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Research Paper

Synthesis, biological investigation and ADME prediction of some novel 2-[1*H*-imidazol-1-yl (phenyl) methyl]-2-phenyl-1, 3 dioxolan-4-yl substituted methanol as antifungal agents

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Novel series of 2-[1*H*-imidazol-1-yl (phenyl) methyl]-2-phenyl-1, 3 dioxolan-4-yl substituted methanol (**5a-f**) were synthesized. The chemical structures of the compound were confirmed by NMR, IR and mass spectral data. The compounds were screened for antifungal activity against pathogenic fungal strains. The synthesized compounds show significant antifungal activity against *Aspergillus niger*, *Aspergillus flavus*as as compared to *Candida albicans*. ADME properties of synthesize compounds were analyzed using Qikprop 3.5 tool of Schrödinger.

Keywords: 1,3-dioxolan, imidazole, antifungal agent, ADME prediction.

INTRODUCTION:

The microbial infection is one of the major problems as far as health is concerned. The incidence of microbial infections is increasing worldwide day by day, even though the active research devoted to the discovery and development of novel antimicrobials. Microbial infections are mainly of three types as micro-organisms are broadly classified as Bacteria, Fungi and virus[1,2]. Of the five fundamental Kingdoms of Life, the Kingdom Fungi is arguably the most diverse and prevalent. Unlike the kingdom Monera (containing bacteria), fungi are eukaryotic organisms whose cellular functions consequently resemble those of plants and animals more closely. Thus the issue of selectivity predominates in the quest for safe and effective chemotherapeutic remedies for diseases caused by fungi[3]. As with all chemotherapy, there is a risk-reward ratio to be taken into account; in the context of fungal infections, this ratio may vary greatly, from minor irritations such as athlete's foot to life-threatening systemic infections such as those caused by *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*[2,4].

The current interest in the development of new antifungal agents can be partially be explained by the dramatic rise in the number of AIDS cases[5,6]. The subsequent suppression of the immune system in patient, they contract a fungal infection during the course of the illness. Other conditions that have spurred the development of new systemic antifungal

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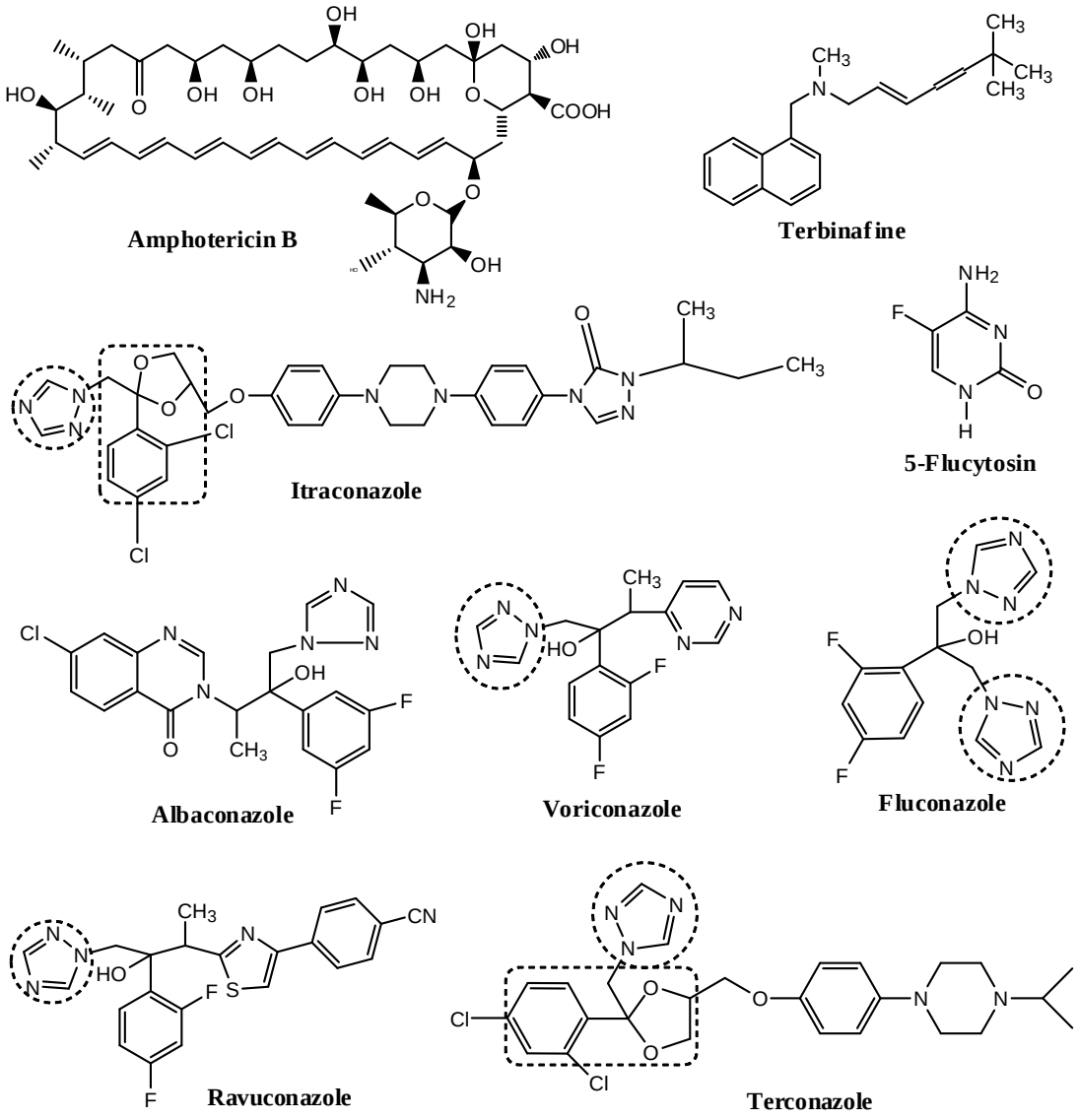


