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Synthesis, evaluation and molecular docking of 1,2,3-triazolyl chalcones as potential antifungal and antioxidant agents

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Abstract: A series of new 1,2,3-triazolyl chalcones were efficiently synthesized and screened for *in vitro* antifungal activity against five different fungal strains such as *Candida albicans*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger* and *Cryptococcus neoformans*. All the synthesized chalcones displayed potential antifungal activity against most of the tested fungal strains. Especially, compounds **9b**, **9c**, **9d** and **9g** are the most active chalcones and displayed excellent MIC values as compared to standard antifungal drug Miconazole. Based on the structural similarity to known triazole inhibitors of sterol 14 α -demethylase (CYP51), molecular docking study was performed to gauge the binding affinity of these chalcones and gains an insight into the plausible mechanism of antifungal action. The synthesized chalcones were also evaluated for *in vitro* antioxidant activity. All compounds exhibited moderate to excellent antioxidant activity, particularly compounds **9e**, **9f**, **9g** and **9h** exhibited excellent antioxidant activity in comparison with standard butylated hydroxytoluene (BHT). Furthermore, the synthesized chalcones were analyzed for ADME properties and showed the potential to build up as good oral drug candidates.

Keywords: 1,2,3-triazole, Chalcone, Antifungal activity, Antioxidant activity, Molecular docking study, ADME prediction.

1. Introduction

The incidences of multidrug-resistant fungal infections in recent years have increased dramatically and commonly seen in patients with weak immune systems ^[1]. An increasing number

of infections with stem cell transplantation, organ transplantation, chemotherapy, and human immunodeficiency virus increases invasive fungal infections^[2]. The major pathogenic strains responsible for systematic fungal infections are *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigates*^[3]. Fluconazole, Voriconazole, Itraconazole and Miconazole are some of the widely used azole based broad-spectrum antifungal drugs^[4] (**Figure 1**). They displayed broad-spectrum antifungal potential against most of the filamentous fungi, however, some of them are not effective against invasive aspergillosis and also suffered from severe drug resistance^[5]. Moreover, the broad use of existing antifungal drugs has caused severe drug resistance. Therefore, it is urgent to develop new antifungal agents with excellent activity against a variety of clinical fungal strains.

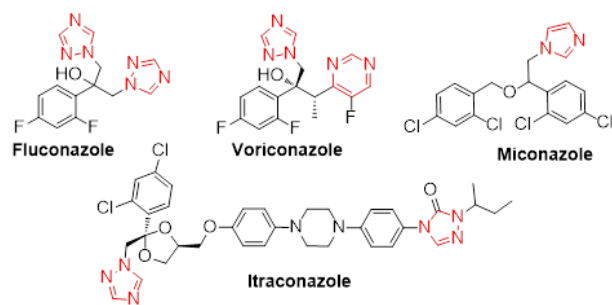


Figure 1. Known azole containing antifungal drugs

Many natural enzymatic and non-enzymatic antioxidants present in the human body that counteract the harmful effect of free radicals and other oxidants^[6]. Although reactive oxygen species (ROS) are essential for various complex mechanisms of the organism's body in various diseases, the excessive production of ROS causes various diseases and oxidative stress. Antioxidant protects the cell from oxidative injury by neutralizing free radicals. Recently, it has been observed that reactive oxygen species participates in the mechanism of action of triazole containing antifungals^[7]. It is important to elucidate possible association

among oxidative stress response and antifungal mechanism of action for developing new targets for novel antifungal agents by rational development of antifungal drugs.

Chalcones i.e. α - β unsaturated ketones are important pharmacophore in many natural and synthetic biologically active compounds^[8–12]. It is used as an effective template in medicinal chemistry and drug discovery process^[13,14]. The double bond in conjugation to carbonyl group is considered as responsible for the pharmacological effect in chalcones. Literature revealed that chalcones exhibited broad spectrum of biological activities like anticancer^[15], antimalarial^[16], antimicrobial^[17], antioxidant^[18], anti-inflammatory^[13] etc.

1,2,3-triazole based natural and synthetic compounds are privileged scaffold in the drug discovery process with a broad spectrum of biological activities^[19–25]. Molecular hybridization of chalcones with 1,2,3-triazole can be an efficient strategy to synthesize chemically diverse and biologically active conjugates. Recent literature revealed that 1,2,3-triazolyl chalcones displayed a broad spectrum of pharmacological activities^[17,22,26–31] (**Figure 2**).

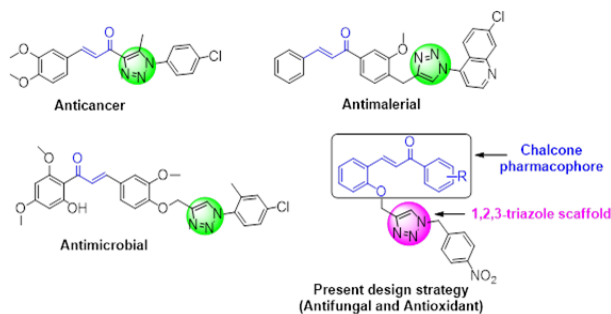


Figure 2. Known 1,2,3-triazolyl-chalcones and our design strategy

In search of the development of new active antifungal and antioxidant agents, combinatorial synthesis of 1,2,3-triazolyl chalcones has

been efficiently carried out by click chemistry and Aldol type condensation reaction. We were encouraged to design and synthesize 1,2,3-triazolyl chalcones from commercially available starting materials with high overall yield and evaluation of their antifungal and antioxidant activities.

Hence, keeping in view the urgent need of potential antifungal and antioxidant agents and in continuation to our earlier work on 1,2,3-triazole based bioactive compounds [21,23,24] and α,β -unsaturated compounds [32–34], we report herein design, synthesis, molecular docking, ADME prediction and bio evaluation of some new 1,2,3-triazolyl chalcones as potential antifungal and antioxidant agents.

