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Comparison of Microwave Irradiation and Conventional Synthesis of 2-thiobarbituric acid Derivatives and *in vitro* Evaluation of Antimicrobial and Cytotoxic activity

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Abstract

The research work was involved in rapid and efficient procedure for the attachment of 2-thiobarbituric acid with arylidene acetophenone under microwave irradiation (MWI) and conventional heating. We found that the preparation time for the compounds was reduced from 24 hours to 5-10 minutes duing microwave heating. The yield also very poor (78-80%) for the compounds during conventional heating, but in MWI methods the yields were observed 96.68-98.50% which was comparatively too high. By using FT-IR, ^{1}H-NMR spectral data, the synthesized compounds were characterized. The cytotoxic and antimicrobial activities of the compounds were also investigated. Staphylococcus aureus, Bacillus megaterium, Pseudomonas aeruginosa and Escherichia coli revealed the inhibition zone were 8-14 mm where sample concentration was 100 µg/disc. However, cytotoxic analysis, the mortality 49-89% were appeared when sample concentration were 0.78-6.25(µg/ml) and more than 12.5 (µg/ml) concentration showed 100% mortality. The presence of a reactive and unsaturated ketone function in synthesized compounds may be responsible for their potential antimicrobial and cytotoxic activity.

Keywords: Microwave irradiation (MWI), *2*-thiobarbituric acid derivatives, Arylidene acetophenone, Antimicrobial and Cytotoxic activity.

Introduction

In the field of synthetic organic chemistry, microwave-assisted synthesis have a large impact in the medicinal/combinatorial chemistry communities. Comparing with traditional process microwave-enhanced synthesis saves significant time and gives maximum amount of yields. In organic synthesis use of microwaves has increased dramatically, receiving widespread acceptance[1]. Generally it is possible to synthesis organic compounds very fast with better yields and high purity by using microwave technology than any other methods[2-4]. Additionally, it is also eco-friendly synthetic method, one-pot syntheses could offer a significant step ahead.

2-thiobarbituric acid, chemically 2-thio-4,6-dioxohexahydropyrimidine is used as the main component to synthesis 2-thiobarbiturates that act as central nervous system depressants. 2-thiobarbituric acid does not give hypnotic and sedative effects by itself but after the substitution with alkyl or aryl group at position 5 provide effects. The derivatives of 2-thiobarbituric acid have especial place in pharmaceutical chemistry. In medical treatments they showed various classical applications such as sedative, hypnotic, anticonvulsant, antiplasmodic and local anaesthetic drugs[5,6]. Recently it has reported that they have applications in anti-tumor, anti-cancer and anti-osteoporosis treatments[7,8].

There are many reports on the reactions of carbonyl compounds-aldehydes, ketones and ester with barbituric acid[9-11]. But there is few study has been reported on the reactions of 2-thiobarbituric acid with α,β-unsaturated carbonyl systems. Although various processes for the preparation of these compounds have been mentioned, the maximum of them produce poor yields[11]. Therefore, considering the necessity of efficient method to synthesis 2-thiobarbituric acid derivatives, we synthesizes the 2-thiobarbituric acid derivatives using MWI which is relatively in good yields and to find out the potential biological activities of these compounds.

Materials and Methods

Instruments: The microwave oven employed for this reaction was specifid as classic white ProLine Micro Chef ST44 (720 W, 2450 MHz) with different power settings. By using electric-melting point apparatus, melting point was measured.

Chemicals: Aromatic aldehydes (benzaldehyde, 4-chlorobenzaldehyde and 4-methoxybenzaldehyde), acetophenones (acetophenone, 4-chloroacetophenone and 4-hydroxyacetophenone), 2-thiobarbituric acid was used for this experiment. 3M NaOH, 95% ethanol, rectified sprit and water were used as solvents.

Product identification: Fourier transform spectrometer (FTIR-8300) was used for FT-IR spectrum (KBr). ^{1}H-NMR was taken using chloroform-d ($CDCl_3$) at room temperature with a JEOL EX 270 spectrophotometer at 270 MHz. The characterization of our products was done by FT-IR, ^{1}H-NMR spectra. Finally, it was confirmed by comparing their melting point with literature.

Rate Enhancements: The rate enhancement was calculated for microwave and conventional method by using the given method:

Rate enhancement = (conventional reaction time/microwave reaction time)

Where, the microwave reaction time and conventional reaction time were taken to the same extent for complete reactions. In the present work, the reactions were done by following a general procedure[12-14].

Synthesis of 2-thiobarbituric acid derivatives (2a-2e): The reflux condition (conventional method): A mixture of arylidene acetophenone (1a-1e) (0.005 mol) and 2-thiobarbituric acid (0.005 mol) were dissolved in 25 ml rectified spirit with equal volume of water in a 250 ml round-bottomed flask. The flask was kept in a paraffin oil bath on a magnetic stirrer by fixing with a refluxing condenser. It was refluxed for 18 hours and the reaction was followed by TLC on silica gel plates (eluting solvent, Pet. ether: Ethyl acetate; 5:1). The mixture was then cool to form solid and dried in air to get the crystal of the compound.

Microwave irradiation methods (MWI): In a 250 ml conical flask an equimolar mixture of 2-thiobarbituric acid (2) (0.005 mol) and arylideneacetphenone (1a-1e) (0.005 mol) were dissolved in 25 ml rectified spirit and 25 ml water. The mixture was irradiated with microwave at different power level for several minutes and the progress of the reaction was followed by TLC on silica gel plate (eluting solvent, Pet. Ether: Ethyl acetate; 5:1). The mixture was then cool to form solid and dried in air to get the crystal of the compound. The purity of the product was checked by TLC.

