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Comparative Conventional and Microwave assisted synthesis of some pyrazoline derivatives and their antimicrobial activity

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ABSTRACT

A novel and simple method have been developed for the synthesis of some Pyrazoline derivatives under microwave irradiation. In addition, these compounds were obtained with conventional heating procedures to compare them with those obtained with microwave irradiation. All the compounds synthesized were characterized by running TLC, Elemental analysis, IR, NMR and MS spectra. Consequently, the microwave irradiation method provided nearly the same or higher product yields in a very short period of time. These results suggest that the microwave irradiation method was more useful than the conventional method due to shorter reaction time and energy savings.

Key words: Synthesis, microwave irradiation; conventional heating Isonicotinohydrazide, ethyl 2-cyanoacetate, hexane-2, 4-dione.

INTRODUCTION

Pyrazoline nucleus shows various biological and pharmacological activities such as antimicrobial,[1-4] antiinflammatory,[5-6] antitumor,[7] anticonvulsant,[8] antiviral,[9]

diuretic,[10] antidiabetic,[11] Analgesic,[12] and anticancer,[13] . The chemistry and antimicrobial activity of some substituted pyrazolone have been investigated in recent years and it was thought worldwide to synthesized novel pyrazolones from easily available starting materials and evaluations of their possible antimicrobial and anti-inflammatory activity. It is well known that the application of microwave irradiation for carrying out chemical transformations offers pollution free and eco-friendly route. Therefore, in the present work techniques are rather slow and create temperature gradients within the sample. So here we are report synthesis of some new pyrazoline derivatives using diethylmalonate, Acetyl acetate, 1- chlorohexane-2-4-dione, ethyl acetate and ethylcynoacetate by conventional and microwave assisted methods has been reported. The reaction carried out in absolute alcohol or DMF in conventional method required about 4-12 hrs, while microwave irradiation method required only 2 to 3.30 min. In conventional method, the yields are lower as compared to microwave irradiation. The route for the synthesis of newer compounds has been depicted in Scheme -1.

EXPERIMENTAL SECTION

All the reactions were carried out in a domestic microwave oven (Kenstar, OM26.EGO). Melting points of synthesis compounds were determined in open capillaries in liquid paraffin are uncorrected. Purity of the compounds in addition to elemental analysis were verified by percolated TLC using silica gel G .as a adsorbent using ethyl acetate: n-hexane (7:3) as a eluent and spot was detected by using iodine vapors. The IR (KBr pellets) spectra were recorded on a Perkin Elimer-1800- spectrophotometer and H^1NMR spectra were recorded on BRUKER DRX-300MHz spectrophotometer, (TMS as a internal reference) and chemical shifts are expressed in δ. MASS spectra were recorded on Jeol D30 spectrophotometer. Elemental analyses for C, H and N were conducted using a Perkin -Elmer C, H, and N analyzer. Their result was found to be an in good agreement with the calculated values (±0.4%).

Synthesis of 1- Isonicotinoylpyrazolidine-3, 5-dione (1)

Conventional method: Isonicotinohydrazide (0.01 mole), diethyl malonate (0.01 mole), acetic acid (0.05 mole) and absolute ethanol (20.0 mL) were taken in a round bottom flask. The mixture was well stirred and refluxed on a water-bath for about 11-12 hr.

Microwave method: Isonicotinohydrazide (0.01 mole), diethyl malonate (0.01 mole) and two to three drops of glacial acetic acid were taken in an Erlenmeyer flask. Then the well-stirred mixture was irradiated in microwave oven for 3 min at 480 W (i.e, 40 % microwave power). The completion of the reaction was monitored by TLC. The brown coloured oily mass obtained was cooled and the crude product was recrystallized from ethanol to give compound **1**. Spectral and analytical data were found to be similar for compounds obtained by both reported methods for conventional and microwave methods.

Synthesis of 1-acetyl-2-isonicotinoylpyrazolidine-3, 5-dione (2)

Conventional method: A mixture of isonicotinohydrazide (0.01 moles) and diethyl malonate (0.01mole) were taken in a round bottom flask and dissolved in (20.00 mL) glacial acetic acid. Then the well-stirred mixture was refluxed for 8-10 hr.

Microwave method: Isonicotinohydrazide (0.01 mole), glacial acetic acid (0.02 mole) and diethyl malonate (0.01mole) was taken in Erlenmeyer flask. Then the well-stirred mixture was irradiated in microwave oven for 2.30 min at 480 W (i.e, 40% microwave power). The completion of the reaction was monitored by TLC. The solid reaction mixtures was cooled and and poured into crushed ice.

