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MICROWAVE ASSISTED RAPID, EFFICIENT SYNTHESIS AND SCREENING OF ACYL HYDRAZONE DERIVATIVES FOR ANTIBACTERIAL ACTIVITY

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Abstract

A series of new N'-(substituted benzylidene)-2-(benzamido)-3-(p-methoxy)-phenyl acrylohydrazides 3 (a-d) have been synthesized with good yields by the efficient, convenient and environmentally benign microwave technique involving the condensation of α-benzamido-(p-methoxy)-cinnamahydrazide (com 2) with aromatic aldehydes. 4-(p-Methoxy)-benzylidene-2-phenyloxazol-5-ones (com 1) was made to undergo hydrozinolysis, yields the key intermediate (com 2). The yields are moderate and purity is high. The structures of all newly synthesized compounds have been established on the basis of analytical and spectral data (IR, NMR & Mass). Of all the compounds screened, compounds 3b,3d showed good antibacterial activity against *Pseudomonas aeruginosa,Staphyloccocus aureus* respectively, which is higher than that produced by the same concentration of standard (streptomycin).

Key Words: Acyl hydrazones, anti bacterial activity, oxazolone, cinnamahydrazide, microwave technique

Introduction

The development of simple, efficient and environmentally benign chemical process (or) methodologies for widely used organic compounds from readily available reagents is one of the major challenges for the chemists in organic synthesis[1]. Microwave enhanced synthesis has attracted substantial attention in recent years, enabling many organic reactions to proceed much faster and with higher yields and superior to conventional method[2-3].The dramatically raising incidence of multi drug resistance microbial infections in past few decades has become a serious health problem. The search for new antimicrobial agents will consequently always remain as an important and challenging task for medicinal chemist[4]. Consequently, there has been an immense research on hydrazones as antibacterial agents[5]. The most important property of hydrazones is the high physiological activity. Extensive studies reveal that the one pair electron on trigonally hybridized nitrogen atom of the azomethine group is responsible for the chemical

and biological activities such as antitumoral[6], antimicrobial[7], antimalarial[8], anticonvulsant[9], antiinflammatory[10], antinociceptive[11] and antiplatelet[12] activities. In the view of these, it was planned to synthesize a new series of hydrazones by microwave irradiation and evaluated the compounds for antibacterial activity.

Materials and Methods

Experimental section

All the melting points reported in this series were determined in open capillaries using Thermonik Pricision Melting Point Cum Boiling Point Apparatus C-PMB and are uncorrected. Homogenity of the compounds was checked by using pre coated TLC plates. The IR spectra were recorded using KBr pellets on a perkin –Elmer 1760 spectrophotometer. ^{1}HNMR spectra were recorded on Bruker Avance 300MHz spectrophotometer, using tetra methyl saline as internal standard. Mass spectra were recorded on an Apex Mass spectrophotometer. Microwave irradiation was carried out in a domestic microwave oven (LGMG, 2450MHz). All the solvents were procured from Sigma Aldrich and used without purification.

Synthesis of 4-(p-methoxy)- benzylidene-2-phenyloxazol-5-ones 1

A mixture of anisaldehyde (10mmol), benzoyl glycine (10 mmol), acetic anhydride (30mmol), catalytic amount of zinc oxide (dry powder 0.49mg, 6mmol) and 10 ml of ethanol were stirred at room temperature (25ºc), the syrupy reaction mixture solidified for a certain period of time as required for completing the reaction. Then the reaction mixture was added to cold ethyl alcohol (30ml) for extraction, the solid obtained after solvent evaporation was washed with hot water (2x10ml), dried and recrystallized (ethanol: water) to afford pure yellow colour crystals of the title compound[13].

Synthesis of α-Benzamido-(p-methoxy)-cinnamahydrazide[14] 2

4-(p-methoxy) benzylidene-2-phenyloxazol-5-ones II (0.03 mmol) was stirred with a solution of hydrazine hydrate (0.06 mmol) in ethanol (25ml) for 20 minutes. The deep yellow colour of oxazolone immediately changed to light yellow colour product, which was filtered, washed and purified by recrystallization from methanol.

General procedure for the synthesis of compounds 3(a-d)

Synthesis of 2-(benzamido)-N-(substituted benzylidene)-3-(p-methoxy)-phenyl acrylohydrazide 3 (a-d):

Equimolar ratios of α-benzamido-(p-methoxy)-cinnamahydrazide and benzaldehyde (0.01 mol) in absolute ethanol with a few drops of glacial acetic acid were subjected to microwave irradiation at 210 W about 60 – 150 sec. The reaction was monitored by TLC and the mixture was allowed to cool at room temperature, filter and purified by recrystallization from methanol. The physical data of the title compounds are given as follows.

4-(p-methoxy)-benzylidene-2-phenyloxazol-5-ones,1: Molecular formula$C_{17}H_{13}NO_3$;Yield: 90%; m.p. 140-142^oC; IR (KBr): 3082cm^{-1} (Ar C-H), 2935cm^{-1}(C-H OCH_3), 1768 cm^{-1}(C=O), 1648cm^{-1} (C=N), 1592 cm^{-1} (C=C), 1260 cm^{-1} (C-O-C).

α-Benzamido-(p-methoxy) cinnamahydrazide, 2 : Molecular formula$C_{17}H_{17}N_3O_3$**;** Yield:75%; m.p. 153-154 ^oC; IR (KBr): 3288 cm^{-1} (NH_2), 3166 cm^{-1} (N-H), 3051cm^{-1} (Ar C-H), 1642cm^{-1} (C=O), 1601cm^{-1} (olefinic ,C=N); ^{1}HNMR (DMSO-d_6): δ 9.78 (1H, s, CO-NH-N), 8.03-6.8(9H, m, Ar C-H), 7.1 (1H, s, C= C-H), 4.51 (2H, s, NH_2), 3.741 (3H, s, OCH_3).

