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The Pharma Innovation

ISSN: 2277- 7695
TPI 2014; 3(10): 05-09

www.thepharmajournal.com
Received: 27-09-2014
Accepted: 08-10-2014

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Microwave assisted synthesis of some pyrazole derivatives and their antibacterial and antifungal activity

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Abstract
In the present investigation, microwave assisted synthesis of 3-(4-substituted phenyl)-1-phenylprop-2-en-1-ones (Chalcones) **(1a-c)** were synthesized by base catalyzed aldol condensation of p-substituted benzaldehyde with acetophenone in the presence of piperidine.
3-(4-substituted phenyl)-1-phenylprop-2-en-1-one (Chalcones) **(1a-c)** was carried out with hydrazine hydrate, phenyl hydrazine, isoniazid, nicotinic hydrazide or thiosemicarbezide in ethanol containing a few drops of glacial acetic acid under microwave irradiation giving 3-(4-substituted phenyl)-5-phenyl-2,3-dihydro-1*H*-pyrazole **(2a-c)**, 3-(4-substituted phenyl)-2-5-diphenyl-2,3-dihydro-1*H*-pyrazole **(3a-c)**, [5-(4-substituted phenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-yl](pyridine-4-yl)methanone **(4a-c),** [5-(4-substituted phenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-yl](pyridine-3-yl)methanone **(5a-c)**, 5-(4-substituted phenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide **(6a-c)** derivatives. The structures of the synthesized compounds were assigned on the basis of elemental analysis, IR and ^{1}H NMR spectral data. The synthesized compounds have been screened for their antibacterial and antifungal activities.

Keywords: 3-(4-Substituted phenyl)-1-phenylprop-2-en-1-one, Antibacterial, Antifungal activities, Microwave irradiation.

1. Introduction
Microwave assisted organic synthesis has opened up some new opportunities. Microwave assisted synthesis is an eco-friendly method, where reactions occurs more rapidly, safely with higher chemical yields compared to the conventional methods of synthesis.
The pyrazole unit is one of the core structures in a number of drugs. More recently extensive studies have been focused on pyrazole derivatives exhibiting anti-inflammatory[1], anti-cancer[2], anti-malarial [3], antihyperglycemic [4], analgesic [5], anticonvulsant [6], antidepressant[7], antiulcer [8], antidiabetic [9], cytotoxic [10], antitubercular [11], antibacterial [12], antifungal [13], herbicidal and insecticidal [14-16].
A synthesis of α,β-unsaturated ketones were achieved by microwave- assisted base catalysed aldol condensation of acetophenone with p-substituted benzaldehyde. Chalcones undergo a cyclization reaction with corresponding hydrazines giving pyrazole derivatives.

2. Materials and Methods
The melting points were determined in open capillary tube and are uncorrected. The IR spectra were recorded on Perkin-Elmer 157 spectrometer using KBr pellets. The ^{1}H NMR spectra were scanned on a DRX-300 MHz spectrometer (300 MHz) in $CDCl_3$/DMSO-d6 using TMS as internal standard and chemical shifts are expressed in δ ppm. Purity of synthesized compounds was checked by TLC using silica gel-G. Spots were exposed in an iodine chamber.

General procedure for preparation of chalcone (1a-c)
A convenient route for the synthesis of *α,β*-unsaturated ketones (Chalcone) was achieved by the reaction of p-substituted benzaldehyde (0.005 mol) with acetophenone (0.005 mol) in the presence of piperidine, under microwave irradiation at 5 sec intervals. The specific reaction time was kept 2 min and then the reaction mixture was cooled in crushed ice. Progress of the reaction was monitored by TLC method. The solid thus obtained was filtered, washed with water, dried and purified by recrystallization from ethanol.