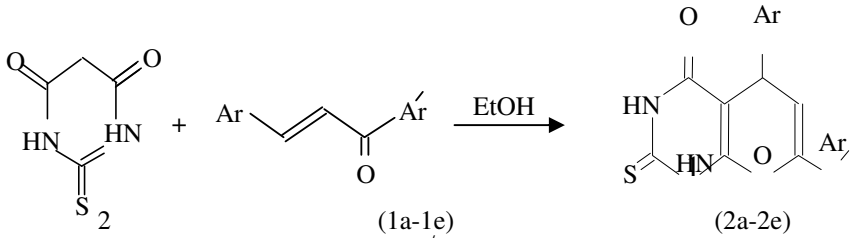
5-phenyl-7-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-2-thioxo-4-oxo-5H-pyrano [2,3-d]pyrimidine (2a): White color in powder form; m.p.: 285-287 ^{0}C; IR, *v*: 3600, 3155, 3028, 1710, 1618, 1446, 1091, 745, 680 (KBr) cm^{-1}; ^{1}HNMR ($CDCI_3$) δ: 10.56 (m, 2H, NH), 7.68-7.07 (m, 9H, Ar-H), 4.82 (s, 1H, Ar-OH), 5.95 (d, 1H, 6-H), 4.42 (d, 1H, 5-H).

5,7-di-(4-chlorophenyl)-1,2,3,4-tetrahydro-2-thioxo-4-oxo - 5H-pyrano [2,3-d]pyrimidine (2b): White color in powder form; m.p.: 246-248^{0}C; IR *v*: 3155, 3010, 1700, 1620, 1591, 1446, 1404, 1317, 1089, 1033, 825, 777, 717 (KBr) cm^{-1}; ^{1}HNMR ($CDCI_3$) δ: 10.42 (m, 2H, NH), 7.72-7.09 (m, 8H, Ar-H), 5.68 (d, 1H, 6-H), 4.41 (d, 1H, 5-H).

5-(4-chlorophenyl)-7-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-2-thioxo-4-oxo-5H-pyrano[2,3-d] pyrimidine (2c): White color in powder form; m.p.: 275-277 ^{0}C; IR *v*: 3700, 3155, 3030, 1710, 1620, 1435, 1100, 775 (KBr) cm^{-1}; ^{1}HNMR ($CDCI_3$) δ: 10.42 (m, 2H, NH), 7.53-7.25 (m, 8H, Ar-H), 4.72 (s, 1H, Ar-OH), 5.93(d, 1H, 6-H), 4.45 (d, 1H, 5-H).

5-(4-methoxyphenyl)-7-phenyl-1,2,3,4-tetrahydro-2-thioxo-4-oxo-5H-pyrano[2,3-d]pyrimidine (2d): White color in powder form; m.p.: 260-262 ^{0}C; IR *v*: 3155, 3030, 1710, 1595, 1444, 1261, 1111, 654 (KBr) cm^{-1}; ^{1}HNMR ($CDCI_3$) δ: 10.96 (m, 2H, NH), 7.79-6.71 (m, 9H, Ar-H), 5.81 (d, 1H, 6-H), 4.33 (d, 1H, 5-H), 3.84 (s, 3H, CH_3O).

5-(4-methoxyphenyl)-7-(4-chlorophenyl)-1,2,3,4-tetrahydro-2-thioxo-4-oxo-5H-pyrano[2,3-d] pyrimidine (2e): White color in powder form; m.p.: 254-256 ^{0}C; IR *v*: 3150, 1700, 1620, 1444, 1423, 1254, 1087, 740. (KBr) cm^{-1}; ^{1}HNMR ($CDCI_3$) δ: 10.63 (m, 2H, NH), 7.56-6.98 (m, 8H, Ar-H), 5.74 (d, 1H, 6-H), 4.27(d, 1H, 5-H), 3.48 (s, 3H, CH_3O).



	Ar	Ar/
2a	C_6H_5	C_6H_4OH
2b	C_6H_4Cl	C_6H_4Cl
2c	C_6H_4Cl	C_6H_4OH
2d	$C_6H_4CH_3O$	C_6H_5
2e	$C_6H_4CH_3O$	C_6H_4Cl

Scheme-1
Synthesis of 2-thiobarbituric acid derivatives (2a-2e)

Bioassay of synthesized compounds: Antimicrobial activities: Microorganisms: Pure culture of the microorganisms were collected from the instituted of Food Science and Technology, BCSIR, Dhaka, Bangladesh. *Aspergillus niger* and *Aspergillus flavus* were taken for the anti-fungal activity test. Potato-dextrose agar (PDA) slants were used to maintain the fungal species and stored at 4°C. The experiments were performed by disc diffusion method[15]. On the other hand, the organisms *Staphylococcus aureus, Bacillus megaterium, Escherichia coli* and *Pseudomonas aeruginosa* were used for anti-bacterial activity test. During experiment the active cultures were prepared by transferring a loopful of cells from stock cultures to flasks and inoculated in Luria-Bertani (LB) broth medium at 37 °C for 24 hours. The bacterial cultures were maintained on LB agar medium at 4 °C[16].

Preparation of discs: The antimicrobial activity was performed as the methods described previously[17]. Three types of discs were used for anti-bacterial and anti-fungal screening. Each test sample was taken in specific volume of solvent to obtain the required concentrations in an aseptic condition. Then discs were soaked with test samples solution and dried. Standard discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known anti-bacterial and anti-fungal agent with that of produced by the test sample. In this study, kanamycin (30 µg/disc) and ketoconazole (30 µg/disc) were used as standard for anti-bacterial and anti-fungal test, respectively. Blank discs were used as negative control which ensures that the residual solvents (left over the discs even after air-drying) and the filter papers were not active themselves.

Diffusion and Incubation: The sample discs, the control discs and the standard antibiotic discs were placed gently on the earlier marked zones in the agar plates with test bacteria and fungi. The plates were then kept in a refrigerator at 4ºC for about 24 hours to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37ºC for 24 hours for bacteria and at 28 ± 2ºC for 48 hours for fungi. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

Cytotoxicity bioassay: The cytotoxic activity was done as described previously[18]. The test samples were dissolved in dimethyl sulfoxide (DMSO) and diluted serialy as 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 µg/ml. Each of the test solution was added to test tubes containing 10 shrimps in simulated brine water (5 ml) for incubation at room temperature. After 24 hours, the mortality percentages of the shrimps were calculated.