2-Benzamido-N'-benzylidene-3-(p-methoxy)-phenylacrylohydrazide, 3a: Molecularformula$C_{24}H_{21}N_3O_3$; Yield: 71 %; m.p. 180-182˚C; IR (KBr): 3201cm^{-1} (N-H), 3044cm^{-1} (Ar C-H), 1634cm^{-1} (C=O), 1602cm^{-1} (olefinic C=N), 1500,1444cm^{-1} (Ar C=C); ^{1}HNMR (DMSO- d_6): δ 11.6 (1H, s, CO-NH-N), 10.03 (1H, s, CO-NH),8.42 (1H, s, HC=N), 6.95-8.06(14H, d, m, Ar C-H), 3.77(3H, s, OCH_3).

2-Benzamido N'-(4-methoxy benzylidene) -3-(p-methoxy)-phenylacrylohydrazide, **3b**:

Molecularformula$C_{25}H_{23}N_3O_4$; Yield: 73%; m.p. 184-186˚C; IR (KBr): 3344cm^{-1} (N-H), 3075 cm^{-1} (Ar C-H), 1659cm^{-1} (C=O), 1261cm^{-1} (C-O), 1599cm^{-1} (olefinic C=C, C=N), 1509,1470cm^{-1} (Ar C=C) ; ^{1}HNMR (DMSO-d_6): δ 11.49 (1H, s, CO-NH-N), 10.02 (1H, s, CO-NH-), 8.33 (1H, s, HC=N), 8.05-6.95(13H, m, Ar C-H), 7.31 (1H, s, C=C-H), 3.809 (3H, s, OCH_3), 3.770 (3H, s, OCH_3) ; Mass: m/z 429 (M ±1).

2-Benzamido-N'-(4-nitro benzylidene)-3-(p-methoxy)-phenylacrylohydrazide, 3c:Molecularformula$C_{24}H_{20}N_4O_5$; Yield: 77%; m.p.222-224 ˚C; IR (KBr): 3189cm^{-1} (N-H), 3006cm^{-1} (Ar C-H), 1632cm^{-1} (C=O), 1600cm^{-1} (olefinic C=C,C=N), 1506,1338cm^{-1} (NO_2), 1413cm^{-1} (C=C); ^{1}HNMR (DMSO- d_6): δ 11.93 (1H, s, CO-NH-N), 10.09 (1H, s, CO-NH-), 8.51 (1H, s, HC=N), 8.0-6.9(13H, m, Ar C-H), 7.18 (1H, s,C=C-H), 3.77(3H, s, OCH_3).

2-Benzamido-N'-(4-hydroxy, 3-methoxy benzylidene)-3-(p-methoxy)-phenylacrylo hydrazide, 3d: Molecularformula$C_{25}H_{23}N_3O_5$; Yield:70 %; m.p. 197-199˚C; IR (KBr): 3363cm^{-1} (OH), 3233cm^{-1} (N-H), 3002cm^{-1} (Ar C-H), 1665 cm^{-1} (C=O), 1600cm^{-1} (C=C,C=N);

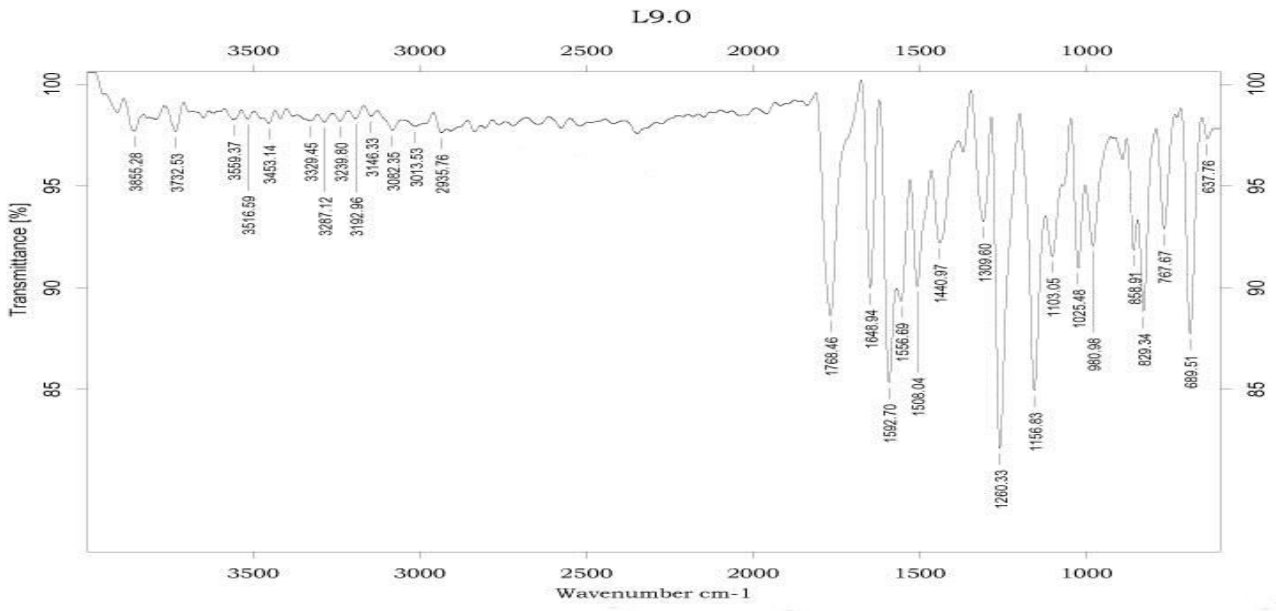
Antibacterial activity[15]:

