Synthesis of novel 1,4-bis-isoxazol/pyrazole and phenylpyrazole scaffolds by efficient green protocol and evaluation of biological properties thereof

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Abstract

Novel 1,4-bis(5-phenyl-1H-pyrazol-3-yl)benzene, 1,4-bis(1,5-diphenyl-1H-pyrazol-3-yl)benzene and 1,4-bis(5-phenylisoxazol-3-yl)benzene have been synthesized by taking $MgFe_2O_4$ MNP's as heterogeneous catalyst to yield reported novel products in good yields in short reaction time.

The synthesized compounds were characterized by analytical techniques and evaluated for antifungal and antibacterial activities. Bis-pyrazole showed significant antibacterial activity while bis-isoxazole showed antifungal activity compared to standard drug ciprofloxacin and clotrimazole respectively.

Keywords: Heterogeneous catalyst, Antimicrobial activities; 1,4-bis(5-phenylisoxazol-3-yl)benzene, 1,4-bis(5-phenyl-1H-pyrazol-3-yl)benzene, 1,4-bis(1,5-diphenyl-1H-pyrazol-3-yl)benzene, Green protocol.

Introduction

Magnetic nanoparticles (MNP's) as heterogeneous catalyst (HC) have lured the researcher's community all over the world for ease of separation from mixture with the help of external magnet. NPs based catalyst has shown excellent catalytic activity as compared to their analogous bulk materials and also enhance their loading function for their excellent antimicrobial activities. The HC has received a significant interest from scientific and industrial perspectives because of its remarkable impact on global economy.

Immobilized magnetic nano catalyst is one of the domains developing enormously. Striking novel catalytic properties including highly enhanced selectivities and reactivities for nanocatalyst compared to bulk counter parts have been reported by researchers. Recently functionalized MNPs have been used as efficient catalyst for many functional group transformation reactions. In last few decades pyrazole and its derivatives have considerably drawn the attention of the researchers owing to their high therapeutic values.

Drugs possess pyrazole as basic moiety in their chemical structure like celecoxib[25], etoricoxib, atorivodine[26] and deracoxib[27]. Recent methodologies in synthesis of pyrazole and its derivatives[1,5,14] possess wide range of pharmacological activities such as antiinflammatory, antipyretic, analgesic, antimicrobial[2,12,24,29,37,40], antitubercular, antiviral, antihypertensive, antiglaucoma, antioxidant, antidepressant, anxiolytic, neuroprotective antidiabetic activity[16-18,20,23,28,30,38] and also significant anticancer activities.[7,8,10,11,33,36,39]

Some arylpyrazole were found to have non nucleoside HIV-I reverse transcriptase inhibitor activity[13]. However, isoxazoles and its derivatives are important components occurring in many natural products e.g. ibotenic acid and muscimol[19] and also in a variety of bioactive compounds showing anti-inflammatory[3,35] monoamine oxidase inhibitors[31], penicillin antibiotic, antimicrobial, antioxidant[34,21] and herbicidal[22].

Moreover, isoxazoles have also shown antitubulin, antinociceptive and anticancer activity[9,15]. Despite of having such spectacular activity and biological and physiological importance, appearance of undesirable side effects of various antibiotics and development of drug resistance has compelled the researchers to synthesize pyrazole and isoxazole[6] analogues antimicrobial agents with new structural frameworks to overcome the shortcomings.

In continuation of research work[4] in the above field, we report an efficient methodology for synthesis of novel compounds 2-4 using cheaper, reusable, non toxic magnetically separable $MgFe_2O_4$ as catalyst with reduced time and in good yields. After complete characterization of compounds, biological activities of newly synthesized compounds were examined thereof.

Material and Methods

Chemicals and reagents: All the reagents and solvents were used without purification and used as such supplied from commercial sources. Schimadzu FTIR 8401 spectrometer was used for FTIR spectra on KBr pallets and for the liquid samples Perkin Elmer version 10.03.06 was used. NMR (both proton and carbon) spectra were recorded on Bruker DRX 300 spectrometer operating at frequency of 300MHz for ^1H and 75MHz for ^{13}C NMR in deuterated chloroform and deuterated DMSO. Waters UPLC-TDQ spectrometer was used for determining molecular mass.

TLC for monitoring progress of reaction was performed on glass plates coated with silica; eluted TLCs were developed in iodine vapor chamber or visualized in UV light. The morphological aspects of $MgFe_2O_4$ MNPs were studied by high resolution electron microscopy (HRTEM-300) KV

Technai G2 30STWIN with Au coating equipped with energy dispersive X-ray spectroscopy.

Preparation of catalyst: $MgFe_2O_4$ NPs were synthesized by chemical method. $Fe(NO_3)_3 \cdot 6H_2O$ (2.0mmol) and magnesium carbonate (1.0mmol) were dissolved in nitric acid. Prepare the uniform solution of it and add monoethanolamine (1.0 mmol) to the reaction mixture followed by sucrose (1.0 mmol) and then excess of HNO_3. The brown colored mixture obtained was kept on hot plate for heating up to dryness to obtain a black residue. The black residue obtained was then kept in furnace at 600°C for 5-6 hrs of heating to get $MgFe_2O_4$ MNPs.

Synthesis of 1,4-bispyrazoles and isoxazoles (2-4): The precursor 1,4-bischalcone 1 (Scheme 1) was first prepared as per the reported literature procedure[32] by using acetophenone and terephthaldehyde in presence of NaOH in ethanol.

To a well stirred solution of bis-chalcone 1 (1 equiv.), corresponding bifunctional nucleophiles 2 (2 equiv.) in 10 ml EtOH, HC (10 mmol %) was charged and the solution was refluxed for the given period of time. Monitoring of reaction was done through TLC. When reaction attained completion, the reaction mixture was cooled at rt, the catalyst was separated by using external magnet and then solvent was removed under vacuum to get a crude solid. The crude obtained was washed with water, filtered, dried and purified by column chromatography using EtOAc/n-hexane solution.

Compound (2): Yellow crystals; melting point>290°C; Yield (%) = 60; R_f = 0.50 (2:8 EtOAc/n-hexanes); IR (cm^{-1}): 3354, 3064, 2885, 1958, 1903, 1820, 1605, 1017; Mass [ESI m/z] (%) M^{++2} = 367; ^{1}H NMR ($CDCl_3$): 6.7-8.0 (m, 7H), 5.1 (s, 1H). ^{13}C NMR ($CDCl_3$): 143.6, 138.0, 136.9, 133.5, 133.3, 129.2, 126.3, 127.2, 125.5, 123.3.

Compound (3): Yellow crystals; melting point = >260°C; Yield (%) = 74; R_f = 0.47 (2:8 EtOAc/n-hexanes); IR (cm^{-1}): 3064, 1960, 1820, 1225, 1037 770; Mass [ESI m/z] (%) M+ = 399; ^{1}H NMR ($CDCl_3$): 7.0-8.0 (m, 13H); ^{13}C NMR ($CDCl_3$): 190.3, 166.4, 152.1, 145.6, 143.7, 138.2, 137.1, 133.1, 129.1, 128.9, 128.7, 123.3. 119.3.

Compound (4): Light yellow solid, melting point >279°C, Yield (%) = 79; R_f = 0.66 (3:7 EtOAc/n-hexanes); IR (cm^{-1}): 3380, 3171, 2677, 2350, 1958, 1820, 1603, 1335, 1079, 835; M^{++2} = 370; 367 (50%); Mass [ESI m/z] (%) M^+ = 369; ^{1}H NMR ($CDCl_3$): 6.73-8.13 (m, 5H); 1.60-2.59 (m, 2H); ^{13}C NMR ($CDCl_3$): 190.42, 143.63, 138.15, 137.1, 133.2, 130.5, 128.8, 127.1, 123.3.

Results and Discussion

Chemistry: The targeted novel compounds 2-4 were synthesized by using $MgFe_2O_4$ (10 mmol%) as catalyst, precursor chalcone (1 mmol), bifunctional nucleophiles (2 mmol) like hydrazine hydrate, phenyl hydrazine, hydroxyl amine hydrochloride at refluxing EtOH for the reported period of time.

The $MgFe_2O_4$ heterogeneous catalyst was easily removed by applying external magnet. The products were obtained in 79-60% yields (Scheme 1). $MgFe_2O_4$ nano catalyst can be very easily separated from the reaction mixture by applying external magnetic field owing to the super magnetic nature of Fe_3O_4 NPs at room temperature.