Results and Discussion

The final products 2a-2e were obtained by the condensation reactions between the primary product (1a-1e) and 2-thiobarbituric acid (2) under conventional heating and were completed in 18 hours with moderate yield, whereas the same reactions under MWI method gave excellent yield within few minutes of irradiation. The structural assignment of 2a-2e was based on spectroscopic data. The FT- IR data of the compounds 2a-2e showed a broad and sharp bands at (ν_{max}) 3155-3100 cm^{-1} for N-H group. The absorption bands at 1710-1680 cm^{-1} represent C=O group. The picks at 1620-1505 cm^{-1} were showing the presence of aromatic C=C rings and C=N of the conjugated form of 2-thiobarbituric acid. 1460-1400 cm^{-1} were indicated to C-C stretching. The bands at 3700-3500 cm^{-1} indicating the presence of Ar-OH group, 800-600 cm^{-1} were specified to aromatic C-Cl group and 1265-1240 cm^{-1} indicates Ar-CH_3O group.

The ^{1}H-NMR spectrum of the synthesized compounds showed the N-H protons were strongly deshielded at δ 10.96-10.42 (d). The proton at position 6 appeared as δ 5.95-5.74 (d), the 5-H proton appeared as δ 4.45-4.27 (d). Ar-H group at δ 7.79-6.71 (m), Ar-OH group at δ 4.82-4.72 (s) and Ar-CH_3O group at δ 3.84-3.48 (s). All the FT-IR, ^{1}HNMR signals are identical to the known compound barbituric acid derivatives[19,20].

The result of conventional heating and microwave irradiation for the synthesis of compound 2a-2e has been compared. Moreover, the time and yield percent on the reaction were also summarized in Table-1. Comparative analysis of total reaction time and percentage yields for all synthesised 2-thiobarbituric acid derivatives by both conventional and microwave irradiation method was carried out to find out if microwave-assisted synthesis of 2-thiobarbituric acid derivatives adds any advantage or not. The result showed that the percentage yields of 2-thiobarbituric acid derivatives improve much better and extreme reduction in total reaction time. By using microwave irradiation, reaction is possible within few minutes; and it also improves the yield. This would be highly advantageous for drug discovery in laboratories where small amounts of different analogues have to be synthesised in short periods of time. This is very useful for combinatorial synthesis of new libraries of compounds. Microwave-assisted synthesis is quicker, environment friendly, high yielding and shows cleaner chemistry.

The synthesised 2-thiobarbituric acid derivatives (2a-2e) were tested for their activity against both Gram positive and Gram negative bacteria by disc diffusion method using Kanamycin as the standard and methanol as the vehicle. *Staphylococcus aureus, Bacillus megaterium, Escherichia coli* and *Pseudomonas aeruginosa* are used as the organisms. Screenings for the newly synthesized compounds were done at concentrations 100 µg $disc^{-1}$. *Staphylococcus aureus and Bacillus megaterium* were found to be resistant to all the compounds. The diameters of zone of inhibition were 8-14 mm. However, The two Gram negative organism namely *Escherichia coli* and *Pseudomonas aeruginosa* were showed zone of

inhibition 6-10 mm resistant to most of the compounds tested (Table-2).

All compounds were also tested for antifungal activity against *Aspergillus niger* and *Aspergillus flavus* by disc diffusion method using ketoconazole (30 µg $disc^{-1}$) as the standard and methanol as the vehicle. As shown in Table-2 both the fungal strains were found to be moderately sensitive to all the tested compounds.

The cytotoxic activities of the synthesized compounds was tested by brine shrimp lethality bioassay. The mortality percentages for all the compounds were found to be very high. Some compounds showed 100% mortality at very low concentration as shown in Table-3. Sample concentration 0.78-6.25 µg ml^{-1} showed the mortality of 49-89%, whereas 12.5-100 µg ml^{-1} concentration showed 100% mortality. From this study, it is evident that all the test samples were lethal to brine shrimp nauplii. These positive results suggested that they may contain antitumor or pesticidal activity.

Table-1
Comparison between two different synthesis methods of 2-thiobarbituric acid derivatives

Compounds	Conventional method		Microwave method		
	Time (hr)	Yield (%)	Time (min)	Power (W)	Yield (%)
2a	18	78.00	8	320	98.00
2b	18	76.00	8	320	97.68
2c	18	80.00	8	320	98.00
2d	18	78.00	8	320	98.27
2e	18	80.00	8	320	98.50

Table 2
Antimicrobial activities of the synthesized compounds

Tested Sample	Name of Bacteria				Name of Fungi	
	S. aureus	*B. megaterium*	*P. aeruginosa*	*E. coli*	*A. niger*	*A. flavus*
	Diameter of Zone of Inhibition (mm)					
2a	14	11	10	8	16	17
2b	12	10	6	9	14	16
2c	12	12	9	7	19	20
2d	12	11	10	10	16	16
2e	11	8	6	-	18	16
Ketoconazole	-	-	-	-	22	26
Kanamycin	28	29	28	27	-	-

Table-3
Cytotoxic activities of the synthesized compounds

Tested Sample	Sample Concentration (µg/ml)							
	0.78	1.56	3.125	6.25	12.5	25	50	100
	Mortality (%)							
2a	49	78	89	89	100	100	100	100
2b	89	89	100	100	100	100	100	100
2c	57	79	89	89	100	100	100	100
2d	68	68	89	89	100	100	100	100
2e	84	87	100	100	100	100	100	100

