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Research Article

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Synthesis and biological investigation of novel 1,3-di(substituted azol)-2-(substituted hetrocycles (substituted aryl)methylene)propane-1,3-dione derivatives as antifungal agents

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ABSTRACT

A novel series of1,3-di(substituted azol)-2-(substituted hetrocycles (substituted aryl)methylene)propane-1,3-dione derivatives were synthesized and tested for their in vitro antifungal activity. These compounds showed moderate activity against strains of Aspergillus species and Candida albicans by Agar diffusion method.

Keywords: Imidazole, triazole, Antifungal

INTRODUCTION

During past two decades fungal infections have emerged as a major cause of disease and mortality in immune compromised patients, cancer or AIDS patients or in organ transplant cases [1,2]. Azole antifungals (e.g. fluconazole, ketoconazole) which act by inhibiting P450 14α-demethylase, a key enzyme in the fungal ergosterol biosynthesis, now become most rapidly expanding group of antifungal compounds. However, their use is limited by high risk of toxicity, development of immediate resistance. In spite of significant research on antifungal agents, the azoles remain the mainstay of therapy for systemic life threatening fungal infections as they have fungistatic, orally active, and broad spectrum activity against most of fungi.

The effects of azoles on fungal biochemistry have been studied extensively, but there is stii much to be learned [3]. At high in vitro concentrations (micromolar), the azoles are fungicidal; at low in vitro concentrations (nanomolar) they are fungistatic. The fungicidal effect is clearly associated with damage to fungal cell membrane, with the loss of essential cellular components such as potassium ions and amino acids. The fungistatic effect of azoles at low concentration has been associated with inhibition of membrane bound enzymes. A cytochrome P450 – class enzyme, lanosterol 14α-demethylase, is likely target for azoles [4]. P450 possesses a heme moiety as a part of its structure, and the basic electron pair of azole rings can occupy a binding site on P450, preventing the enzyme from turning over. The function of lanosterol 14α-demethylase is to oxidatively remove methyl group from lanosterol during ergosterol biosynthesis.

When demethylation is inhibited, the 14α-sterol accumulates in the membrane, causing destabilization. As this happens, repair mechanisms, such as chitin synthesis, are initiated to patch the damage. This degrades membrane function further. Lanosterol 14α-demethylase is also required for mammalian biosynthesis of cholesterol, and the

azoles are known to inhibit cholesterol biosynthesis [5]. In general, higher concentrations of the azoles are needed to inhibit mammalian enzyme. This provides selectivity for antifungal action.

Prompted by observations and in continuation of our search for bioactive molecules, we designed the synthesis of novel 1,3-di(substituted azol)-2-(substituted hetrocycles (substituted aryl)methylene)propane-1,3-dione derivatives (Fig-1) which showed high antifungal activity.

EXPERIMENTAL SECTION

Physical measurement

Melting points were determined on scientific melting point apparatus in open capillaries and were uncorrected. ^{1}H 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. FT-IR spectra were recorded on JASCO FT-IR 4000 using KBr powder.

Synthesis of Diethyl 2-substituted benzylidene malonate [6] (1a-1b)

To a solution of diethyl malonate (15 g, 93 mmol) in 40 ml of ethanol, were added the respective aldehyde (100 mmol), 1.5 ml of piperidine and 1 ml of glacial acetic acid. Then the mixture was stirred at refluxing temperature for 12 hrs, or until thin-layer chromatography indicated the complete consume of the starting material. After removing solvent, the crude product was washed with a saturated solution of sodium bisulfite (20 ml). The product (Table-1) was extracted by diethyl ether (2 x 20 ml), dried with sodium sulphate and evaporated to give the respective pure oil.

Synthesis of Diethyl 2-(substituted hetrocycles (substituted aryl) methylene) malonate (2a-2e)

To a solution of the Diethyl 2-substituted benzylidene malonate (8.1 mmol) in water (25 ml) was added the respective secondary amine (6 mmol) at the presence or absence of acetic acid (0.1 ml) and the mixture was stirred at room temperature until the complete consume of the starting materials. After removing solvent, the crude products were dissolved in diethyl ether (2 × 40 ml) and washed with water until the pH became neutral. The organic solvent was dried with sodium sulphate and then evaporated to give the respective pure compound (Table-2).