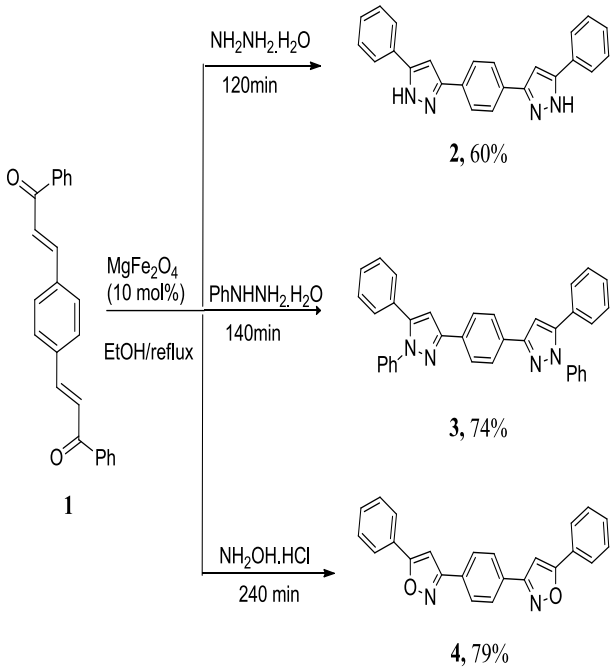


Figure 1: Synthesis of novel compounds 2-4

Recovery and Reusability of catalyst: On investigation of reusability of magnetic ferrite nano particles, it was found that even on repeated use of recovered catalyst it did not showed any substantial deactivation in surface activity even long after 5 cycles of reactions (Chart 1).

Antimicrobial activity: Newly synthesized compounds (2-4) were scanned for their antibacterial (MTCC No.) property against *E. coli* (118), *S. typhi* (98), *B. subtilis* (121) and *S. aureus* (96) bacteria whereas antifungal scanning was performed against *C. albicans* (2479) and *A. niger* (281) using disc diffusion method as in table 1.

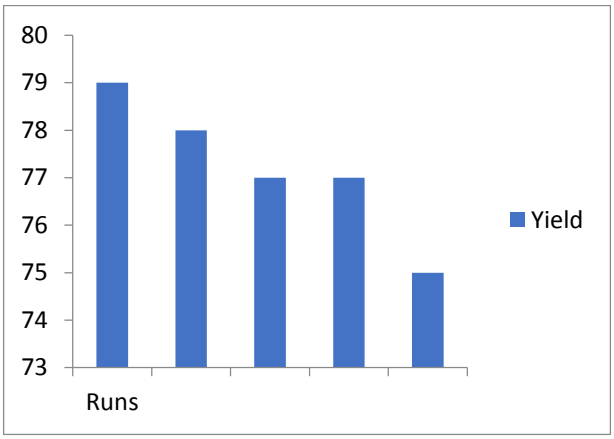


Chart 1: Reusability of $MgFe_2O_4$ heterogeneous catalyst

Table 1
Antifungal and Antibacterial scanning results for compounds (2-4)

Inhibition Zone ± S.D.									
Compounds	**Antibacterial**					**Compounds**	**Antifungal**		
	Conc. ug/mL	***B.subtilis***	***S.typhi***	***S.aureus***	***E.coli***		**Conc. ug/mL**	***A.niger***	***C.albicans***
2	100	6.13±0.52	19.85±0.49	17.15±0.45	8.25±0.71	2	100	3.37±0.62	1.80±0.12
	200	8.43±2.07	20.87±0.35	18.78±0.57	8.57±0.44		200	4.37±0.25	2.14±0.15
	300	10.00±1.49	22.00±0.57	21.05±0.20	11.25±1.36		300	5.40±0.27	4.89±0.79
	400	13.80±1.11	22.12±0.57	22.310±0.80	14.22±1.38		400	7.00±0.40	5.45±0.48
3	100	3.92±0.67	3.57±0.72	2.97±0.27	4.62±0.85	3	100	7.30±0.40	5.02±0.45
	200	3.11±0.77	3.852±0.74	3.51±0.66	4.37±1.93		200	8.75±0.28	6.15±0.78
	300	3.92±0.55	4.96±0.37	2.47±0.47	4.87±0.47		300	12.87±0.47	8.66±0.85
	400	4.70±0.56	5.12±0.47	3.05±0.78	4.12±0.42		400	14.12±0.47	11.02±0.87
4	100	3.25±0.50	2.57±0.47	3.25±0.50	3.00±0.57	4	100	3.67±0.69	3.06±0.77
	200	3.75±0.5	3.87±0.47	4.00±0.57	3.12±0.75		200	4.88±0.65	3.39±0.73
	300	3.87±0.47	4.87±0.47	4.87±0.47	3.37±0.25		300	5.17±0.47	4.39±0.65
	400	4.12±0.47	6.0±0.57	6.87±0.47	3.62±0.25		400	7.37±0.12	6.88±0.89
Control (DMSO)*		***	***	***	***	Control (DMSO)*		***	***
Ciprafloxacin[a]	100	10.03±0.75	20.00±0.89	18.25±0.98	9.04±0.45	Clotrimazole[b]	100	7.50±0.89	5.12±0.45

On calculating the inhibition zone in the range of 2.56-19.85 mm in terms of average diameter indicates significant antimicrobial activities. Compound 2 showed comparable antibacterial properties to that of standard ciprofloxacin drug, while compound 3 and 4 exhibited moderate sensitivity. The decreasing order of antimicrobial activity among the compounds is 2>3>4.

On the other hand, the average zone of inhibition ranging from 1.80-7.30 indicates encouraging antifungal activity for the same compounds when compared to that of clotrimazole with inhibition zone (5.12-7.5mm). Compound 3 expressed substantial antifungal bioassay while compound 4 and 2 registered moderate. Thus the order of antifungal activities among the compounds is 3>4>2.

The minimum inhibition conc. (MIC) was also examined for all the newly synthesized heterocyclic compounds. The values recorded for compounds 4-6 are depicted in table 2. *S. typhi* showed the best MIC for compound 2 whereas compound 4 gave the lowest MIC.

Table 2
Minimum Inhibition Concentration (MIC) of compounds (2-4)

MIC (ug/mL) of compounds			
Bacteria	**2**	**3**	**4**
Bacillus subtilis	1.2	1.1	0.91
Staphylococcus aureus	8.3	1.4	1.1
Salmonella typhi	10.89	1.70	1.32
Escherchia coli	3.3	1.2	0.99