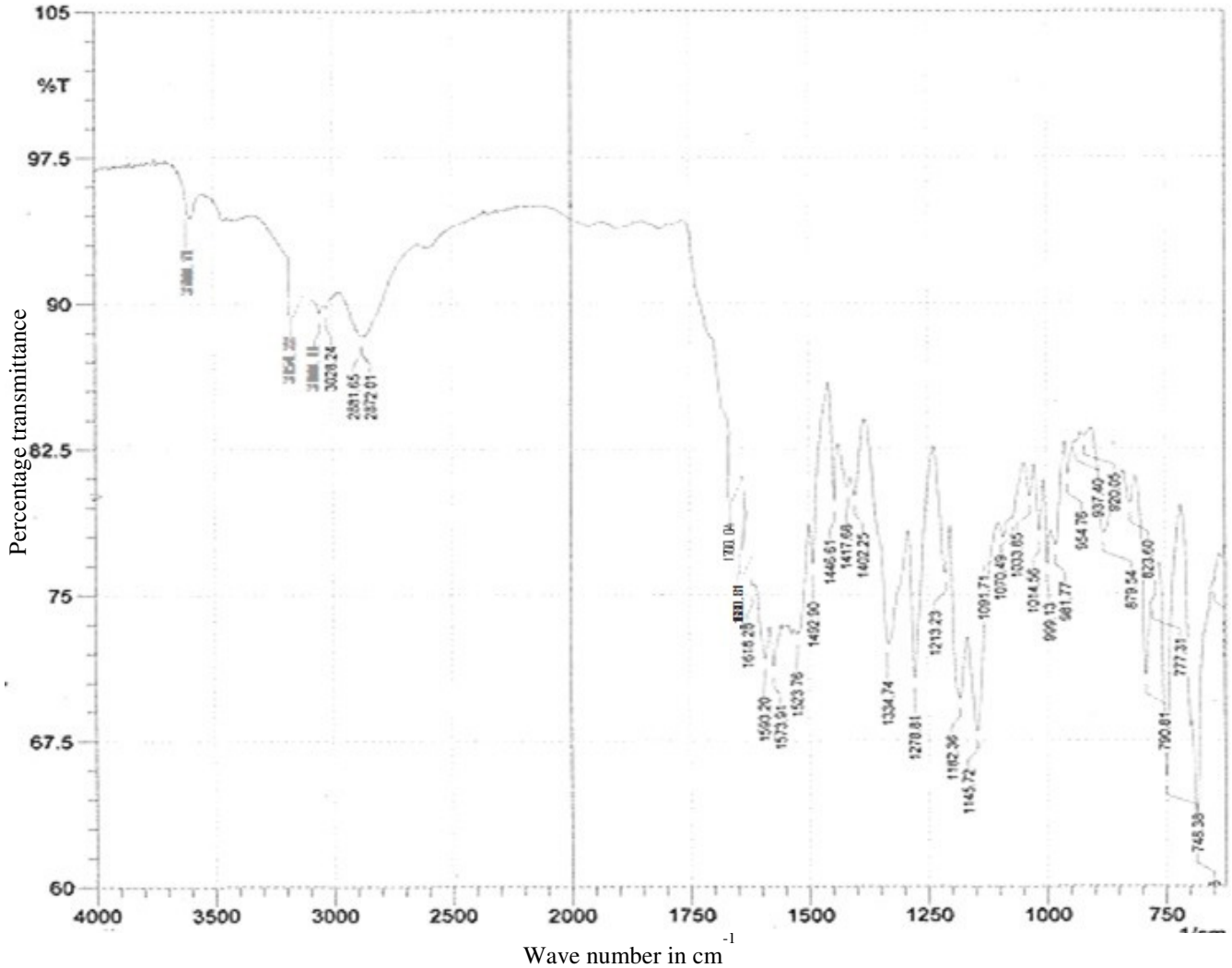


Figure-1
IR spectrum of 5-phenyl-7-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-2-thioxo-4-oxo-5*H*-pyrano [2,3-d]pyrimidine

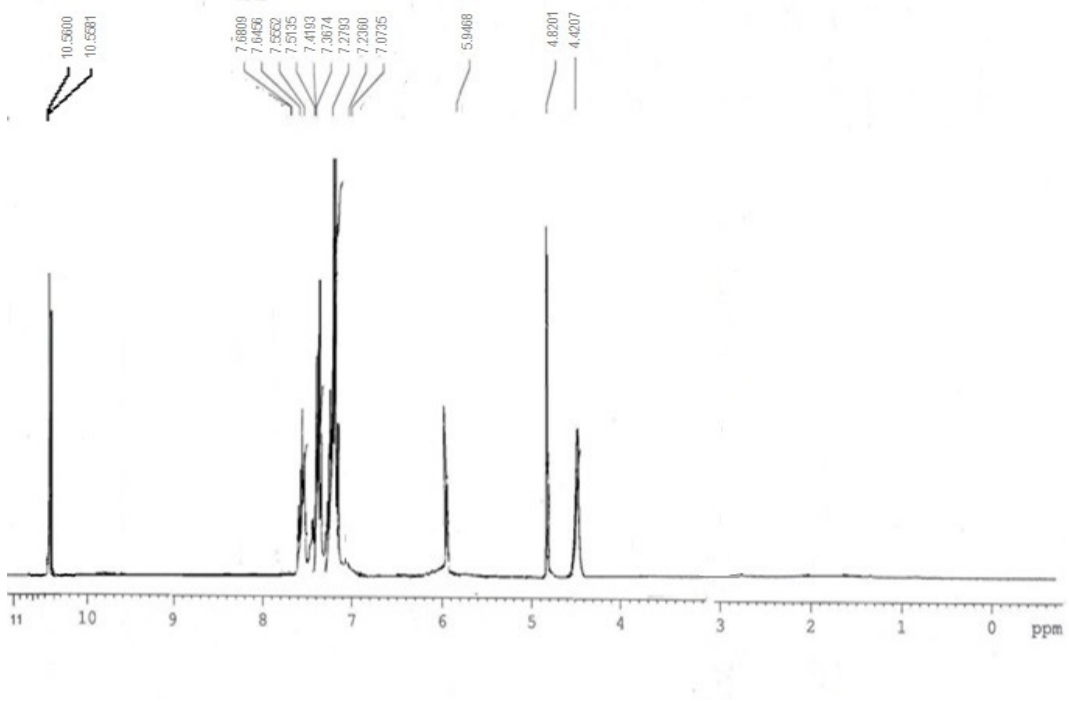


Figure-2
NMR spectrum of 5-phenyl-7-(4-hydroxyphenyl)-1,2,3,4-tetra- hydro-2-thioxo-4-oxo-5*H*-pyrano [2,3-d]pyrimidine