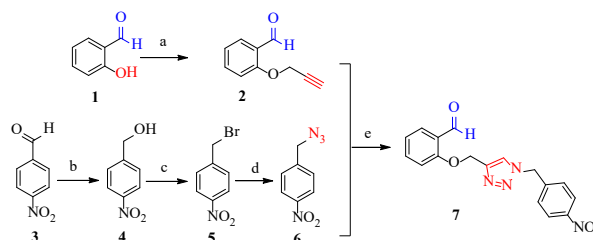
2 Result and discussion

2.1 Chemistry

In present work, we have described the syntheses of new substituted 1,2,3-triazolyl chalcones **9a-i** from commercially available starting materials (**Scheme 2**). 1,2,3-triazole based benzaldehyde **7** was formed by the fusion of benzyl azide and the terminal alkyne group of substituted benzaldehyde **2** via the click chemistry approach (**Scheme 1**).

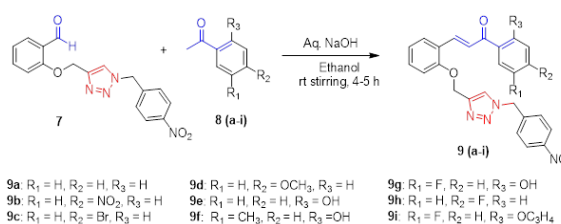
The treatment of *o*-hydroxy benzaldehyde **1** with propargyl bromide in the presence of K_2CO_3 as a base in *N,N*-dimethylformamide (DMF) at room temperature afforded 2-(prop-2-yn-1-yloxy)benzaldehyde **2** in 89% yield (**Scheme 1**). The *p*-nitrobenzyl azide **6** was prepared from the *p*-nitrobenzaldehyde via $NaBH_4$ reduction, bromination, and nucleophilic substitution reaction of sodium azide, according to the reported procedure [35]. (**Scheme 1**). Furthermore, 1,3-dipolar cycloaddition reaction of benzyl azide **6** and terminal alkyne group of benzaldehyde **2** using a catalytic amount of copper diacetate $Cu(OAc)_2$ in *t*-BuOH- H_2O

(3:1) at room temperature for 20 h afforded regioselective 1,4-disubstituted-1,2,3-triazole incorporated benzaldehyde **7** in 88% yield (**Scheme 1**).



Scheme 1. Reagents and conditions: (a) Propargyl bromide, K_2CO_3 , DMF, r.t., 2.5 h; (b) $NaBH_4$, methanol, 0 °C to r.t., 2 h; (c) PBr_3 , CH_2Cl_2 , 0 °C, 0.5 h; (d) NaN_3 , acetone: H_2O (3:1), r.t., 24 h; (e) $Cu(OAc)_2$ (20 mol%), *t*-BuOH/ H_2O (3:1), r.t., 19-27 h.

Finally, newly synthesized 1,2,3-triazole based benzaldehyde **7** was condensed with various substituted acetophenone (**8a-i**) in aqueous NaOH, resulted into the corresponding 1,2,3-triazolyl chalcones (**9a-i**) in quantitative isolated yield (85-90%) (**Scheme 2**).



Scheme 2. Synthesis of 1,2,3-triazolyl-chalcones

The structures of 1,2,3-triazolyl-chalcones **9a-i** were confirmed by FT-IR, ^1H NMR, ^{13}C NMR and LC/ESI-MS analysis. Structures of the synthesized 1,2,3-triazolyl-chalcones are given below in **Figure 3**.

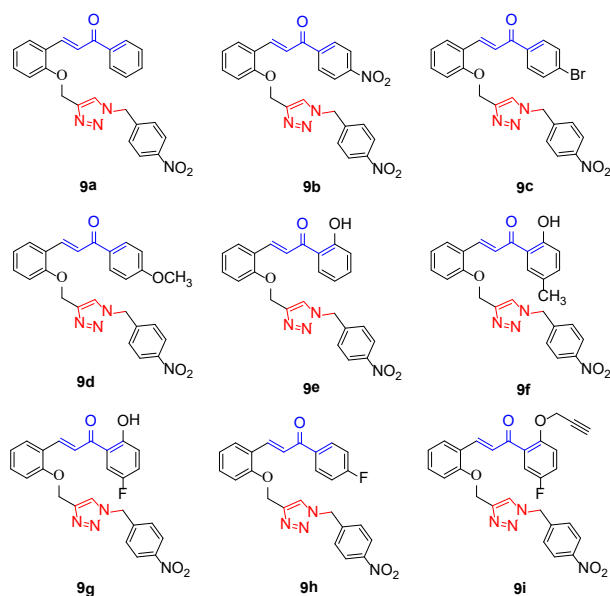


Figure 3. Structures of 1,2,3-triazolyl chalcones

2.2 Antifungal activity

All the synthesized 1,2,3-triazolyl-chalcones were screened for their *in vitro* antifungal activity. The antifungal activity was evaluated against five different pathogenic fungal strains such as *Candida albicans*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, and *Cryptococcus neoformans*. Minimum inhibitory concentration (MIC) values were determined using a standard agar plate method [36,37]. MIC value of an antimicrobial compound is its lowest concentration that prevents visible growth of the microorganism. It is a measure of the effective ability of the antimicrobial against the concerned microbe. Miconazole was used as a standard for the comparison of antifungal activity. Dimethyl sulfoxide was used as solvent control.

Table 1. *In vitro* antifungal, antioxidant data and *in silico* molecular docking score of 1,2,3-triazolyl chalcones (9a-i).

Entry	Antifungal Activity MIC (µg/mL)					Antioxidant activity IC ₅₀ (µg/mL)	Molecular Docking Score
	CA	FO	AF	AN	CN		
9a	25	25	25	25	6.25	50.17± 0.35	-7.944
9b	6.25	12.5	12.5	25	12.5	40.32± 0.46	-8.947
9c	12.5	50	12.5	50	12.5	27.11± 0.25	-8.154
9d	12.5	12.5	25	25	12.5	21.12 ± 0.19	-8.447
9e	25	12.5	50	50	12.5	16.34 ± 0.91	-7.902
9f	12.5	25	50	75	12.5	15.66± 0.45	-8.015
9g	25	25	50	50	50	16.37± 0.87	-7.458
9h	25	25	75	50	100	16.06 ± 0.18	-7.263
9i	75	50	75	50	50	31.46 ± 0.88	-7.039
MA	25	25	12.5	25	25	-	-
BHT	-	-	-	-	-	16.47± 0.18	-