Synthesis of 2-(substituted hetrocycles (substituted aryl) methylene) malonic acid [7] (3a-3e)

Dissolve sufficient quantity of KOH (2.78 mole) in same amount of water in 500 ml RBF and add 10 ml of ethanol to produce homogenous solution. Introduce 1 mole of Diethyl 2-(substituted hetrocycles (substituted aryl) methylene) malonate slowly with shaking. Attach reflux condenser and reflux the mixture for 3 hours. Hydrolysis is then complete i.e. a test portion dissolves in excess of water. Distilled off as much ethanol as possible on water bath and dissolved residue in comparatively small volume of water. Cool the solutions in large beaker surrounded by ice and add dil. H_2SO_4 slowly. Stirred vigorously with mechanical stirrer, until solution is acidic to congo red paper. Extract solution with sufficient quantity diethyl ether, dried ethereal extract with Sodium sulp1hate and distilled off ether on water bath (Table-3).

Synthesis of 2-(substituted hetrocycles (substituted aryl) methylene) malonyl dichloride (4a-4e)

In necked flask add 2-(substituted hetrocycle(substituted aryl)methylene)malonic acid and thionyl chloride (1:2). Heat the solution in fuming chamber. Monitor the completion of reaction on TLC. Then above mixture is distilled for removal of thionyl chloride and the separated product was used immediately for final step (Table-4).

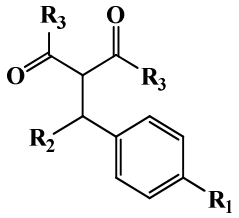
Synthesis of 1, 3-di(substituted azol)-2-(substituted hetrocycles(substituted aryl)methylene)propane-1,3-dione (5a-5j)

Dissolve required quantity of imidazole or triazole in sufficient quantity of N, N-dimethyl formamide and stirred the mixture until all imidazole or triazole dissolved. Then, slowly add 2-(substituted hetrocycle (substituted aryl) methylene) malonyl dichloride product from burette drop by drop. Stirred the mixture until completion of reaction. Add the mixture in sufficient quantity of water and filter off the product (Table-5).

2-((4-chlorophenyl)(morpholino)methyl)-1,3-di(1H-imidazol-1-yl)propane-1,3-dione

IR (KBr): 2972, 1783, 1704, 1634, 761 cm^{-1}; ^{1}H NMR (400MHz, DMSO): δ = 8.1-7.2 (m, 10H), 4.3 (d, 1H), 3.8 (d, 1H), 3.4-2.6 (m, 8H); MS (TOF, 1.99 e4): *m/z* = 414.20[M+H]$^+$; $C_{20}H_{20}ClN_5O_3$ (413.86);

Figure 1: General structure of 1, 3-di(substituted azol)-2-(substituted hetrocycles(substituted aryl)methylene)propane-1,3-dione