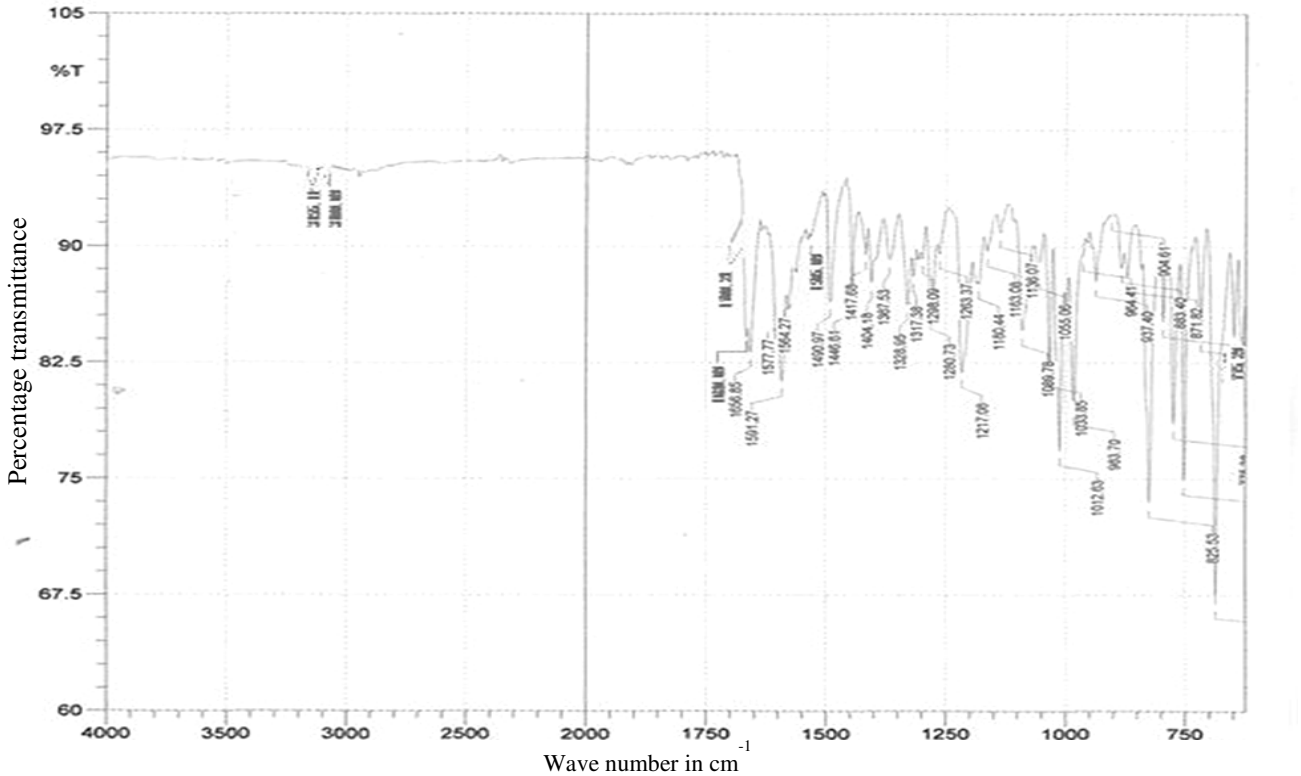


Figure-3
IR spectrum of 5,7-di-(4-chlorophenyl)-1,2,3,4-tetrahydro-2-thioxo-4-oxo-5*H*-pyrano [2,3-d]pyrimidine

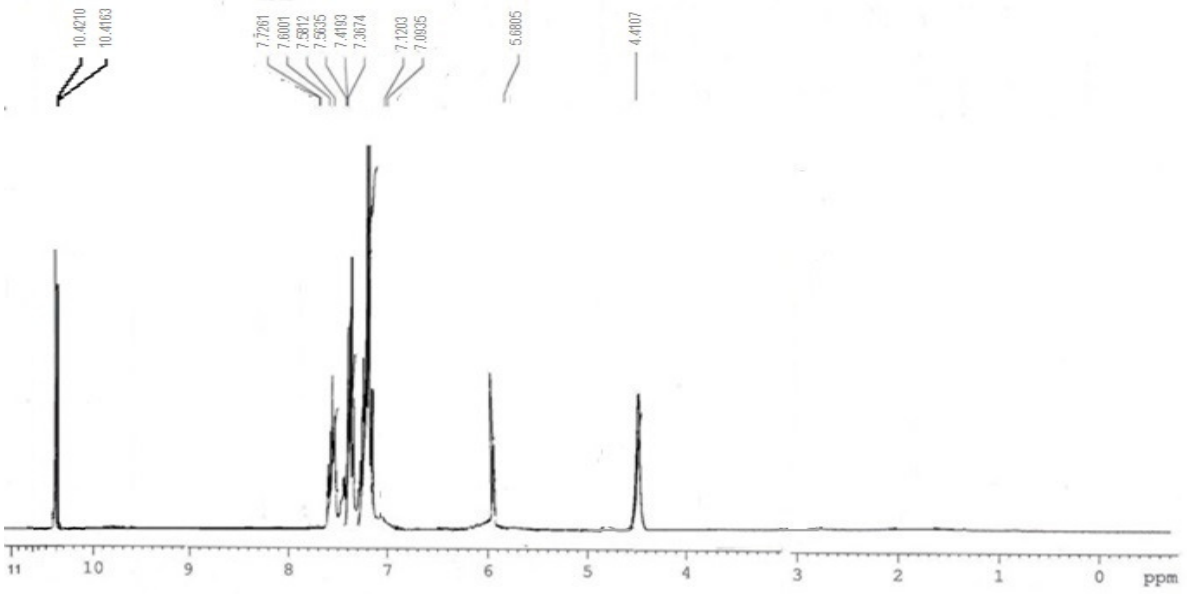


Figure-4
NMR spectrum of 5,7-di-(4-chlorophenyl)-1,2,3,4-tetrahydro-2- thioxo-4-oxo-5*H*-pyrano [2,3-d]pyrimidine