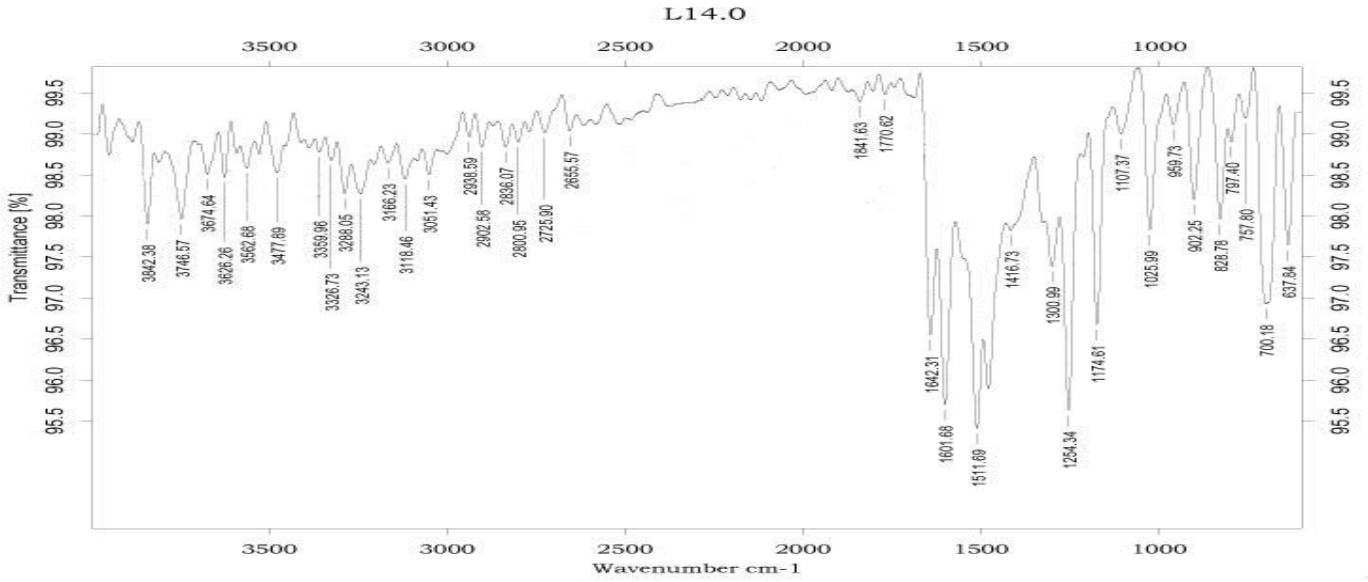
The antibacterial activity was evaluated by employing 24 hrs cultures of *B. subtilis, S. aureus, Pseudomonas aeruginosa* and *E.coli* using nutrient agar medium. The bacterial strains were transferred to sterile plates aseptically. The plates were left at room temperature and allowed for solidification. In each plate two wells of 4mm diameter

were made using a sterile borer. Accurately different dilutions of compounds (50µg/ml, 100µg/ml) were transferred to wells aseptically and labelled accordingly. The plates were incubated at 37˚C for 24 hrs. The diameter of zones of inhibition surrounding each well was recorded.

Results and Discussion

The synthesis of N-(Substituted benzylidene)-2-benzamido-3-(p-methoxy)-phenyl acrylohydrazides 3 (a-d) was carried out in three steps under green protocols first by the condensation of hippuric acid with anisaldehyde in presence of acetic anhydride and zinc oxide as catalyst by stirring at room temperature, yields 4-(p-Methoxy)-benzylidene-2-phenyl oxazol-5-ones (com 1), is the precursor for the synthesis of title compounds. Later compound 1 undergoes hydrozinolysis with hydrazine hydrate by stirring at room temperature to give key intermediate (com 2).The intermediate upon nucleophilic addition with functionalised aromatic aldehydes in ethanol by microwave irradiation produce title compounds 3(a-d) with shorter reaction time (1-2.3min). The reaction is simple, clean, rapid, efficient and devoid of any side products. The structures of synthesized compounds were confirmed on the basis of ^{1}HNMR, IR & Mass spectral data. The ^{1}HNMR spectrum of title compounds was more informative and structure of the title compounds was confirmed by the absence of a singlet at δ 4.51, appearance of a singlet in the region of δ 8.4-8.7 is attributable to C=N group and evaluated in vitro to determine their antibacterial activity against four bacterial strains. Antibacterial activity results have revealed that synthesized acylhydrazones exhibits good activity in comparison to the standard drug streptomycin against *Staphyloccocus aureous, Psedomonas aeruginosa* and minimal activity against *E.coli, Bacillus subtilis* which have been shown in table I.