CA-*Candida albicans*, FO-*Fusarium oxysporum*, AF-*Aspergillus flavus*, AN-*Aspergillus Niger*, CN-*Cryptococcus neoformans*, MA- Miconazole, BHT- Butoxy hydroxy toluene, Values are the average of three readings (n=3) ± standard deviation

MIC values of the tested compounds are presented in **Table 1**. Most of the newly synthesized 1,2,3-triazolyl chalcones were found to show excellent antifungal activity as compared to standard antifungal drug Miconazole. From the antifungal activity data (**Table 1**), it is observed that almost all the synthesized chalcones were displayed promising antifungal activity against fungal strain *C. Albicans*. Particularly, chalcones **9b**, **9c**, **9d**, and **9g** are the most active among all tested compounds against most of the fungal strains. Structure-activity relationship revealed that introduction of nitro (-NO₂) group at *para*- position of acetophenone moiety in compound **9b** showed a significant rise in activity as compared to **9a** (activity increases by two-fold as reflected in lowered MIC value). Compound **9a** is equipotent to Miconazole against *C. albicans* (MIC value 25 µg/mL), *A. Niger* (MIC value 25 µg/mL), *F. oxysporum* (MIC value 25 µg/mL), *A. flavus* (MIC value 25 µg/mL) and most active than Miconazole in case of fungal strain *C. neoformans* (MIC value 12.5

$\mu\text{g/mL}$). Introduction of alkyne group in case of **9i** reduces the antifungal activity compared to other tested compounds. Introduction of electron-withdrawing groups and halogens on acetophenone shows a significant enhancement in an activity. Graphical representation of antifungal activity data of the synthesized compounds is represented in **figure 4**.

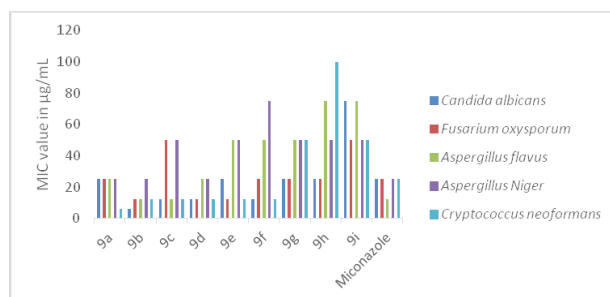


Figure 4. Antifungal activity of synthesized analogues as compared to standard Miconazole

2.3 Antioxidant activity

All the synthesized chalcones displayed good to moderate radical scavenging activity when compared with the standard drug BHT (**Table 1**). Particularly compounds **9e**, **9f**, **9g** and **9h** exhibit significant IC_{50} values ($\text{IC}_{50} = 16.34 \pm 0.91$, 15.66 ± 0.45 , 16.37 ± 0.87 , 16.06 ± 0.18 in $\mu\text{g/mL}$ respectively) as compared to standard antioxidant BHT. Structure-activity data revealed that the hydroxy group is important for the enhancement of antioxidant potential as reflected in IC_{50} values. Also, the substitution of electron-donating groups on acetophenone increases the antioxidant activity of 1,2,3-triazole substituted chalcones as compared to standard antioxidant BHT. The antioxidant activity of these compounds may be related to their redox properties, which allow them to act as reducing agents or hydrogen atom donors and scavenge-free radicals.

2.4 Molecular docking

Molecular docking study revealed the binding

orientations of 1,2,3-triazolyl-chalcones in the active sites of CYP51. It was observed that all the synthesized chalcones could snugly fit into the active site of CYP51 with varying degrees of affinities at co-ordinates close to a native ligand. Their docking scores varied from -8.947 for the most active analogue to -7.039 for moderately active with average docking score of -7.907 signifying a promising binding affinity towards CYP51 (**Table 2**, **Fig 5** and **Fig 6-13**). To gain a better understanding on the potency and types of interactions contributing to the enhanced binding affinity towards the target, a detailed investigation of the per-residue interactions for the most active analogues (**9b**, **9c**, **9d** and **9f**) was carried out which is elaborated in detail for one of the most active compound **9b** while it summarized in **Table 2** for other active compounds (**9c**-**Fig 6**, **9d**- **Fig 7** and **9f**- **Fig 9**).

The best-docked conformation of **9b** was seen to be deeply embedded into the active pocket of sterol 14 α -demethylase (CYP51). The lower interaction energy (Glide binding energy -62.896 kcal/mol, Glide score of from -8.947) rationalizes the tighter binding of this chalcone analogue (**Figure. 5**) into the CYP51 active site.

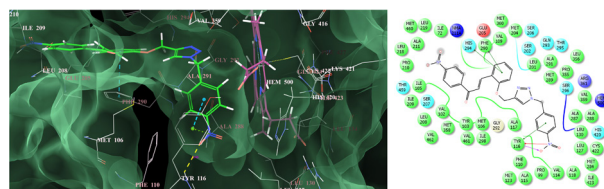


Figure 5: Binding mode of **9b** into the active site of sterol 14 α -demethylase (CYP51) (on the right side: the pink lines signify hydrogen bonding interaction, green lines signify π - π stacking while red line represent cation- π stacking interactions)

The per-residue interaction analysis revealed that the enzyme-inhibitor complex was stabilized by a series of favourable van der Waals interactions observed with Hem500 (-6.776 kcal/mol), Val461 (-2.929 kcal/mol), Val359 (-2.431 kcal/

Table 2. The per-residue interaction analysis based on molecular docking for 1,2,3-triazolyl-chalcones with sterol 14 α -demethylase (CYP51).

