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GREEN SYNTHESIS, SPECTRAL CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF NOVEL ACETYL PYRAZOLE DERIVATIVES

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ABSTRACT

Compounds 1-acetyl-4, 5-dihydro-5-aryl-3-(thiophen-2yl)pyrazoles (**5-8)** have been synthesized by microwave followed by green chemistry principle and convectional method. The Microwave irradiation method is the best method over than conventional method. Because eco-friendly, less solvent, time saving and good yields compared to the conventional method. All the synthesized compounds are characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectral studies and elemental analysis. Compounds are screened for bacterial activity against *S.aureus* and *S.pyogenes* (Gram positive) and *P.aeroginosa* and *K.pneumoniae* (Gram negative) strains and *C.albicans* is the fungal strains. The bromo substituted phenyl ring compound shows an excellent antibacterial activity against the bacterial strains and the methoxy group substituted compound shows the excellent antifungal activity against the fungal strains when compared with the standard drug Ciprofloxacin's for both antifungal and antibacterial activity. The drug ability of the compounds was confirmed by Lipinski's Rule (Rule 5).

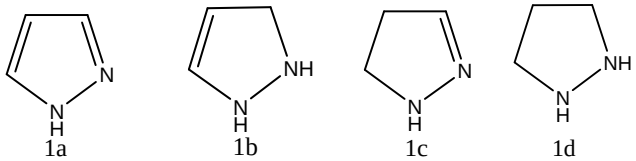
KEYWORDS: Microwave irradiation, Pyrazole, Hydrazine hydrate, Antimicrobial activity, Lipinski's rule.

INTRODUCTION

Hetero cyclic compounds have gained immense important in human life because of their variety of applications, particularly these compounds have been successfully tested against several diseases and therefore have medicinal importance in recent years.[1] In recent years, greener organic synthesis is one of the most important in medicinal compound synthesis.

Microwave assisted organic synthesis has opened up new opportunities for the synthetic chemists by providing novel routes not by practical conventional methods. Microwave assisted synthesis is an eco-friendly and efficient method for synthesis of organic compounds as compared to the conventional method of synthesis. In this method the reaction occurs more rapidly, safety and with higher chemical yields and therefore, this method become superior to the conventional method. The conventional method, requiring a longer time and larger quantity of solvents and reagents, causes environmental pollution and contributes to health hazards.

Pyrazoles (1a) are the important members of heterocyclic compounds with two adjacent nitrogens in a five-membered ring system. Among the two nitrogen atoms; one is basic and the other is neutral in nature. These are aromatic molecules due to their planar conjugated ring structures with six delocalized π-electrons. The aromatic nature arises from the four π electrons and the unshared pair of electrons on the –NH nitrogen. The partially reduced forms of pyrazole are named as pyrazolines (**1b** or **1c**); while completely reduced form is pyrazolidine (**1d**).[2]



1a , 1b , 1c , 1d

The Pyrazole unit is one of the core structures in a number of drugs. Pyrazoles and its derivatives, a class of well known nitrogen heterocycles, occupy a prime position in medicinal and pesticide chemistry for their diverse biological activities. More recently extensive studies have been focused on pyrazole derivatives exhibiting anti-inflammatory [3], anti-cancer[4], antimalarial[5], antihyperglycemic[6], analgesic[7], anticonvulsant[8], antidepressant[9], antiulcer [10], antidiabetic [11], cytotoxic[12], antitubercular[13],antibacterial[14], antifungal[15],herbicidal and insecticidal.[16-18] Pyrazole derivatives with a phenyl group at the 5-position exhibit excellent characteristics of blue photoluminescence and electroluminescence.[19-21]

Herein, as a continuation of our research work on the development of novel methods to construct biologically important heterocyclic compounds[22], we report the synthesis of thiophenyl pyrazole derivatives bearing substituent on the 4-position via Suzuki cross

coupling method under conventional and microwave irradiation method. When compared to conventional method microwave irradiation method achieved the title compounds in very short in time with excellent yields. The synthesized compounds are characterized by IR, ^{1}H NMR, ^{13}C NMR, Mass, elemental analysis and antimicrobial activities.