SCHEME OF SYNTHESIS

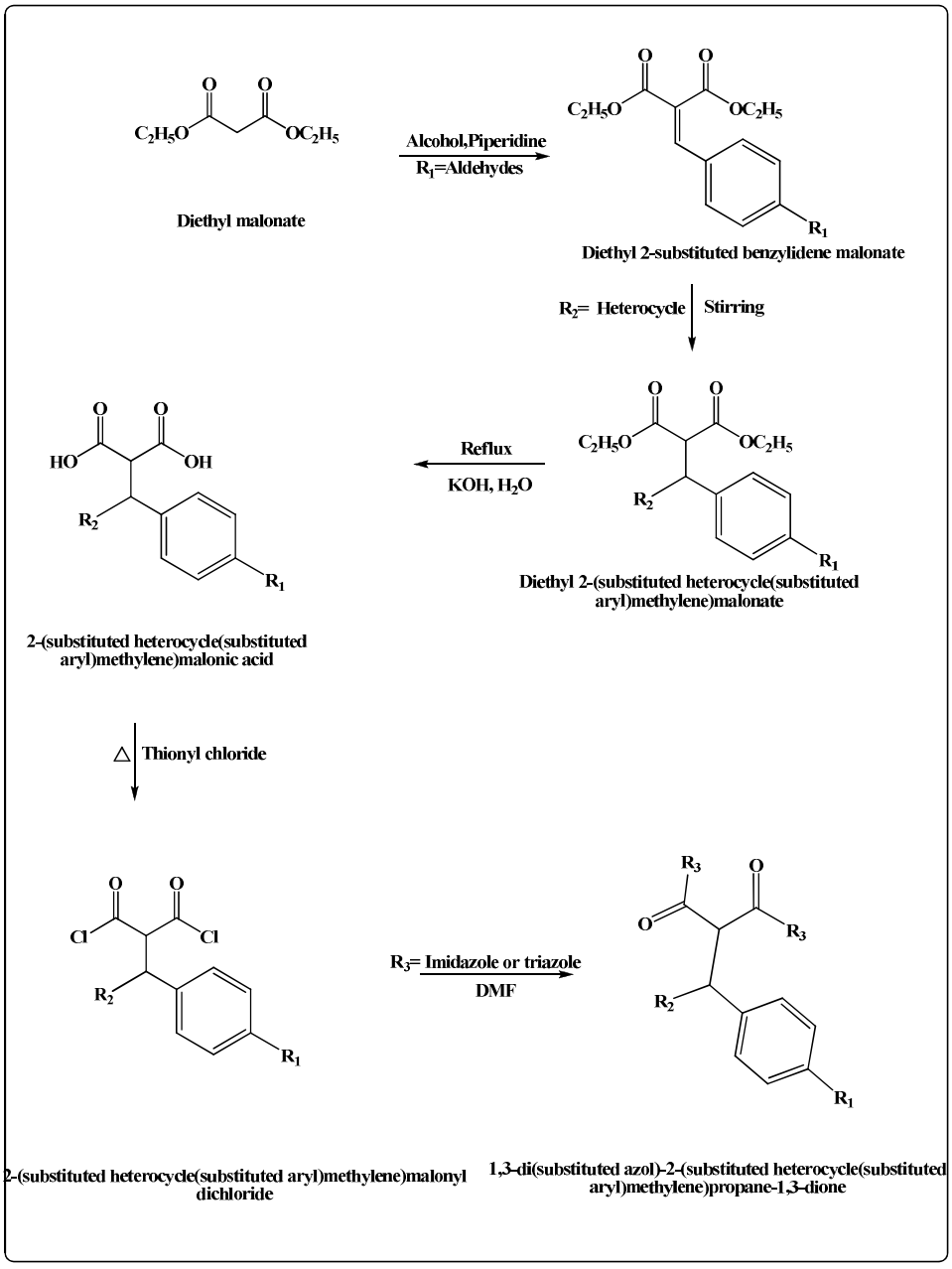


Table -1: Experimental data of synthesized compounds 1a- 1b

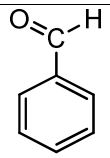
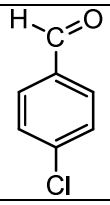
Sr. No.	R_1	Molecular formula	Molecular Weight	% Yield	Boiling Point* (^{0}C)	Rf Value**
1.		$C_{14}H_{16}O_4$	248.27	84 % w/v	222-224	0.79
2.		$C_{14}H_{15}ClO_4$	282.72	71.3%w/v	188-190	0.72

Table -2: Experimental data of synthesized compounds 2a-2e

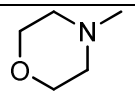
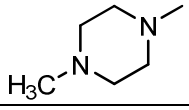
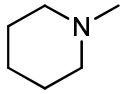
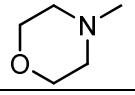
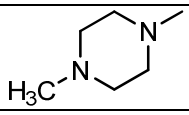
Comp	R_1	R_2	Molecular Formula	Molecular Weight	% Yield	Melting Point* (^{0}C)	Rf Value**
1	Cl.		$C_{18}H_{24}ClNO_5$	369.84	72.57	56	0.49
2	Cl.		$C_{19}H_{27}ClN_2O_4$	382.88	78.76	48	0.44
3	Cl.		$C_{19}H_{26}ClNO_4$	367.87	75.98	72	0.48
4	H.		$C_{18}H_{25}NO_5$	335.39	75.10	50	0.42
5	H.		$C_{19}H_{28}N_2O_4$	348.44	72.00	60	0.39