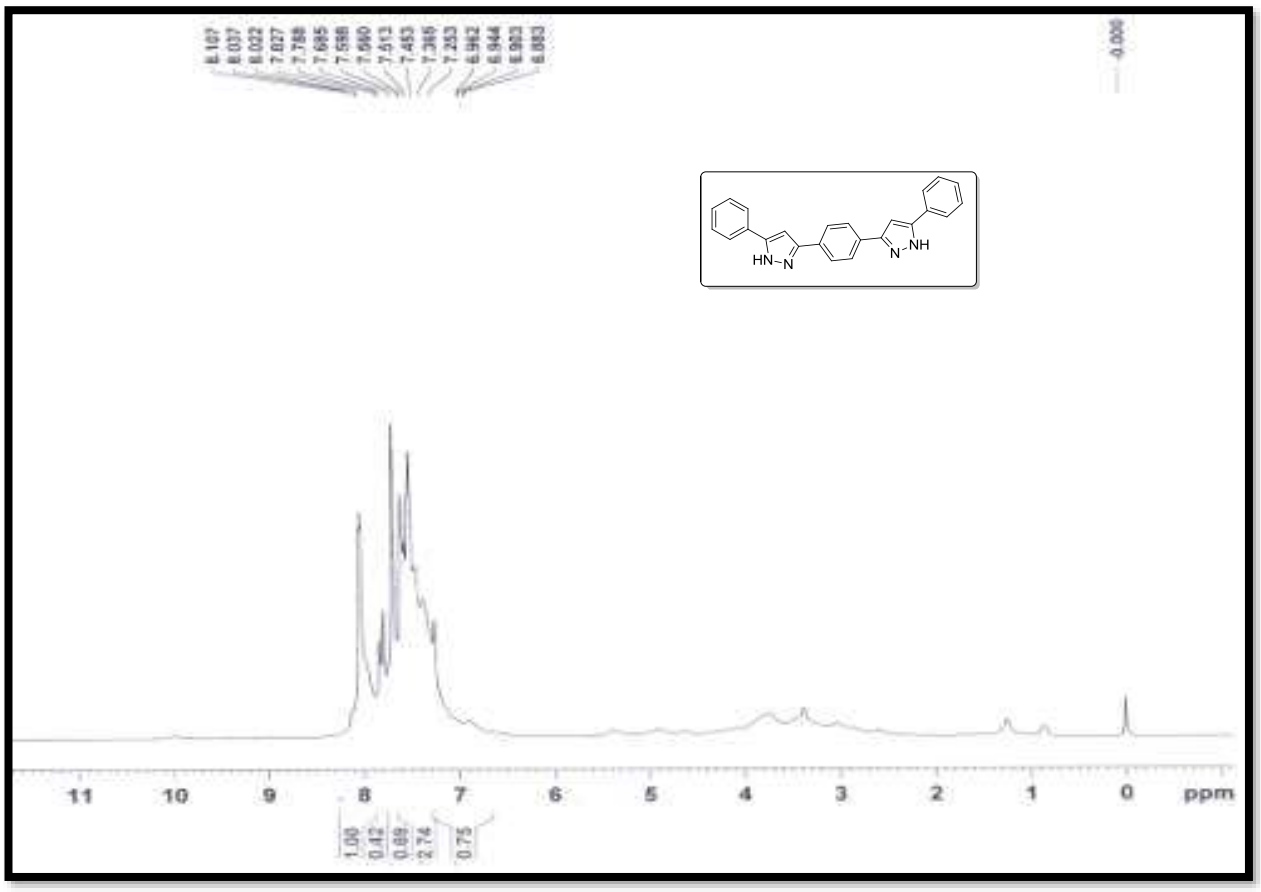
Supplementary Information

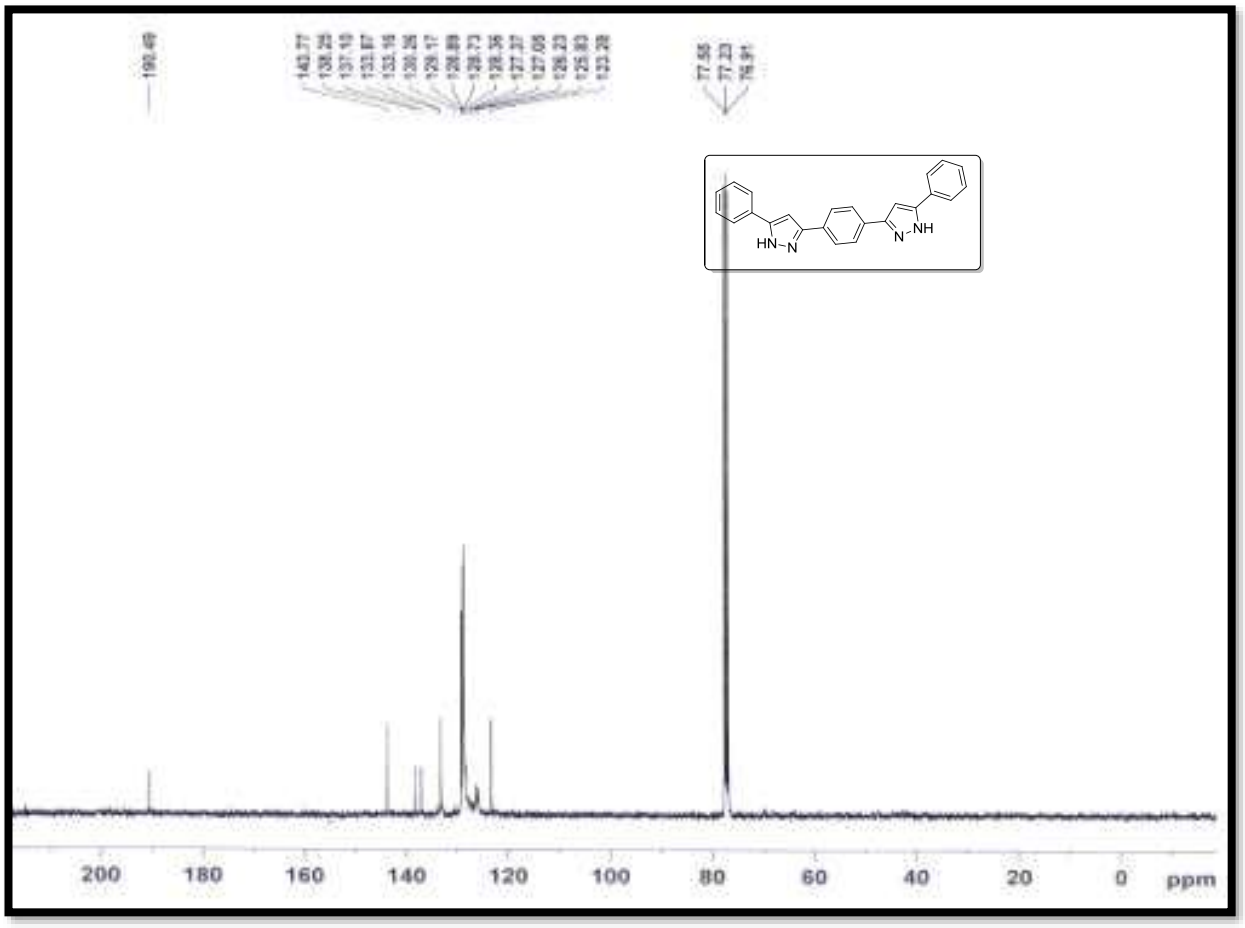
Preparation of the magnesium ferrite MNPs: Nanoparticle of $MgFe_2O_4$ was synthesized by combustion technique. 2.0 mmol of $Fe(NO_3)_3 \cdot 9H_2O$ and 1.0 mmol of $MgCO_3$ were dissolved in HNO_3 and mixed uniformly. 1.0 mmol of monoethanolamine was added to the reaction mixture followed by 1.0 mmol of each of sucrose and excess of nitric acid respectively. The resulting mixture was then put on a hot plate at 80°C till it completely dried to a black residue. This black residue was then kept in muffle furnace at 600°C for 6-8h to obtain nanoparticle of $MgFe_2O_4$.

Characterization of catalyst: The high resolution transmission electron microscopy (HRTEM) of NP'S is shown in figure 1. The particle size of the magnesium ferrite NP's sample is typically in the range of 100-200nm. They are irregular in shape and are attached to each other along the grain boundaries. The material was verified by XRD data which matched very well with standard data (JCPDS file no.737960). The peaks are indexed as (220), (311), (222), (400), (331) and (440) as in figure 2. Size of crystallite was found be 100 nm from analysis of XRD profile by Debye Sheerer equation $D = (\frac{0.9\lambda}{\beta Cos\theta})$.