General procedure for preparation of compound (2a-c), (3a-c), (4a-c), (5a-c) and (6a-c)

A mixture of the chalcone (0.004 mol.) in ethanol (10 mL) with the appropriate amount of corresponding hydrazines (hydrazine hydrate, phenyl hydrazine, isoniazid, nicotinic hydrazide or thiosemicarbezide) (0.004 mol) in glacial acetic acid (2 drop) was irradiated under microwave for a specific time of 1 min. Then the reaction mixture was poured into crushed ice and kept overnight at room temperature. The solid obtained was filtered, washed and recrystallized from ethanol. The completion of reaction was monitored by TLC. (Eluent: $CHCl_3$-MeOH (7:3)).

3-(4-Methylphenyl)-5-phenyl-2,3-dihydro-1*H*-pyrazole (2a)

Yield 77 %, m.p. 178ºC ; IR (KBr) cm^{-1}: 3380, 3410 (N-H pyrazole); 3044 (–Ar-CH); ^{1}H NMR (DMSO d_6) δ: 9.96, 9.81 (2H, NH); 4.65 (N-CH); 6.61-7.20 (Ar-H); Anal. Calcd. for $C_{16}H_{16}N_2$: C, 81.32; H, 6.82; N, 11.85%. Found: C, 81.18; H, 6.70; N, 11.74%.

3-(4-methoxyphenyl)-5-phenyl-2,3-dihydro-1*H*-pyrazole (2b)

Yield 76%, m.p. 174ºC; IR (KBr) cm^{-1}: 3381, 3415 (N-H pyrazole); 3045 (–Ar-CH); ^{1}H NMR (DMSO d_6) δ: 9.94, 9.77 (2H, NH); 4.65 (N-CH); 6.55-7.25 (Ar-H); 2.25 (Ar-OCH_3); Anal. Calcd. for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10%. Found: C, 76.05; H, 6.30; N, 11.02%.

3-(4-Nitrophenyl)-5-phenyl-2,3-dihydro-1*H*-pyrazole (2c)

Yield 78%, m.p. 182 ºC; IR (KBr) cm^{-1}: 3381, 3410 (N-H pyrazole); 3039 (Ar-CH); 1520, 1310 (Ar-NO_2 str.); ^{1}H NMR (DMSO d_6) δ: 9.94, 9.83 (-NH, pyrazole); 4.64 (N-CH); 6.47-7.20 (Ar-H); Anal. Calcd. for $C_{15}H_{13}N_3O_2$: C, 67.40; H, 4.90; N, 15.72%. Found: C, 67.31; H, 4.79; N, 15.65%.

3-(4-Methylphenyl)-2,5-diphenyl-2,3-dihydro-1*H*-pyrazole (3a)

Yield 74%, m.p. 188ºC; IR (KBr) cm^{-1}: 3412 (N-H, pyrazole); 3055 (Ar-CH); ^{1}H NMR (DMSO d_6) δ: 9.79 (NH, pyrazole); 5.44 (N-CH); 6.56-6.96 (Ar-H); Anal. Calcd. For $C_{22}H_{20}N_2$: C, 84.58; H, 6.45; N, 8.97%. Found: C, 54.37; H, 6.34; N, 8.81%.

3-(4-Methoxyphenyl)-2,5-diphenyl-2,3-dihydro-1*H*-pyrazole (3b)

Yield 79%, m.p. 179ºC; IR (KBr) cm^{-1}: 3419 (N-H, pyrazole); 3053 (Ar-CH); ^{1}H NMR (DMSO d_6) δ: 9.76 (NH, pyrazole); 5.54 (N-CH); 6.54-7.10 (Ar-H); Anal. Calcd. for $C_{22}H_{20}N_2O$: C, 80.46; H, 6.14; N, 8.53%. Found; C, 80.55; H, 6.11; N, 8.44%.

3-(4-Nitrophenyl)-2,5-diphenyl-2,3-dihydro-1*H*-pyrazole (3c)

Yield 72%, m.p. 189ºC; IR (KBr) cm^{-1}: 3421 (N-H pyrazole); 3048 (Ar-CH); 1530, 1315 (Ar-NO_2 str.); ^{1}H NMR (DMSO d_6) δ: 9.75 (NH, pyrazole); 5.57 (N-CH); 6.52-7.08 (Ar-H); Anal. Calcd. for $C_{21}H_{17}N_3O_2$: C, 73.45; H, 4.99; N, 12.24%. Found: C, 73.38; H, 4.87; N, 12.18%.

