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RESEARCH ARTICLE

Ferric (III) Chloride-Catalyzed Synthesis of 3, 4-Dihydropyrimidine-2(1H) Ones / Thiones for Biginelli Reaction and Characterization of Their Anticancer Activity

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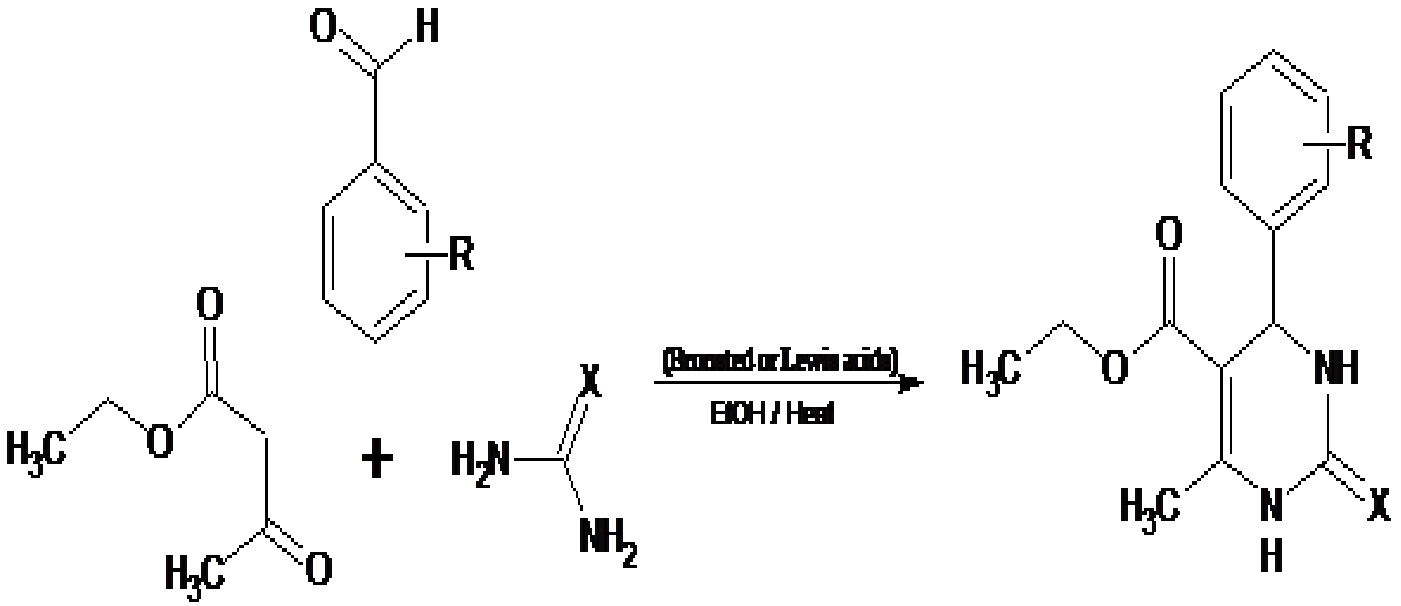
Abstract

We describe the syntheses of 3,4-Dihydropyrimidine-2(1H)Ones/Thiones by a one-pot cyclocondensation of urea or thiourea, aldehydes and ethylacetoacetates using Ferric (III) Chloride as catalyst, this method has the advantage of excellent yields (55–93%) and short reaction time (6-7 hours). Furthermore, we have studied the antioxidant activities of these synthesized 3,4-Dihydropyrimidine-2(1H)Ones/Thiones. Some the synthesized compounds appear the Important antioxidant properties, these properties studied by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assays. The cellular toxicity of the compound 1 was studied on (MCF7) cell lines using MTT assay that revealed the presence of a toxic effect of the cells with the highest activity of the compound 1 on (MCF7) cell lines. This indicates that compound 1 has an antioxidant and anticancer effect and can be used as a new chemical compound but this needs further investigation in vivo to confirm our results in vitro. The compounds were established on the basis of the spectral studies using IR, 1H-NMR, ^{13}C-NMR, and Mass spectra.