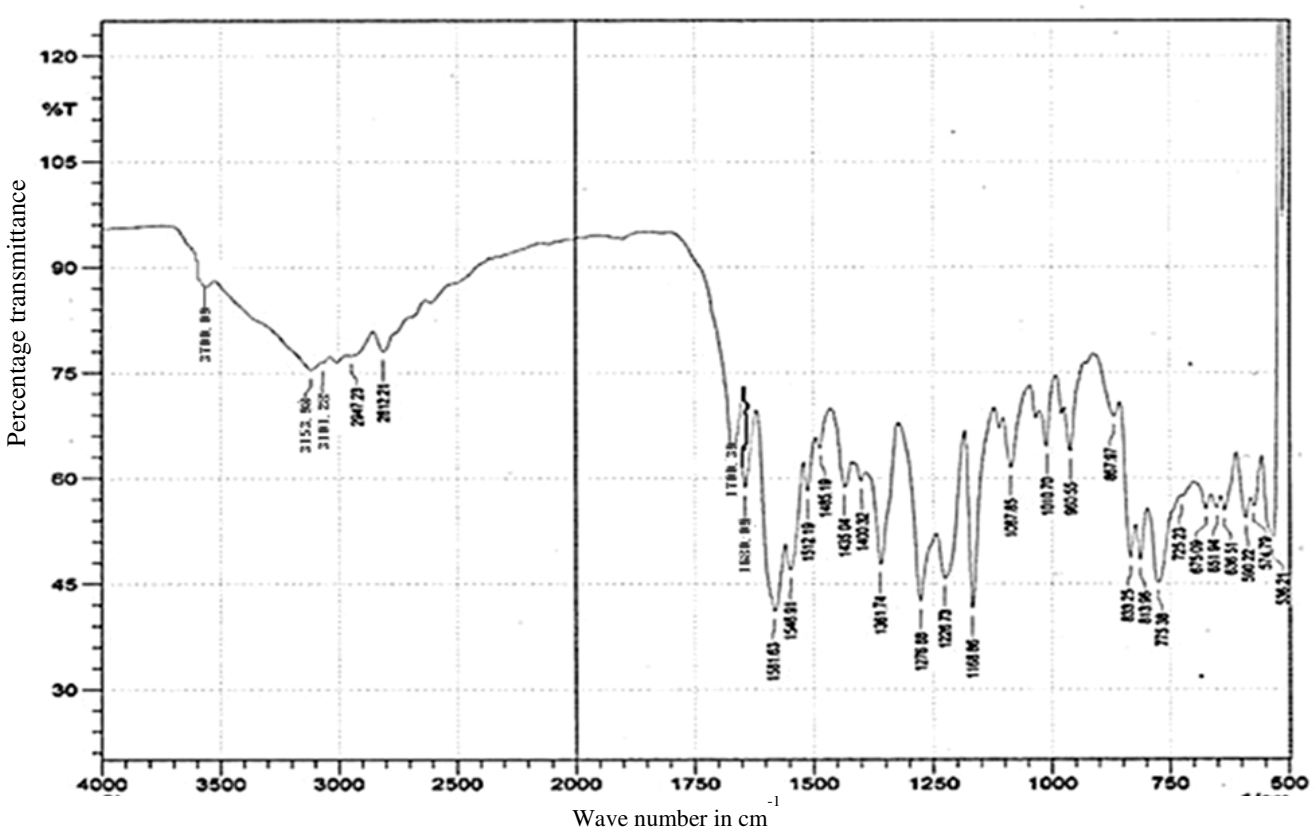


Figure-5
IR spectrum of 5-(4-chlorophenyl)-7-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-2-thioxo-4-oxo-5*H*-pyrano [2,3-d]pyrimidine

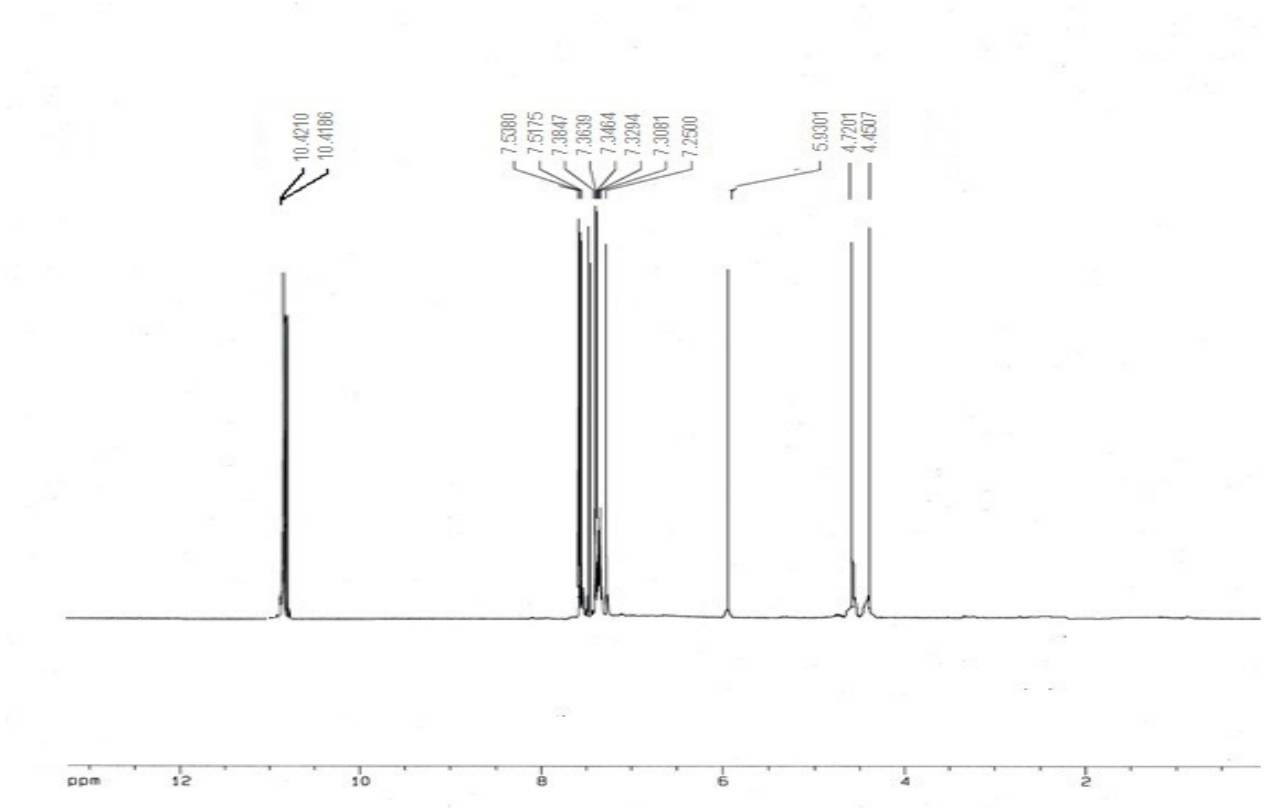


Figure-6
NMR spectrum of 5-phenyl-7-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-2-thioxo-4-oxo-5*H*-pyrano [2,3-d]pyrimidine