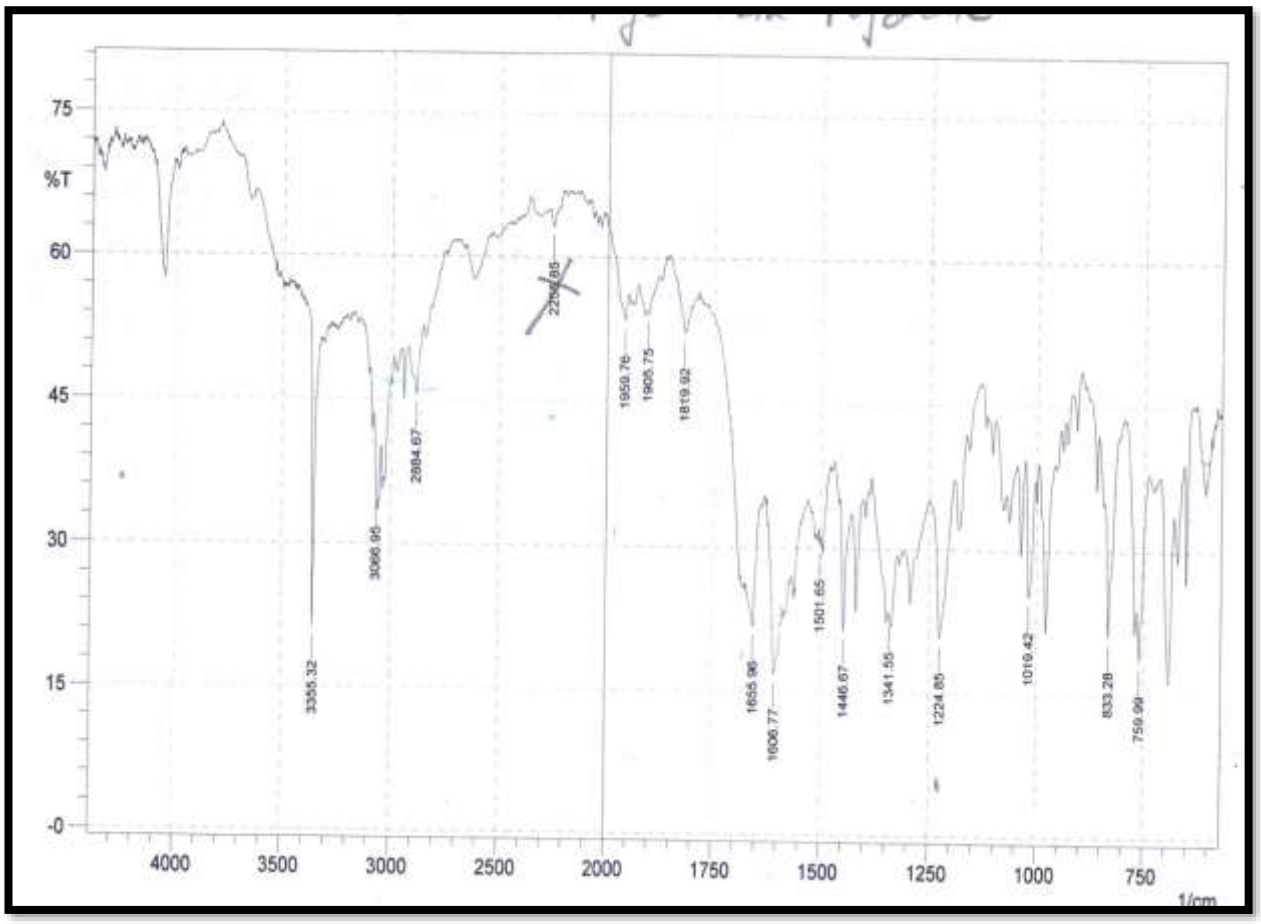
Culture media for antimicrobial activity: Culture media for antimicrobial activity were prepared by taking nutrient agar-agar media (15 g), peptone (5 g), yeast extract (2 g), beef extract (1 g) (which dissolved in distilled water with the application of heat). pH of the solution was adjusted to 6.5-7.0 with the help of 0.1%w/v sodium chloride solution. The culture media, disc and glass wares were sterilized by autoclaving at 15 lb/sq.inch pressure for 15 minutes and the synthesized compounds were tested for antimicrobial activity.

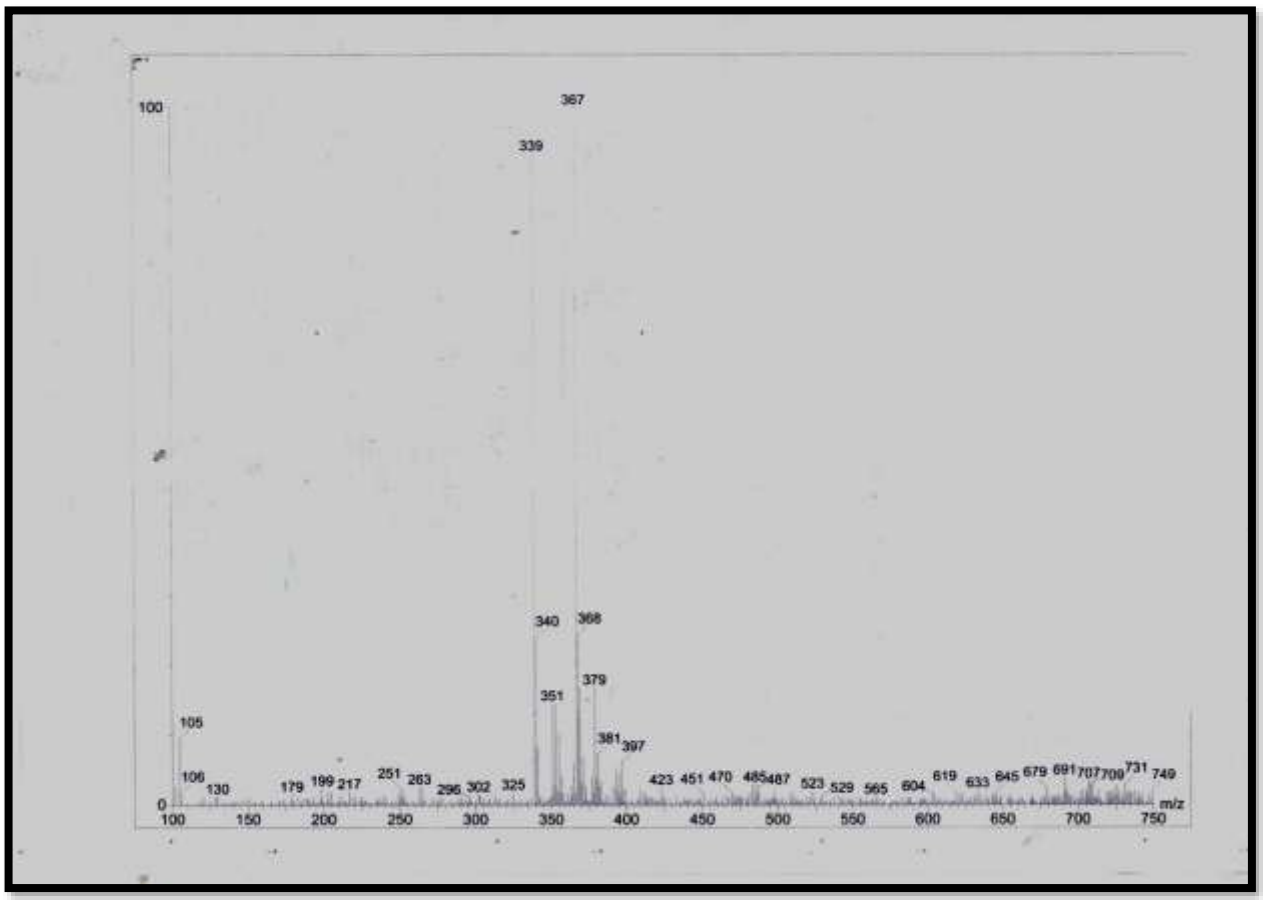
Culture Media for Antimicrobial activity: Culture media for antifungal activity was performed by taking nutrient agar-agar media (15 g), peptone (10 g), glucose (40 g) (dissolved in distilled water with the application of heat), pH of the solution was adjust to 6.5-7.0 with the help of 0.1%w/v sodium chloride solution. The culture media, disc and glass wares was sterilized by autoclaving at 15 lb/sq. inch pressure for 1 5 min and the synthesized compounds were tested for antifungal activity.