MATERIALS AND METHODS

Chemistry: Melting points (uncorrected) were determined using a Guna melting point apparatus. FT-IR spectra were carried out on a Perkin-Elmer1650 spectrophotometer and noteworthy absorption values (cm^{-1}) alone are listed ^{1}H and^{13}C NMR spectra are recorded at 400 MHz and 100 MHz respectively on Bruker AMX 400 NMR spectrometer using $CDCl_3$ as solvent. The ESI +ve MS spectra are recorded on Varian Saturn 2200 MS spectrometer. Satisfactory micro analyses are obtained on Carlo Erba1106 CHN analyser. Performing TLC assessed the reaction and the purity of the products. By adopting the literature precedent, chalcones are prepared.

General procedure for the synthesis of chalcones (1-4)

The Chalcones (1-4) was prepared by literature survey method.[22]

General procedure for the synthesis of 1-acetyl-4, 5-dihydro-5-phenyl-3-(thiophen-2yl)pyrazoles(5-8)in conventional method.

Chalcones (1-4) (0.01mol), hydrazine hydrate (0.01 mol), anhydrous sodiumacetate(0.01mol) and acetic anhydride taken in a round bottom flask and the reaction mixture flask was refluxed until the products are formed .The reaction is monitored by TLC. The time required for the formation of various pyrazole is shown in Table 1. The reaction mixture is poured in to crused ice and left overnight. The precipitate is separated by filtration washed well with water, dried and obtained solids are purified by column chromatography using tolune and ethylacetate(1:1) mixture as eluent which afford the title compounds (5-8) in excellent yields.[22]

General procedure for the synthesis of 1-acetyl-4, 5-dihydro-5-phenyl-3-(thiophen-2yl) pyrazoles (5-8)in microwave irradiation method.

Chalcones(1-4) (0.01mol), hydrazine hydrate (0.01mol), acetic anhydride catalytic amount of K_2CO_3 taken in a beaker. The reaction mixture was stirred well and microwave irradiation for 10-15 minute at a power of 320 watts. The completion of the reaction was monitored by TLC. The time required for the formation of various pyrazole is shown in **Table 1**.The reaction

mixture is poured in to crushed ice and left overnight. The precipitate is separated by filtration washed well with water, dried and obtained solids are purified by column chromatography using tolune and ethylacetate(1:1) mixture as eluent which afford the title compounds (5-8) in excellent yields.

BIOLOGICAL ACTIVITY

Antimicrobial Activity: Four concentrations of tests compounds were prepared i.e 0.25 mg/ml, 0.50 mg/ml, 0.75 mg/ml, 1.00 mg/ml. Antimicrobial activity of the compounds has been evaluated using standard methods.[23-25]

Antibacterial Activity: The nutrient agar medium was prepared and autoclaved at15.1 Ibs pressure for 20 minutes. This medium was poured in to Petri plates and allowed to solidify. On the surface of media microbial suspension was spread with the help of sterilized cotton swab. Cups were made by boring into agar surface with a previously sterilized cork borer and scooping out the punched part of agar. Six cups were made in each Petri plate and into these cups was added the concentration (0.25 mg/ml, 0.50 mg/ml, 0.75 mg/ml, 1.00 mg/ml) of the test compounds, fifth was filled with the standard, Ciprofloxacin (CIP^5), and the sixth was filled with the control (DMSO). The plates were kept in cold for one hour to allow the diffusion of test compounds and then incubated at 37± 0.5°C for 24 hours for antibacterial activity. The zone of inhibition formed around the cups after respective incubation was measured and percentage inhibition of the compounds were calculated.

Antifungal Activity: *Fo*r anti fungal screening, spore suspension (5 ml) of each test microorganism (72 hour culture) was added to sterilize Saboured dextrose agar medium at 35-40°C with shaking. The Petri dishes were seeded with the mixture and the filter paper discs of the test compounds (concentration 0.25 mg/ml, 0.50 mg/ml, 0.75 mg/ml, 1.00 mg/ml), reference drug, Ciproflaxacin control (DMSO) were placed in the same manner as in antibacterial activity determination. These Petri dishes were incubated at 30 ± 1°C for 48 hours. The zone of inhibition of growth was considered as an indicator for the antifungal activity.