Code	Docking Score	Glide Interaction Energy (kcal/mole)	Per-Residues interactions			π - π / Cation- π Stacking
			Van der Waals (kcal/mol)	Columbic (kcal/mol)	H-bond (Å)	
9b	-8.947	-62.896	Hem 500(-6.776), Val461 (-2.929), Met460 (-3.151), Thr459 (-2.898), His458 (-2.543), Met360 (-3.233), Val359 (-2.431), Met358 (-2.981), Leu356 (-2.779), Thr295 (-2.358), His294 (-2.277), Ala291 (-3.531), Phe290 (-3.236), Ala287 (-2.528), Pro210 (-2.614), Ile209 (-2.643), Leu208 (-3.157), Glu205 (-2.827), Leu127 (-2.789), Tyr116 (-3.788), Phe110 (-2.735), Met106 (-3.434), Ile105 (-2.742), Tyr103 (-2.39), Val102 (-2.825)	Hem500 (-3.959), Arg361 (-2.414), Phe290 (-2.362), Leu208 (-2.522), Glu205 (-3.569), Arg124 (-2.223), Ile105 (-2.293), Val102 (-3.201)	Tyr116 (2.65)	π - π Stacking: His294 (2.168), Phe290 (2.374), Tyr116 (2.651) Cation- π Stacking: Tyr116 (2.651)
9c	-8.154	-60.358	Hem500 (-6.458), Val461 (-2.463), Met460 (-2.793), Thr459 (-2.264), His458 (-2.129), Met360 (-2.233), Val359 (-2.143), Met358 (-2.280), Leu356 (-2.261), Thr295 (-2.157), His294 (-2.082), Ala291 (-2.191), Phe290 (-2.467), Ala287 (-2.269), Pro210 (-2.600), Ile209 (-2.204), Leu208 (-3.072), Glu205 (-2.523), Leu127 (-2.114), Tyr116 (-2.878), Phe110 (-2.034), Met106 (-3.247), Ile105 (-2.558), Tyr103 (-2.095), Val102 (-2.216)	Hem500 (-3.548), Arg361 (-2.239), Phe290 (-2.203), Leu208 (-2.136), Glu205 (-3.122), Arg124 (-2.183), Ile105 (-2.150), Val102 (-2.872)	Tyr116 (2.50)	π - π Stacking: His294 (2.271), Phe290 (2.303), Tyr116 (2.498) Cation- π Stacking: Tyr116 (2.498)
9d	-8.447	-61.216	Hem500 (-6.702), Val461 (-2.751), Met460 (-2.901), Thr459 (-2.727), His458 (-2.241), Met360 (-2.929), Val359 (-2.230), Met358 (-2.328), Leu356 (-2.388), Thr295 (-2.377), His294 (-2.191), Ala291 (-2.796), Phe290 (-2.866), Ala287 (-2.324), Pro210 (-2.628), Ile209 (-2.315), Leu208 (-3.096), Glu205 (-2.617), Leu127 (-2.187), Tyr116 (-2.892), Phe110 (-2.128), Met106 (-3.338), Ile105 (-2.729), Tyr103 (-2.123), Val102 (-2.221)	Hem500 (-3.667), Arg361 (-2.548), Phe290 (-2.226), Leu208 (-2.254), Glu205 (-3.417), Arg124 (-1.110), Ile105 (-2.025), Val102 (-2.442)	Tyr116 (2.63)	π - π Stacking: His294 (2.175), Phe290 (2.369), Tyr116 (2.632) Cation- π Stacking: Tyr116 (2.632)
9f	-8.015	-59.091	Hem500 (-5.137), Val461 (-2.644), Met460 (-2.481), Thr459 (-2.282), His458 (-2.230), Met360 (-2.233), Val359 (-1.834), Met358 (-1.928), Leu356 (-2.037), Thr295 (-2.171), His294 (-1.871), Ala291 (-1.897), Phe290 (-2.569), Ala287 (-2.022), Pro210 (-1.911), Ile209 (-1.798), Leu208 (-2.316), Glu205 (-1.899), Leu127 (-2.017), Tyr116 (-2.838), Phe110 (-1.969), Met106 (-2.010), Ile105 (-2.167), Tyr103 (-2.154), Val102 (-2.111)	Hem500 (-2.299), Arg361 (-1.324), Phe290 (-1.732), Leu208 (-2.001), Glu205 (-2.970), Arg124 (-1.537), Ile105 (-2.023), Val102 (-2.219)	--	π - π Stacking: His294 (2.126), Phe290 (2.549), Tyr116 (3.176) Cation- π Stacking: Tyr116 (3.176)

mol), Leu356 (-2.779 kcal/mol), Thr295 (-2.358 kcal/mol), Ala291 (-3.531 kcal/mol), Ala287 (-2.528 kcal/mol), Leu127 (-2.789 kcal/mol), Tyr116 (-3.788 kcal/mol), Phe110 (-2.735 kcal/mol) and Met106 (-3.434 kcal/mol) residues through 1-(4-nitrobenzyl)-1*H*-1,2,3-triazole core while the other half of the molecule i.e. 4-nitro-phenylprop-2-en-1-one was seen to be engaged significant van der Waals interactions with Met460 (-3.151 kcal/mol), Thr459 (-2.898 kcal/mol), His458 (-2.543 kcal/mol), Pro210 (-2.614 kcal/mol), Ile209 (-2.643 kcal/mol), Leu208 (-3.157 kcal/mol), Ile105 (-2.742 kcal/mol), Tyr103 (-2.39 kcal/mol) and Val102 (-2.825 kcal/mol) residues. The central phenyl ring linking these two halves of the molecule also showed very favourable van der Waals interactions with Met360 (-3.233), Met358 (-2.981), His294 (-2.277), Phe290 (-3.236) and Glu205 (-2.827) residues. The enhanced binding affinity observed for **9b** is also attributed to significant electrostatic interactions observed with Hem500 (-3.959 kcal/mol), Arg361 (-2.414 kcal/mol), Phe290 (-2.362 kcal/mol), Leu208 (-2.522 kcal/mol), Glu205 (-3.569 kcal/mol), Arg124 (-2.223 kcal/mol), Ile105 (-2.293 kcal/mol) and Val102 (-3.201 kcal/mol) residues of active site. Since CYP51 is a Heme enzyme, it is expected for a ligand to show significant steric and electrostatic interactions with Heme moiety and in the present study, it was consistently observed for all the chalcone analogues investigated. Furthermore, **9b** showed a very prominent hydrogen bonding interactions via the nitro (-NO₂) functional group with Tyr116 residue having a bonding distance of 2.65 Å. The compound was also seen to be engaged in very close pi stacking interactions, firstly a pi-pi stacking interaction was observed between the central phenyl ring and His294 (2.168 Å), Phe290 (2.374 Å) residues and secondly through the 4-nitro phenyl ring and Tyr116 (2.651 Å) residue. A Cation-Pi stacking type of interaction was also observed through the same nitro functional group and Tyr116 (2.651 Å) residue.