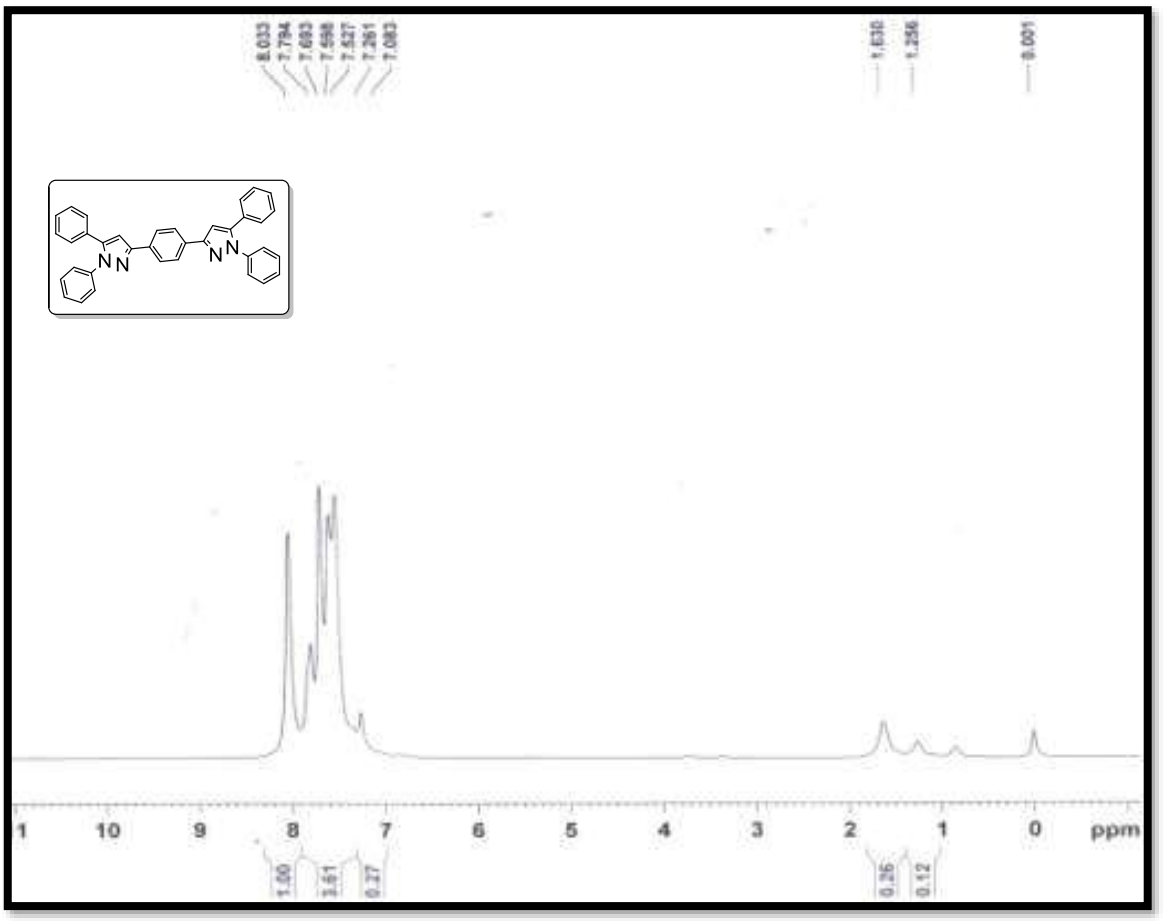


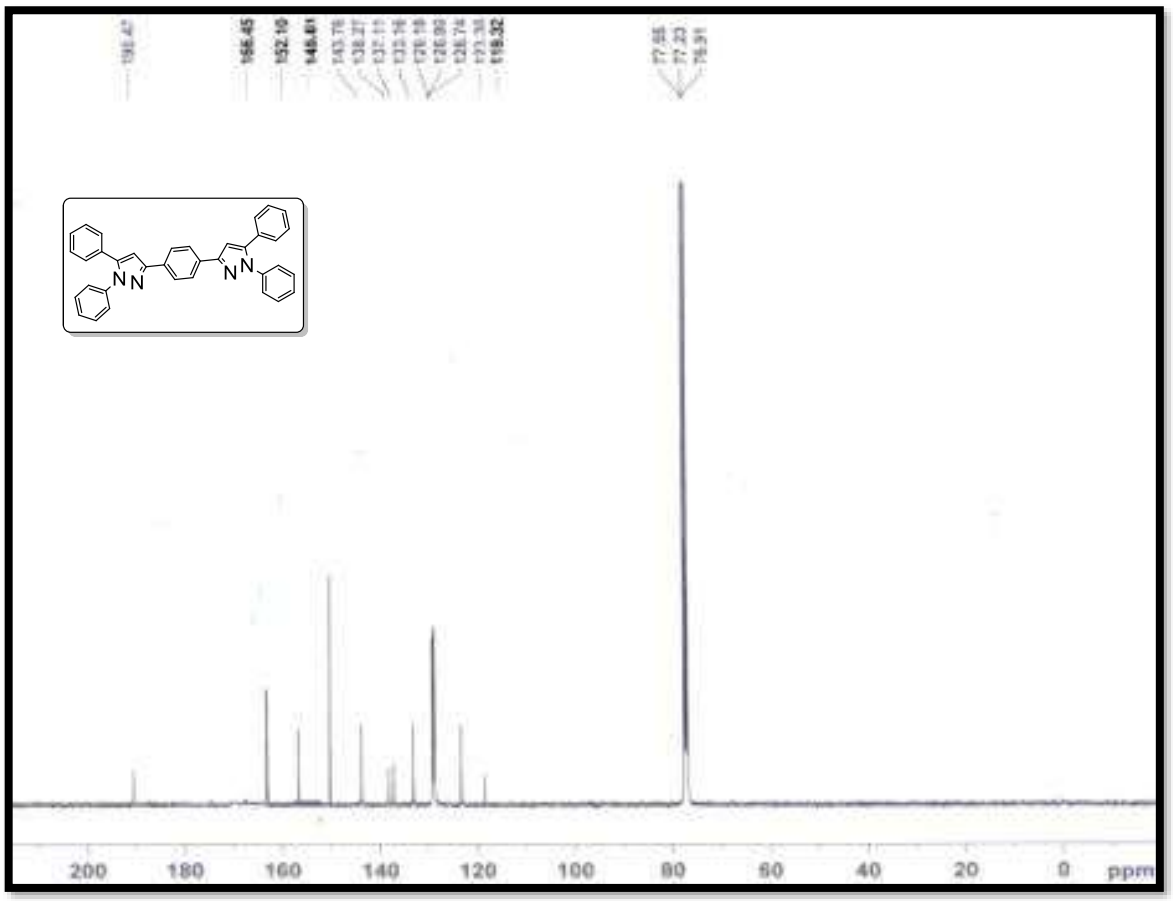
S1: ^{1}H NMR (300MHz) and ^{13}C NMR (75 MHz) of Compound 2



