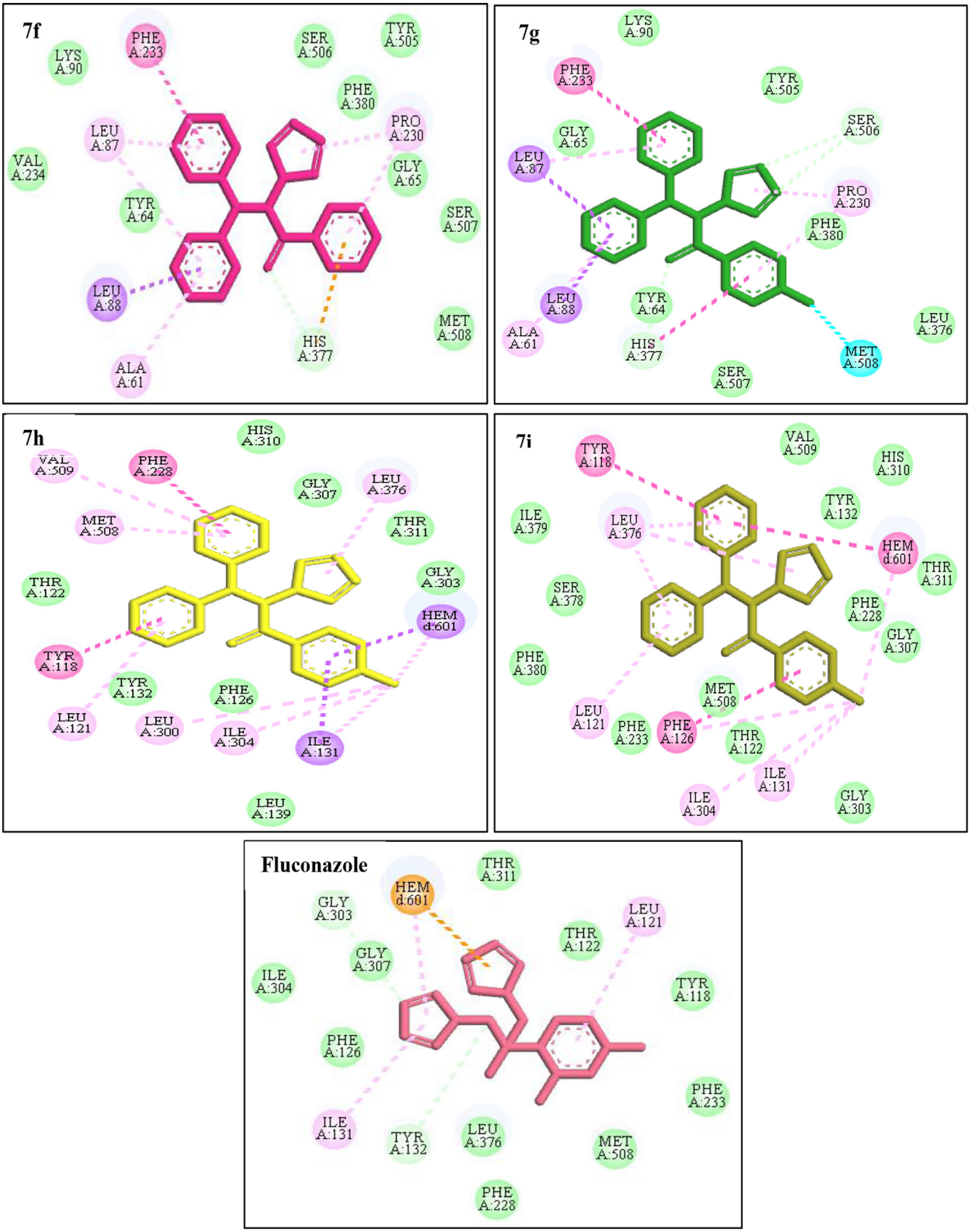


Fig. 5. 2D interactions of **7f-i** and Fluconazole in cytochrome P_{450} 14α-demethylase (CYP51) active site (green: Vander waals, light green: carbon hydrogen bond, dark pink: pi-pi, light pink: pi-alkyl, purple: pi-sigma, orange: pi-cation, blue: halogen bond).