Fig 1: Antifungal agents

agents are increase the frequency of bone marrow, organ transplant and long term use of corticosteroid. The emergence of fungi resistant to currently available agents especially the azoles has made the need for new and effective antifungal agents more urgent[7,8]. Currently, drugs which are used to treat fungal infection having different pharmacophore are shown in **Fig. 1.**

From the literature survey it is observed that no efforts have been made for developments of a molecular scaffold containing these two important cores i.e. phenylacetophenone and imidazole ring. In view of this we have attempted the synthesis novel of series of 2-[1H-imidazol-1-yl (phenyl) methyl]-2-phenyl-1,3 dioxolan-4-yl substituted methanol (Fig. 2).

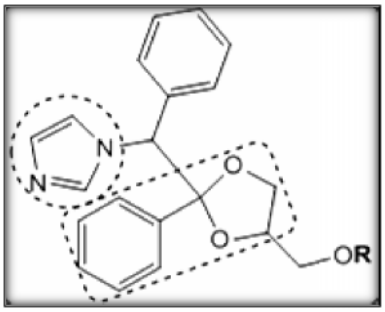


Fig. 2: Designed pharmacophore

2. MATERIALS AND METHODS

2.1. Chemistry

The compounds were synthesized by conventional (solvent phase) methods. Melting points were determined on scientific melting point apparatus in open capillaries and were uncorrected. TLC was performed on Silica Gel GF254 for TLC (Merck) and spots were visualized by iodine vapors or by irradiation with UV light (254 nm). Solvents were reagent grade and, when necessary, were purified and dried by standard methods. FT-IR spectra were recorded on JASCO FT-IR 4000 using KBr powder. ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER AVANCE II 400 spectrometer (400MHz) with TMS as internal standard and DMSO as a solvent. Mass spectra were recorded on Time of flight mass spectrometer.

2.2. Synthesis of 2-bromo-1,2-diphenylethanone (2)

Suspend 0.103 mol phenylacetophenone in glacial acetic acid in a 50ml flask, warm gently on a water bath, until a clear solution results, then cool as per as possible without the formation of crystal. To this solution add 0.184 mol of bromine; do not allow the temperature to raise above 45°C, during addition the brominated product separate from the solution when about three quarters of the bromine has been added[7,9]. After 2 hours, cool the flask in a bath of ice and salt, filter the product wash with little cold glacial acetic acid followed by small volume of water until all the acid has been removed and recrystallized from hot rectified spirit.