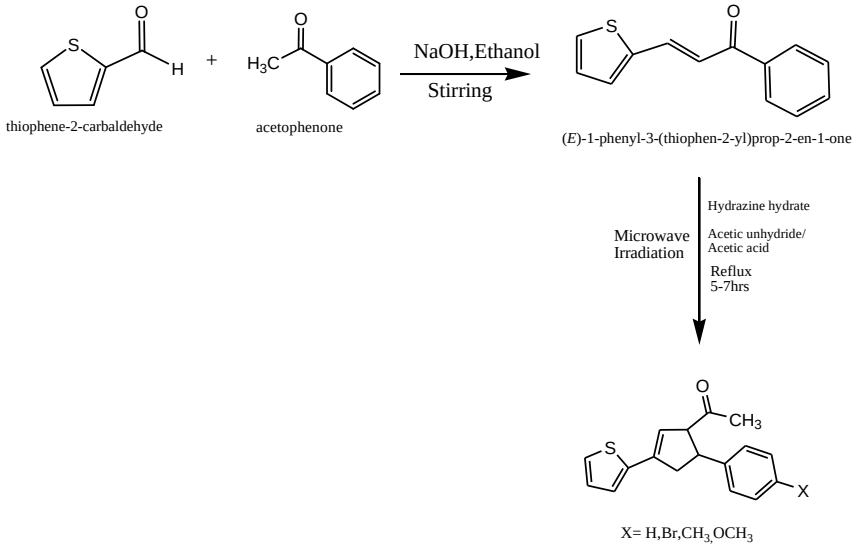
RESULTS AND DISCUSSION

Physico-Chemical Characters

1-acetyl-4, 5-dihydro-5-phenyl-3-(thiophen-2yl)pyrazoles(5)

Yield 70%; m.p 94°C; Molecular Formula $C_{15}H_{16}N_2SO$; %Calcd.(Found); C%=66.17 (66.24), H% =5.88 (5.93), N% = 10.28 (10.29), S%=11.78 (11.74), m/z $(M)^+$: 272. **FT-IR**

(KBr, cm^{-1}): 3082.25,3022.45 cm^{-1}(Aromatic CH stretching), 2910.58cm^{-1} (Aliphatic CH stretching), 1647.21 cm^{-1}(Amide C=O stretching), 1423.47 cm^{-1}(C=N Stretching), 626.87,702.09,736.81,763.81 cm^{-1}(Aromatic ring stretching).**^{1}H NMR** ($CDCl_3$,δ,ppm,400MHz) 3.37(dd,H_{4a}), 3.75(dd,H_{4e}), 5.93(dd,H_{5a}), 2.43(acetyl methyl groups), 7.79-6.93(Aromatic protons and thiophene protons). **^{13}C NMR** ($CDCl_3$, δ ppm,100 MHz); 55.26(C-5 of pyrazole moiety), 42.04 (C-4 of pyrazole moiety), 153.87 (C-3 of pyrazole moiety), 21.98 (acetyl methyl carbon), 168.96 (amide cabonyl carbon), 128.59-124.59 (Aromatic and thiophenecarbons) and 144.32(Ipso carbon).



Scheme-1: Synthetic route for the reaction of acetyl pyrazole.

1-acetyl-4, 5-dihydro-5-Bromophenyl-3-(thiophen-2yl) pyrazoles (6)

Yield 70%; m.p 100°C; Molecular Formula $C_{15}H_{15}N_2SOBr$; %Calcd. (Found); C%= 51.42 (51.47), H% =4.28 (4.31), N% = 7.8 (8.0) S%=9.16 (9.21), m/z (M) $^{+}$:349,347. **FT-IR** (KBr,cm^{-1}) : 3066.11 cm^{-1}(Aromatic CH stretching),2885.51cm^{-1} (Aliphatic CH stretching),1658.78 cm^{-1}(Amide C=O stretching), 1417.68 cm^{-1}(C=N Stretching), 642.3,709.8,763.81 cm^{-1}(Aromatic ringstretching). **^{1}HNMR**($CDCl_3$, δppm,400MHz), 3.33(dd,H_{4a}), 3.71(dd, H_{4e}), 5.93(dd,H_{5a}), 2.41(acetyl methyl groups), 7.64-6.93(Aromatic protons and thiophene protons).**^{13} C NMR** ($CDCl_3$, δ ppm,100 MHz); 55.44 (C-5 of pyrazole moiety),41.89 (C-4 of pyrazole moiety), 152.78 (C-3 of pyrazole moiety), 21.97 (acetyl methyl carbon), 168.94 (amide cabonyl carbon),128.04-124.67 (Aromatic and thiophenecarbons)144.09,132.01,130.25(Ipso carbon).