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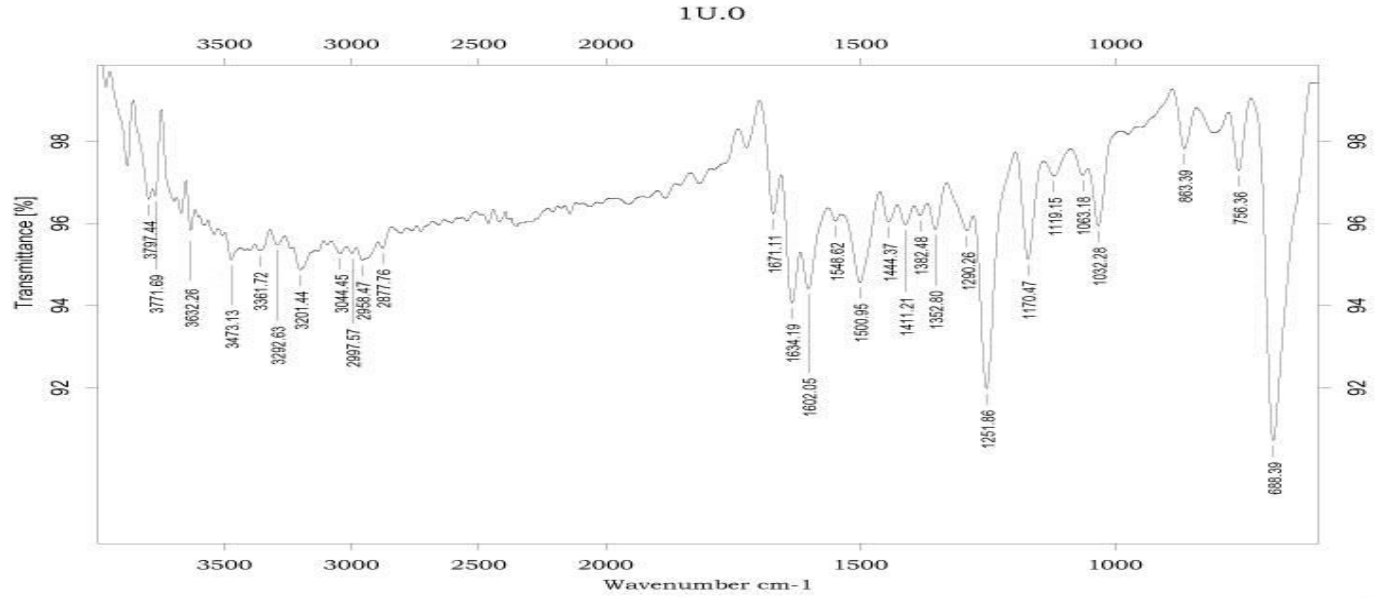
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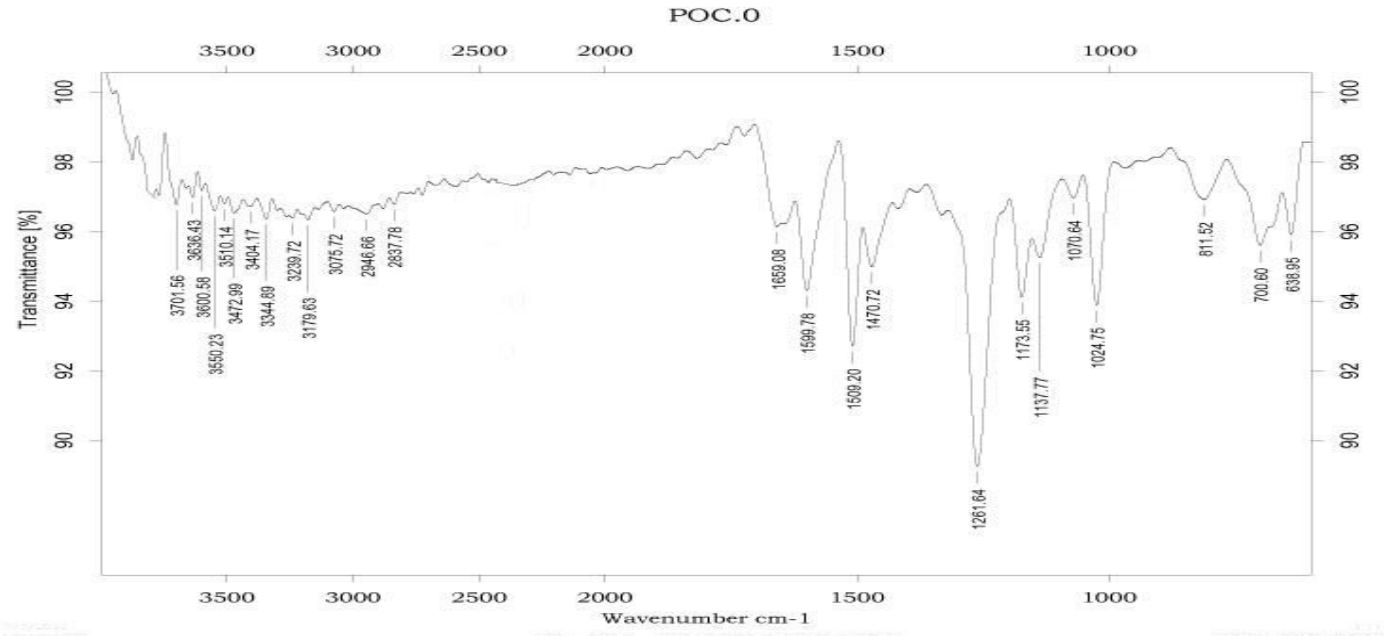
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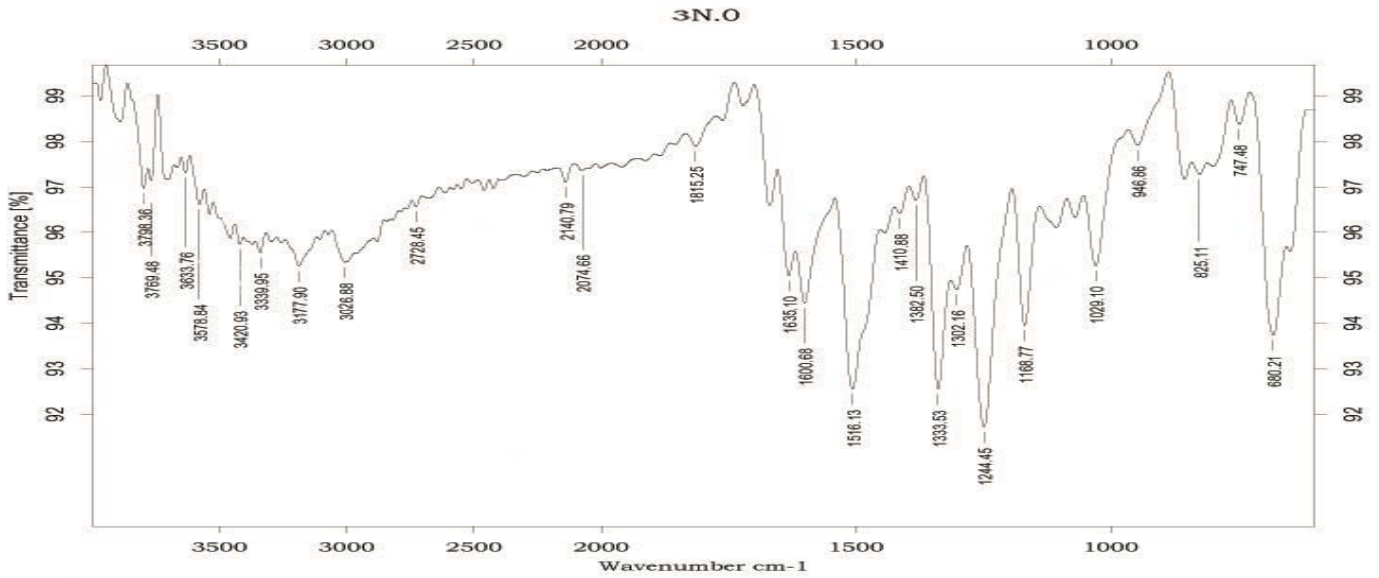