Synthesis of (3, 5-dimethyl-1*H*-pyrazol-1-yl) (pyridin-4-yl) methadone (3)

Conventional method: A mixture of isonicotinohydrazide (0.01 mole) and acetyl acetone (0.01 mol) in 20 mL of absolute alcohol was heated under reflux for 5-6 hr with stirring. The completion of the reaction was monitored by TLC.

Microwave method: A mixture of isonicotinohydrazide (0.01 mole) and acetyl acetone(0. 01 mol) was taken in Erlenmeyer flask. The mixture was well stirred and irradiated in microwave oven for a period 2 min. with intermitted irradiation for 30 sec. interval. The reaction mixture was then allowed to stand at room temperatures. The solid product obtained was filtered off, washed with water and dissolved in methanol then filtered, dried and recrystallized from ethanol to afford compound **3**. The physical data and R_f value are recorded in **Table 1**. Elemental analysis and spectral data are recorded in **Table-2** and **Table-3**

Synthesis of 3-(chloro-methyl)-1-isonicotinyl-1*H* pyrazole-5 (4*H*)-one (4)

Conventional method: A mixture of isonicotinohydrazide (0.01 mole) and 1-chloro-hexane 2-4 dione (0.01 mole) was taken in absolute alcohol (20.00 mL). The mixture was well stirred and refluxed for 12-14 hours.

Microwave method: Mixture of isonicotinohydrazide (0.01 mole) and 1-chloro-hexane 2-4-dione (0.012 mole) was taken in an Erlenmeyer flask. The well-stirred mixture was irradiated in microwave oven for 3.30 min at 480 W (i.e, 40 % microwave power) with intermitted irradiation for 30 sec. interval.

Synthesis of 1- isonicotinoyl-3methyl-1*H*-pyrazol-5 (4*H*)-one (5)

Conventional method: An equimolar mixture (0.01 mole) of isonicotinohydrazide, hexane-2, 4-dione and acetic acid (0.005 mole) were taken in a round bottom flask and dissolved in absolute alcohol (15 mL). Then the reaction mixture was refluxed for about 10-11 hr. The progress of reaction was observed by TLC.

Microwave method: A mixture of isonicotinohydrazide (0.01 mole), hexane-2, 4-dione and acetic acid (0.005 mole) were taken in an Erlenmeyer flask and mixed thoroughly. The mixture was then irradiated under microwave oven for 3.00 min at 480 W (i.e, 40 % microwave power) with intermitted irradiation for 30 sec. interval. Upon completion of the reaction (monitored by TLC), the reaction mixture was poured onto the crushed ice. The solid mass obtained was filtered and washed several times with water. Purification by recrystallization with alcohol gave product **5**.

Synthesis of 3-Amino-1-isonicotinoyl-1H-pyrazol-5 (4H)-one (6)

Conventional method: Isonicotinohydrazide, (0.01 mole) and ethyl 2-cyanoacetate (0.01 mole) was taken 20.0 mL of DMF and the mixture was refluxed for 10-11 hours. The reaction mixture

was allowed to attain the room temperature. The mixture was then poured into the ice-cold water. The resulting solid product **6** was filtered, dried, and recrystallized from methanol.

Microwave method: A mixture of isonicotinohydrazide (0.01 mole), and (0.01 mole) ethyl 2-cyanoacetate was taken in an Erlenmeyer flask and mixed thoroughly. The mixture was irradiated under microwave for 2.00 min at 600 W (i.e, 50 % microwave power) power with intermittent radiation of 30 sec. interval. The progress of the reaction was monitored by the TLC. The mixture was then poured into the ice-cold water, filtered, dried, and recrystallized from methanol to give product **6**. The physical data and R_f values are reported in Table-1. Elemental analysis and spectral data are recorded in Table-2 and Table-3.