1-acetyl-4, 5-dihydro-5-methylphenyl-3-(thiophen-2yl)pyrazoles (7)

Yield 70%; m.p 126°C; Molecular Formula $C_{16}H_{18}N_2SO$; %Calcd. (Found); C%= 67.13 (67.19) ,H% =6.29 (6.34), N% = 9,78(9.79), S%=11.18(11.21), m/z (M)+:286. **FT-IR** (KBr, cm^{-1}): 3086.11, 3001.24 cm^{-1}(Aromatic CH stretching), 2885.51cm^{-1} (Aliphatic CH stretching), 1658.78cm^{-1}(Amide C=O stretching), 1417.68 cm^{-1}(C=N Stretching), 628.79, 642.3, 709.8, 763.81 cm^{-1}(Aromatic ring stretching).**^{1}H NMR** ($CDCl_3$, δ ppm,400 MHz) 3.35(dd,H_{4a}),3.71(dd,H_{4e}),5.91(dd,H_{5a}),2.42(acetyl methyl groups),7.68-6.93(Aromatic protons and thiophene protons),2.42(Phenyl methyl proton).**^{13}C NMR** ($CDCl_3$, δ ppm,100 MHz)55.16(C-5 of pyrazole moiety),42.07 (C-4 of pyrazole moiety), 153.98 (C-3 of pyrazole moiety), 21.98 (acetyl methyl carbon), 168.86 (amide cabonyl carbon),128.48-124.54 (Aromatic and thiophene carbons)144.42,140.78(Ipso carbons),21.52(Phenyl methyl carbon).

1-acetyl-4, 5-dihydro-5-methoxyphenyl-3-(thiophen-2yl)pyrazoles (8)

Yield 70%; m.p 90°C; Molecular Formula $C_{16}H_{18}N_2SO_2$; %Calcd. (Found); C%= 63.57 (63.63) ,H% = 5.96(6.0), N% = 9.23(9.27), S%=10.59(10.61), m/z (M)+:302 FT-IR(KBr, cm^{-1}): 3026.31 cm^{-1}(Aromatic CH stretching),2899.01cm^{-1} (Aliphatic CH stretching),1660.71 cm^{-1}(Amide C=O stretching), 1425.4 cm^{-1}(C=N Stretching), 636.51,709.8 cm^{-1}(Aromatic ring stretching). ^{1}H NMR($CDCl_3$,δ ppm,400 MHz) 3.33(dd,H_{4a}),3.70(dd,H_{4e}),5.90(dd,H_{5a}),2.41(acetyl methyl groups),7.72-7.03(Aromatic protons and thiophene protons),3.87(Phenyl methoxy proton).^{13}C NMR ($CDCl_3$, δ ppm,100 MHz)55.42(C-5 of pyrazole moiety),42.17 (C-4 of pyrazole moiety), 153.70 (C-3 of pyrazole moiety), 21.96 (acetyl methyl carbon), 168.74 (amide cabonyl carbon),128.23-114.20 (Aromatic and thiophenecarbons)144.48(Ipso carbon),55.13(Phenyl methoxy carbon).

Spectral analysis

FT-IR Spectrum of Compound 5

FT-IR spectrum of compound **5** shows characteristic absorption frequencies at 3082 cm^{-1} due to aromatic CH stretching vibration. The absorption bands at 2910.58 cm^{-1} are attributed to the aliphatic CH stretching vibration. The absorption frequency at 1647.21 cm^{-1} is assigned to amide carbonyl stretching vibration. The absorption band at 1423.47cm^{-1} is assigned to C=N stretching vibration. The absence of carbonyl band clearly supported for the formation of compound **5**, besides the disappearance of NH stretching vibration, which conforms the situ acetylation reaction due to acetic anhydride solvent.