Table 6
Physiochemical properties of the synthesized compounds **7a-i**.

Entry	MW[a]	LogP[b]	HBD[c]	HBA[d]	TPSA (Å)[e]	RB[f]	Lipinski/Veber violation
7a	277.32	2.39	0	3	47.78	5	0
7b	295.31	2.78	0	4	47.78	5	0
7c	311.77	2.90	0	3	47.78	5	0
7d	356.22	3.01	0	3	47.78	5	0
7e	313.30	3.17	0	5	47.78	5	0
7f	353.42	3.53	0	3	47.78	6	0
7g	371.41	3.91	0	4	47.78	6	0
7h	387.86	4.01	0	3	47.78	6	0
7i	432.31	4.11	0	3	47.78	6	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).

3. Conclusion

In order to find effective antifungal agents, a new set of 1,2,4 triazole derivatives (**7a-i**) were designed and synthesized via a three-step pathway. The chemical structures of these compounds were confirmed using IR, ^{1}H NMR, ^{13}C NMR and Mass spectra. The synthesized compounds were evaluated for their antifungal activities against several yeasts, filamentous fungi and clinical strains using CLSI method. According to the biological results, among all the tested compounds, **7g** and **7h** showed significant activity against all tested yeast strains (except *C. krusei*) with MIC values of 0.5 µg/mL and also, against Fluconazole-sensitive strains with MIC values ranging from 0.5-.8 µg/mL. Compound **7c** had MIC value ranging from 1-32 µg/mL against all Fluconazole-resistant strains. In addition, compound **7f** were better at inhibiting *A. flavus* than Fluconazole with MIC value of 0.5 µg/mL. Toxicity test was also performed and the results represented that these compounds had safety therapeutic indexes. SAR studies indicated that the presence of one or two halogen atom (F, Cl, Br) at the ortho or para position of C-1 phenyl ring or the presence of one or two phenyl ring at C-3 position has significant effect on the antifungal activity of the tested derivatives. The binding energies and interactions of active compounds were in good agreement with biological data. The outcome results indicated that these active derivatives may serve as a lead compound with little structural modifications can be considered as antifungal agents.

4. Experimental section

4.1. Chemistry

Melting points were determined using an Electro-thermal IA 9100 apparatus and the values given were uncorrected. IR spectra (KBr, cm^{-1}) were determined on a Perkin Elmer IR. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker 500 MHz spectrophotometer using TMS as an internal standard. Chemical shift values were recorded in ppm on δ scale. Mass spectra were recorded on an Agilent spectrometer. Thin layer chromatography (TLC) sheets pre-coated with UV fluorescent silica gel Merck F_{254} were used to monitor the progress of the reactions. The spots were visualized using UV lamp.

4.2. General procedure for the synthesis of intermediates 3a-e

To a solution of phenyl halides (50 mmol) in dichloromethane (30 ml), $AlCl_3$ (60 mmol) was added and the resulting mixture was stirred at room temperature for 30 min. Then, the mixture was cooled to 0 ° C and a solution of chloroacetyl chloride (54 mmol) in dichloromethane (20 ml) was added dropwise to it. The reaction mixture was stirred at room temperature for 24 h. After completion of the reaction, 50 ml of HCl solution (5 %) was slowly added and the reaction mixture was extracted with dichloromethane (3 × 30 ml) and then washed with $NaHCO_3$ (20 ml), water (2 × 20 ml) and brine (20 ml), respectively. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the residue was recrystallized from *n*-hexane to afford the title compounds **3a-e**.