Synthesis of 1–isonicotinoyl-4-phenylthiosemicarbazide (7)

Conventional method: A mixture of isonicotinohydrazide (0.01 mole) and phenyl isothiocynate (0.01 mole) was dissolved in DMF (20.00 mL) in a round bottom flask and two to three drops of morpholine was added as a catalyst Then the well-stirred mixture was refluxed for 4-5 hours. The reaction mixture was then allowed to stand at room temperatures. After that the mixture was poured in ice-cold water. The resulting solid product was filtered, dried, and recrystallized from benzene to give **7**

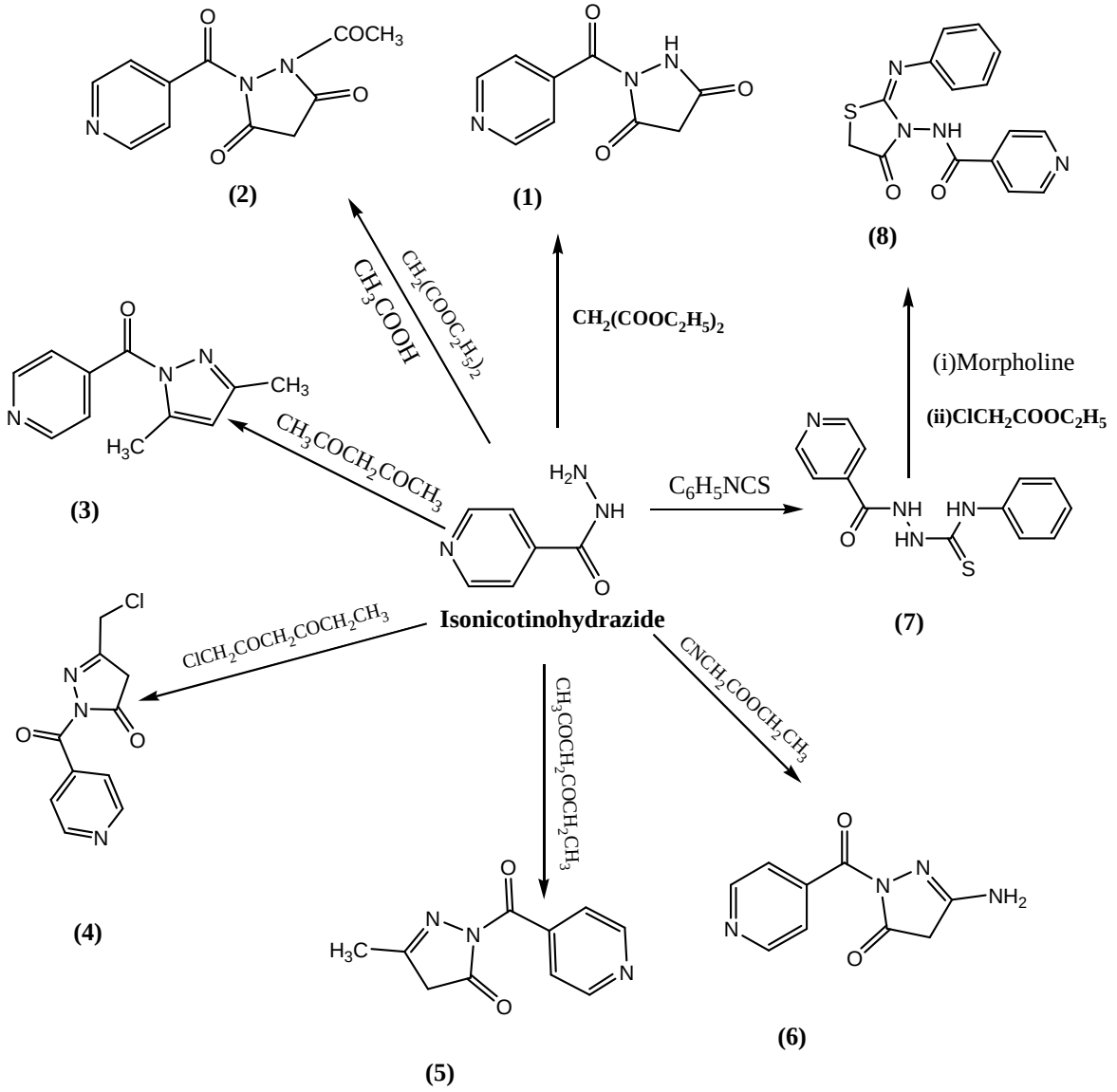
Microwave method: A mixture of isonicotinohydrazide (0.01 mole) and phenyl isothiocynate (0.01 mole) in a 20.0 mL DMF was taken in an Erlenmeyer flask. In the reaction mixture two to three drops of morpholine was added as a catalyst. The mixture was irradiated under microwave for 2.00 min at 400 W power with intermittent radiation of 15 sec interval. The resulting solid was filtered, dried and recrystallized from benzene to give product **7**.

Synthesis of N- (4-oxo-2- (phenylimino) thiazolidin-3-yl) isonicotinamide (8)

Conventional method: To a mixture of 6 (0.01 mole), ethylchloroacetate (0.01 mole) and 2-3 drops of morpholine were taken in a round bottom flask and dissolved in ethanol (15 ml). After then reaction mixture was refluxed for 7 hours. The resulting solid product **8** was filtered, dried, and recrystallized from methanol.