Table 1: Physical and analytical data of the compounds (5-8)

Compound	X	Time Δ (h)/minutes		Yield %		m.p (°C)	Elemental analysis % [Found% (Calc .%)]				m/z (M)+ Molecular formula
		Conventional method (h)	Micro Wave irradiation method (minutes)	Conventional method	Micro Wave irradiation method		C	H	N	S	
5	H	7	15	70	86	94	66.24 (66.17)	5.93 (5.88)	10.29 (10.28	11.74 (11.78)	272, $C_{15}H_{16}N_2SO$
6	Br	7	15	75	90	100	51.47 (51.42)	4.31 (4.28)	8.0 (7.8)	9.21 (9.16)	347,349 $C_{15}H_{15}N_2SOBr$
7	CH_3	5	9	70	88	126	67.19 (67.13)	6.34 (6.29)	9.79 (9.75)	11.19 (11.12)	286, $C_{16}H_{18}N_2SO$
8	OCH_3	5	10	70	86	90	63.63 (63.57)	6.0 (5.96)	9.27 (9.23)	10.56 (10.61)	302, $C_{16}H_{18}N_2SO_2$

Elemental analysis of compound 5(C_{cal} 66.17, C_{obs} 66,24 ; H_{cal} 5.88 , H_{obs} 5.93 ;N_{cal} 10.28, N_{obs} 10.29;S_{cal} 11.78, S_{obs}11.74 are consistent with the proposed molecular formula($C_{15}H_{16}N_2SO$) of compound **5**.

The mass spectrum of the compound 6 shows the proposed molecular formula of the compound **6**. The m/z value $M^{+\cdot}$ 347 & 349.

^{1}H NMR Spectrum of Compound 5

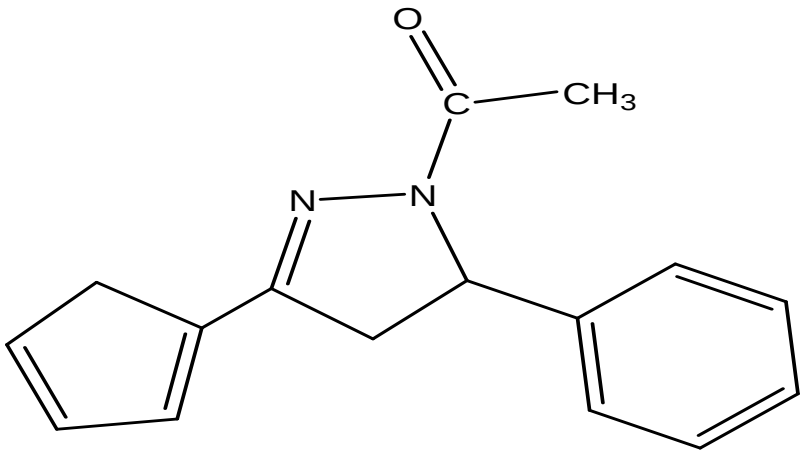
In the H NMR spectrum of compound 5, the methylene protons (H-4a and H-4e) of the pyrazoline moiety appeared as two doublets of doublets due to multiple coupling involving both geminal and vicinal protons. The signals for H-4a and H-4e are observed at 3.37 and 3.75 ppm. The doublet of doublet at 3.37ppm ($J_{4a,5a=}$17.5 Hz and $J_{4a,4e}$=4.0 Hz)is assigned to H-4a proton of the pyrazoline moiety. Likewise, the doublet of doublet at 3.75 ppm($J_{4e,4a}$ =17.5 and $J_{4e,5a}$ =11.5 Hz) is assigned to H-4e proton of the pyrazoline moiety. Similarly, the methine proton (H-5) of the pyrazole moiety is expected to give signal as a doublet of doublet due to vicinal coupling with the two magnetically nonequivalent protons of the methylene group (H-4a-H4e) of the pyrazoline moiety and the signals are observed at 5.93 ppm ($J_{5a,4a}$= 11.5Hzand $J_{5a,4e}$ =4.0 Hz).Also the acetyl methyl protons of pyrazoline moiety gives signal as a singlet at 2.43 ppm. The aromatic protons appear as a multiplet in the range of 6.93-7.79 ppm. The ^{1}H NMR coupling constant (J,Hz) values of 5-8 are given in **Table-2.**