While the non-bonded interactions (steric and electrostatic) were observed to be the major driving force for mechanical interlocking of the chalcone analogues, the high binding affinity was strongly facilitated by these hydrogen bonding and Pi-stacking interactions which serve as an "anchor" to guide the orientation of inhibitor into the 3D space of enzyme's active site.

Overall, the binding affinity data derived from the docking study suggest that the 1,2,3-triazolyl chalcones possess a promising affinity for the fungal CYP51 enzyme qualifying them as a pertinent starting point for structure-based lead optimization to generate compounds with high selectivity and potency against CYP51.

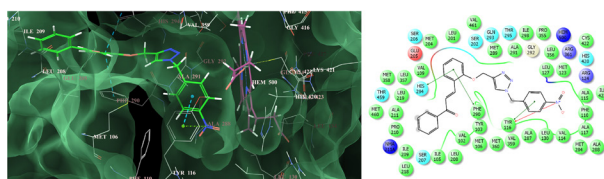


Figure 6: Binding mode of **9a** into the active site of sterol 14 α -demethylase (CYP51) (on the right side: the pink lines signify hydrogen bonding interaction, green lines signify π - π stacking while red line represent cation- π stacking interactions)

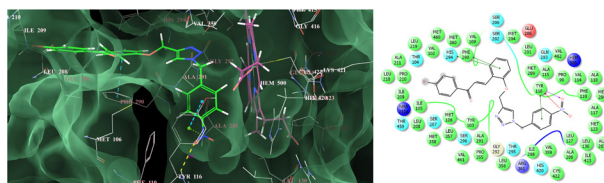


Figure 7: Binding mode of **9c** into the active site of sterol 14 α -demethylase (CYP51) (on the right side: the pink lines signify hydrogen bonding interaction, green lines signify π - π stacking while red line represent cation- π stacking interactions)

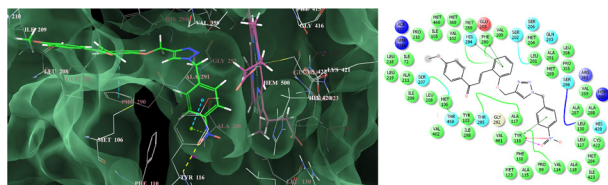


Figure 8: Binding mode of **9d** into the active site of sterol 14 α -demethylase (CYP51) (on the right side: the pink lines signify hydrogen bonding interaction, green lines signify π - π stacking while red line represent cation- π stacking interactions)

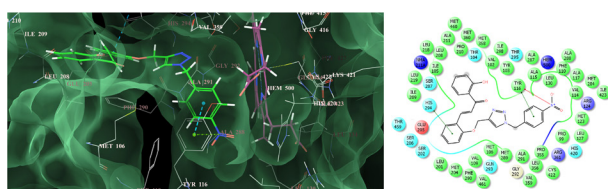


Figure 9: Binding mode of **9e** into the active site of sterol 14 α -demethylase (CYP51) (on the right side: the pink lines signify hydrogen bonding interaction, green lines signify π - π stacking while red line represent cation- π stacking interactions)

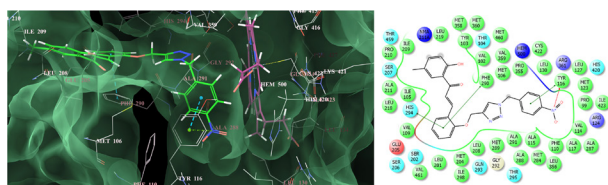


Figure 10: Binding mode of **9f** into the active site of sterol 14 α -demethylase (CYP51) (on the right side: the pink lines signify hydrogen bonding interaction, green lines signify π - π stacking while red line represent cation- π stacking interactions)

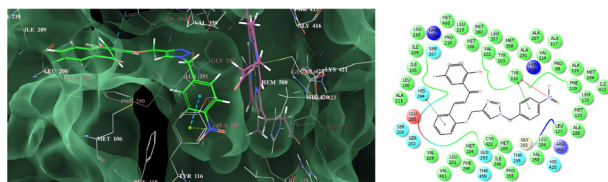


Figure 11: Binding mode of **9g** into the active site of sterol 14 α -demethylase (CYP51) (on the right side: the pink lines signify hydrogen

bonding interaction, green lines signify π - π stacking while red line represent cation- π stacking interactions)

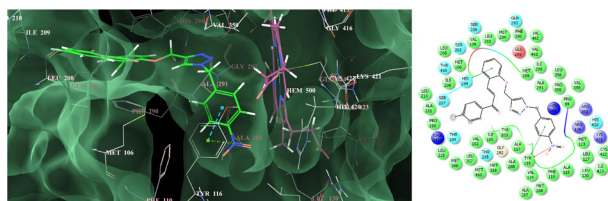


Figure 12: Binding mode of **9h** into the active site of sterol 14 α -demethylase (CYP51) (on the right side: the pink lines signify hydrogen bonding interaction, green lines signify π - π stacking while red line represent cation- π stacking interactions)

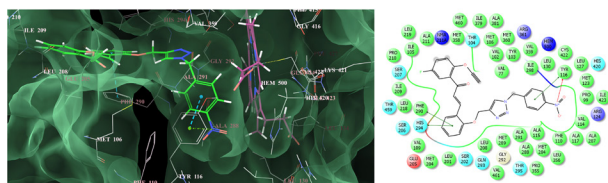


Figure 13: Binding mode of **9i** into the active site of sterol 14 α -demethylase (CYP51) (on the right side: the pink lines signify hydrogen bonding interaction, green lines signify π - π stacking while red line represent cation- π stacking interactions)

2.5 ADME Properties

Pharmacokinetic parameters of the synthesized 1,2,3-triazolyl-chalcones are summarised in **table 3**. It is observed that only compounds **9c** and **9i** violated Lipinski's rule of five ($\text{miLogP} \leq 5$), remaining all other compounds did not show more than one violation of Lipinski's rule of five. Also, compounds exhibited a good % ABS (% absorption) ranging from 57.70% to 73.51%. Hence, All the tested compounds except **9c** and **9i** followed the criteria for orally active drug and therefore, these newly synthesized 1,2,3-triazolyl-chalcones may have a good potential to develop as good oral active drug candidates.