Yellow crystals, mp 124-125°C; percentage yield: 83%.

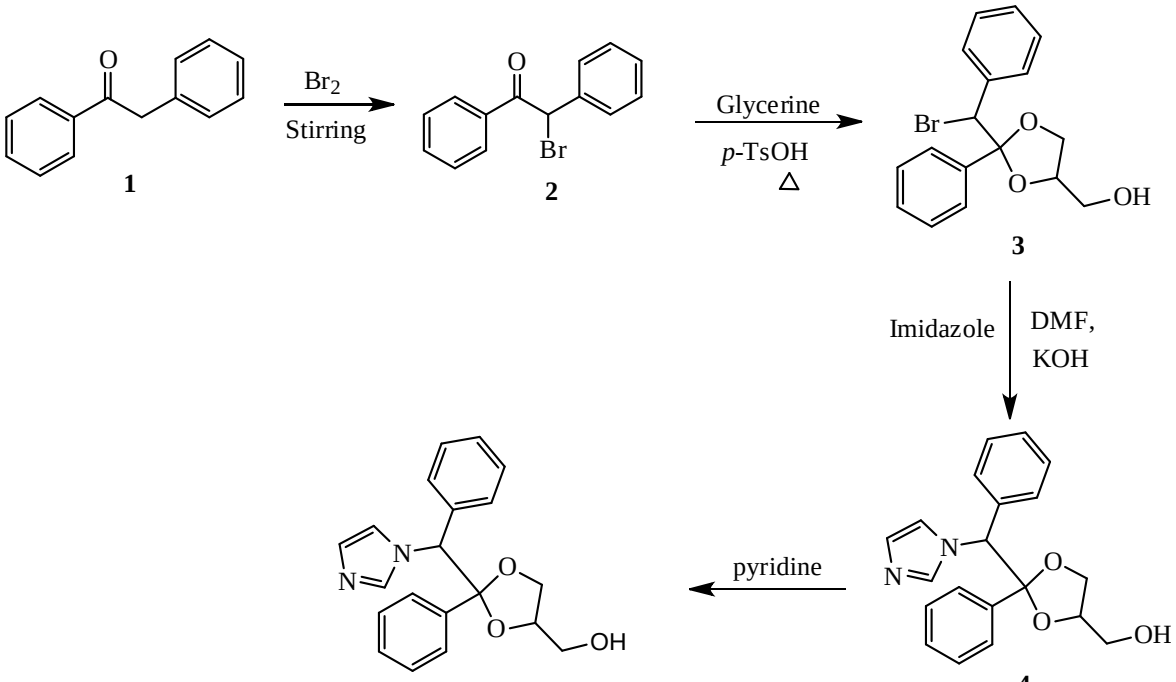
2.3. Synthesis of 2-[bromo (phenyl) methyl]-2-phenyl-1, 3-dioxolan-4 yl methanol (3)

Glycerine (10 mol) and 2-bromo-1, 2-diphenylethanone (10 mol) in benzene were refluxed in the presence of p-toluenesulphonic acid with azeotropic removal of water. The residue was dissolved in EtOAc, washed with water, filters the product and dried[7,10].

Colourless crystals, mp 210-212°C; percentage yield: 68%.

2.4. Synthesis of 2-[1*H*-imidazol-1-yl(phenyl)methyl]-2-phenyl-1,3dioxolan-4-yl methanol (4)

The 2-[bromo (phenyl) methyl]-2-phenyl-1, 3-dioxolan-4yl methanol (**3**) (0.01 mol), imidazole (0.01mole) and KOH was dissolved in DMF, reflux for 4 hours and then the reaction mixture was poured



1 **2** **3** **4**

5a-f
(2-((1*H*-imidazol-1-yl)(phenyl)methyl)-2-phenyl-1,3-dioxolan-4-yl)methanol

Fig. 3: **Synthesis of designed compounds (Scheme 1)**

the reaction mixture was poured into the cold water. The product obtained was filtered and recrystallized with rectified spirit[8-10].Colorless crystals, mp 250-252°C; percentage yield: 59%.