Table 2: Coupling constant values of compound 5-8

Compound	Coupling constant J (Hz)					
	$J_{4a,4e}$	$J_{4a,5a}$	$J_{4e,4a}$	$J_{4e,5a}$	$J_{5a,4a}$	$J_{5a,4e}$
5	4.0	17.5	17.5	11.5	11.5	4.0
6	4.0	17.5	17.5	11.5	11.5	4.0
7	4.0	17.5	17.5	11.5	11.5	4.0
8	3.75	17.25	17.5	11.0	11.25	3.75

^{13}C NMR Spectrum of Compound 5

In the ^{13}C NMR spectrum of compound 5, 1-acetyl-4, 5-dihydro-5-phenyl-3-(thiophen-2yl)pyrazoles, ^{13}C resonance at 55.26 ppm is assigned to C-5 of pyrazole moiety. The ^{13}C resonance observed at 42.04 ppm is due to C-4 of pyrazole moiety. The ^{13}C resonance observed at 153.84 ppm is assigned to C-3 of pyrazole moiety. The aromatic carbons are observed in the region of 124.59-128.79 ppm. The ^{13}C resonance observed at 21.98 ppm is due to acetyl methyl carbon. The remaining ^{13}C signal at 144.32 is due to ipso carbon. From the above Spectral studies the synthesized compounds were confirmed. The proposed structure of the synthesized compound is 1-acetyl-4, 5-dihydro-5-phenyl-3-(thiophen-2yl)pyrazoles.



1-acetyl-4, 5-dihydro-5-phenyl-3-(thiophen-2yl)pyrazoles

LIPINSKI'S RULE

The above synthesized compounds (**5-8)** obey the Lipinski's Rule of Five, because they have not (1). No more than 5 Hydrogen bond donors (The total number of Nitrogen-Hydrogen and Oxygen-Hydrogen bonds). (2). Not more than 10 Hydrogen bond acceptors (all Nitrogen's and Oxygen atoms). (3). A molecular mass of our synthesized compounds are not exceeding 500 Daltons. (Molecular masses of Compound **5**- 272 , Compound **6**-349,347, Compound **7**-286 ,Compound **8**- 302).(4). Melting point of our synthesized compounds are not exceeding 500°C (Melting points of Compound **5**- 94°C, Compound**6**- 100°C, Compound**7**-126°C ,Compound**8**- 90°C).(5). The log p value also not exceeding greater than 5(log p values of Compound 5- 2.04, Compound6- 2.87, Compound7- 2.53 , and Compound 8- 1.92).So the above synthesized compounds are obeyed the Lipinski's Rule[26] of Five. Therefore our synthesized compounds (**5-8)** are drug molecules. The Values are shown **Table 3**.

Table 3: Lipinski's Rule For Compounds (5-8)

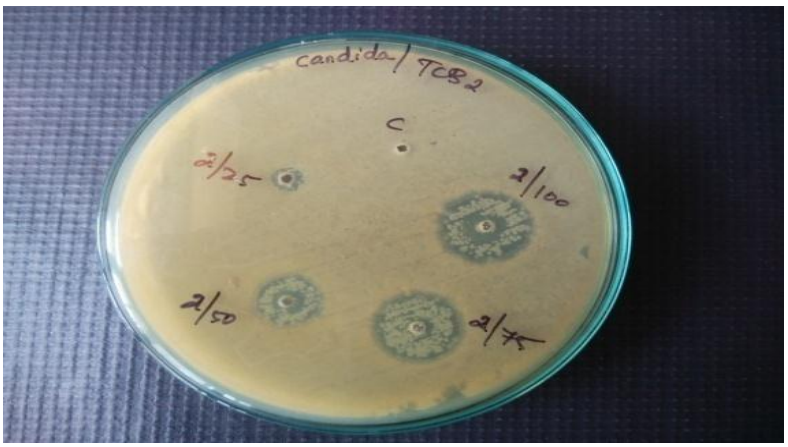
Compound	No. Of H-bond donors	No. of H-bond acceptors	Molecular mass m/z	Melting point in °C	log p
5	<5	<10	272	94	2.04
6	<5	<10	349,347	100	2.87
7	<5	<10	286	126	2.53
8	<5	<10	302	90	1.92