[5-(4-Methylphenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-yl](pyridine-4-yl)methanone (4a)

Yield 80%, m.p. 171 ºC; IR (KBr) cm^{-1}: 3419 (N-H pyrazole); 3056 (-Ar-CH); 1593 (C=N); 1656 (C=O); ^{1}H NMR (DMSO d_6) δ: 9.70 (NH, pyrazole); 5.56 (N-CH); 6.52-7.30 (Ar-H); Anal. Calcd. for $C_{22}H_{19}N_3O$: C, 77.40; H, 5.61; N, 12.31%. Found: C, 77.32; H, 5.52; N, 12.26%.

[5-(4-Methoxyphenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-yl](pyridine-4-yl)methanone (4b)

Yield 77%, m.p. 173 ºC; IR (KBr) cm^{-1}: 3417 (N-H pyrazole); 3051 (-Ar-CH); 1590 (C=N); 1657 (C=O); ^{1}H NMR (DMSO d_6) δ: 9.76 (NH, pyrazole); 5.50 (N-CH); 6.55-7.27 (Ar-H); Anal. Calcd. for $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.76%. Found: C, 73.85; H, 5.30; N, 11.69%.

[5-(4-Nitrophenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-yl](pyridine-4-yl)methanone (4c)

Yield 74%, m.p. 177 ºC; IR (KBr) cm^{-1}: 3415 (N-H pyrazole); 3049 (-Ar-CH); 1595 (C=N); 1655 (C=O); 1540, 1335 (Ar-NO_2 str.); ^{1}H NMR (DMSO d_6) δ: 9.78 (NH, pyrazole); 5.54 (N-CH); 6.53-7.23 (Ar-H); Anal. Calcd. for $C_{21}H_{16}N_4O_3$: C, 67.73; H, 4.33; N, 15.05 %. Found: C, 67.71; H, 4.27; N, 15.00%.

[5-(4-Methylphenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-yl](pyridine-3-yl)methanone (5a)

Yield 76%, m.p. 173 ºC; IR (KBr) cm^{-1}: 3421 (N-H pyrazole); 3055 (Ar-CH); 1592 (C=N); 1659 (C=O); ^{1}H NMR (DMSO d_6) δ: 9.75 (NH, pyrazole); 5.53 (N-CH); 6.56-7.27 (Ar-H); Anal. Calcd. for $C_{22}H_{19}N_3O$: C, 77.40; H, 5.61; N, 12.31%. Found: C, 77.31; H, 5.53; N, 12.25%.

[5-(4-Methoxyphenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-yl](pyridine-3-yl)methanone (5b)

Yield 74%, m.p. 174 ºC; IR (KBr) cm^{-1}: 3419 (N-H pyrazole); 3052 (-Ar-CH); 1589 (C=N); 1658 (C=O); ^{1}H NMR (DMSO d_6) δ: 9.71 (NH, pyrazole); 5.51 (N-CH); 6.53-7.22 (Ar-H); Anal. Calcd. for $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.76%. Found: C, 73.84; H, 5.30; N, 11.68%.

[5-(4-Nitrophenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-yl](pyridine-3-yl)methanone (5c)

Yield 76%, m.p. 179 ºC; IR (KBr) cm^{-1}: 3417 (N-H pyrazole); 3051 (-Ar-CH); 1596 (C=N); 1659 (C=O); ^{1}H NMR (DMSO d_6) δ: 9.79 (NH, pyrazole); 5.56 (N-CH); 6.51-7.21 (Ar-H); Anal. Calcd. for $C_{21}H_{16}N_4O_3$: C, 67.73; H, 4.33; N, 15.05 %. Found: C, 67.68; H, 4.25; N, 15.01%.