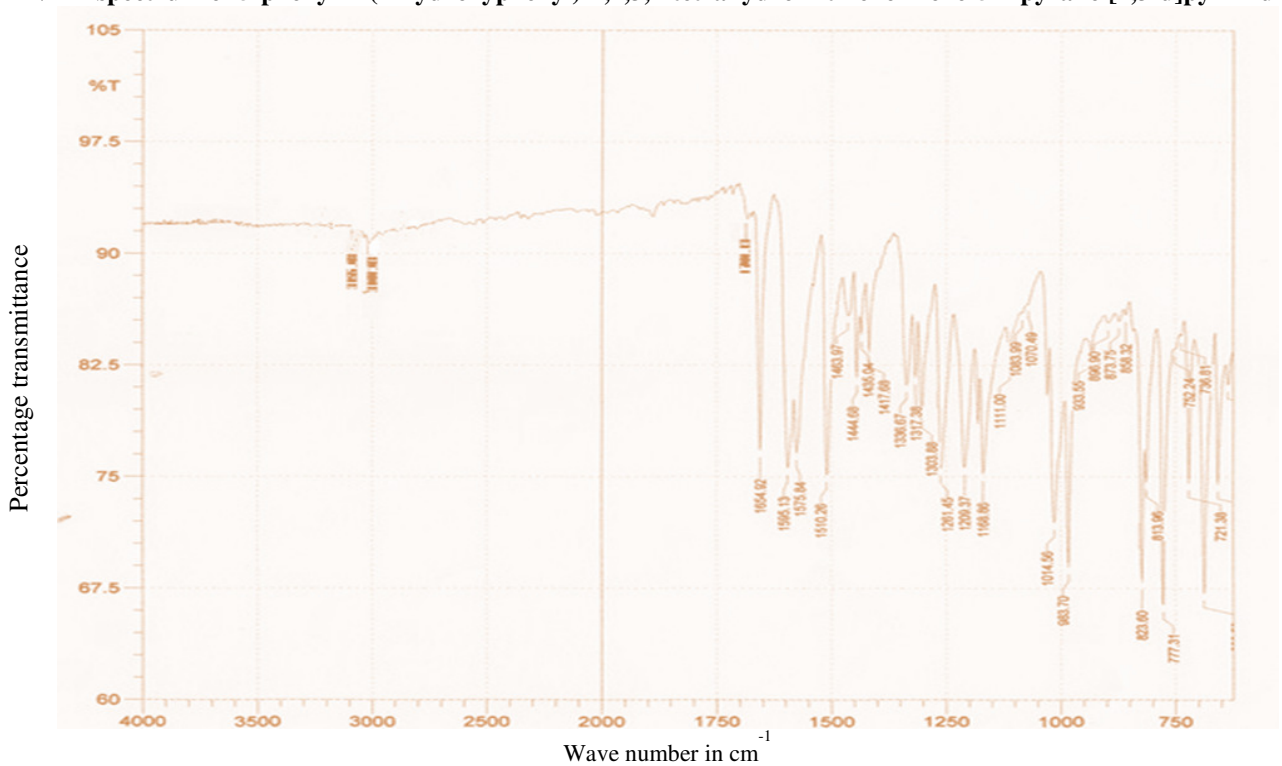


Figure-7
IR spectrum of 5-(4-methoxyphenyl)-7-(4-hydroxyphenyl)-1,2,3,4- tetrahydro-2-thioxo-4-oxo-5*H*-pyrano [2,3-d]pyrimidine

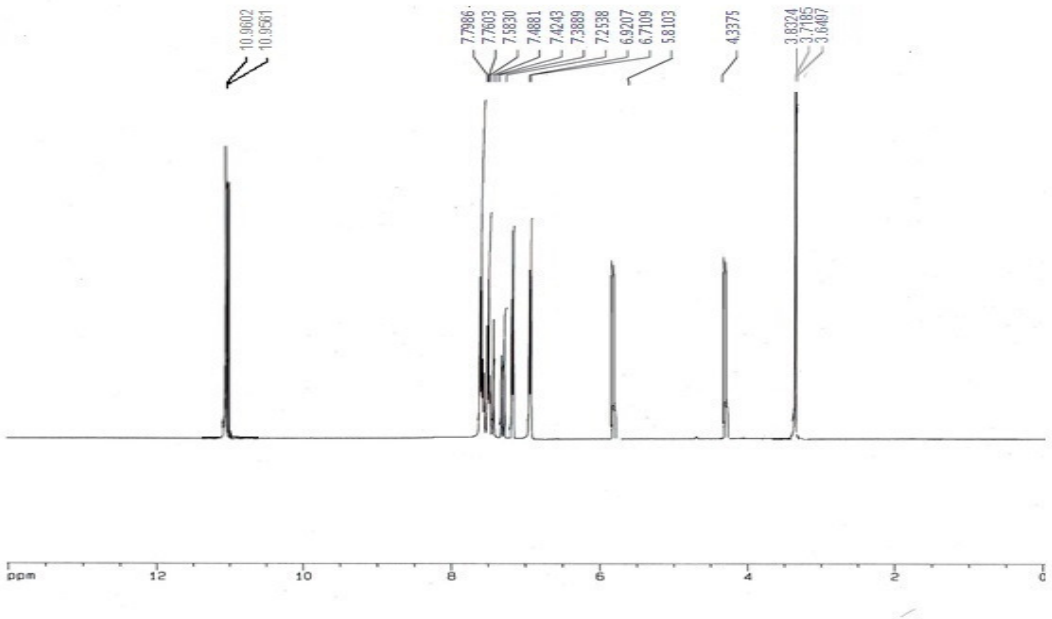


Figure-8
NMR spectrum of 5-(4-methoxyphenyl)-7-(4-hydroxyphenyl)-1,2,3,4-tetrahydro-2-thioxo-4-oxo-5*H*-pyrano [2,3-d]pyrimidine