Biological Activity

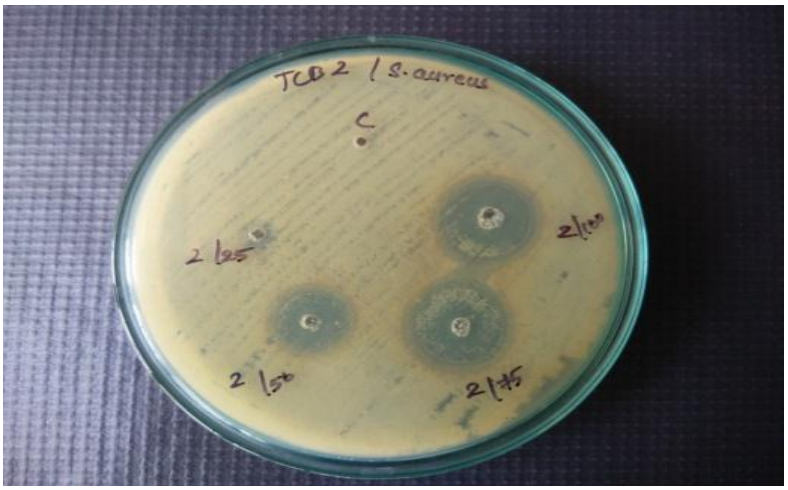
From the **Table: 4 & Fig-1**.shows, moderate (0.25mg/ml) to good (1.00mg/ml) bacterial activity against the Gram positive and Gram negative bacterial strains for all the compounds **(5-8)**.

1. Structure activity relationships (SAR) of the synthesized compounds were studied and it was given in the **Table-4**. The bromo substituted compound has more activity than others. All the compounds showed better inhibition in 1.00mg/ml concentration. From the above studies we concluded that the electron withdrawing group enhances the antibacterial activity. So the bromo substituted phenyl ring is having an excellent antibacterial activity of these compounds (**5-8)**.

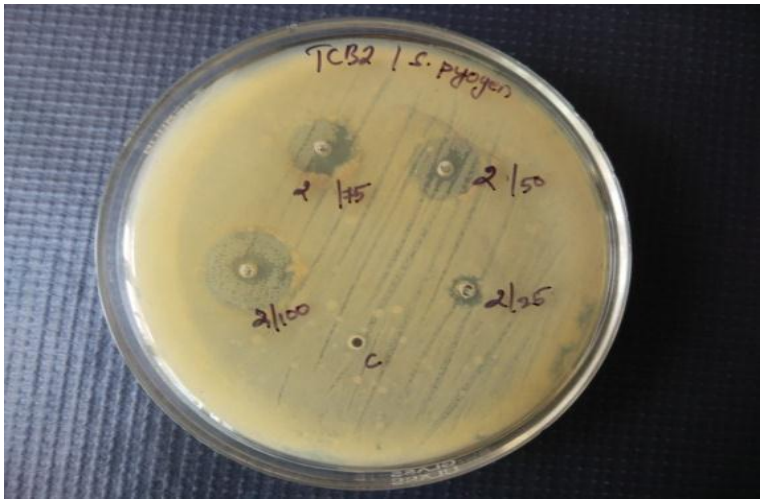
In Antifungal activities, all the synthesized compounds show a good to excellent antifungal activity against the fungal stains C.*albicans*. From the above studies we concluded that the electron donating group enhances the antifungal activity. So the methoxy substituted phenyl ring is having an excellent antibacterial activity of these compounds (**5-8)**. The bromo substituted and methyl substituted compounds having a similar antifungal activity of these compounds (**5-8)**.