Table- 1. The physical data and R_f values of synthesized compounds

Compound.	Reactions condition	Molecular Formulal	Mol. Wt.	Conventional Method		Microwave Method		m.p °C	R_F
				Yield (%)	Time (Hrs)	Yield %	Time (min)		
1	Acetic acid	$C_9H_7N_3O_3$	205	48	11-12	88	3.00	145	0.63
2		$C_{11}H_9N_3O_4$	247	42	8-10	87	2.30	175	0.67
3		$C_{11}H_{11}N_3O$	201	52	5-6	88	2.00	172	0.68
4		$C_{10}H_8ClN_3O_2$	237	53	12-14	86	3.30	168	0.65
5	Acetic acid	$C_{10}H_9N_3O_2$	203	50	10-11	87	3.00	162	0.59
6		$C_9H_8N_4O_2$	204	51	10-11	88	2.00	116	0.73
7		$C_{13}H_{12}N_4OS$	272	50	4-5	83	2.00	138	0.67
8	Morpholine	$C_{15}H_{12}N_4O_2S$	312	48	6-7	84	2.30	168	0.61



Scheme-1

Microwave method: A mixture of 7 (0.01 mol) and ethylchloroacetate in presence of catalytic amount of morpholine were taken in Erlenmeyer flask and mixed thoroughly. Then the mixture was irradiated under microwave oven for 2.30 min at 480 W (i.e. 40% microwave power) with an intermitted irradiation for 30sec. interval.

Table: 2 Elemental analysis data of compounds

Compd.	Mol. Formula	Elemental Analysis %					
		Found			Calculated		
		C	H	N	C	H	N
1	$C_9H_7N_3O_3$	52.53	3.24	20.77	52.69	3.44	20.48
2	$C_{11}H_9N_3O_4$	53.14	4.01	17.24	53.44	3.67	17.00
3	$C_{11}H_{11}N_3O$	63.36	5.31	21.08	66.66	5.51	20.28
4	$C_{10}H_8ClN_3O_2$	50.24	3.59	18.08	50.54	3.39	17.68
5	$C_{10}H_9N_3O_2$	59.34	4.75	21.00	59.11	4.46	20.68
6	$C_9H_8N_4O_2$	52.92	4.24	27.77	52.92	3.95	27.44
7	$C_{13}H_{12}N_4OS$	57.55	4.24	20.70	57.34	4.44	20.57
8	$C_{15}H_{12}N_4O_2S$	57.35	4.14	18.20	57.28	3.87	17.94

Antimicrobial activity

All the synthesis compounds 1-8 were screened for In *vitro* antimicrobial study. It was carried out on Muller Hinton agar (Hi-media) plates (37 ^{0}C, 24 h) by agar diffusion cup plate method [14-15]. All the compounds were screened for antimicrobial activity at, 200,100 and 50 µg/ml concentration against the bacterial strains: *Escherichia coli, Staphylococcus aureus, Pseudomonas eruginosa* and *Staphylococcous aureus*, Antifungal activity was tested on Sabouraud dextrose agar (Hi-media) plates (26 0C, 48-72 h) by cup plate method[16] against *Candida albicans* and *Aspergillus niger* at the concentration level of 200,100 and 50µg/ml, Ciprofloxacin and Griseofulvin were used as a standards for comparison of antibacterial and antifungal activity under the similar conditions. DMSO was used as a solvent control for both antibacterial and anti fungal activities. The results are summarized in **Table- 4** and **Table-5** that include the activity of reference compound Ciprofloxacin and Griseofulvin respectably. The tested compounds exhibited mild to moderate antibacterial activity against all four strains of bacteria. The compounds, 1, 2,4 and 8 are active on *E. coli* where as 5 and 8 are active on *P. aeruginosa*. It has also been observed that compounds **3** and **4** showed activity against *B.subtitis*, and 2 and 4 are active on *S. aureus*.