Table -3: Experimental data of synthesized compounds 3a-3e

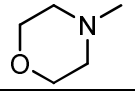
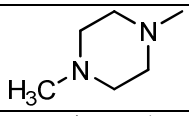
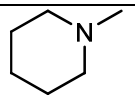
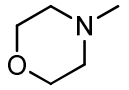
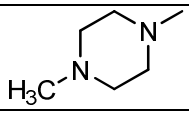
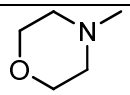
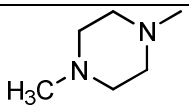
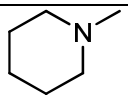
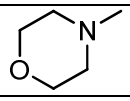
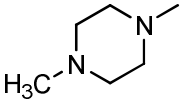
Comp	R_1	R_2	Molecular Formula	Molecular Weight	% Yield	Melting Point* (^{0}C)	Rf Value**
1	Cl.		$C_{14}H_{16}ClNO_5$	313.73	70.00	240	0.40
2	Cl.		$C_{15}H_{19}ClN_2O_4$	326.78	76.76	200	0.33
3	Cl.		$C_{15}H_{18}ClNO_4$	311.76	72.00	184	0.42
4	H.		$C_{14}H_{17}NO_5$	279.29	73.56	80	0.32
5	H.		$C_{15}H_{20}N_2O_4$	292.33	67.00	68	0.34

Table -4 Experimental data of synthesized compounds 4a-4e

Comp	R_1	R_2	Molecular Formula	Molecular Weight	% Yield	Boiling Point* (^{0}C)	Rf Value**
1	Cl.		$C_{14}H_{14}Cl_3NO_3$	350.62	65.00	180	0.48
2	Cl.		$C_{15}H_{17}Cl_3N_2O_2$	363.67	74.87	222	0.39
3	Cl.		$C_{15}H_{16}Cl_3NO_2$	348.65	71.14	340	0.49
4	H.		$C_{14}H_{15}Cl_2NO_3$	316.18	69.62	320	0.39
5	H.		$C_{15}H_{18}Cl_2N_2O_2$	329.22	62.79	310	0.37

2-((4-chlorophenyl)(morpholino)methyl)-1,3-di(1H-1,2,4-triazol-1-yl)propane-1,3-dione
IR (KBr): 3131, 1785, 1702, 1562, 784 cm^{-1}; ^{1}H NMR (400MHz, DMSO): δ = 8.6-8.2 (m, 4H), 7.6-7.4 (m, 4H), 4.4 (d, 1H), 3.9 (d, 1H), 3.4-2.6 (m, 8H); MS (TOF, 1.99 e4): *m/z* = 416.20[M+H]$^+$; $C_{18}H_{18}ClN_7O_3$ (415.83);

2-((4-chlorophenyl)(4-methylpiperazin-1-yl)-1,3-di(1H-imidazol-1-yl)propane-1,3-dione
IR (KBr): 2987, 1700, 1489, 1425, 761 cm^{-1}; ^{1}H NMR (400MHz, DMSO): δ = 8.1-7.2 (m, 10H), 4.3 (d, 1H), 3.8 (d, 1H), 3.5 (s, 3H), 3.4-2.6 (m, 8H); MS (TOF, 1.99 e4): *m/z* = 427.3[M+H]$^+$; $C_{21}H_{23}ClN_6O_2$ (426.90);

2-((4-chlorophenyl)(4-methylpiperazin-1-yl)-1,3-di(1H-1,2,4,-triazol-1-yl)propane-1,3-dione
IR (KBr): 2986, 1720, 1689, 1591, 820 cm^{-1}; ^{1}H NMR (400MHz, DMSO): δ = 8.6-8.2 (m, 4H), 7.6-7.4 (m, 4H), 4.4 (d, 1H), 3.9 (d, 1H), 3.5 (s, 3H), 3.4-2.6 (m, 8H); MS (TOF, 1.99 e4): *m/z* = 429.9 [M+H]$^+$; $C_{19}H_{21}ClN_8O_2$ (428.88);