Table 3. Pharmacokinetic parameters important for good oral bioavailability

Entry	%ABS	n-atoms	TPSA (Å ²)	n-ROTB	MV	MW	mllog p	n-ON	n-OHNH	Lipinski's violations	Drug-likeness model score
Rule	-	-	-	-	-	<500	≤5	<10	<5	≤1	-
9a	73.51	33	102.85	09	387.65	440.46	4.94	8	0	0	-0.50
9b	57.70	36	148.67	10	410.98	485.46	4.90	11	0	1	-0.44
9c	73.51	34	102.85	09	405.53	519.36	5.75	08	0	2	-0.35
9d	70.33	35	112.08	10	413.19	470.49	5.00	09	0	1	-0.35
9e	66.54	34	123.08	09	395.66	456.46	4.88	09	1	0	+0.25
9f	66.54	35	123.08	09	412.23	470.49	5.31	09	1	1	+0.00
9g	66.54	35	123.08	09	400.60	474.45	5.02	09	1	1	+0.16
9h	73.51	34	102.85	09	392.58	458.45	5.11	08	0	1	-0.19
9i	70.33	38	112.08	11	440.62	512.50	5.23	09	0	2	-0.03

ABS: absorption; TPSA: topological polar surface area; n-ROTB: number of rotatable bonds; MV: molecular volume; MW: molecular weight; lmiLog P: logarithm of partition coefficient; n-ON: number of hydrogen bond acceptors; n-OHNH: number of hydrogen bonds donors.

3 Conclusion

In conclusion, we have synthesized some new 1,2,3-triazolyl-chalcones and subsequently screened for their *in vitro* antifungal and antioxidant activity. Most of the synthesized chalcones were displayed potential antifungal activity against most of the fungal strain used in the experiment which indicates the broad-spectrum antifungal activity of the synthesized chalcones. Molecular docking studies further supported the strong inhibitory activity which was further enriched using per-residue interaction analysis that could provide a deeper insight into the specific residues and their associated type of thermodynamic interactions influencing the binding affinity for the crucial fungal enzyme CYP51. Also, synthesized 1,2,3-triazolyl-chalcones showed potential antioxidant properties. Furthermore, ADME

prediction shows better chances of these derivatives to evolve as a potential antifungal and antioxidant drug candidates. This inference derived from the *in vitro*/ *in silico* experiments is now fruitfully utilized to carry out site-specific modifications around the scaffold to improve the selectivity and potency of the 1,2,3-triazolyl-chalcones.

4 Experimental

4.1 Analytical methods

All chemicals and reagents used were of analytical grade. The progress of the reactions was monitored by thin-layer chromatography (TLC) on aluminium plates coated with silica gel 60 F₂₅₄, 0.25 mm thickness (Merck). The detection of the components was made by exposure to iodine vapours or UV light. Melting points were determined by an open capillary method and are uncorrected. ¹H-NMR spectra were recorded in DMSO-*d*₆ on a Bruker DRX-200 MHz spectrometer. ¹³C NMR spectra were recorded in DMSO-*d*₆ on a Bruker DRX-100 MHz instrument. IR spectra were recorded using a Bruker ALPHA ECO-ATR FTIR spectrometer. Mass spectra were recorded on

LC/ESI-MS spectrometer.

4.2 Chemistry

General procedure for the preparation of 1,2,3-triazolyl-chalcones:

A mixture of 1,2,3-triazole incorporated benzaldehyde **7** (1 mmol) and various substituted acetophenone (**8a-i**) (1 mmol) in ethanolic NaOH was taken in a 50 mL round bottom flask, equipped with a mechanical stirrer. The reaction mixture was stirred at room temperature for 4-5hrs. The progress of the reaction was monitored by TLC. The reaction mixture was poured into 50 mL ice-water and the obtained solid was filtered, dried and recrystallized from ethanol-DMF to obtain a dark brown solid product (**Scheme 2, 9a-i**).

Spectral data of synthesized compounds:

4.2.1 (E)-3-(2-((1-(4-Nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenylprop-2-en-1-one (9a):

Yellow colored solid, Yield: 86%, mp. 178-180 °C; IR (cm⁻¹): 1655 (C=O), 1588 (C=C), 1506 (C=C Ar), 1341 (N-O), 999 (N-N); ¹H NMR (200 MHz, DMSO-*d*₆ ppm): 8.83 (s, 1H), 8.66 (d, *J* = 8.0 Hz, 2H), 8.30 (d, *J* = 8.0 Hz, 5H), 7.96 (d, *J* = 8.0 Hz, 4H), 7.66 (d, *J* = 8.0 Hz, 4H), 6.24 (s, 2H), 5.72 (s, 2H); ¹³C NMR (100 MHz, DMSO-*d*₆ ppm): 191.0, 158.3, 148.3, 144.9, 143.7, 140.9, 138.3, 133.0, 130.4, 128.8, 128.5, 124.5, 125.0, 122.3, 122.2, 121.9, 115.7, 58.0 and 50.2; ESI-MS: Calcd for C₂₅H₂₀N₄NaO₄ [M+Na]⁺, 463.4, found: 463.0

4.2.2 (E)-3-(2-((1-(4-Nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-(4-nitrophenyl)prop-2-en-1-one (9b):

Yellow colored solid, Yield: 85%, mp. 182-184 °C; IR (cm⁻¹): 1592 (C=O), 1511 (C=C), 1392 (C=C Ar), 1339 (N-O), 1009 (N-N); ¹H NMR (200 MHz, DMSO-*d*₆ ppm): 8.88 (s, 1H), 8.72

(d, *J* = 8.0 Hz, 3H), 8.35 (d, *J* = 8.0 Hz, 4H), 8.01 (d, *J* = 8.0 Hz, 3H), 7.70 (d, *J* = 8.0 Hz, 4H), 6.28 (s, 2H), 5.76 (s, 2H); ¹³C NMR (100 MHz, DMSO-*d*₆ ppm): 191.6, 158.9, 151.3, 148.9, 145.5, 144.3, 143.8, 141.5, 129.7, 129.4, 129.2, 125.1, 124.6, 122.8, 116.3, 58.6 and 50.8; LC-MS: Calcd for C₂₅H₂₀N₅O₆ [M+H]⁺, 486.5, found: 486.2