2.5. Synthesis of 2-[1*H*-imidazol-1-yl (phenyl) methyl]-2-phenyl-1,3dioxolan-4-yl substituted methanol (5a-h)

The 2-[1*H*-imidazol-1-yl(phenyl)methyl]-2-phenyl-1,3dioxolan4-yl methanol (**4**) (0.1 mol) was taken in pyridine in 100 ml of conical flask then add of substituted chloride. After the initial reaction has subsided warm mixture over a small flame for a minute and pour, with vigorous stirring into 8-7 ml of water. Allow the precipitate to settle, decant the supernatant liquid, filter and recrystallized with ethanol.

2.5.1(2-((1H-imidazol-1-yl)(phenyl)methyl) -2-phenyl-1,3-dioxolan-4-yl)methyl acetate (5a)

IR (KBr): 3059, 1730, 1670, 1210, 1012 cm^{-1};

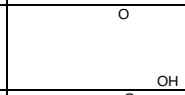
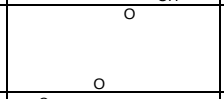
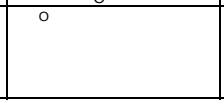
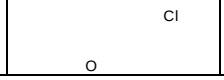
^{1}H NMR (400MHz, DMSO): δ= 7.3-7.9 (m, 12H), 6.1 (s, 1H), 4.5 (s, 1H), 3.7-3.5 (m, 5H), 2.8 (s, 3H); ^{13}C NMR (400MHz, DMSO): δ= 171.2, 145.3, 137.5, 136.9, 132.4, 128.2, 126.2, 125.9, 120.5, 76.6, 70.2, 63.8, 20.1;

MS *m/z* = 379.2 [M+1].

2.5.2. 1-((4-((benzyloxy) methyl)-2-phenyl-1, 3-dioxolan-2-yl) (phenyl) methyl)-1H-imidazole(5b)

IR (KBr): 3049, 1673, 1215, 1020 cm^{-1};

Table 1: Characterization data of synthesized derivatives (5a-5f)

Comp.	R'	Molecular Formula	Molecular Weight	% Yield	Melting Point[a]
5a		$C_{22}H_{22}O_4N_2$	378	66.28	290-291
5b		$C_{27}H_{26}O_3N_2$	426	49	293-294
5c		$C_{22}H_{22}O_5N_2$	394	44.14	296-297
5d		$C_{24}H_{26}O_5N_2$	422	51.85	288-287
5e		$C_{27}H_{24}O_4N_2$	440	49.19	284-286
5f		$C_{22}H_{21}O_4N_2Cl$	412	51	271-273

[a]Melting points were uncorrected

^{1}H NMR (400MHz, DMSO): δ= 7.2-7.9 (m, 17H), 6.1 (s, 1H), 4.7 (s, 2H), 4.5 (s, 1H), 3.8-3.5 (m, 5H); ^{13}C NMR (400MHz, DMSO): δ= 144.3, 137.4, 131.5, 129.6, 128.1, 125.4, 114.8, 79.7, 76.6, 71.2, 64.5;

MS *m/z* = 427.1 [M+1].

2.5.3. 2-((2-((1H-imidazol-1yl)(phenyl) methyl)-2-phenyl-1,3-dioxolan-4-yl) methoxy)acetic acid (5c)

IR (KBr): 3508, 3070, 1711, 1671, 1228, 1020 cm^{-1};

^{1}H NMR (400MHz, DMSO): δ= 11.0 (s, 1H), 7.3-8.0 (m, 13H), 6.0 (s, 1H), 4.8 (s, 2H), 3.7-3.5 (m, 5H); ^{13}C NMR (400MHz, DMSO): δ= 172.9, 144.6, 136.8, 134.1, 131.7, 128.9, 127.5, 125.6, 121.3, 76.2, 62.9,21.3;

MS *m/z* = 395.2 [M+1].

2.5.4. Ethyl 2-((2-((1H-imidazol-1-yl) (phenyl)methyl)-2-phenyl-1,3- dioxolan -4yl)methoxy)acetate (5d)

IR (KBr): 3090, 1722, 1661, 1220, 1011 cm^{-1};

^{1}H NMR (400MHz, DMSO): δ= 7.1-7.9 (m, 13H), 5.9 (s, 1H), 4.6-4.2 (m, 5H), 3.8-3.5 (m, 4H), 2.8 (s, 3H); ^{13}C NMR (400MHz, DMSO): δ= 169.2, 144.1, 137.8, 135.4, 130.5, 127.8, 125.9, 123.3, 77.3, 63.4, 20.7;

MS *m/z* = 423.3 [M+1].