2-((4-chlorophenyl)(piperidin-1-yl)methyl)-1,3-di(1H-imidazol-1-yl)propane-1,3-dione
IR (KBr):3096, 1728, 1696, 1534,821 cm^{-1}; ^{1}H NMR (400MHz, DMSO): δ = 8.1-7.2 (m, 10H), 4.3 (d, 1H), 3.8 (d, 1H), 3.4-2.6 (m, 10H); MS (TOF, 1.99 e4): *m/z* = 412.1 [M+H]$^+$; $C_{21}H_{22}ClN_5O_2$ (411.88);

2-((4-chlorophenyl)(piperidin-1-yl)methyl)-1,3-di(1H-1,2,4-triazol-1-yl) propane-1,3-dione
IR (KBr): 3131, 1722, 1702, 1550, 824 cm^{-1}; ^{1}H NMR (400MHz, DMSO): δ = 8.6-8.2 (m, 4H), 7.6-7.4 (m, 4H), 4.4 (d, 1H), 3.9 (d, 1H), 3.4-2.6 (m, 10H); MS (TOF, 1.99 e4): *m/z* = 414.8 [M+H]$^+$; $C_{19}H_{20}ClN_7O_2$ (413.86);

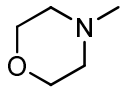
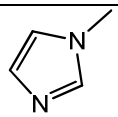
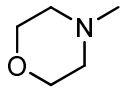
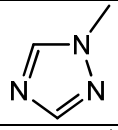
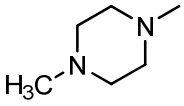
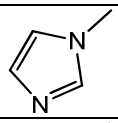
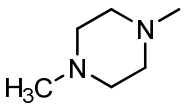
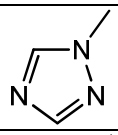
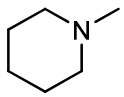
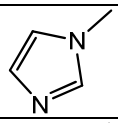
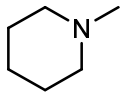
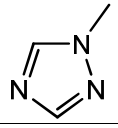
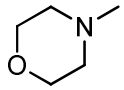
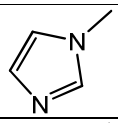
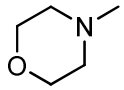
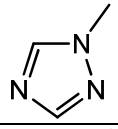
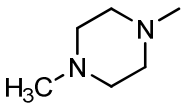
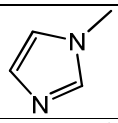
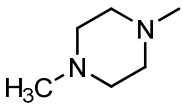
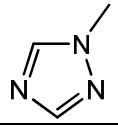
1,3-di(1H- imidazol-1-yl)-2-(morpholino(phenyl)methyl)propane-1,3-dione
IR (KBr): 2916, 1898, 1709, 1581, 845 cm^{-1}; ^{1}H NMR (400MHz, DMSO): δ = 8.2-7.2 (m, 11H), 4.2 (d, 1H), 3.8 (d, 1H), 3.4-2.5 (m, 8H); MS (TOF, 1.99 e4): *m/z* = 380.4 [M+H]$^+$; $C_{20}H_{21}N_5O_3$ (379.41);

2-(morpholino(phenyl)methyl)-1,3-di(1H-1,2,4,-triazol-1-yl)propane-1,3-dione
IR (KBr): 3026, 1716, 1690, 1449, 763cm^{-1}; ^{1}H NMR (400MHz, DMSO): δ = 8.6-8.3 (m, 4H), 7.6-7.4 (m, 5H), 4.4 (d, 1H), 3.9 (d, 1H), 3.4-2.6 (m, 8H); MS (TOF, 1.99 e4): *m/z* = 382.5 [M+H]$^+$; $C_{18}H_{19}N_7O_3$ (381.41);

1,3-di(1H- imidazol-1-yl)-2-((4-methylpiperazin-1-yl)(phenyl)methyl) propane-1,3-dione
IR (KBr): 3097, 1765, 1698, 1490, 847cm^{-1}; ^{1}H NMR (400MHz, DMSO): δ = 8.3-7.3 (m, 11H), 4.3 (d, 1H), 3.8 (d, 1H), 3.4-2.5 (m, 8H), 2.4 (s, 3H); MS (TOF, 1.99 e4): *m/z* = 393.5 [M+H]$^+$; $C_{21}H_{24}N_6O_2$ (392.45);