Key words: *Aldehydes, urea/thiourea, Ethyl acetoacetates, Anticancer, Antioxidant, MCF7.*

Introduction

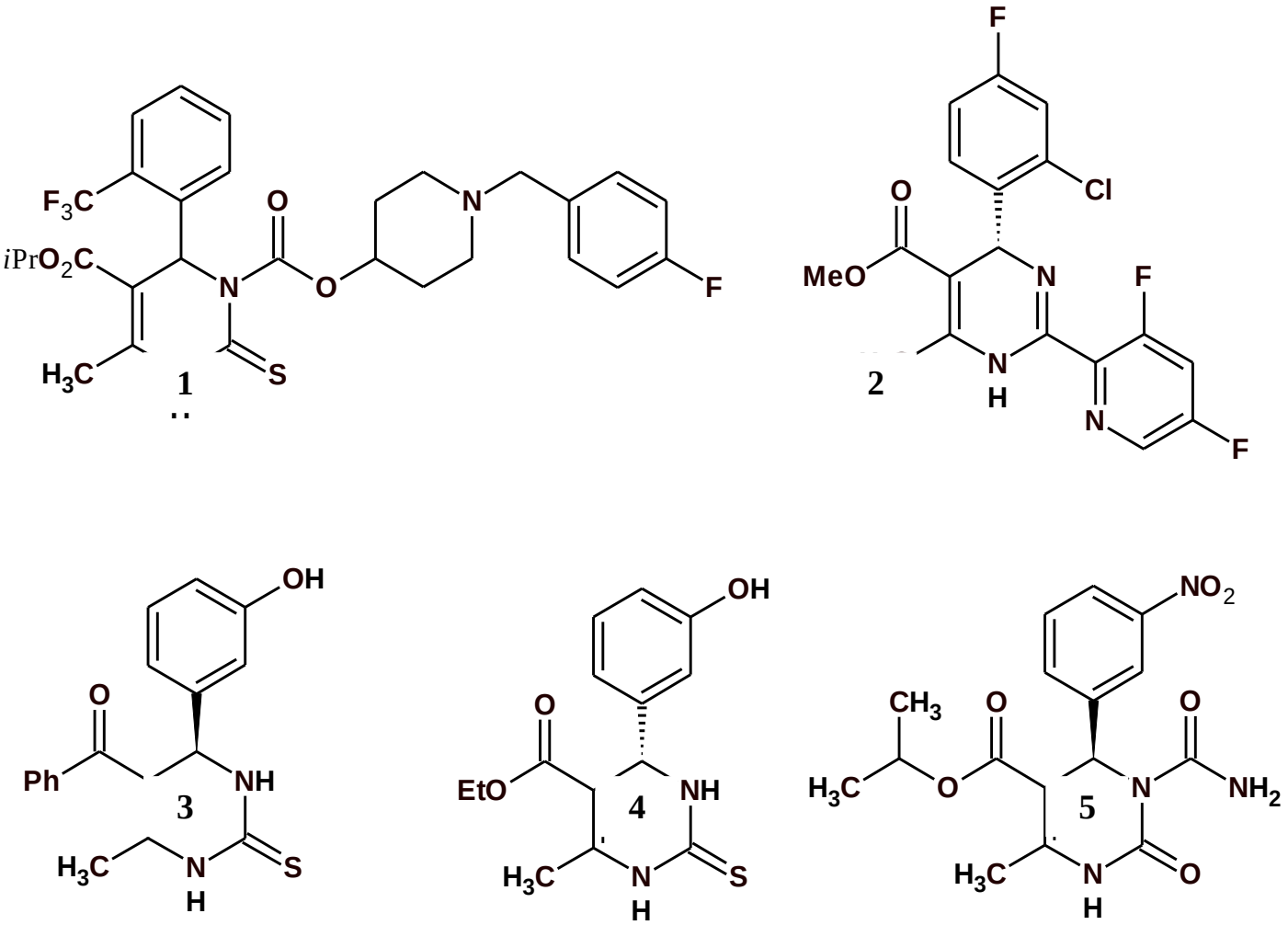
In 1891, an Italian chemist, P. Biginelli has reported the three component Multi component Reactions (MCR) using ß-keto esters such as ethyl acetoacetate, aromatic aldehydes such as benzaldehyde, and ureas (or thioureas) in the presence of acid catalyst (Brönsted or Lewis acids), affording dihydropyrimidinone derivatives [1](Scheme1).