The antifungal activity of the compounds was studied for the two pathogenic fungi. It was observed that compounds 2 and 4 had highest activity against *C. albicans* and *A. niger*. It has also been observed that compound 8 showed good activity against *C. albicans.*

RESULT AND DISCUSSION

In conventional method, the yield of all the products is lower as compared to the yield obtained by synthesis by microwave irradiation technique. Microwave irradiation method facilitates the polarization of the reacting molecule causing reactions to occur at higher rate. A comparative study in terms of yield and reaction time is shown in Table-I. The compounds **(1-7)** were synthesized by condensation of isonicotinohydrazide and diethyl malonate or in glacial acetic acid or acetyl acetone or 1-chloro-hexane 2-4 dione or hexane-2, 4-dione and acetic acid or ethyl 2-cyanoacetateor phenyl isothiocynate respectively. Compound **8** was synthesized by the condensation reaction of **7** and ethylchloroacetate in presence of morpholine. The above-mentioned compounds were also synthesized by the conventional method. It is noteworthy that the reaction which required 5-12 hr in conventional method was completed within 2.00-3.30 min in Microwave system at power level of 480-600 W. Yield have been remarkably improved from 42-53% to 83- 90 %. Their IR, ^{1}H NMR and mass spectral studies established structure of

Table: 3 Spectral data of synthesized compounds

Comp.1	**1-Isonicotinoylpyrazolidine-3, 5-dione**	
	IR(KBr cm^{-1})	3303(-NH), 1666, 1635(C=O), 1555 (C=N), 1219 (N-N)
	^{1}H NMR (δ ppm)	7.7-7.9 (m, 4H, pyridine), 8.70 (s, H, NH) 3.35 (s, 2H, CH_2);
	MS: (m/z)	205 $[M]^+$. 205$[C_9H_6N_3O_3]^+$, 162 $[C_8H_6N_2O_2]^+$, 135 $[C_6H_5N_3O]^+$, 127 $[C_4H_3N_2O_2]^+$, 106 $[C_6H_4NO]^+$, 99 $[C_3H_3N_2O_2]^+$, 78 $[C_5H_4N]^+$
Comp.2	**1-Acetyl-2-isonicotinoylpyrazolidine-3, 5-dione**	
	IR(KBr cm^{-1})	1730, 16931666, 1635 (C=O), 1553 (C=N),1226 (N-N));
	^{1}H NMR (δ ppm)	3.49 (s, 2H, CH_2), 7.79-7.81(m, 4H, pyridine), 2.40 (s, 3H, CH_3).
	MS: (m/z)	232 $[C_{10}H_6N_3O_4]^+$, 204 $[C_9H_6N_3O_3]^+$, 169 $[C_6H_5N_2O_4]^+$, 141 $[C_5H_5N_2O_3]^+$,127$[C_4H_3N_2O_3]^+$, 106 $[C_6H_4NO]^+$, 98 $[C_3H_2N_2O_3]^+$ 78 $[C_5H_4N]^+$
Comp.3	**(3, 5-dimethyl-1H-pyrazol-1-yl) (pyridin-4-yl) methanone**	
	IR(KBr cm^{-1})	1693, (C=O), 1553 (C=N), 1232 (N-N)