4.3. General procedure for the synthesis of intermediates 5a-e

To a solution of compounds **3a-e** (40 mmol) in toluene, 1,2,4-triazole (48 mmol) and $NaHCO_3$ (48 mmol) were added and the reaction mixture was refluxed for 20 h. After completion of the reaction, the mixture was quenched by an ice bath and then extracted with ethyl acetate (3 × 30 ml). The organic layer was washed with water (2 × 20 ml) and brine (10 ml), respectively. Afterwards, the organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuum and the residue was recrystallized from diethyl ether to yield the title compounds **5a-e**.

4.4. General procedure for the synthesis of compounds 7a-i

To a suspension of NaH (8 mmol) in acetonitrile (30 ml), a solution of compound 5a-e (6 mmol) in acetonitrile (10 ml) was added and the resulting mixture was stirred at room temperature for 1 h. Then, a solution of benzhydryl bromide or benzyle bromide (6 mmol) in acetonitrile (10 ml) was added dropwise and the mixture was refluxed for 24 h. After this time, the reaction mixture was cooled to room temperature and the solvent was evaporated in vacuo. The crude product was washed with water (50 ml) and the aqueous layer was separated and extracted with dichloromethane (3 × 30 ml). The organic layers were dried over Na_2SO_4. The crude product was purified by column chromatography on silica gel eluting with ethyl acetate and petroleum ether (3:1) to yield pure products 7a-i.

4.4.1. 1,3-diphenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (7a)

Yield: 75 %, mp: 145-148°C. IR (KBr) ν (cm^{-1}): 3101 (C-H, aromatic), 2928.8 (C-H, aliphatic), 1690.0 (C=O, ketones), 1594.2 (C=N), 1283.7 (C–N stretch, aromatic).^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.28 (s, 1H, triazole), 7.92 (d, J = 7.5 Hz, 2H, Ar-H-CO), 7.91 (s, 1H, triazole), 7.59 (t, J = 7.5 Hz, 1H, Ar-H-CO), 7.46 (t, J = 7.5 Hz, 2H, Ar-H-CO),7.18-7.24 (m, 3H, Ar-H) 7.01 (d, J = 6.8 Hz, 2H, Ar-H), 6.24 (dd, J = 8.8, 5.8 Hz, 1H, CH), 3.55 (dd, J = 14.3, 5.8 Hz,

1H, CH_2), 3.41 (dd, J = 14.3, 9.0 Hz, 1H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 193.8, 151.4, 143.6, 135.6, 134.8, 134.7, 129.5, 129.4, 129.3, 129.1, 127.9, 65.5, 39.0; MS m/z (%): 277.2 (5) [M^+].

4.4.2. 1-(4-fluorophenyl)-3-phenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (7b)

Yield: 61%, mp: 65-68°C. IR (KBr) ν (cm^{-1}): 3108.7 (C–H stretch, aromatic), 2915.8 (C-H, aliphatic), 1699.3, (C=O, ketone), 1597.1 (C=N), 1241.7 (C–N stretch, aromatic), 1270.4 (Ar-F). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.24 (s, 1H, triazole), 7.93 (dd, J = 8.3, 5.5 Hz, 2H, 4-F-Ar-H, H-3 and H-5), 7.89 (s, 1H, triazole), 7.34-7.37 (m, 3H, Ar-H) 7.19-7.23 (m, 2H, Ar-H), 7.10 (t, J = 8.3 Hz, 2H, 4-F-Ar-H, H-2 and H-6), 6.17 (dd, J = 8.3, 6.4 Hz, 1H, CH), 3.53 (dd, J = 14.1, 6.1 Hz, 1H, CH_2), 3.40 (dd, J = 14.1, 8.6 Hz, 1H, CH_2). MS m/z (%): 295.1(6) [M^+].