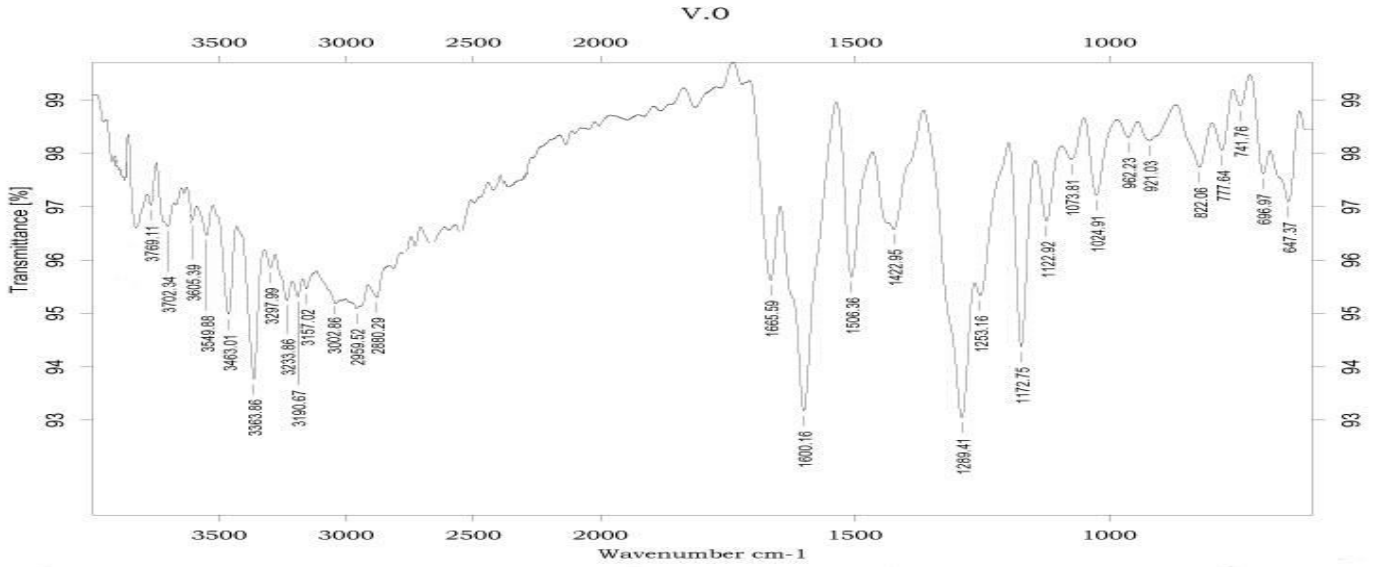

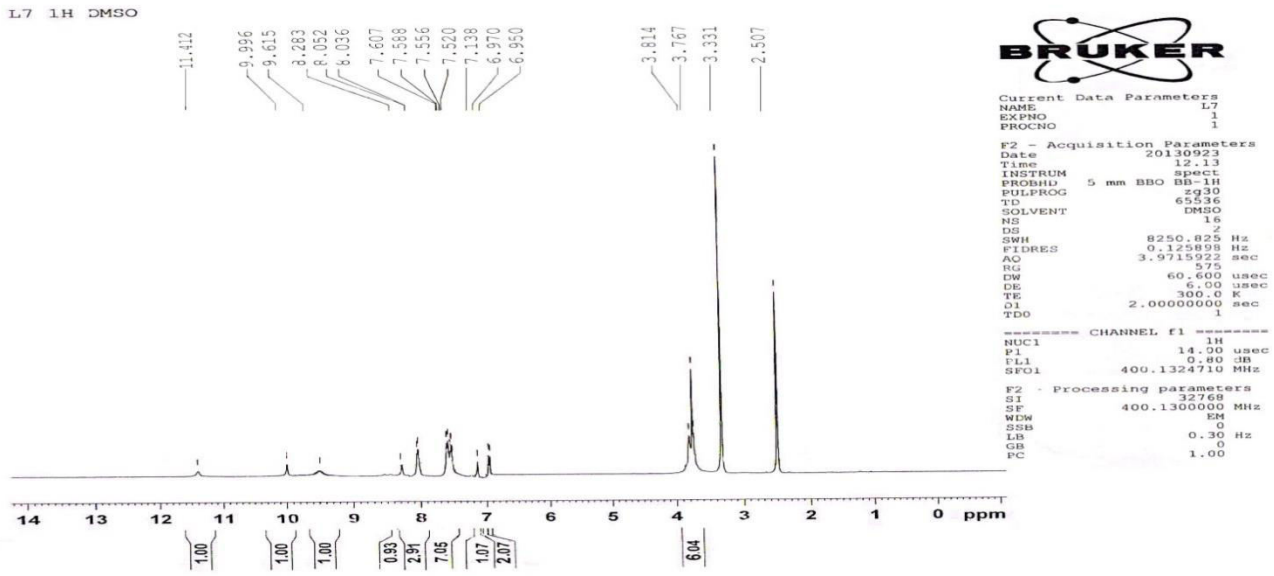

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