5-(4-Methylphenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide (6a)

Yield 82%, m.p. 190 ºC; IR (KBr) cm^{-1}: 3308 (NH_2), 3377 (N-H pyrazole); 3047 (-Ar-CH); 1245 (C=S); ^{1}H NMR (DMSO d_6) δ: 7.36 (2H, NH_2); 9.81 (NH, pyrazole); 4.65 (N-CH); 6.56-7.29 (Ar-H); Anal. Calcd. for $C_{17}H_{17}N_3S$: C, 69.12; H, 5.80; N, 14.22%. Found: C, 69.03; H, 5.65; N, 14.10%.

5-(4-Methoxyphenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide (6b)

Yield 77%, m.p. 178 ºC; IR (KBr) cm^{-1}: 3310 (NH_2), 3375 (N-H pyrazole); 3054 (-Ar-CH); 1239 (C=S); ^{1}H NMR (DMSO d_6) δ: 7.41 (2H, NH_2); 9.74 (NH, pyrazole); 4.67 (N-CH); 6.54-7.29 (Ar-H); Anal. Calcd. for $C_{17}H_{17}N_3OS$: C, 65.57; H, 5.50; N, 13.49%. Found: C, 65.49; H, 5.45; N, 13.44%.

5-(4-Nitrophenyl)-3-phenyl-2,5-dihydro-1*H*-pyrazole-1-carbothioamide (6c)

Yield 72%, m.p. 171 ºC; IR (KBr) cm^{-1}: 3311 (NH_2), 3378 (N-H pyrazole); 3048 (Ar-CH); 1247 (C=S); ^{1}H NMR (DMSO d_6) δ: 7.42 (2H, NH_2); 9.81 (NH, pyrazole); 4.69 (N-CH); 6.55-

7.27 (Ar-H); Anal. Calcd. for $C_{16}H_{14}N_4O_2S$: C, 58.88; H, 4.32; N, 17.17%. Found: C, 58.81; H, 4.26; N, 17.10%.

3. Results and discussion

The starting compounds 3-(4-substituted phenyl)-1-phenylprop-2-en-1-one (Chalcones) (**1a-c)** react with corresponding hydrazides in ethanol containing a few drops of glacial acetic acid under microwave irradiation to give (**2a-c)-(6a-c**), respectively.

The structure was established though IR and ^{1}H NMR spectral data. The IR spectra of (**2a-c)** exhibited absorption bands for primary amine (-NH) at 3380-3381, 3410-3415 cm^{-1}, (-N-N) at 1240-1245 cm^{-1} and (-C-N) at 1082-1087 cm^{-1}. The ^{1}H NMR spectra of these compounds revealed signals at δ = 9.94-9.96, 9.77-9.83 ppm for (-NH) ring proton, a singlet at δ = 4.64-4.65 ppm for (-N-CH) at pyrazole ring and a multiplet at δ = 6.47-7.25 ppm for the aromatic protons.

The IR of (**3a-c)** exhibited absorption bands for primary amine (-NH) at 3412-3421 cm^{-1} and (–N-N) at 1245-1249 cm^{-1} and (-C-N) at 1105-1114 cm^{-1}. The ^{1}H NMR spectra of these compounds revealed signals at δ = 9.75- 9.79 ppm for (-NH) proton, a singlet at δ = 5.44-5.57 ppm for (-N-CH) at pyrazole ring and a multiplet at δ = 6.52-7.10 ppm for the aromatic proton.

The IR of (**4a-c)** exhibited absorption bands for primary amine (-NH) at 3415-3419 cm^{-1}, (-C=N) at 1590-1595 cm^{-1}, (-C=O) at 1655-1657, (–N-N) at 1246-1249 cm^{-1} and (-C-N) at 1082-1114 cm^{-1}. The ^{1}H NMR spectra of these compounds revealed signals at δ = 9.70-9.78 ppm for (-NH) ring proton, a singlet at δ = 5.50-5.56 ppm for (-N-CH) at pyrazole ring and a multiplet at δ = 6.52-7.30 ppm for the aromatic proton.