4.4.3. 1-(4-chlorophenyl)-3-phenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (7c)

Yield: 72%, mp: 116-119°C. IR (KBr) ν (cm^{-1}): 3088.0 (C–H stretch, aromatic), 2926.1 (C-H, aliphatic), 1690.8, (C=O, ketone), 1587.7 (C=N), 1279.8 (C–N stretch, aromatic), 1090.9 (Ar-Cl). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.24 (s, 1H, triazole), 7.89 (s, 1H, triazole), 7.82 (d, J = 8.5 Hz, 2H, 4-Cl-Ar-H, H-2 and H-6), 7.40 (d, J = 8.5 Hz, 2H, 4-Cl-Ar-H H-3 and H-5), 7.18 -7.24 (m, 3H, Ar-H) 7.00 (d, J = 6.8 Hz, 2H, Ar-H), 6.16 (dd, J = 8.5, 6.1 Hz, 1H, CH), 3.53 (dd, J = 14.2, 6.1 Hz, 1H, CH_2), 3.40 (dd, J = 14.2, 8.6 Hz, 1H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 192.8, 151.7, 143.6, 141.3, 135.4, 133.2, 130.4, 129.8, 129.4, 129.3, 128.0, 65.3, 38.9. MS m/z (%): 311.1 (5) [M^+].

4.4.4. 1-(4-bromophenyl)-3-phenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (7d)

Yield: 69 %. IR (KBr) ν (cm^{-1}): 3112.3 (C–H stretch, aromatic), 2972.8 (C-H, aliphatic), 1690.8, (C=O, ketone), 1584.4 (C=N), 1287.0 (C–N stretch, aromatic), 1011.0 (Ar-Br). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.35 (s, 1H, triazole), 7.93 (s, 1H, triazole), 7.76 (d, J = 8.3 Hz, 2H, 4-Br-Ar-H H-2 and H-6), 7.59 (d, J = 8.3 Hz, 2H, 4-Br-Ar-H H-3 and H-5), 7.19-7.25 (m, 3H, Ar-H) 7.00 (d, J = 7.4, 2H, Ar-H), 6.19 (dd, J = 8.2, 6.3 Hz, 1H, CH), 3.54 (dd, J = 14.2, 6.0 Hz, 1H, CH_2), 3.41 (dd, J = 14.2, 8.6 Hz, 1H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 192.9, 151.1, 135.2, 133.6, 132.9, 130.5, 130.2, 129.4, 129.3, 128.0, 65.5, 38.9. MS m/z (%): 355.0 (7) [M^+].

4.4.5. 1-(2,4-difluorophenyl)-3-phenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (7e)

Yield: 56 %, mp: 78-80°C. IR (KBr) ν (cm^{-1}): 3121.1 (C–H stretch, aromatic), 2952.3 (C-H, aliphatic), 1681.7, (C=O, ketone), 1607.7 (C=N), 1240.4 (C–N stretch, aromatic), 1282.9 (Ar-F). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.97 (s, 1H, triazole), 7.94 (dd, J = 15.0, 8.3 Hz, 1H, 2,4-F-Ar-H, H-6), 7.87 (s, 1H, triazole), 7.21-7.22 (m, 3H, Ar-H) 7.00 (t, J = 9.0 Hz, 1H, 2,4-F-Ar-H, H-3), 6.96 (d, J = 5.8 Hz, 2H, Ar-H), 6.84 (t, J = 9.0 Hz, 1H, 2,4-F-Ar-H, H-5), 5.91 (dd, J = 10.1, 4.1 Hz, 1H, CH), 3.56 (dd, J = 14.1, 3.5 Hz, 1H, CH_2), 3.35 (dd, J = 13.5, 10.5 Hz, 1H, CH_2). MS m/z (%): 313.2 (2) [M^+].