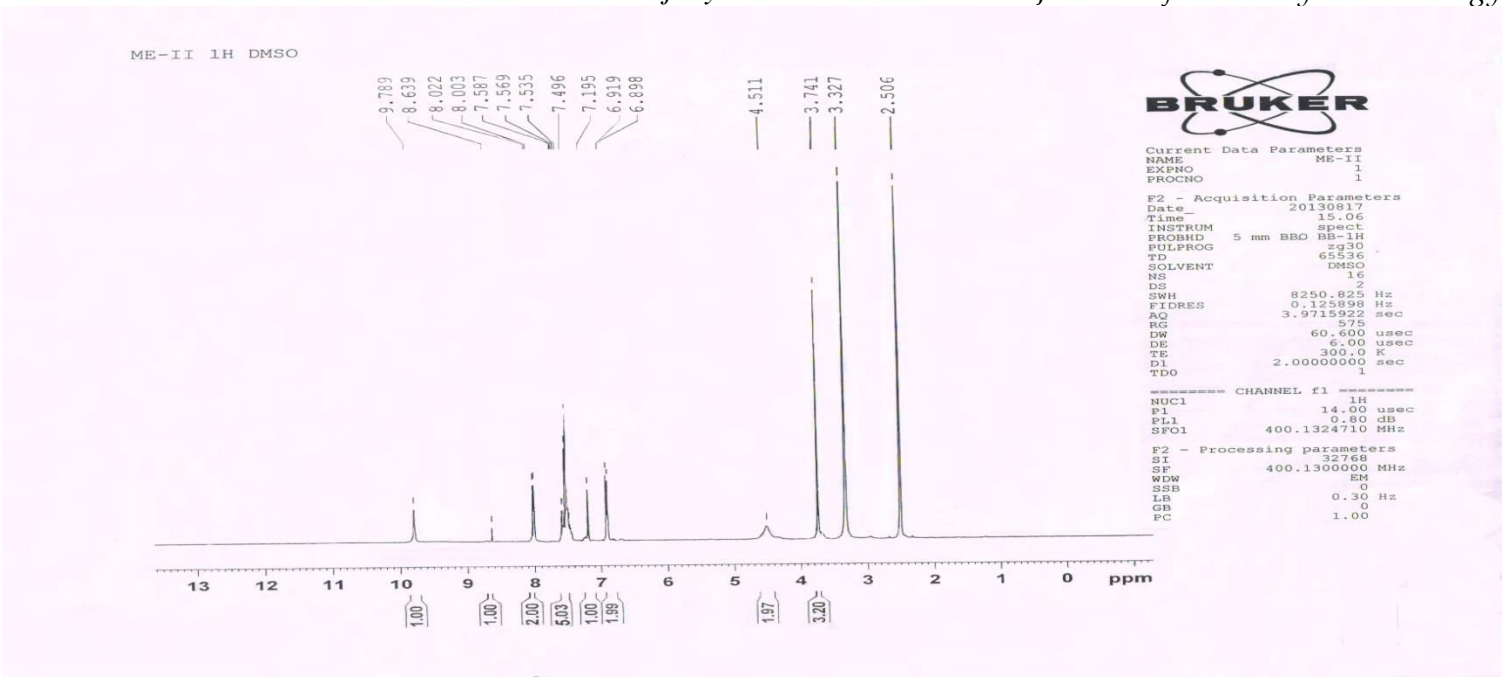
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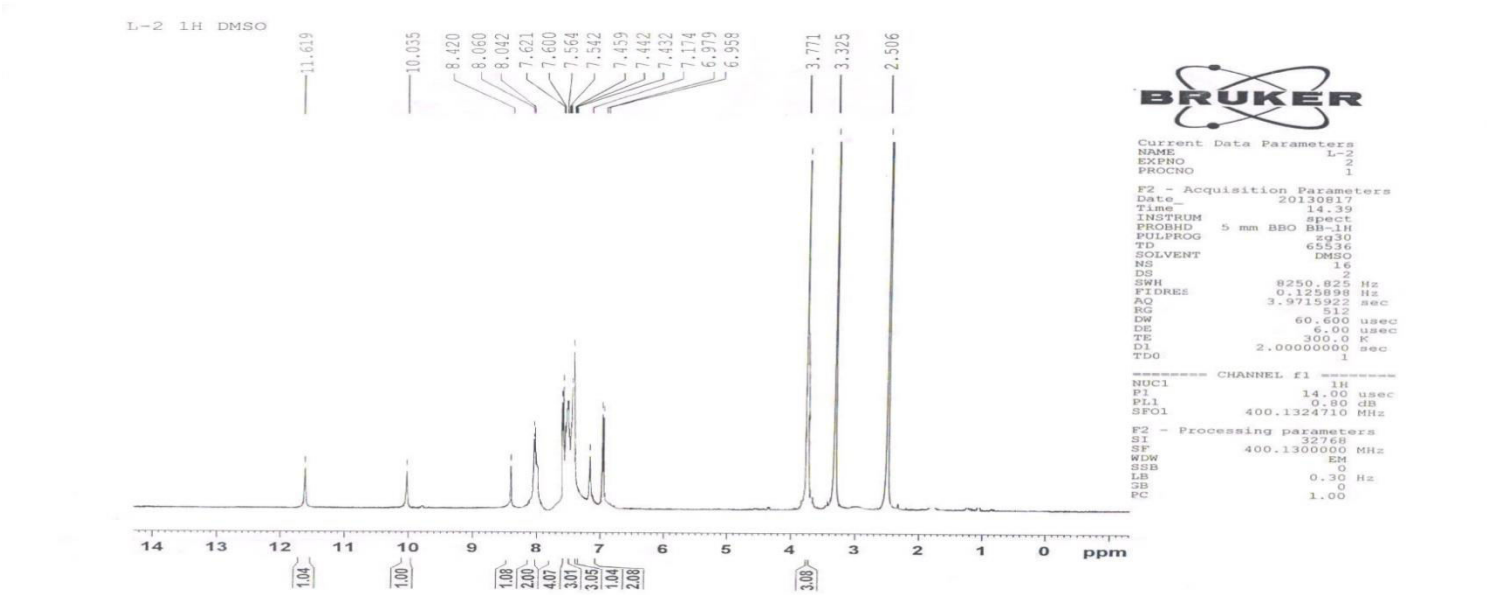
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