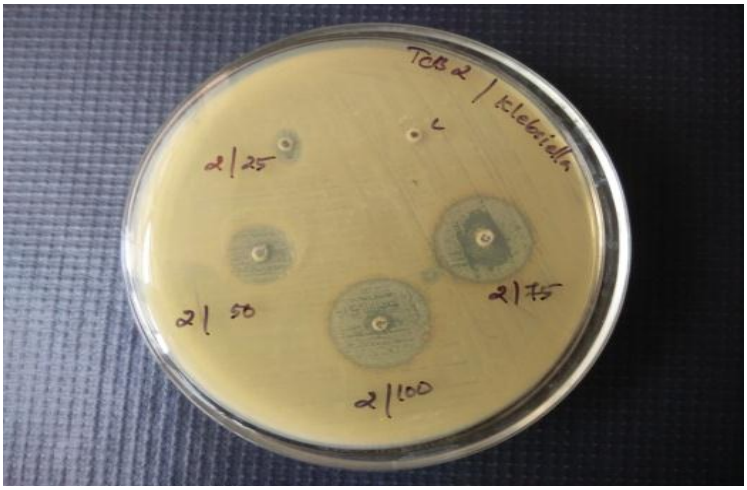
***Candida* species for compound-6**



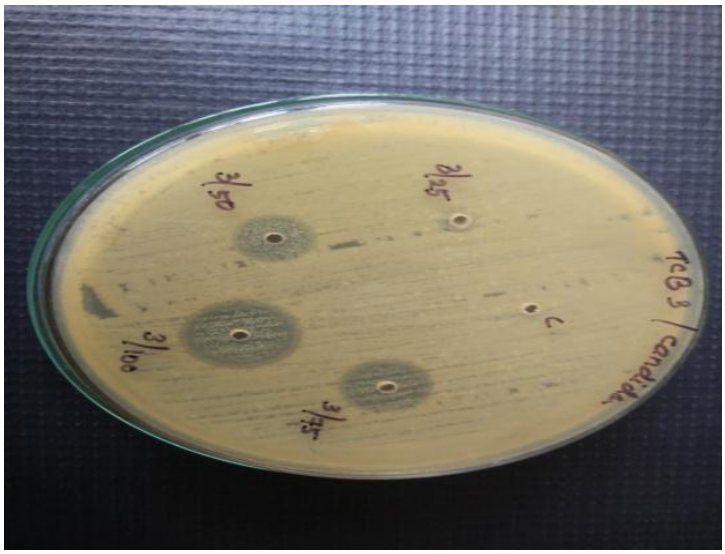
***S.aureus* for compound-6**



S.Pyogeses **for compound-6**



Klebsiella.Pneumoniae **for compound-6**



Candida **species for compound-7**

Fig 1: Antimicrobial activity of compound 6 and 7

Table 4: Antimicrobial activities of compound 5-8

Compound	Zone of Inhibition(mm in diameter)																			
	Bacterial Strains																Fungal Strains			
	S.aureus				*S.pyogenes*				*P.aeruginosa*				*K.pneumoniae*				*C.albicans*			
	0.25	0.50	0.75	1.00	0.25	0.50	0.75	1.00	0.25	0.50	0.75	1.00	0.25	0.50	0.75	1.00	0.25	0.50	0.75	1.00
5	5	8	12	15	6	10	12	15	5	9	15	19	7	12	15	19	5	10	15	17
6	5	12	15	20	6	10	13	18	6	10	20	23	5	10	18	20	5	10	15	19
7	10	13	1	18	8	10	14	16	-	12	17	20	14	16	18	20	7	12	15	19
8	8	15	18	19	7	10	15	20	6	10	17	18	-	12	14	20	6	12	15	20

5-9 - less activity (inhibition mm in diameter)
10-14 - moderate activity (inhibition mm in diameter)
15-19 - good activity (inhibition mm in diameter)

Above 20 -Excellent activity (inhibition mm in diameter)

CONCLUSION

Microwave irradiation method was the best method for the synthesis of compound **(5-8)** followed by green chemistry. All the synthesized compounds are characterized by IR, Mass, ^{1}H NMR, ^{13C} NMR spectral studies, elemental analysis and antimicrobial activities. All the synthesized compounds **(5-8)** show a good to excellent antimicrobial activity in the concentration of 1.00 mg/ml and also these compounds shows a poor activity in the concentration of 0.25 mg/ml. The electron withdrawing group enhances the antibacterial activity and the electron donating group enhances the antifungal activity.

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