X= O, S

Scheme1

Biginelli reaction allows the synthesis of important building blocks and versatile synthons such as 3, 4 dihydropyrimidin-2- (1H) -one (DHPM) derivatives[2], which are present motives in organic synthesis owing to their biological and pharmacological properties [3,4]. DHPMs can act as antihypertensive, antiviral, antibacterial, anti-inflammatory, or anticancer agents and potent calcium channel blockers, as examples of the wide range of biological activities that they can exhibit[5]. Moreover, some marine alkaloids containing a dihydropyrimidinone-5-carboxylate core (the batzelladine alkaloids) have been isolated, and these alkaloids were found to be potent HIV gp 120-CD4 inhibitors [6]. Furthermore, DHPMs could be obtained as chiral compounds, and the control of the stereochemistry at C(4) is crucial to determine their biological properties such as (*R*)-SQ 32547 (1)antihypertensive agent[7] ,Bay 41-4109 (2) (antiviral) [8], (*S*)-monastrol (3) (mitotic kinesinEg5 inhibitor) [9] ,(*R*)-mon97 (4) (anticancer agent) [10] and (*R*)-SQ 32926 (5)(antihypertensive)[11].As in the structures disclosed in (Fig. 1).



Figure

Due to the significance of the Biginelli reaction produce, much action on improving the yields and reaction conditions has been actively pursued. For epitome, Lewis acid catalysts, like $GaCl_3$[12], Co $(NO_3)_2.6H_2O$[13], TMSCl ($H_2C_2O_4$ or H3BO3)[14], $FeCl_3.6H_2O$/TMSBr[15] and L-proline nitrate[16].

Materials and Methods

Instruments and Reagents

All reagents and all solvents were purchased from Sigma–Aldrich, fluka and Merck. Human cancer cell lines; MCF-7 cell line were obtained from the Iraq biotech Cell Bank Unit.

Nuclear Magnetic Resonance (^{1}HNMR and ^{13}CNMR) spectra were recorded on Bruker AVANCE 500 MHz (500 MHz for proton, 125 MHz for carbon) In the Department of Chemistry, University of Tehran, Iran. Spectrometer with tetramethylsilane (TMS) as the internal reference using (DMSO-*d6*) as solvent, and chemical shifts were reported in parts per million (ppm). ESI-MS was recorded at 3 kV.

Synthesis of ethyl-4-(Substituted)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (1-7) (General Method) [17]

In 100 ml RBF (0.015 mol) urea or thiourea and 25 ml of ethanol was added and heated until clear solution was observed.

Then It was cooled to room temperature. (0.01 mol) ethyl acetoacetate and (0.01 mol) aldehyde and Ferric (III) chloride hydrous (25% mol) was added into above solution and stirred for (6-7) hours at reflux temperature.The progress of the reaction was followed by TLC using chlorofom :methanol 7:3 as eluent. In some cases solid products were obtained. It was filtered and washed with cold H_2O (3 x 30 mL) and a mixture of EtOH-H_2O 1:1 (3 x 20 mL).

If solid product is not obtained, the reaction mixture was poured onto crushed ice (50 g). Stirring was continued for (10-15) min, the solid products were filtered, washed with cold H_2O (3 x 30 mL) and a mixture of EtOH-H_2O 1:1 (3 x 20 mL), the solids were dried and recrystallised from a suitable solvent.

Ethyl-4- (2-hydroxy-3-methoxyphenyl)- 6-methyl- 2- oxo- 1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (a)

The compound was prepared from (1.52g, 0.01 mol) of o-vanillin and (1.3 ml,0.01 mol) of ethylaceto acetate with(0.9 g, 0.015mol) urea. Yield= 85%, m.p.= 205-206 ˚C. ^{1}H-NMR (500 MHz, DMSO-*d6*, δ, ppm): 1.032 (*t*, 3H, CH_3), 2.262 (*s*, 3H, CH_3), 3.921 (*m*, 2H, -OCH_2-), 5.518 (*d*, J=2.5 Hz, 1H, C_4-H ring), 6.60-6.84 (3H, Ar-H), 7.05 (*s*,1H, -NH-), 8.72(*s*,1H, -NH-)and9.08 (*s*, 1H, OH).ESI-MS m/z calcd. For $C_{15}H_{18}N_2O_5$ ([M]$^+$) 306.314, found 306.

Ethyl-4- (2- hydroxy-3-methoxyphenyl)-6-methyl- 2- thioxo-1, 2, 3, 4-tetrahydropyrimidine- 5- carboxylate (b)

The compound was prepared from (1.52 g,0.01 mol) of o-vanillin and (1.3 ml,0.01 mol) of ethyl acetoacetate with(1.14 g, 0.015mol) thiourea. Yield= 71%, m.p.=189-190˚C. ^{1}H-NMR (500 MHz, DMSO-*d6*, δ,ppm): 1.198 (*s*, 3H, CH_3), 2.258 (*s*, 3H, CH_3), 4.131 (*s*, 2H, -OCH_2-), 5.522 (*s*, 1H, C_4-H ring), 6.581-6.857 (3H, Ar-H), 9.04 (*s*,1H, -NH-), 9.09(*s*,1H, -NH-) and 10.17 (*s*, 1H, OH).