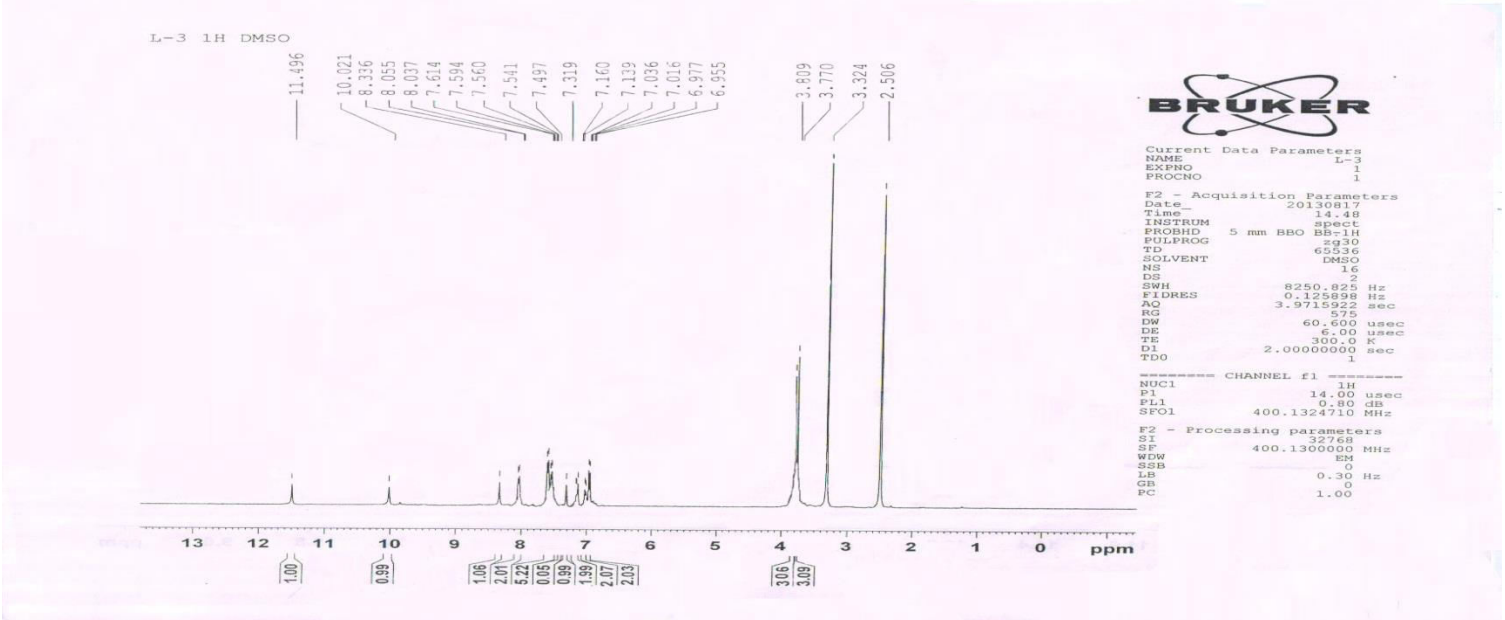
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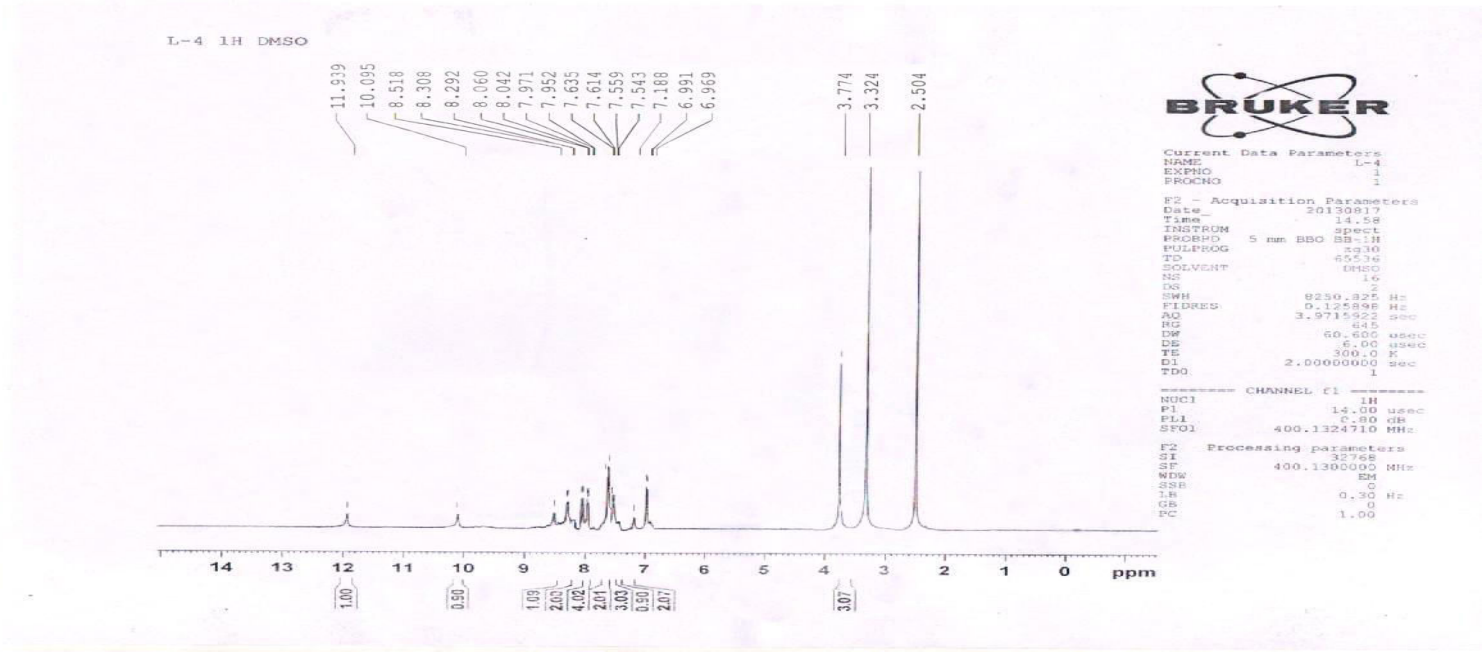