2-((4-methylpiperazin-1-yl)(phenyl)methyl)- 1,3-di(1H-1,2,4,-triazol-1-yl)propane-1,3-dione
IR (KBr): 2916, 1716, 1689, 1557, 876 cm^{-1}; ^{1}H NMR (400MHz, DMSO): δ = 8.7-8.3 (m, 4H), 7.7-7.4 (m, 5H), 4.2 (d, 1H), 3.9 (d, 1H), 3.4-2.6 (m, 8H), 2.5 (s, 3H); MS (TOF, 1.99 e4): *m/z* = 395.6 [M+H]$^+$; $C_{19}H_{22}N_8O_2$ (394.43).

Table -5 Experimental data of synthesized compounds 5a-5j

No.	R_1	R_2	R_3	Molecular Formula	Mol. Wt	% Yield	M. P* (^{0}C)	Rf Value**
P_1	Cl			$C_{20}H_{20}ClN_5O_3$	413.86	61.34	196	0.46
P_2	Cl			$C_{18}H_{18}ClN_7O_3$	415.83	63.68	130	0.34
P_3	Cl			$C_{21}H_{23}ClN_6O_2$	426.90	64.56	139	0.35
P_4	Cl			$C_{19}H_{21}ClN_8O_2$	428.88	70.12	202	0.31
P_5	Cl			$C_{21}H_{22}ClN_5O_2$	411.88	68.54	140	0.44
P_6	Cl			$C_{19}H_{19}ClN_7O_2$	413.86	68.00	112	0.39
P_7	H			$C_{20}H_{21}N_5O_3$	379.41	64.79	98	0.36
P_8	H			$C_{18}H_{19}N_7O_3$	381.41	63.13	94	0.33
P_9	H			$C_{21}H_{24}N_6O_2$	392.45	60.00	90	0.31
P_{10}	H			$C_{19}H_2 2N_8O_2$	394.43	62.54	96	0.28

Biological Investigation

Three test organisms were selected for testing of new compounds, including *Candida albicans* (NCIM 3471), *Aspergillus fumigatus* (NCIM 902) and *Aspergillus niger* (NCIM 596).

The susceptibility of *Candida albicans* and *Aspergillus* species to the newly synthesized compounds was determined by means of the Agar diffusion technique, according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 1995)[8]. Stock solutions of chemicals were prepared in DMSO at a concentration 3 mg/ml; MICs were determined in Sabouraud quadrant agar plates containing different dilutions of tested compounds. Controls were prepared without chemicals and with reference antifungal drug Clotrimazole. All the plates were then placed at 35^0 C and read after 24 and 48 hours incubation.

RESULTS AND DISCUSSION

Chemistry

1, 3-di(substituted azol)-2-(substituted hetrocycles(substituted aryl)methylene)propane-1,3-dione derivatives were synthesized from the commercially available Diethyl malonate(Scheme). Diethyl malonate was condensed with aldehyde to give Diethyl 2-substituted benzylidene malonate (1a-1b) (Table-1). This was subjected to Aza-Michael reaction to give Diethyl 2-(substituted hetrocycles (substituted aryl) methylene) malonate. (2a-2e) (Table-2). This product on treatment with alcoholic KOH gives 2-(substituted hetrocycles (substituted aryl) methylene) malonic acid (3a-3e). The product was subjected to nucleophilic substitution reaction with thionyl chloride to give 2-(substituted hetrocycles (substituted aryl) methylene) malonyl dichloride. (4a-4e) and this product was immediately used for attachment of imidazole or triazole in DMF to give1,3-di(substituted azol-1-yl)-2-substituted hetrocycle(substituted aryl)methylene)propane 1,3-dione (5a-5j) (Table-5).

Biological Investigation

The Biological Investigation of the synthesized compounds was studied for Antifungal activity by calculating MICs by Agar diffusion method (Table-6).