2.5.5. 3-((2-((1H-imidazol-1-yl)(phenyl) methyl)-2-phenyl-1,3-dioxolan-4-yl) methoxy)-3-oxopropanoic acid (5e)

IR (KBr): 3509, 3033, 1711, 1665, 1221, 1016 cm^{-1};

^{1}H NMR (400MHz, DMSO): δ= 10.8 (s, 1H), 7.2-6.8 (m, 13H), 6.0 (s, 1H), 4.5-4.3 (m, 3H), 3.7-3.4 (m, 4H); ^{13}C NMR (400MHz, DMSO): δ= 171.2, 168.4, 144.4, 137.8, 134.2, 130.6, 128.9, 127.1, 125.4,

120.2, 76.5, 62.3;

MS *m/z* = 423.2 [M+1].

2.5.6. (2-((1H-imidazol-1-yl) (phenyl) methyl)-2-phenyl-1, 3-dioxolan-4-yl) methyl-2-chloroacetate (5f)

IR (KBr): 3029, 1720, 1661, 1220, 1012, 749 cm^{-1};

^{1}H NMR (400MHz, DMSO): δ= 7.4-8.1 (m, 13H), 6.2 (s, 1H), 4.8-4.5 (m, 5H), 3.7 (m, 2H); ^{13}C NMR (400MHz, DMSO): δ= 168.7, 144.5, 138.3, 134.1, 130.6, 127.7, 125.8, 121.3, 74.4, 56.8, 51.2;

MS *m/z* = 414.2 [M+2].

2.6. Biological activity

Determination of Minimal Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the test substances against *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* was determined by liquid broth method of two fold serial dilution technique. In this assay, the minimum concentration of each test substance required to inhibit the growth of microorganism was determined.

In this assay, a series of assay tubes were prepared which contains uniform volume (1ml) of sterile SD broth and same volume of known concentration of test substance was added. Starting from first tube up to sixth tube was serially diluted in twofold decreasing concentrations and seventh tube was positive control and left without test substance. The tubes with the test substance inoculated with 1 ml of inoculum ($1x10^{6}$ CFU per ml). The final concentration of test substance ranged from 1000 to 31.25 µg per ml. In the experiment, solvent control and sterility controls were maintained. The tubes were incubated at 28°C for 48 h. The standard antibiotic; Clotrimazole was tested as standard drug having concentrations ranges from 100 to 3.12 µg per ml. The turbidity of culture medium was observed visually which is indicative of the presence of a large number of cells. The turbidity of culture medium was directly proportional to the inhibition of organism. The MIC values then calculated by observing the turbidity of the test culture medium.

2.7. ADME prediction

ADME properties were calculated using Qikprop v3.5 tool of Schrödinger. It predicts both physicochemically significant descriptors and pharmacokinetic relevant properties. QikProp provides ranges for comparing a particular molecule's properties with those of 95% of known drugs. Qikprop evaluates the acceptability of analogs based on Lipinski's rule of five[11], which is essential to ensure drug-like pharmacokinetic profile while using rational drug design[12]. All the analogs were neutralized before being used by Qikprop.

Table 2: Minimum Inhibitory Concentration of test drug against selected fungal strains

Compounds	MIC in µg/ml		
	A. niger	***A. fiavus***	***C. albicans***
5a	31.25	31.25	500
5b	62.5	500	1000
5c	62.5	62.5	500
5d	31.25	62.5	500
5e	31.25	62.5	500
5f	62.5	62.5	500
Clotrimazole	25	12.5	25

3. RESULTS AND DISCUSSION

3.1. Chemistry

The target compounds 2-[1*H*-imidazol-1-yl (phenyl) methyl]-2-phenyl-1, 3 dioxolan-4-yl substituted methanol were synthesized from the commercially available phenylacetophenone (**Scheme 1**). The phenyl acetophenone was brominated to gives 2-bromo-1, 2-diphenylethanone **2**, which on treatment with p-toluene sulphonic acid and glycerine gives dioxolone derivatives **3**. Bromine of compound **3** was replaced with imidazole by using KOH and DMF as solvent. Final derivatives were synthesized by using different substitutes in pyridine as solvent (**5a-f**). The proposed derivatives were synthesized and their structures have been verified by IR, ^{1}H-NMR, ^{13}C-NMR, and LC-MS spectroscopy.