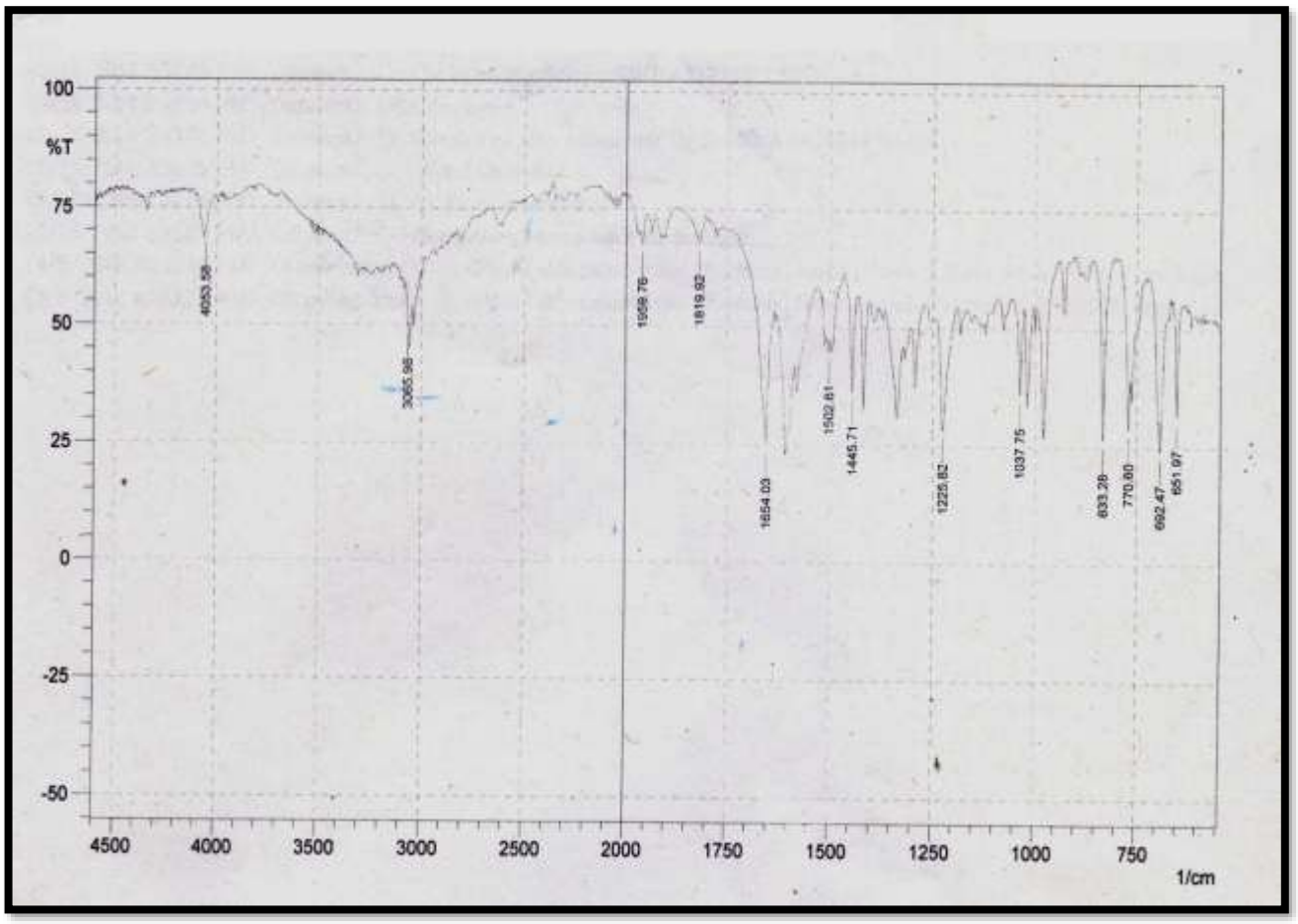


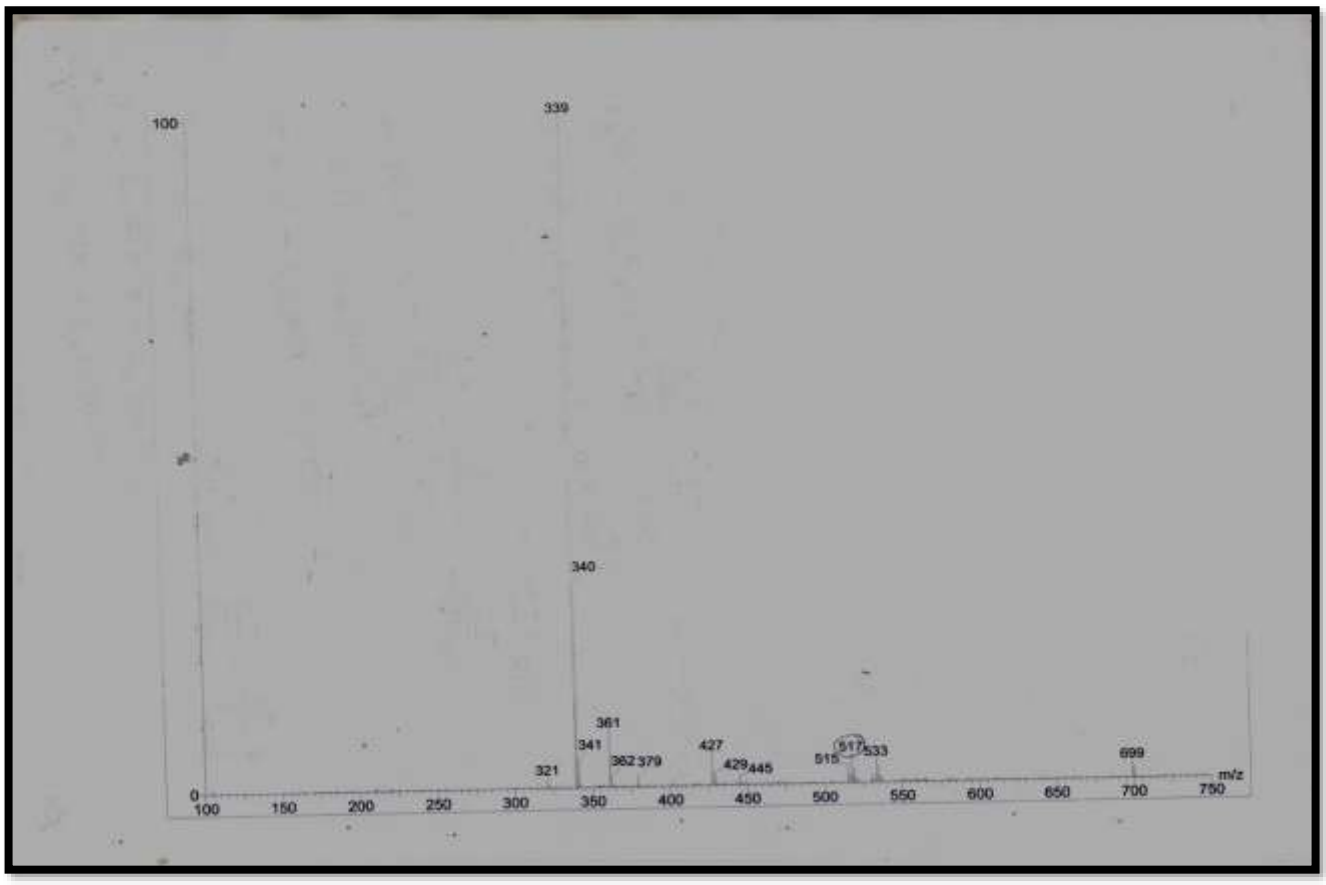
S2: FTIR and Mass spectrum of compound 2