	^{1}H NMR (δ ppm)	7.79-8.79 (m, 4H, pyridine), 5.71(s, 1H, CH) 2.10 (s, 3H, CH_3) 3.35 (s, 3H, CH_3).
	MS: (m/z)	201 $[M]^+$, 186 $[C_{10}H_8N_3O]^+$, 172 $[C_9H_6N_3O]^+$, 160 $[C_9H_8N_2O]^+$, 123$[C_6H_7N_2O]^+$, 120$[C_6H_4N_2O]^+$, 106 $[C_6H_4NO]^+$, 95 $[C_4H_3N_2O]^+$, 78 $[C_5H_4N]^+$
Comp.4	**3-(Chloromethyl)-1-isonicotinoyl-1H-pyrazol-5(4H)-one**	
	IR(KBr cm^{-1})	1695,1666 (C=O), 1606(C=N), 1239 (N-N)
	^{1}H NMR (δ ppm)	7.7-7.8 (m, 4H, pyridine), 3.45 (s, 2H, CH_2), 2.80 (s, 2H, CH_2),
	MS: (m/z)	237$[M]^+$, 202$[C_{10}H_8N_3O]^+$, 195 $[C_8H_6ClN_3O]^+$, 188 $[C_9H_6N_3O_2]^+$, 162 $[C_8H_6N_2O_2]^+$, 158$[C_5H_4ClN_2O_2]^+$, 111$[C_4H_3N^2O_2]^+$, 106 $[C_6H_4NO]^+$, 78 $[C_5H_4N]^+$
Comp.5	**1-isonicotinoyl-3-methyl-1H-pyrazol-5(4H)-one**	
	IR(KBr cm^{-1})	1695, 1606(C=O), 1530, 1510(C=N), 1301(N-N)
	^{1}H NMR (δ ppm)	7.79-7.81(m, 4H, pyridine), 3.45 (s, 2H, CH_2), 2.06 (s, 2H, CH_3)
	MS: (m/z)	203 $[M]^+$, 188 $[C_9H_6N_3O_2]^+$, 162 $[C_8H_6N_2O_2]^+$, 148 $[C_7H_7N_2O_2]^+$, 125 $[C_5H_5N_2O_2]^+$,106$[C_6H_4NO]^+$, 111$[C_4H_3N_2O_2]^+$, 97$[C_4H_5N_2O]^+$, 83$[C_3H_3N_2O]^+$, 78 $[C_5H_4N]^+$
Comp.6	**3-Amino-1-isonicotinoyl-1H-pyrazol-5(4H)-one**	
	IR(KBr cm^{-1})	3334, 3218(-NH_2), 1698, 1672(C=O), 1554(C=N), 1290 (N-N)
	^{1}H NMR (δ ppm)	7.20-7.77 (m, 4H,pyridine); 5.12(s, 2H, NH_2), 3.33(s2H, CH_2);
	MS: (m/z)	204 $[M]^+$, 188 $[C_9H_6N_3O_2]^+$, 162 $[C_7H_6N_4O]^+$, 148 $[C_7H_7N_2O_2]^+$, 126 $[C_4H_4N_3O_2]^+$,111 $[C_4H_3N_2O_2]^+$, 106$[C_6H_4NO]^+$, 98$[C_3H_4N_3O]^+$, 83$[C_3H_3N_2O]^+$, 78 $[C_5H_4N]^+$
Comp.7	**1-Isonicotinoyl-4-phenylthiosemicarbazide**	
	IR(KBr cm^{-1})	3416,3372,3312(-NH), 1689(C=O), 1568(C=N), 1275(N-N); 1100 (C=S),
	^{1}H NMR (δ ppm)	2.6 (s, 2H, CH2), 7.8-8.1(m, 4H, pyridine), 6.46-7.10. (m, 5H, Ar-H) 10.20 (s,H,HN-CO), 4.34 (s,H,HN-Ph), 2.20 (s,H,HN-N)
	MS: (m/z)	272 $[M]^+$, 195 $[C_7H_7N_4OS]^+$, 166 $[C_7H_8N_3S]^+$, 151 $[C_7H_7N_2S]^+$, 136$[C_7H_6N_2S]^+$, 121$[C_6H_5N_2O]^+$, 106$[C_6H_6NO]^+$, 92 $[C_6H_6N]^+$. 78 $[C_5H_4N]^+$,
Comp.8	**1-Isonicotinoyl-4-phenylthiosemicarbazide**	
	IR(KBr cm^{-1})	1698, 1649 (C=O), 1554 (C=N), 1235 (N-N) 740 (C-S-C)
	^{1}H NMR (δ ppm)	9.67 (s, H, HN) 7.6-7.8 (m, 4H, pyridine), 6.46-7.10. (m, 5H, Ar-H),
	MS: (m/z)	312 $[M]^+$,235 $[C_9H_7N_4O_2S]^+$, 234 $[C_{10}H_8N_3O_2S]^+$, 206 $[C_9H_8N_3OS]^+$, 191 $[C_9H_7N_2OS]^+$, 156$[C_4H_3N_2O_2S]^+$, 121$[C_6H_5N_2O]^+$, 106$[C_6H_6NO]^+$, 78 $[C_5H_4N]^+$,