Ethyl-4-(5-bromo-2-hydroxyphenyl)-6-methyl- 2- oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate(c)

The compound was prepared from (2.01g, 0.01mol) of 5-bromo-2-hydroxybenzaldehyde and (1.3ml, 0.01 mol) of ethyl acetoacetate with (0.9g, 0.015mol) urea. Yield= 81%, m.p.= 212-213˚C. ^{1}H-NMR (500 MHz, DMSO-*d6*, δ,ppm): 1.053 (*t*, 3H, CH_3), 2.264 (*s*, 3H, CH_3), 3.662 (*m*, 2H, -OCH_2-), 5.40 (*d*, J=2.5 Hz, 1H, C_4-H ring), 6.75-7.22 (3H, Ar-H), 7.05 (*d*, J=2.5 Hz, 1H, -NH-), 9.15(*s*,1H, -NH-)and9.94 (*s*, 1H, OH).^{13}C-NMR(DMSO-*d6*, δ,ppm):14.44 (-CH_3), 18.20 (-CH_3), 50.07 (C_4-ring), 59.53 (-OCH_2-), 97.54 (C_5-ring), 152.4 (C_6-ring), 110.8-149.3(Ar-C), 154.7 (C=O amide)and 165.7(C=O ester).ESI-MS m/z calcd. For $C_{14}H_{15}BrN_2O_3S$ ([M - H]$^+$) 355, found 354.

Benzene-1, 4-diyl-bis-(ethyl-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropy- rimidine-5-carboxylate) (d)

The compound was prepared from (1.34g, 0.01mol) of terephthal- aldehyde and (2.6 ml, 0.02 mol) of ethyl acetoacetate with (1.8 g, 0.03mol) urea. Yield= 93 %, m.p.= 290-291˚C.^{1}H-NMR (500 MHz, DMSO-*d6*, δ,ppm): 1.093 (*t*, 6H, CH_3), 2.232 (*s*, 6H, CH_3), 3.974 (*m*, 4H, -OCH_2-), 5.108 (*d*, J=2 Hz, 2H, C_4-H ring), 6.717 (*s*, 4H, Ar-H), 7.70 (*s*, 2H, -NH-)and9.18(*s*, 2H, -NH-).^{13}C-NMR(DMSO-*d6*, δ,ppm):14.52 (-CH_3), 18.22 (-CH_3), 54.19 (C_4-ring), 59.64 (-OCH_2-), 99.75 (C_5-ring), 148.7 (C_6-ring), 126.7-144.4 (Ar-C), 152.5(C=O amide)and 165.7 (C=O ester).ESI-MS m/z calcd. For $C_{22}H_{26}N_4O_6$ ([M] $^+$) 442.

4,4'-benzene-1,4- diyl- bis-(ethyl- 6- methyl-2- thioxo-1, 2, 3, 4- tetrahydro- pyrimidine-5-carboxylate) (5e)

The compound was prepared from (1.34g, 0.01mol) of terephthal- aldehyde and (2.6 ml, 0.02mol) of ethylacetoacetate with (2.8g, 0.03mol) thiourea. Yield= 55 %, m.p.= 210-211˚C.^{1}H-NMR (500 MHz, DMSO-*d6*, δ,ppm): 1.100 (*t*, 6H, CH_3), 2.274 (*s*, 6H, CH_3), 4.003 (*m*, 4H, -OCH_2-), 5.138 (*d*, J=2 Hz, 2H, C_4-H ring), 7.184 (*s*, 4H, Ar-H), 9.62 (*s*, 2H, -NH-)and10.3(*s*, 2H, -NH-). ^{13}C-NMR (DMSO-*d6*, δ, ppm):14.47 (-CH_3), 17.63 (-CH_3), 54.22 (C_4-ring), 60.08 (-OCH_2-), 101.7 (C_5-ring), 145.5 (C_6-ring), 127.07-143.4 (Ar-C), 165.5(C=O ester) and 174.6 (C=S).