3.2. Biological activity

The biological screening was done by determining the Minimum Inhibitory Concentration (MIC) of the test substances against *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* was determined by liquid broth method of two fold serial dilution technique. The synthesized compounds show significant antifungal activity against *Aspergillus niger* & *Aspergillus flavus* as compared to *Candida albicans*.

Compound **5a** was shown better inhibitory activity against A. niger and A. flavus where as compounds **5d** and **5e** shows better inhibitory activity against A. niger as compared to A. flavus. Phenyl dioxalan core found to be important to inhibit fungal growth.

3.3. ADME prediction

Approximately 40%-60% of developing drugs failed during the clinical trials because of ADME/Tox deficiencies. Virtual screening should not be restricted to optimize binding affinity and improve selectivity; and the pharmacokinetic

properties should also be included as important filters in virtual screening. Total forty four descriptors and pharmaceutically relevant properties of substituted Imidazole analogs were analyzed using Qikprop (QikProp, version 3.5, Schrödinger, LLC, New York, NY, 2012). Significant descriptors required for predicting the drug-like properties of molecules are reported here. These properties are as follows,

1. Molecular weight (mol_MW) (150–650)
2. Octanol/water partition coefficient (Log Po/w) (-2–6.5)
3. Aqueous solubility (QPlogS) (-6.5–0.5) 4. Apparent MDCK cell permeability (QPPMDCK) (<25 poor,>500 great)
5. Brain/blood partition coefficient (QPlogBB) (-3.0–1.2)
6. Percent human oral absorption (C80% is high, B25% is poor).
7. Prediction of binding to human serum albumin. (QPlogKhsa) (-1.5 – 1.5)
8. Number of likely metabolic reactions. (#metab‡) (1 - 8)

All the structures showed significant values for the properties analyzed (**Table 3**) and showed drug-like characteristics based on Lipinski's rule of 5. The ADME values of design compound inhibitors are given in **Table 3**.

The first three properties are based on Lipinski rule of five, molecular weight (mol_MW) less than 650, partition coefficient between octanol and water (logPo/w) between -2 and 6.5 and solubility (QPlogS) greater than -5.

Brain/blood partition coefficient (QPlogBB) parameter indicated about the ability of the drug to pass through the blood–brain barrier. Whereas QPPMDCK predicted apparent MDCK cell permeability in nm/s. MDCK cells are considered to be a good mimic for the blood–brain barrier.

Higher the value of MDCK cell, higher the cell permeability. #metab parameter indicated about the metabolic reaction of compound like aromatic OH oxidation, amine dealkylation, ether dealkylation, etc. All designed compounds had showed ADME properties in acceptable range.

Table 3: Prediction of ADMET properties

Comd No.	Mol_MW	Log Po/w	Log S	Log BB	PMDCK	Log Khsa	#metab	% Oral Absorption
5a	378.427	4.18	-4.39	-0.420	1001.57	0.327	2	100.0
5b	426.514	6.26	-6.73	-0.209	2713.16	0.942	4	100.0
5c	394.426	3.94	-4.64	-1.256	57.06	0.068	4	86.4
5d	422.480	4.66	-5.83	-0.951	656.30	0.371	4	100.0
5e	422.437	4.16	-4.16	-1.306	41.21	0.054	3	85.3
5f	412.872	4.68	-5.37	-0.430	1356.08	0.507	3	100.0

4. CONCLUSION

The new series of 2-[1*H*-imidazol-1-yl (phenyl) methyl]-2-phenyl-1,3 dioxolan-4-yl substituted methanol derivatives were synthesized and their antifungal activities were examined. The biological assay results indicated that compounds showed moderate antifungal activity against *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*. ADME properties can be taken as best hit molecules and can be considered for further studies like QSAR, synthetic studies, and biological studies. Thus this study will provide basis for design of new analogs more effective against clinically relevant antifungal agents.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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