The IR of (**5a-c)** exhibited absorption bands for primary amine (-NH) at 3417-3421 cm^{-1}, (-C=N) at 1589-1596 cm^{-1}, (-C=O) at 1658-1659, (–N-N) at 1245-1249 cm^{-1} and (-C-N) at 1079-1112 cm^{-1}. The ^{1}H NMR spectra of these compounds revealed signals at δ = 9.71-9.79 ppm for (-NH) proton, a singlet at δ = 5.51-5.56 ppm for (-N-CH) at pyrazole ring and a multiplet at δ = 6.51-7.27 ppm for aromatic proton.

The IR of (**6a-c)** exhibited absorption bands for primary amine (-NH) at 3375-3378cm^{-1}, 3308-3311 cm^{-1} for (-NH_2), 1239-1247 cm^{-1} for (–C=S). The ^{1}H NMR spectra of these compounds revealed signals at δ = 9.74-9.81 ppm for (-NH) proton, a singlet at δ = 4.65-4.69 ppm for (-N-CH) at pyrazole ring and a multiplet at δ = 6.54-7.29 ppm for aromatic proton.

4. Antimicrobial activity

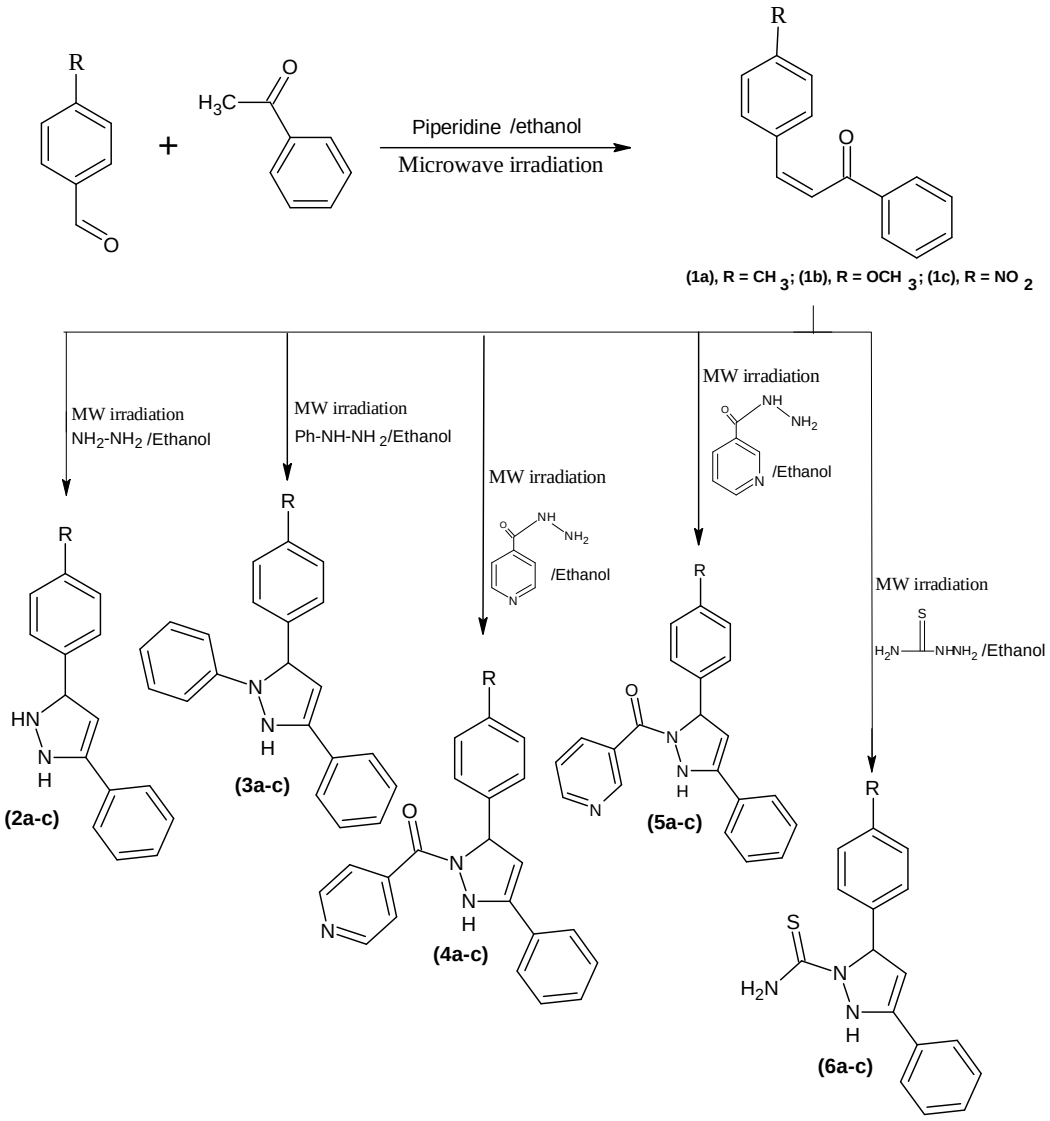
All the compounds (**2a-c), (3a-c), (4a-c), (5a-c)** and **(6a-c)** were tested for antibacterial activity against *Escherichia coli (Gram -ve), Staphylococcus aureus (Gram +ve), Pseudomonas aeruginosa (Gram +ve)* bacteria and antifungal activity against three fungal strains *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus*. Ampicillin and griseofulvin were used as standard drugs for antibacterial and antifungal activity, respectively.