Ethyl- 4- (2-hydroxynaphthalen-1-yl)- 6-methyl- 2-oxo-1, 2, 3, 4- tetrahydropyrimidine- 5- carboxylate (f)

The compound was prepared from (1.72g, 0.01mol) of 2-hydroxy-1-naphthaldehyde and (1.3ml, 0.01 mol) of ethylacetoacetate with (0.9g, 0.015mol) urea. Yield=80%, m.p.= 254-255˚C. ^{1}H-NMR (500 MHz, DMSO-*d6*, δ, ppm): 1.277 (*t*, 3H,

CH_3), 1.81 (*s*, 3H, CH_3), 4.219 (*m*, 2H, -OCH_2-), 5.077 (*dd*, J= 3, 4.5 Hz, 1H, C_4-H ring), 7.04-8.04 (6H, Ar-H), 7.63 (*s*,1H, -NH-), 7.64(*s*,1H, -NH-).ESI-MS m/z calcd. for $C_{18}H_{18}N_2O_4$([M]$^+$) 342.412.

***E*ethyl- 4- (2-hydroxynaphthalen-1-yl)- 6-methyl- 2- thioxo-1, 2, 3, 4- tetrahydropyrimidine-5-carboxylate (5g)**

The compound was prepared from (1.72 g, 0.01mol) of 2-hydroxy-1-Na- phthaldehyde and (1.3 ml, 0.01 mol) of ethyl acetoacetate with (1.4 g, 0.015mol) thiourea. Yield=71%, m.p.=201-202˚C. ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm): 1.276 (*t*, 3H, CH_3), 1.855 (*s*, 3H, CH_3), 4.206 (*m*, 2H, -OCH_2-), 5.190 (*dd*, J=2.5, 5.5 Hz, 1H, C_4-H ring), 7.08-8.16 (6H, Ar-H), 9.16 (*s*,1H, -NH-) and 9.50 (*s*,1H, -NH-).

Antioxidant Activity[18]

DPPH Radical Scavenging Activity

Methanolic solutions of 1,000 ppm concentration of DHPMs (a, b, c, f, g) were prepared. Varying amounts (5, 10, 15, 20and 25µL) of each methanolic solution of DHPMs 5(a, b, c, f, g) were taken in separate test tubes containing5 ml of 0.004% methanolic solution of DPPH. All the test solutions were prepared in triplicate.

The mixtures were shaken vigorously and placed in dark for 2 h, or until stable values were obtained. The absorbance of the samples was measured at 517 nm. The percent DPPH radical scavenging activity of each sample and standard was calculated using the following equation:

% DPPH radical scavenging activity = [1 - (A_t / A_o)] x 100

Where, at is the absorbance of the sample, and $\mathbf{A_o}$ is the absorbance of the control. Mean values from three independent samples were calculated for each compound and ascorbic acid were used as a standard.

Reducing Power Activity

Different amounts of methanolic solutions of DHPMs 5(a, b, c, f, g) i.e., (0.1, 0.2, 0.3, 0.4 and 0.5) mg/ml were mixed with 2.5 ml of the phosphate buffer (200 mmol and pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixtures were incubated at 50 °C. After incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 rpm for 10 min. The upper layer was separated, and 5 ml of it was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride. Absorbance of the resultant solutions was measured at 700 nm.

Anticancer Activity

Maintenance of Cell Cultures[19, 20]

MCF-7 cell line were obtained from the Iraq biotech Cell Bank Unit This human cell line was Maintained in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were pass aged using Trypsin-EDTA reseeded at 50% confluence twice a week, and incubated at 37 °C.

Cytotoxicity Assays (MTT assay) [21]

To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates. Cell lines were seeded at 1 × 10^4cells/well. After 24 hrs. Or a confluent monolayer was achieved; cells were treated with tested compounds5a.

Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT (and incubating the cells for 1.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells was solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking.

The absorbency was determined on a micro plate reader at 492 nm (test wavelength); the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:-