compounds **1-8**. All the reaction has also been performed under classical heating conditions. The comparison of the results for all the compounds indicates that the reaction are efficiently promoted by microwave irradiating .The reaction time was striking shortened 8 to 10 hours to 2 to 3 min and quantatively yields were obtained. The yield and the physical constants of the Compounds synthesized by the conventional and microwave irradiation methods are given in Table- 1

Table 4: *In vitro* antibacterial activity of synthesized compounds

Compd.	Inhibition zone diameter in mm											
	E.coli			*P. aeruginosa*			*B.subtitis,*			*S.aureus*		
	50 µg/mL	100 µg/mL	200 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
1	14.45	22.20	27.60	14.68	20.10	24.60	14.12	22.20	24.68	14.24	22.20	24.20
2	10.45	22.46	28.40	10.54	22.40	27.44	15.46	20.46	26.44	20.34	20.46	28.40
3	14.24	21.34	27.50	14.35	21.30	24.50	18.20	22.30	28.50	15.18	20.34	20.34
4	14.26	20.46	28.20	14.45	23.20	24.20	16.60	23.40	27.70	20.34	24.46	28.46
5	19.56	24.24	27.20	19.34	22.10	26.00	15.00	24.24	25.26	18.26	24.24	26.50
6	1023	22.22	25.20	10.24	23.22	25.20	16.40	22.22	26.20	09.45	22.22	25.20
7	18.34	23.14	25.14	18.34	22.14	24.14	19.45	24.14	26.14	21.34	24.14	24.14
8	18.20	24.12	28.10	18.45	20.12	26.10	21.50	24.12	24.12	19.22	24.12	24.12
Ciprofloxacin	31.34	31.34	31.34	30.45	30.45	30.45	31.40	31.40	31.40	31.11	31.11	31.11

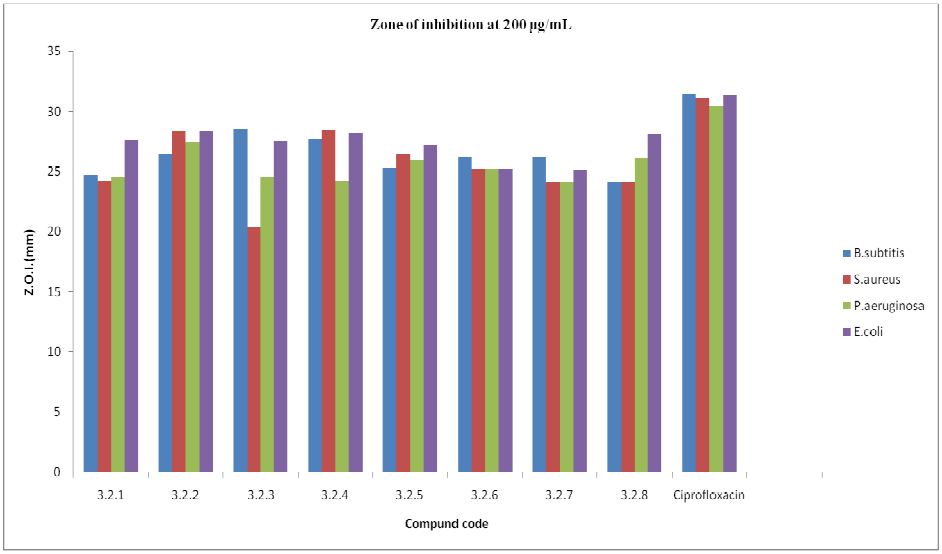


Fig. 1: Graphical representation of Zone of Inhibition for antibacterial activity at 200 µg/mL

Table 5: Antifungal activity of the synthesized compounds

Compd.	Antifungal activity (zone of inhibition in mm)					
	C.albicans			*A. niger*		
	50 µg/mL	100 µg/mL	200 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
1	06.20	12.20	20.40	05.35	11.40	12.50
2	08.10	24.30	26.40	11.35	17.40	28.20
3	06.10	14.30	20.45	06.40	15.50	20.60
4	10.20	25.30	18.30	08.30	16.20	22.30
5	11.30	12.20	13.30	11.20	11.20	11.20
6	15.10	16.40	20.10	12.30	15.10	16.20
7	11.20	12.30	13.10	11.20	11.10	11.30
8	07.20	22.20	26.10	06.20	12.20	20.20
Griseofulvin	29.20	29.20	29.20	30.10	30.100	30.10