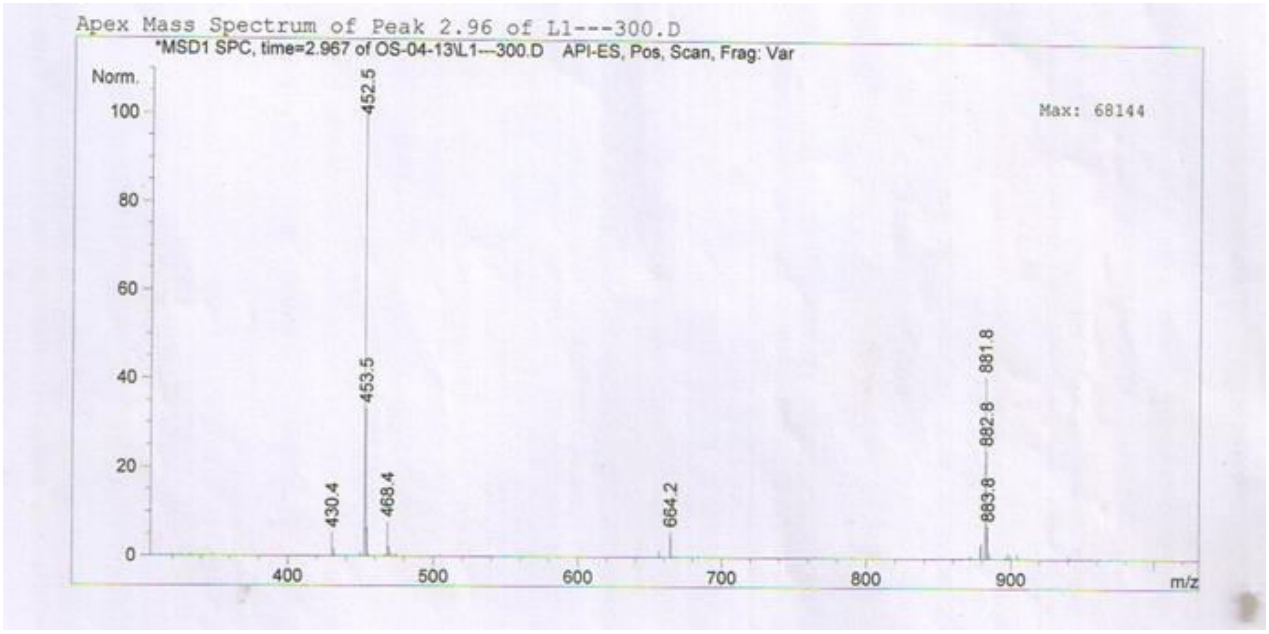
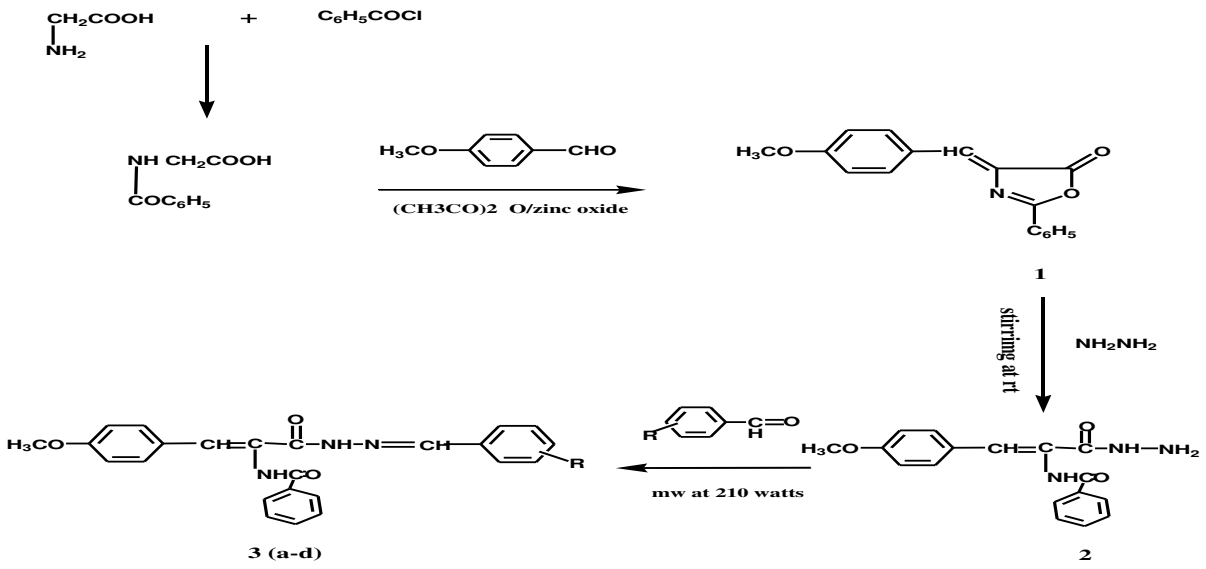


Figure- I: General method of synthesis of compounds 3(a-d).



compound	a	b	c	d
R	H	4-OCH_3	4-NO_2	4-OH,3-OCH_3

Table I: antibacterial activities of compounds 3(a-d) at two different concentrations against four different bacterial strains.

Sno	Compound	R	Zone of inhibition in mm							
			E.coli		***P.aeruginosa***		***S.aureus***		***B.subtilis***	
			50µg/ml	**100µg/ml**	**50µg/ml**	**100µg/ml**	**50µg/ml**	**100µg/ml**	**50µg/ml**	**100µg/ml**
1	3a	H	13	18	11	14	4	10	NS	10
2	3b	4-OCH_3	11	11	12	18	10	12	14	20
4	3d	4-NO_2	4	8	8	13	9	12	16	21
5	3e	4-OH,3-OCH_3	6	10	12	20	12	15	18	20
6	Std	Streptomycin	14	18	10	12	8	14	20	24

NS: Not significant

Conclusion

We developed a facile and microwave assisted synthesis for acylhydrazones. It has been observed that substitution alters the antibacterial activities of the compounds. Among all those, Compound 3b,3d showed good anti bacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus than other derivatives*. Compound 3a showed comparable activity as that of standard against all four organisms.

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