Inhibition rate = A-B/A

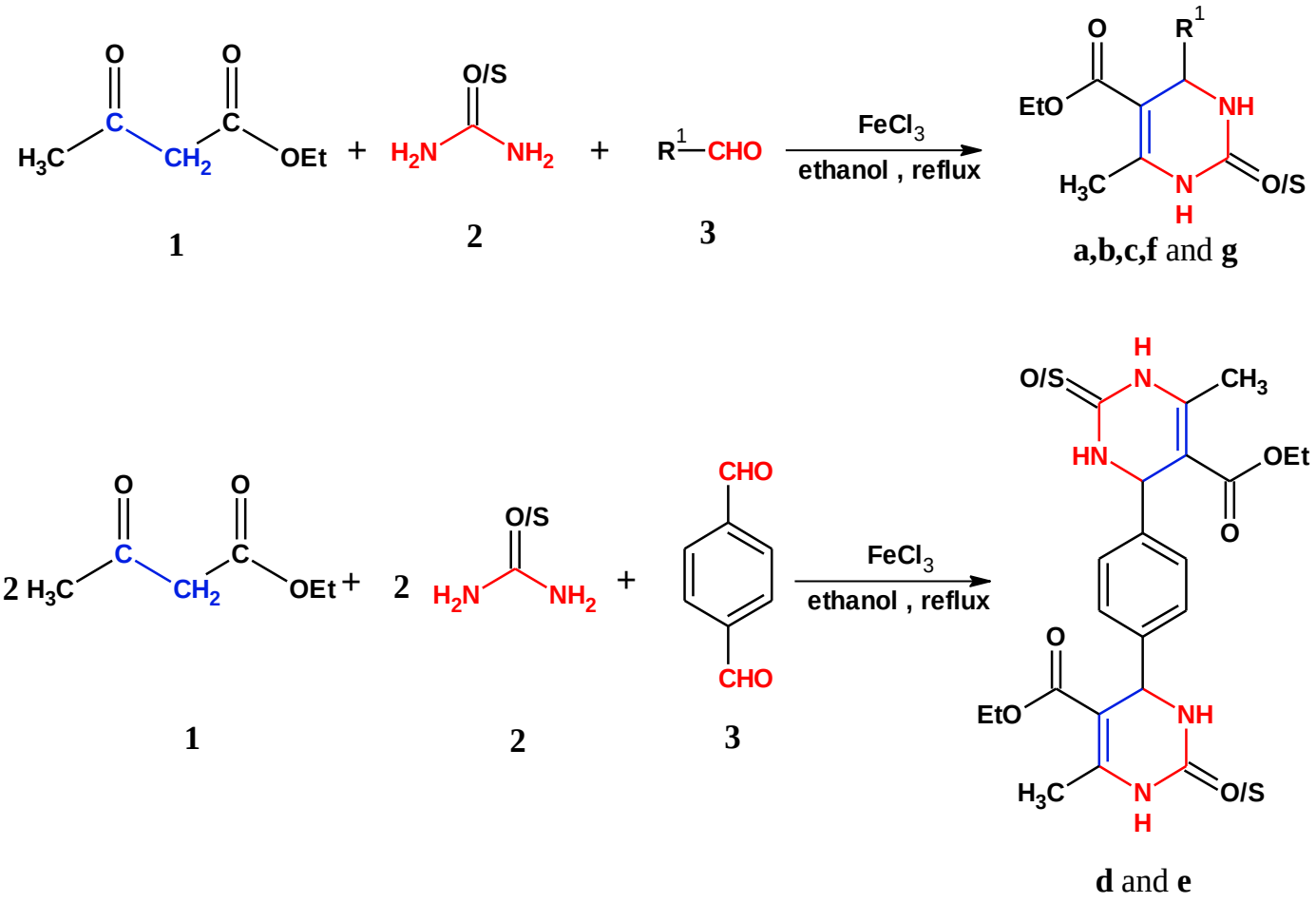
Where A and B are the optical density of control and the optical density of test, respectively.

Results and Discussion

Chemical

3, 4- dihydropyrimidin- 2 (1H)- one/thione derivatives a-g were synthesized by one-pot three-component condensation reaction (Biginelli reaction) of ethyl acetoacetate 1, urea/thiourea2 and aromatic aldehydes 3in the presence of ethanol as solvent and $FeCl_3$ as catalyst. Ferric (III) chloride considered as an appropriate catalyst for the Biginelli reaction, and was used to synthesize a series 3, 4-dihydropyrimidin-2 (1H) -one/thione derivatives (a-g) by reacting ethyl acetoacetate1 (2 mmol), urea/thiourea2 (3 mmol) withbenzaldehydes3 (2 mmol) at 80°C

under ethanol as solvent (Scheme 1) and these results are listed in Table 1.