4.4.6. 1,3,3-triphenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (7f)

Yield: 73 %, mp: 161-164°C IR (KBr) ν (cm^{-1}): 3105.1 (C-H, aromatic), 2924.1 (C-H, aliphatic), 1693.5 (C=O, ketones), 1597.0 (C=N stretch, aromatic).^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.25 (s, 1H, triazole), 7.88 (d, J = 7.5 Hz, 2H, Ar-H-CO, H-2 and H-6), 7.79 (s, 1H, triazole), 7.51 (t, J = 7.4 Hz, 1H, Ar-H-CO, H-4), 7.37 (t, J = 7.7 Hz, 2H, Ar-H-CO H-3 and H-5), 7.29 (d, J = 7.5 Hz, 2H, Ar-H), 7.14 -7.223 (m, 7H, Ar-H) 7.08 (t, J = 7.3 Hz, 1H, Ar-H), 6.84 (d, J = 11.5 Hz, 1H, CH-N), 5.09 (d, J = 11.5 Hz, 1H, CH-$(Ph)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 194.2, 151.2, 143.3, 139.6, 139.4, 135.7, 134.5, 129.4, 129.35, 129.28, 129.26, 129.09, 128.6, 128.4, 127.9, 127.8, 65.5, 53.9. MS m/z (%): 353.2 (7) [M^+].

4.4.7. 1-(4-fluorophenyl)-3,3-diphenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (7g)

Yield: 58 %, mp: 149-152°C. IR (KBr) ν (cm^{-1}): 3104.3 (C-H, aromatic), 3028.9 (C-H, aliphatic), 1695.9 (C=O, ketones), 1599.5 (C=N stretch, aromatic). 1243.7 (C–N stretch, aromatic), 1273.0 (Ar-F). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.30 (s, 1H, triazole), 7.91(dd, J = 8.7, 5.3 Hz, 2H,4-F-Ar-H-CO, H-3 and H-5), 7.80 (s, 1H, triazole), 7.27 (d, J = 7.5 Hz, 2H, Ar-H, H-2 and H-6), 7.23-7.13 (m, 7H, Ar-H) 7.09 (t, J = 7.5 Hz, 1H, Ar-H), 7.04 (t, J = 8.7 Hz, 2H, 4-F-Ar-H-CO, H-2 and H-6), 6.80 (d, J = 11.6 Hz, 1H, CH-N), 5.08 (d, J = 11.6 Hz, 1H, CH-$(Ph)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 192.7, 150.8, 143.2, 139.4, 139.1, 132.1, 131.97, 131.90, 129.41, 129.33, 128.6, 128.3, 127.9, 116.6, 116.4, 100.4, 65.5, 53.9. MS m/z (%):371.2 (7) [M^+].

4.4.8. 1-(4-chlorophenyl)-3,3-diphenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (7h)

Yield: 67 %, mp: 140-142°C. IR (KBr) ν (cm^{-1}): 3101.6 (C-H, aromatic), 2926.1 (C-H, aliphatic), 1691.4 (C=O, ketones), 1588.7 (C=N stretch, aromatic). 1275.4 (C–N stretch, aromatic), 1093.1 (Ar-Cl). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.33 (s, 1H, triazole), 7.81(d, J = 8.2 Hz, 2H, Ar-H-CO, H-2 and H-6), 7.81 (s, 1H, triazole), 7.34 (d, J = 8.3 Hz, 2H, Ar-H-CO, H-3 and H-5), 7.27 (d, J = 7.5, 2H, Ar-H), 7.23 -7.13 (m, 7H, Ar-H) 7.10 (t, J = 7.3 Hz, 1H, Ar-H), 6.79 (d, J = 11.6 Hz, 1H, CH-N), 5.09 (d, J = 11.6 Hz, 1H, CH-$(Ph)_2$). MS m/z (%): 387.1 (12) [M^+].

4.4.9. 1-(4-bromophenyl)-3,3-diphenyl-2-(1H-1,2,4-triazol-1-yl)propan-1-one (7i)

Yield: 63 %, mp: 110-113°C. IR (KBr) ν (cm^{-1}): 3103.3 (C-H, aromatic), 2973.0 (C-H, aliphatic), 1692.8 (C=O, ketones), 1583.1 (C=N stretch, aromatic). 1287.8 (C–N stretch, aromatic), 1012.4 (Ar-Br). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.13 (s, 1H, triazole), 7.77 (s, 1H, triazole), 7.71 (d, J = 8.4 Hz, 2H, Ar-H-CO, H-2 and H-6), 7.51 (d, J = 8.4 Hz, 2H, Ar-H-CO, H-3 and H-5), 7.26-7.11 (m, 10 H, Ar-H), 6.73 (d, J = 11.6 Hz, 1H, CH-N), 5.07 (d, J=11.6 Hz, 1H, CH-$(Ph)_2$). MS m/z (%): 431.1(5) [M^+].