MBC and MFC were determined using Broth dilution method. Serial dilution for primary and secondary screening, material and method was followed as per NCCLS-1992 manual [17].

A stock solution was prepared of each drug (2000 µg/mL concentration). In primary screening, 1000, 500, 250 and 125 µg/mL concentrations of the synthesized drugs were taken. The synthesized drugs were found active in this primary screening. These were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were diluted to obtain 100, 50, 25, 12.5, 6.250, 3.125 and 1.5625 µg/mL concentrations. The standard drug used in the present study is ampicillin for evaluating antibacterial activity which showed 50, 50 and 100 µg/mL MBC against *S. aureus*, *E. coli* and *P. aeruginosa*, respectively. Griseofulvin is used as the standard drug for antifungal activity, which showed 100 µg/mL MFC against all the species, used for the antifungal activity. The results of antimicrobial and antifungal activities of synthesized compounds are shown in Table 1.

Table 1: Antibacterial and antifungal activity of all the synthesized compounds.

Sr. No.	Minimal Bactericidal Concentration (MBC) (µg/mL)			Minimal Fungicidal Concentrations (FBC) (µg/mL)		
	Gram negative		Gram positive	*C. albicans*	*A. niger*	*A. clavatus*
	E. coli	*P. aeruginosa*	*S. aureus*			
2a	250	100	50	500	250	500
2b	100	250	50	1000	500	250
2c	50	500	250	250	1000	500
3a	500	500	500	250	250	100
3b	250	250	500	500	500	1000
3c	50	100	1000	500	500	500
4a	1000	250	250	1000	100	250
4b	250	500	500	500	250	500
4c	500	100	250	500	500	250
5a	250	250	100	250	250	100
5b	50	100	50	250	100	500
5c	100	500	250	500	500	250
6a	250	500	50	100	250	500
6b	250	500	250	500	500	100
6c	500	250	250	250	100	500
S.D.	50	100	50	100	100	100



Scheme 1: Synthesis of compounds (2a-c), (3a-c), (4a-c), (5a-c) and (6a-c).