4.2.3 (E)-1-(4-Bromophenyl)-3-(2-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)prop-2-en-1-one (9c):

Yellow colored solid, Yield: 85%, mp. 190-193 °C; IR (cm⁻¹): 1655 (C=O), 1587 (C=C), 1407 (C=C Ar), 1343 (N-O), 998 (N-N); ¹H NMR (200 MHz, DMSO-*d*₆ ppm): 8.79 (s, 1H), 8.43 - 8.35 (m, 4H), 7.93-7.73 (m, 9H), 7.48 (t, *J* = 8.0 Hz, 1H) 6.08 (s, 2H), 5.72 (s, 2H); ¹³C NMR (100 MHz, DMSO-*d*₆ ppm): 188.0, 155.3, 137.9, 133.6, 129.1, 127.4, 126.7, 125.8, 125.7, 125.6, 125.4, 121.5, 119.4, 119.2, 118.9, 112.7, 55.0 and 47.2; ESI-MS: Calcd for C₂₅H₁₉BrN₄NaO₄ [M+Na]⁺, 542.3, found: 542.0

4.2.4 (E)-1-(4-Methoxyphenyl)-3-(2-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)prop-2-en-1-one (9d):

Yellow colored solid, Yield: 87%, mp. 175-178 °C; IR (cm⁻¹): 1655 (C=O), 1588 (C=C), 1477 (C=C Ar), 1340 (N-O), 1014 (N-N); ¹³C NMR (100 MHz, DMSO-*d*₆ ppm): 187.8, 160.5, 155.1, 145.1, 141.7, 140.5, 137.7, 127.6, 125.6, 125.4, 121.3, 119.2, 119.0, 112.5, 110.7, 54.8, 52.8 and 47.0; LC-MS: Calcd for C₂₆H₂₃N₄O₅ [M+H]⁺, 471.5, found: 471.2

4.2.5 (E)-1-(2-Hydroxyphenyl)-3-(2-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)prop-2-en-1-one (9e):

Yellow colored solid, Yield: 86%, mp. 198-200 °C; IR (cm⁻¹): 1766 (C=O), 1594 (C=C), 1402 (C=C Ar), 1343 (N-O), 1008 (N-N); ¹H NMR (200 MHz, DMSO-*d*₆ ppm): 8.83 (s, 1H), 8.67 (d, *J* = 8.0 Hz, 2H), 8.30 (d, *J* = 8.0 Hz, 2H), 7.96 (d, *J* = 8.0 Hz, 4H), 7.65 (d, *J* = 8.0 Hz, 6H),

6.23 (s, 2H), 5.71 (s, 2H); ^{13}C NMR (100 MHz, DMSO- d_6 ppm): 190.0, 158.7, 155.1, 145.1, 141.7, 140.5, 136.7, 132.3, 127.2, 126.8, 125.6, 125.5, 125.3, 121.3, 119.8, 119.1, 119.0, 116.6, 114.7, 112.5, 54.7 and 47.0; LC-MS: Calcd for $\text{C}_{25}\text{H}_{21}\text{N}_4\text{O}_5$ $[\text{M}+\text{H}]^+$, 457.5, found: 457.2

4.2.6 (E)-1-(2-Hydroxy-5-methylphenyl)-3-(2-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)prop-2-en-1-one (9f):

Yellow coloured solid, Yield: 88%, mp. 188-192 °C; IR (cm^{-1}): 1660 (C=O), 1515 (C=C), 1433 (C=C Ar), 1344 (N-O), 1044 (N-N); ^1H NMR (200 MHz, DMSO- d_6 ppm): 8.41 (s, 1 H), 8.25 (d, J = 8.0 Hz, 3H), 7.88 (d, J = 8.0 Hz, 4H), 7.54 (d, J = 8.0 Hz, 3H), 7.24 (d, J = 8.0 Hz, 3H), 5.81 (s, 2H), 5.29 (s, 2H), 1.90 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6 ppm): 186.4, 153.0, 152.2, 142.2, 138.8, 137.6, 133.8, 128.9, 124.9, 124.3, 123.3, 122.7, 122.6, 122.5, 118.4, 117.4, 116.9, 116.3, 116.1, 112.5, 109.6, 51.9, 44.1 and 15.1; LC-MS: Calcd for $\text{C}_{26}\text{H}_{23}\text{N}_4\text{O}_5$ $[\text{M}+\text{H}]^+$, 471.5, found: 471.2

4.2.7 (E)-1-(5-Fluoro-2-hydroxyphenyl)-3-(2-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)prop-2-en-1-one (9g):

Yellow coloured solid, Yield: 87%, mp. 177-180 °C; IR (cm^{-1}): 1659 (C=O), 1593 (C=C), 1440 (C=C Ar), 1337 (N-O), 1026 (N-N); ^1H NMR (400 MHz, DMSO- d_6 ppm): 8.60 (d, J = 8.0 Hz, 3H), 8.34-8.21 (m, 4H), 7.90 (d, J = 8.0 Hz, 4H), 7.51 (d, J = 8.0 Hz, 3H), 6.15 (s, 2H), 5.68 (s, 2H); ^{13}C NMR (100 MHz, DMSO- d_6 ppm): 192.5, 158.3, 157.8, 155.5, 148.3, 144.9, 143.7, 139.9, 130.4, 128.8, 128.5, 124.5, 123.0, 122.3, 121.8, 120.7, 119.7, 118.3, 115.7, 58.0 and 50.2; ESI-MS: Calcd for $\text{C}_{25}\text{H}_{20}\text{FN}_4\text{O}_5$ $[\text{M}+\text{H}]^+$, 475.4, found: 476.0

4.2.8 (E)-1-(4-Fluorophenyl)-3-(2-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)prop-2-en-1-one (9h):

Yellow colored solid, Yield: 90%, mp. 197-200 °C; IR (cm^{-1}): 1655 (C=O), 1597 (C=C), 1445

(C=C Ar), 1333 (N-O), 1008 (N-N); ^{13}C NMR (100 MHz, DMSO- d_6 ppm): 191.0, 165.9, 163.8, 158.3, 148.3, 144.9, 143.7, 140.9, 134.9, 131.0, 130.4, 128.8, 128.5, 124.5, 122.3, 121.9, 116.0, 115.7, 58.0 and 50.2; LC-MS: Calcd for $\text{C}_{25}\text{H}_{19}\text{FN}_4\text{O}_4$ $[\text{M}]^+$, 458.4, found: 458.1