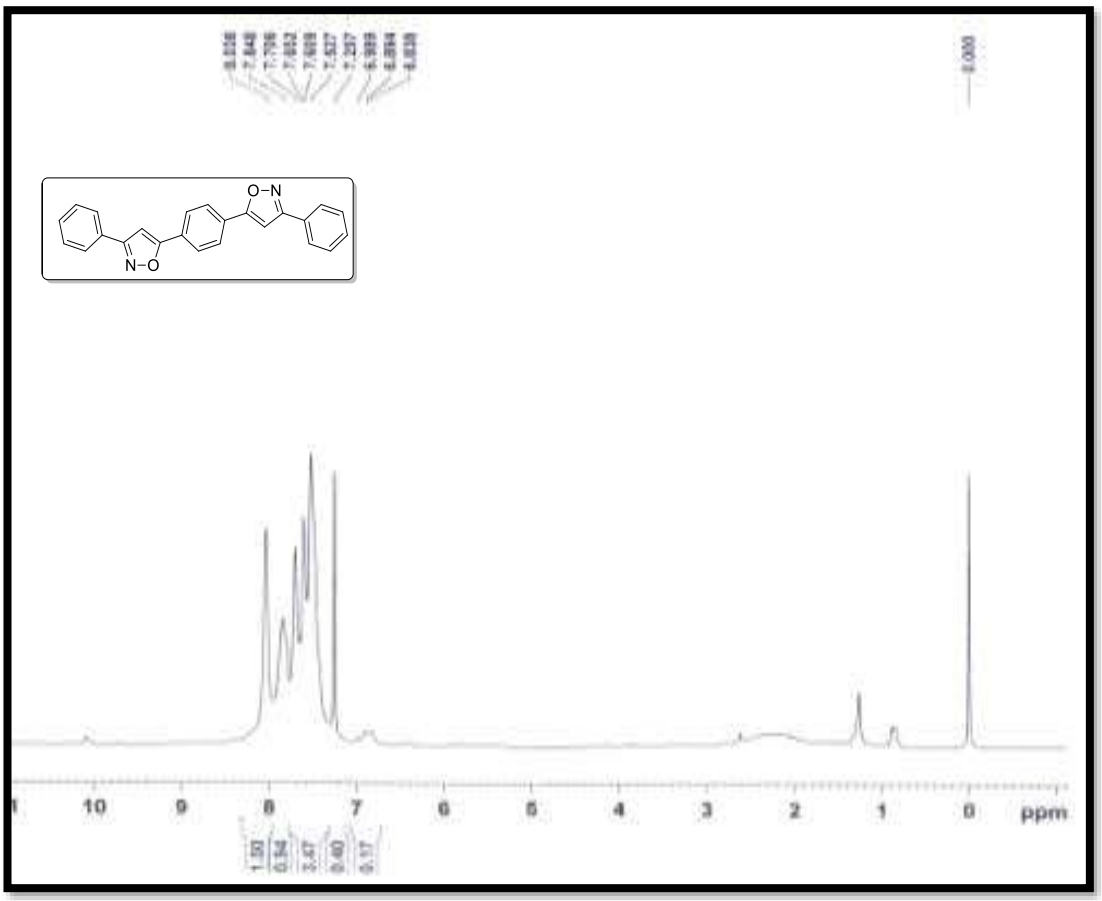


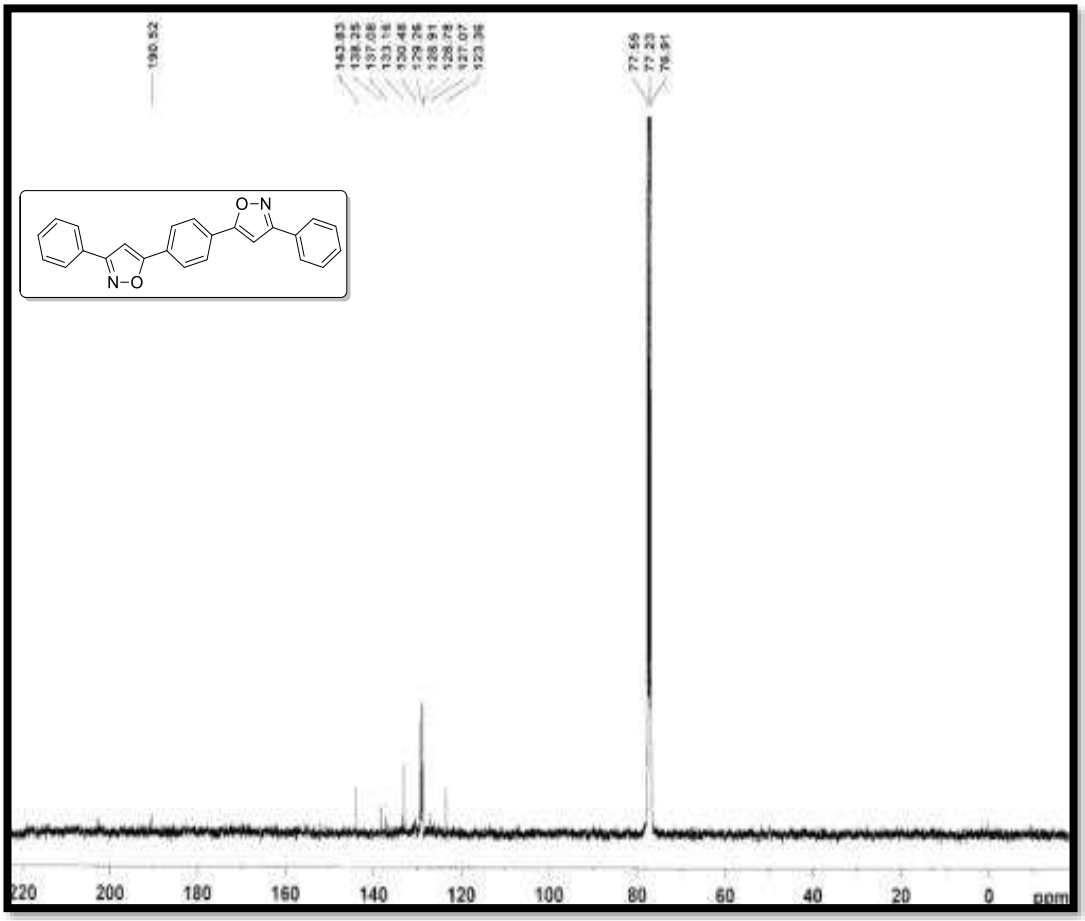
S3: ^{1}H NMR (300MHz) and ^{13}C NMR (75 MHz) of Compound 3