Scheme 1: Synthesis of 3, 4-dihydropyrimidin-2 (1H) -one/thione derivatives a-g

Table 1: Properties of DHPMs a-g

Entry	R^1	O/S	Time (h)	m.p°C	Yield %
a	2-OH-3-OCH_3-C_6H_3	O	6	205-206	85
b	2-OH-3-OCH_3-C_6H_3	S	7	189-190	71
c	2-OH-5-Br-OCH_3-C_6H_3	O	7	212-213	81
d	——	O	3	290-291	93
e	——	S	4	210-211	55
f	2-OH –$C_{10}H_7$	O	5	254-255	80
g	2-OH –$C_{10}H_7$	S	6	201-202	71

The results are listed in Table 1. It was found that benzaldehyde and both electron withdraw groups or electron donating groups could implement Biginelli reaction with urea/thiourea and ethyl acetoacetate in good yields at 80°C (entries a–g, Table 1).

The position of the substitution group seldomly had an effect on reaction yields, although it was necessary to prolong the reaction time when ortho- and meta substituted substrates was used for this reaction (entries a, b, c, f, and g, Table 1), which may be caused by the increase in steric hindrance around the carbonyl group and the effect of electron withdrawing substituent, respectively.

However, aromatic aldehydes substituted by electron-withdrawing group gave higher yields of DHPMs than by electron-donating group at the same position (entried, Table 1). Finally, 2-hydroxy naphthaldehyde could afford the corresponding DHPMs in high yields under the same conditions smoothly (entries f and g, Table 1).

Antioxidant Activity

We monitored the antioxidant activities of these compounds. The antioxidant activity is determined by using DPPH assay. Due to its simplicity and accuracy, DPPH assay is the most widely used method to assess antioxidant potential of compounds. Therefore; the antiradical activities of test compounds Table (2) have been determined using DPPH assay. During this assay, antioxidant is used to reduce the alcoholic solution of DPPH resulting in the

formation of the non-radical form DPPH-H in the reaction. And, the dark colored DPPH radical solution in the presence of an antioxidant compound turned yellow-colored diphenylpicrylhydrazine in the presence of antioxidants and thus absorbance of the solution decreases. The DPPH assay is commonly used to assess free radical scavenging activity of antioxidants. (Fig. 1) shows a noteworthy decline in the concentration of DPPH radical in terms of % inhibition Table (3) due to the scavenging ability of test compounds.

The change in absorbance was measured at 517 nm. The inhibition percentage of all tested samples showed a concentration-dependent pattern as evident from (Fig. 1) the inhibition percentages of the test compounds range from 67.37% to 18.16%. The Vit C exhibited inhibition percentages of 95.06% at 25 µL in 1000 ppm concentration whereas compound ethyl-4-(2-hydroxy-3-methoxyphenyl)- 6- methyl- 2-oxo-1,2, 3, 4- tetrah- ydropyrimidine-5-carboxylate a. showed highest inhibition percentage as 67.37% in comparison to all test compounds at 25 µL in 1000 ppm.

Similarly, whereas all the test compounds show lower percentage inhibition at this concentration. Overall all the compounds showed different antioxidant activity in comparison to the standard compound and among the entire compound.

Table 2: Absorption values of the compounds

Comp.	Conc. 1000 ppm																			
	5 µL				10 µL				15 µL				20 µL				25 µL			
	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a
a	0.287	0.288	0.287	0.287	0.235	0.237	0.237	0.236	0.218	0.218	0.22	0.218	0.211	0.213	0.211	0.211	0.199	0.201	0.2	0.2
b	0.469	0.47	0.47	0.469	0.411	0.411	0.41	0.410	0.386	0.387	0.387	0.386	0.375	0.377	0.376	0.376	0.326	0.325	0.325	0.325
c	0.553	0.554	0.554	0.553	0.545	0.545	0.546	0.545	0.526	0.525	0.527	0.526	0.51	0.511	0.511	0.510	0.501	0.502	0.502	0.501
f	0.453	0.454	0.453	0.453	0.434	0.433	0.436	0.434	0.415	0.414	0.415	0.414	0.391	0.392	0.391	0.391	0.384	0.383	0.383	0.383
g	0.445	0.446	0.445	0.445	0.373	0.375	0.374	0.374	0.342	0.344	0.343	0.343	0.34	0.339	0.339	0.339	0.294	0.295	0.295	0.294
Vit. C	0.059	0.058	0.059	0.058	0.042	0.043	0.043	0.042	0.036	0.035	0.035	0.035	0.034	0.034	0.034	0.034	0.031	0.031	0.032	0.031