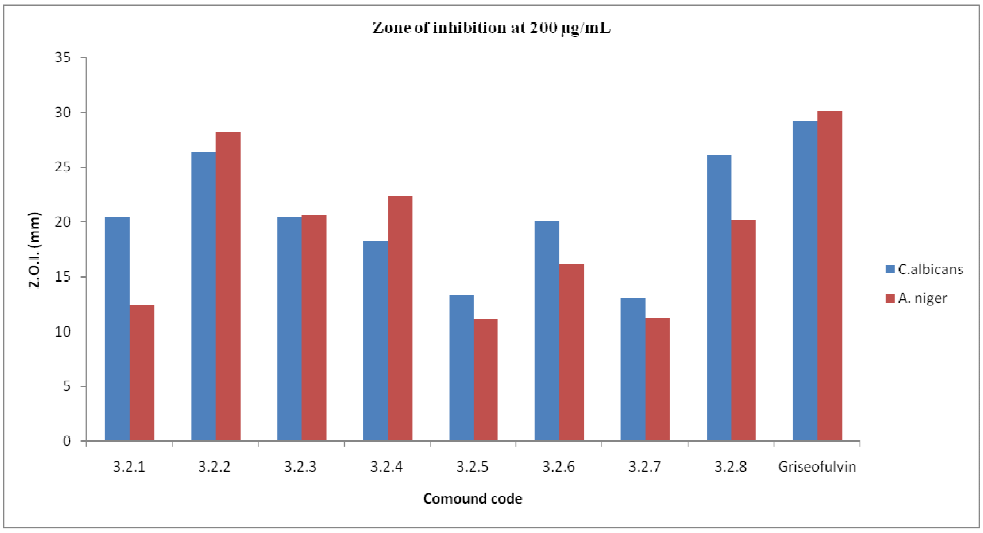


Fig 2: Graphical representation of Zone of Inhibition for antifungal activity

Table.4.7: *in vitro* Antimicrobial activity

Compound	MIC in (µg/ml)					
	E.coli	***P.aeruginosa***	***B. Subtilis***	***S.aureus***	***C.albicans***	***A.niger***
.1	250	250	250	250	250	500
.2	500	250	500	500	125	125
.3	250	250	250	500	250	250
.4	250	250	250	250	500	125
.5	125	250	250	125	250	--
.6	250	250	125	125	250	500
.7	250	250	125	125	500	--
.8	125	250	250	250	125	--
Ciprofloxacin	6.25	6.25	6.25	6.25	NT	NT
Griseofulvin	NT	NT	NT	NT	6.25	3.125

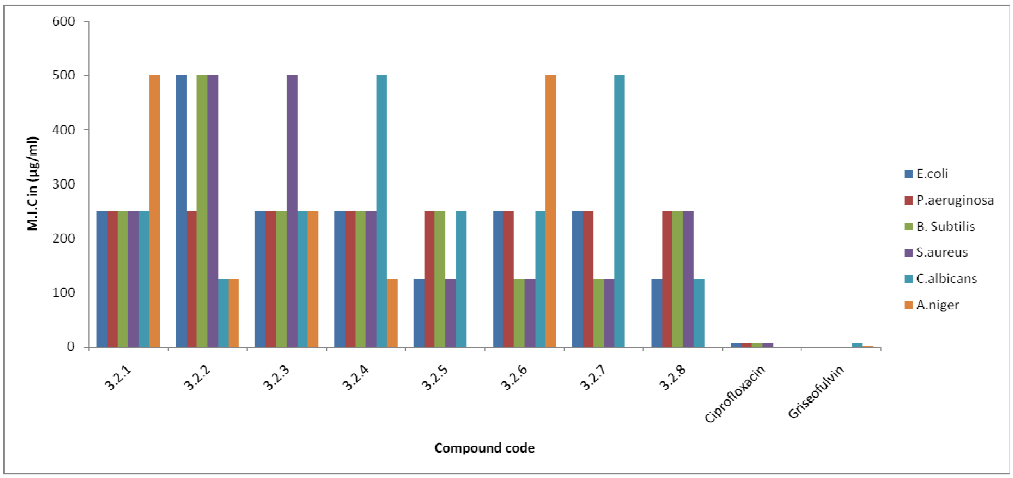


Fig 3. Graphical representation of M.I.C. for antibacterial activity

All the synthesized compounds were tested for *in vitro* antimicrobial activity. The tested compounds exhibited mild to moderate antibacterial activity against all four strains of bacteria. The compounds, **1 to 5 and 8** are active on *E. coli* whereas **5** and **8** is active on *P. aeruginosa*. It has also been observed that compounds **3** and **4** showed activity against *B.subtitis*, and. **2** and **4** are active on *S. aureus*. It was observed that compound **5** showed 125 (µg) MBC for *E. coli* and *S. aureus* while compounds **6** and **7** have given 125 MBC for bacteria *B.subtitis and S. aureus* .Similar 125 MBC have been recorded by the compound .8.for *E. coli* . Most of compounds showed 250 MBC for all the bacteria stains.

Griseofulvin and Amphotericin- B was used as reference drug for inhibitory activity against fungi. It was observed that compounds **2** and **4** had highest activity against *C. albicans* and *A. niger*. It has also been observed that compound **8** showed good activity against *C. albicans* . On the basis of biological activity results. It was observed that compounds **2** had 125 for MIC for *C. albicans* and *A. niger* fungi. Similarlr compounds **3.2.4** showed 125 MIC for *A. niger* and 8 for *C. albicans.* It was observed that most of compounds showed 250 MIC value for both the fungi.

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