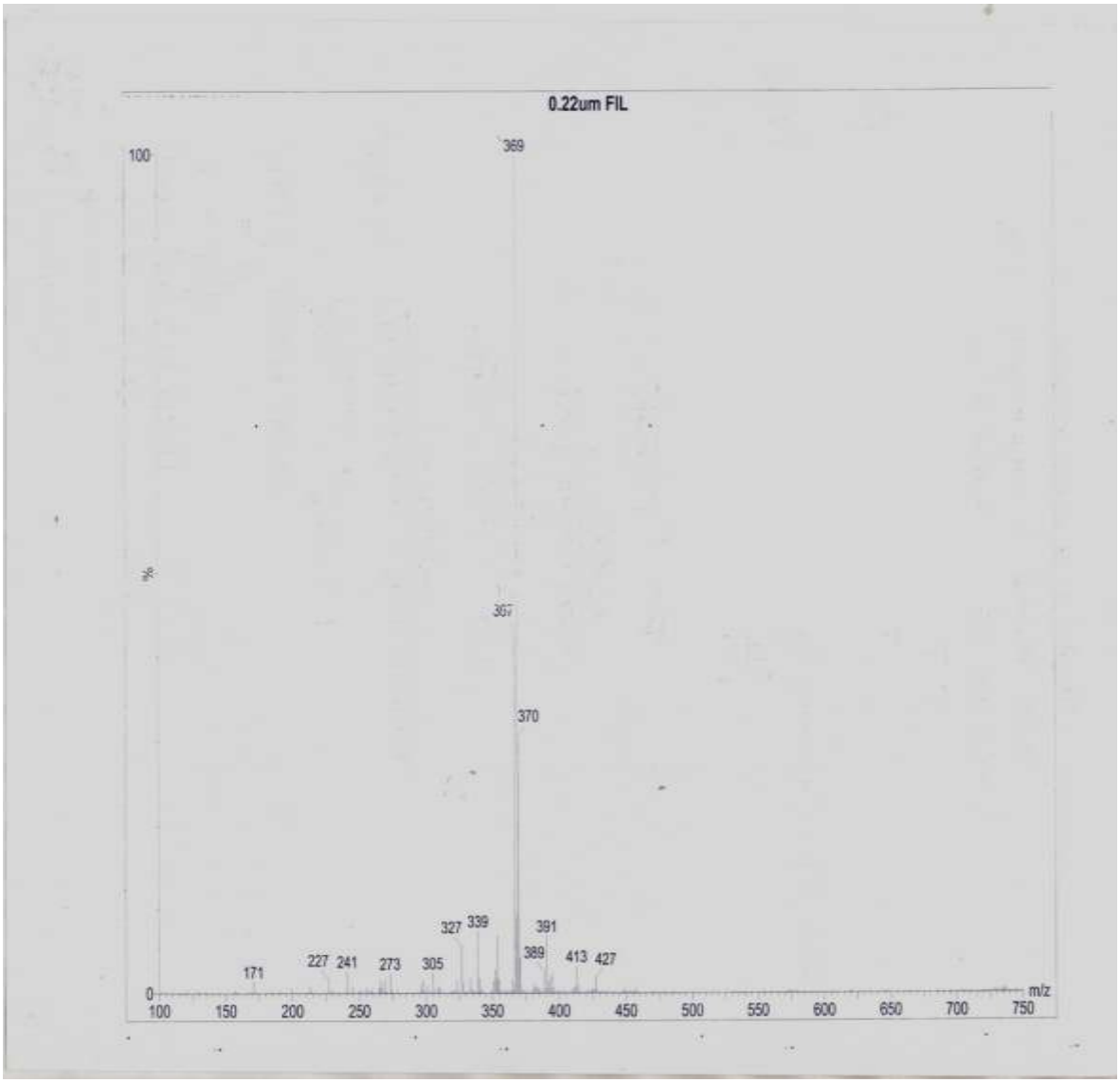


S4: FTIR and Mass spectrum of compound 3





S5: ^{1}H NMR (300MHz) and ^{13}C NMR (75 MHz) of Compound 4



S6: Mass spectrum of compound 4

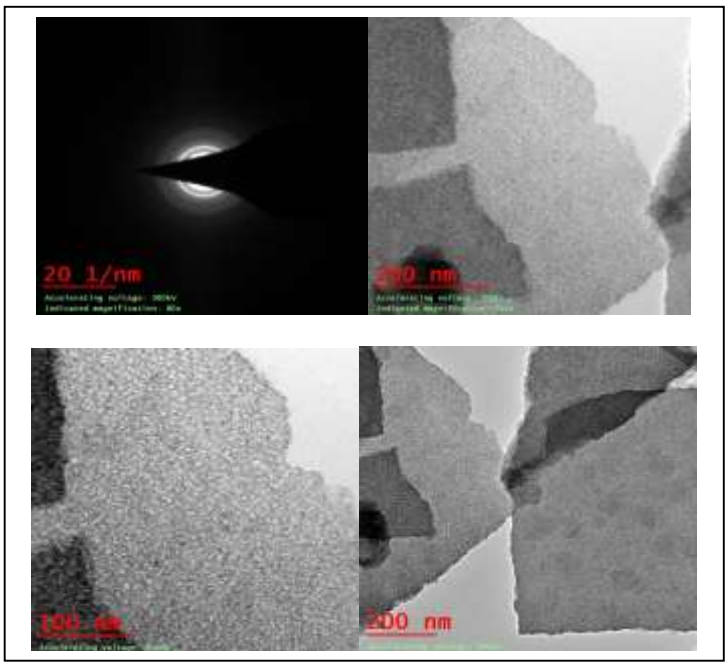


Figure 1: X-ray diffraction pattern and HRTEM image of $MgFe_2O_4$ MNP's

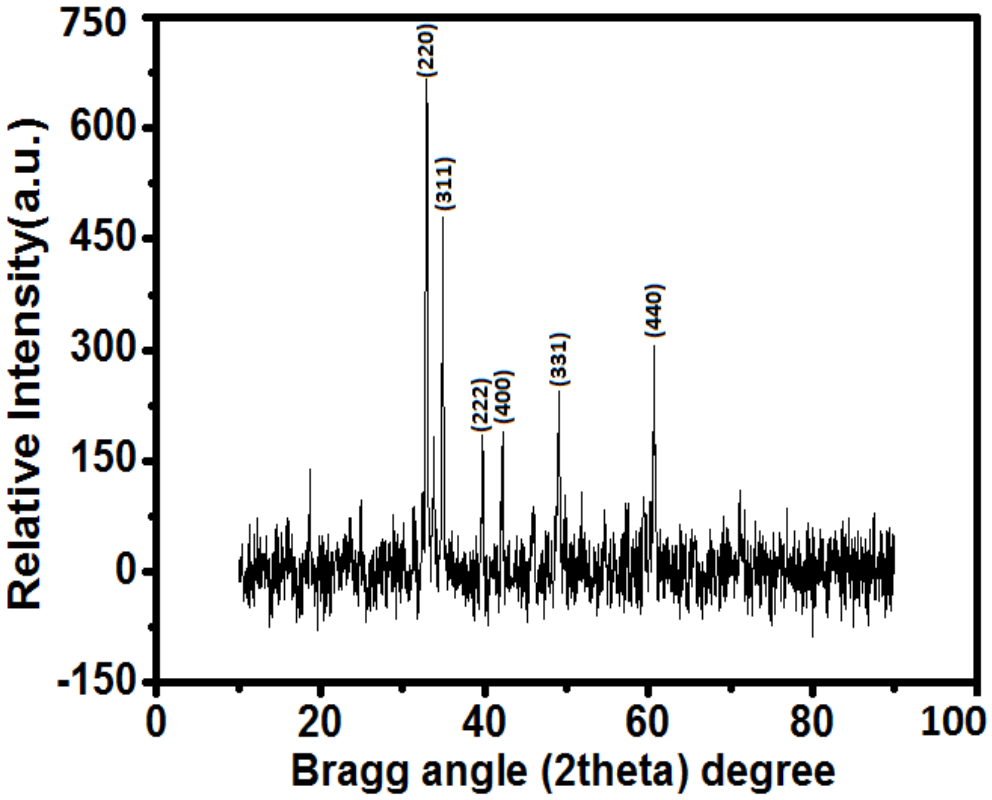


Figure 2: Powder XRD pattern for the $MgFe_2O_4$ nanoparticle

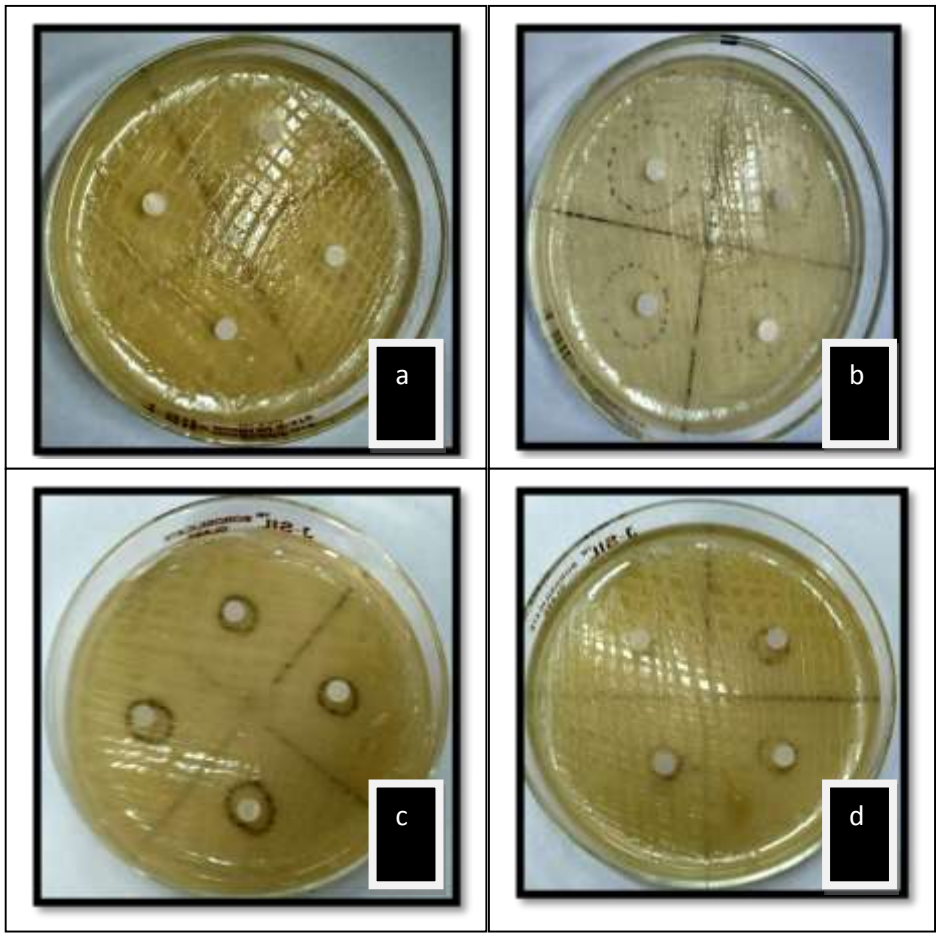


Figure 3: Antibacterial sctivity against (a) *B. subtilis* (b) *S. aureus* (c) *S. typhi* (d) *E. coli* at concn. 100ug/mL for compound 2.

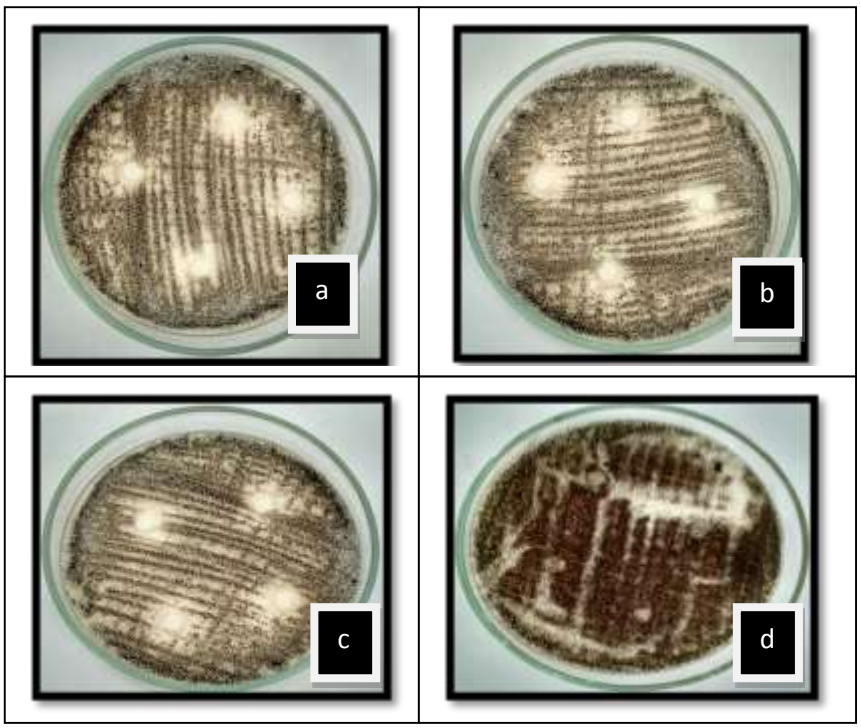


Figure 4: Antifungal activity against *A.niger* for (a) Clotrimazole (standard) (b) compound 2 (c) compound 3 and (d) compound 4 at conc. 100 µg/mL

Conclusion

Thus we have developed protocol for synthesis novel compounds 2-4 by using cheaper heterogeneous catalyst working in milder reaction conditions, shorter reaction time, simple and cleaner workup procedure. On comparing the antimicrobial activities of synthesized novel compounds, compound 2 exhibited the significant antibacterial property with that of ciprofloxacin as standard drug.

Compound 3 registered comparable antifungal activities to that of clotrimazole as standard drug opening the doors of new possibilities in the arena of antibiotics. The compounds 2-4 are under investigation in our laboratory for scanning of cytotoxicity.

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