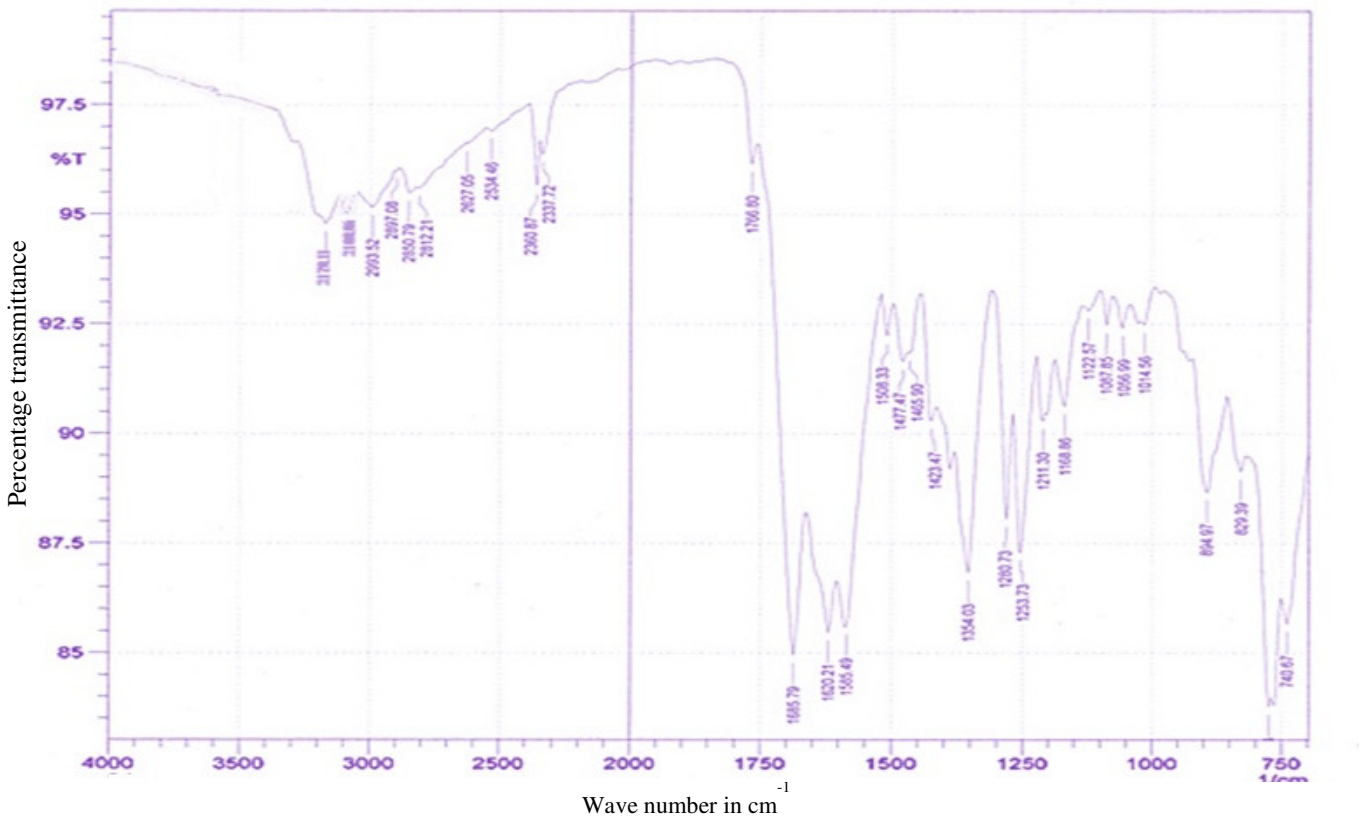


Figure-9
IR spectrum of 5-(4-methoxyphenyl)-7-(4-chlorophenyl)-1,2,3,4-tetrahydro-2-thioxo-4-oxo-5*H*-pyrano (2,3-d)pyrimidine

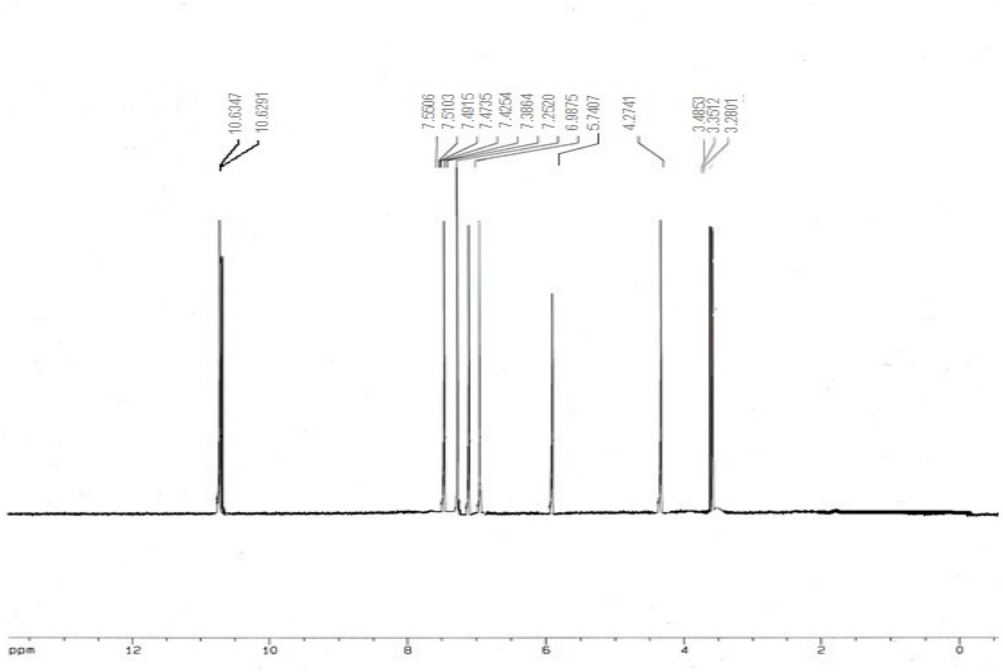


Figure-10
NMR spectrum of 5-(4-methoxyphenyl)-7-(4-chlorophenyl)-1,2,3,4-tetrahydro-2-thioxo-4-oxo-5*H*-pyr

Conclusion

From this study, it is concluded that the microwave-assisted syntheses offers reduction in reaction time, excellent yields without any undesirable side products, cleaner reaction and operation simplicity. Microwave synthesis method also reduce the reaction time from hours to minutes with improved yield as compared to the conventional heating. The use of low amount of chemicals prove this synthesis method as environmental friendly. In other words, as a recent work of green chemistry, it is very useful for performing the synthesis of drug, intermediates and chemicals.

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