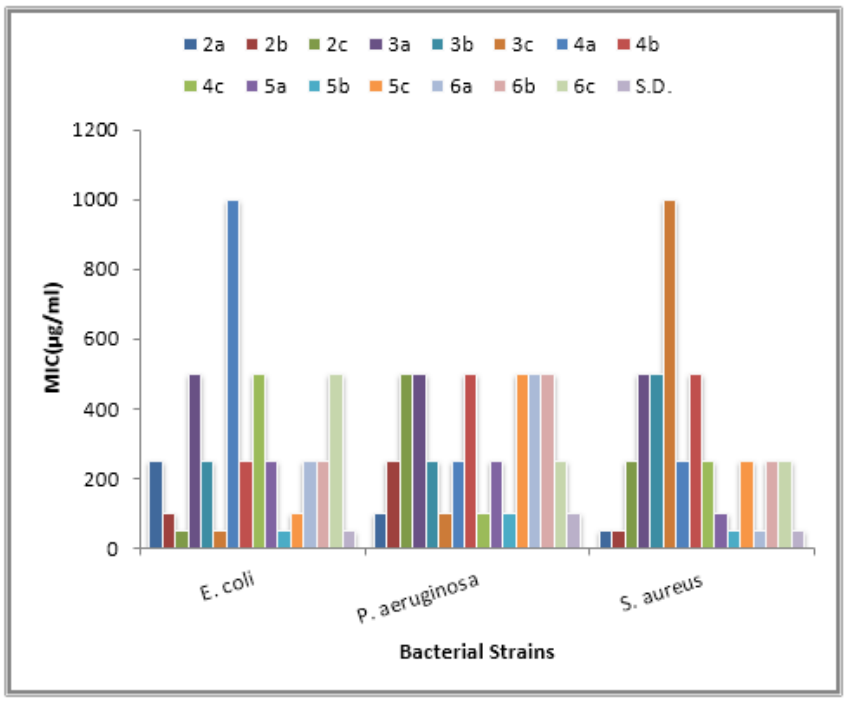


Fig 1: Antibacterial Activity of Synthesized Compounds

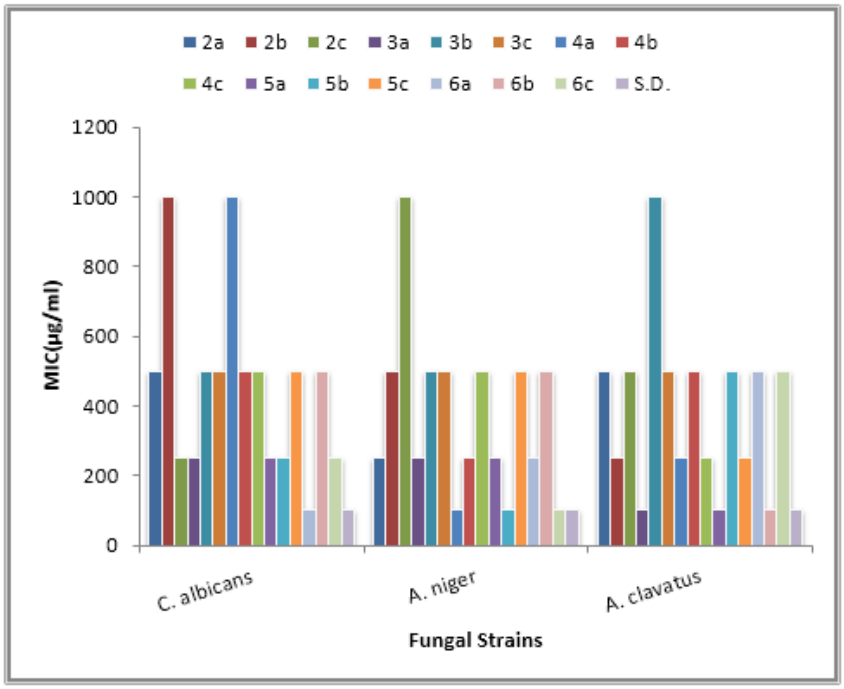


Fig 2: Antifungal Activity of Synthesized Compounds

5. Acknowledgement
Authors are thankful to the Head, Department of Chemistry, PAHER University, Udaipur (Rajasthan.) for providing laboratory facilities and the Head, Department of Pharmacy for providing spectral and analytical data.

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