4.5. Antifungal assay

Microorganisms were obtained from the Mycology and Parasitology Departments of Shiraz University of Medical Sciences. The *in-vitro* antifungal activity of synthesized compounds **7a-i** was evaluated against several types of yeast and filamentous fungi including *C. albicans* (ATCC 10261, CBS 1912), *C. krusei* (ATCC 6258), *C. glabrata* (ATCC 90030), *C. parapsilosis* (ATCC 4344), *C. dubliniensis* (CBS 8501), *C. tropicalis* (ATCC 750), *C. neoformans* (ATCC 9011), *A. flavus* (ATCC 64025), *A. clavatus* (ATCC 20542), *A. fumigatus* (ATCC 14110), *P. boydii* (CBS 329093), *E. dermatitidis* (CBS 120549) and six clinical isolates of yeasts identified by PCR-RFLP [37]. The broth microdilution (CLSI) method was used to determine the minimal inhibitory concentrations (MIC) of the tested compounds [38]. The stock solution of the tested compounds was prepared in dimethyl sulfoxide (DMSO) at a concentration of 40 mg/mL. Subsequently, the compounds were diluted in broth media (RPMI 1640 media) to prepare final concentrations (0.25 to 256 µg/ml). For preparation of stock inoculums, several colonies of the yeasts and filamentous fungi were suspended in sterile PBS. Then, inoculum suspensions were adjusted to concentration of 1-5 $\times$ 10^6 cells/mL at 530 nm. MICs were defined as the lowest concentration of the antimicrobial agents that created ≥50 growth inhibitions. All experiments were repeated three times and the mean MICs were reported as MIC_{50}.

4.6. Molecular docking study

Firstly, all synthesized compounds were drawn, optimized and converted to PDBQT format according to our previous protocol

by MGL tools 1.5.6 [39]. The three dimensional crystal structure of lanosterol 14-α demethylase (PDB ID: 5TZ1) was achieved from Protein Data Bank (http://www.rcsb.org). The co-crystal ligand and water molecules were removed and missing hydrogens were added. The missing atoms of PDBs were then corrected with MODELLER 9.17 and converted to PDBQT format. The docking procedure was done in a grid box with a size of 70 × 70 × 70 and a center of x = 69.292, y = 69.427, z = 3.640 using AutoDock Tools. The molecular grid was kept around Ala 61, Tyr 64, Gly 65, Leu 87, Leu 88, Lys 90, Tyr 118, Leu 121, Thr 122, Phe 126, Ile131, Tyr, 132, Leu 139, Phe 228, Pro 230, Phe 233, Val 234, Leu 300, Gly 303, Ile 304, Gly 307, His 310, Thr 311, Leu 376, His 377, Ser 378, Ile 379, Phe 380, Tyr 505, Ser 506, Ser 507, Met 508, Val509 [40]. Total 100 distinct poses of ligands were generated using the Lamarckian genetic algorithm. Gasteiger charges for **7a** (-0.0001), **7b** (-0.0002), **7c** (-0.0001), **7d** (-0.0002), **7e** (0.0001), **7f** (-0.0003), **7g** (-0.0004), **7h** (-0.0003), **7i** (-0.0004) and also, kolman charge of 5TZ1 (13.24) were applied using AutoDock Tools. The docking was run by AutoDock Tools using an in-house batch script (DOCKFACE) [32,41]. All interactions were visualized and evaluated on the basis of the best docking pose using discover studio client 2016 [42].

Credit author statement

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2022.134039.

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