Table -6 In vitro antifungal activity of compounds using MIC

Comp.	*A. niger*	*A. fumigatus*	*C. albicans*
P1	40	40	40
P2	50	50	50
P3	40	30	40
P4	30	40	40
P5	40	50	40
P6	60	60	60
P7	40	60	50
P8	50	60	50
P9	40	60	50
P10	40	60	50

Graph No. 1: Graph of % Zone of inhibition Vs compounds

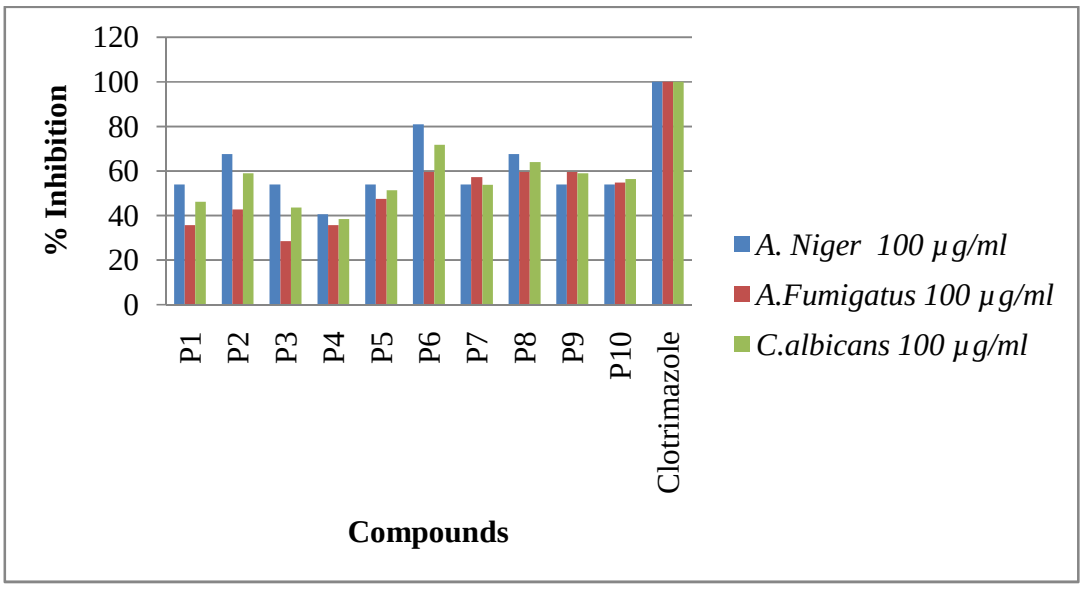


Table-7: % Zone of Inhibition (mm) against the *A. Niger, A. Fumigatus and C. albicans.*

Sr. No.	Compound	*A. Niger* 100 µg/ml	*A.Fumigatus* 100 µg/ml	*C.albicans* 100 µg/ml
1	P1	54.05	35.71	46.15
2	P2	67.56	42.85	58.97
3	P3	54.05	28.57	43.58
4	P4	40.54	35.71	38.46
5	P5	54.05	47.61	51.28
6	P6	81.04	59.52	71.79
7	P7	54.05	57.14	53.86
8	P8	67.56	59.52	64.10
9	P9	54.05	59.52	58.97
10	P10	54.05	54.76	56.41
11	Clotrimazole	100	100	100

Structure Activity Relationship (SAR)

-The basic structural requirement of members of the azole class is weakly basic imidazole or 1,2,4-triazole ring (pKa-6.5-6.8) bonded by nitrogen –carbon linkage to the rest of structure.

-At the molecular level, the amidine nitrogen atom (N-3 in the the imidazoles, N-4 in the the triazoles) is believed to bind to the heme iron of enzyme bound cytochrome p-450 to inhibit activation of molecular oxygen and prevent oxidation of steroidal substrates by the enzymes.

-The most potent antifungal azoles possess two or three aromatic rings, at least one of which is halogen substituted and other nonpolar functional group.

-The large nonpolar portion of these molecules mimics the nonpolar steroidal part in substrates for lanosterol 14α-demethylase.

-The nonpolar functionality confers high lipophilicity to the antifungal azoles.The free bases are typically insoluble in water but are soluble in organic solvents.

CONCLUSION

Novel1,3-di(substituted azol-1-yl)-2-substituted hetrocycle(substituted aryl)methylene)propane 1,3-dione derivatives were prepared, structurally characterized using spectroscopic techniques and their Biological Investigation as Antifungal activity was screened by calculating MICs. Among the synthesized compounds and was found to be the most active agents as compared with standard.

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