4.2.9 (E)-1-(5-Fluoro-2-(prop-2-yn-1-yloxy)phenyl)-3-(2-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)prop-2-en-1-one (9i):

Yellow colored solid, Yield: 86%, mp. 200-204 °C; IR (cm^{-1}): 1674 (C=O), 1564 (C=C), 1412 (C=C Ar), 1341 (N-O), 1008 (N-N); ^{13}C NMR (100 MHz, DMSO- d_6 ppm): 190.6, 160.9, 158.8, 158.3, 154.0, 148.3, 144.9, 143.7, 139.9, 130.4, 128.8, 128.5, 126.8, 124.5, 123.0, 122.3, 120.1, 119.6, 118.6, 115.7, 80.0, 78.8, 59.7, 58.0 and 50.2; ESI-MS: Calcd for $\text{C}_{28}\text{H}_{21}\text{FN}_4\text{NaO}_5$ $[\text{M}+\text{Na}]^+$, 535.5, found: 535.0

4.3 Antifungal activity assay

Antifungal activity was determined by standard agar dilution method as per CLSI (formerly, NCCLS) guidelines^[38]. The synthesized compounds and standard Miconazole were dissolved in DMSO solvent. The medium yeast nitrogen base was dissolved in phosphate buffer pH 7 and it was autoclaved at 110°C for 10 min. With each set a growth control without the antifungal agent and solvent control DMSO were included. The fungal strains were freshly sub cultured onto Sabouraud dextrose agar (SDA) and incubated at 25°C for 72 h. The fungal cells were suspended in sterile distilled water and diluted to get 10⁵ cells/mL. Ten microliters of standardized suspension was inoculated onto the control plates and the media incorporated with the antifungal agents. The inoculated plates were incubated at 25°C for 48 h. The readings were taken at the end of 48 and 72 h. The MIC was the lowest concentration of drug preventing growth of macroscopically visible colonies on drug containing plates when there was visible

growth on the drug free control plates.

4.4 Antioxidant activity assay

Antioxidant activity of the synthesized compounds has been assessed *in vitro* by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay [39]. Results were compared with standard antioxidant BHT (Butylated Hydroxy Toluene). The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple coloured methanol solution of 1, 1-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1mL of various concentrations of the test compounds (5, 10, 25, 50 and 100 mg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was measured against blank at 517 nm. The per cent inhibition (I %) of free radical production from DPPH was calculated by the following equation.

$$\% \text{ of scavenging} = [(A \text{ control} - A \text{ sample}) / A \text{ blank}] \times 100$$

Where 'A control' is the absorbance of the control reaction (containing all reagents except the test compound) and 'A sample' is the absorbance of the test compound. Tests were carried at in triplicate.

4.5 Molecular docking

To gain insight into the plausible mechanism of action by which the 1,2,3-triazolyl-chalcones studied herein could exhibit the antifungal activity and guide further SAR based on our experimental studies, molecular docking against sterol 14 α -demethylase (CYP51) was performed. Molecular docking has emerged as a reliable tool to identify the biological target for the bioactive molecules and gain an insight

into crucial ligand-protein thermodynamic interactions. Especially in the absence of available resources to perform enzyme-based assays, molecular docking is considered as complementary to the *in vivo* and *in vitro* biological study for understanding the ligand-receptor interactions at the atomic level. With this purpose, the 3D X-ray crystal structure of sterol 14 α -demethylase (CYP51) enzyme complexed with its inhibitor-Miconazole (pdb code: 3KHM) was retrieved from the Protein Data Bank (PDB) (<http://www.rcsb.org/pdb>). Sterol 14 α -demethylase (CYP51) is a crucial fungal enzyme that catalyses the conversion of lanosterol to ergosterol in the cell and thus its inhibition could lead to accumulation of 14 α -methyl sterols in the cell causing impaired cell growth. The GLIDE (Grid-based Ligand Docking with Energetics) program integrated into the Schrödinger Molecular modelling package (Schrodinger, LLC, New York, NY, 2018) [40-43] was used to perform the docking study. The preliminary crystal structure of CYP51 was refined using with the protein preparation wizard to remove the experimental errors in the structure. This involved eliminating the crystallographic water molecules since no conserved interactions with the enzyme are reported; adding the missing hydrogen/side-chain atoms corresponding to pH 7.0, assignment of appropriate charge and protonation state followed by energy minimization (applying the OPLS-2005 force field) of the obtained structure to relieve the steric clashes among the amino acid residues till RMSD for the heavy atoms reached 0.30Å. The 3D structures of the 1,2,3-triazole incorporated chalcone were sketched using the build panel and optimized using Ligand Preparation tool in Maestro which involves adjusting realistic bond lengths and angles, ascribing the partial charges using OPLS-2005 force-field followed by energy minimization until its average RMSD reached 0.001Å. The active site of CYP51 enzyme for docking was defined using Receptor Grid Generation panel

wherein a grid box of 10X10X10Å dimensions centred on centroid of the co-crystallized ligand was generated. With this automatic setup, the lowest energy conformation of each ligand was docked against defined active site of CYP51 using extra precision (XP) Glide scoring function to gauge the binding affinities of triazole analogues towards CYP51. For each compound, the most stable docking pose was selected according to best-scored conformation and analysed quantitatively for the most significant interactions with residues lining the active site using Maestro's Pose Viewer utility.

4.6 ADME Prediction

All the synthesized 1,2,3-triazolyl-chalcones were tested for Lipinski's rule of five. According to this rule, a molecule likely to be developed as an orally active drug should not exhibit more than one violation of following four criteria: molecular weight ≤ 500 , number of hydrogen bond donors ≤ 5 , miLogP (octanol-water partition coefficient) ≤ 5 , number of hydrogen bond acceptors ≤ 10 . ADME properties were predicted by using molinspiration online property calculation toolkit. A collective property of physicochemical properties, pharmacokinetics and pharmacodynamics of a compound is represented by a numerical value known as the drug-likeness model score was computed by using MolSoft software.

Conflict of interest

The authors declare that they have no conflict of interest.

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