Table 3: Values of inhibition of compounds

Comp.	Conc. 1000 ppm														
	5 µL			10 µL			15 µL			20 µL			25 µL		
	A_a	A_o	(I%)	A_a	A_o	(I%)	A_a	A_o	(I%)	A_a	A_o	(I%)	A_a	A_o	(I%)
a	0.287	0.613	53.12	0.236	0.613	61.44	0.218	0.613	64.32	0.211	0.613	65.47	0.2	0.613	67.37
b	0.469	0.613	23.38	0.410	0.613	33.00	0.386	0.613	36.92	0.376	0.613	38.66	0.325	0.613	46.92
c	0.553	0.613	9.67	0.545	0.613	11.03	0.526	0.613	14.19	0.510	0.613	16.69	0.501	0.613	18.16
f	0.453	0.613	26.04	0.434	0.613	29.14	0.414	0.613	32.35	0.391	0.613	36.16	0.383	0.613	37.46
g	0.445	0.613	27.35	0.374	0.613	38.98	0.343	0.613	44.04	0.339	0.613	44.64	0.294	0.613	51.93
Vit. C	0.058	0.635	90.76	0.042	0.635	93.28	0.035	0.635	94.43	0.034	0.635	94.64	0.031	0.635	95.06

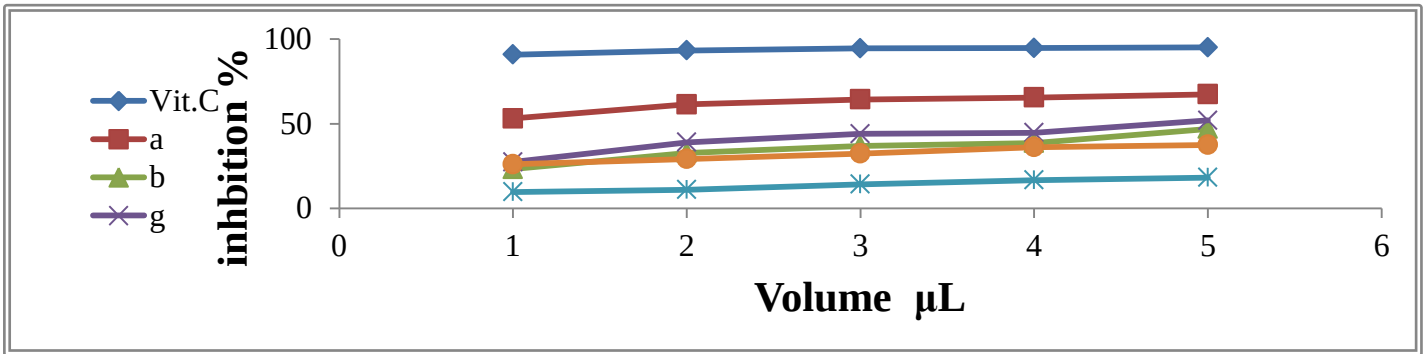


Figure 1: DPPH radical scavenging activities of test compounds (a, b, c, f, g) and standard antioxidant

Reducing Power Assay

Figure (2) and Table (4) show the reducing power of (a, b, g) as a function of their concentration. In this assay, the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers (i.e. antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the Fe^{2+} concentration. The reducing power of the compounds increased with concentration. The reducing power of a was excellent Figure (2); at 0.5 mg/ml of the reducing power of a was higher than 0.46. At 0.1 mg/ml, the reducing powers ofawas 0.21, and at 0.3 mg/ ml were 0.34. Reducing power of Vit.C at 0.5 mg/ml was 0.841.

Table 4: Absorption values of the compounds

Comp.	Concentration (mg/ml)																			
	0.1				0.2				0.3				0.4				0.5			
	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a	A_1	A_2	A_3	A_a
a	0.218	0.224	0.213	0.218	0.262	0.258	0.263	0.261	0.345	0.341	0.337	0.341	0.412	0.419	0.415	0.415	0.461	0.465	0.463	0.463
b	0.185	0.178	0.179	0.181	0.213	0.221	0.216	0.217	0.252	0.246	0.251	0.25	0.321	0.328	0.323	0.324	0.364	0.358	0.359	0.36
g	0.191	0.198	0.189	0.193	0.238	0.236	0.225	0.233	0.283	0.288	0.281	0.284	0.364	0.358	0.354	0.359	0.421	0.42	0.425	0.422
Vit. C	0.328	0.324	0.329	0.327	0.451	0.448	0.453	0.451	0.573	0.579	0.571	0.574	0.698	0.689	0.7	0.696	0.844	0.838	0.841	0.841

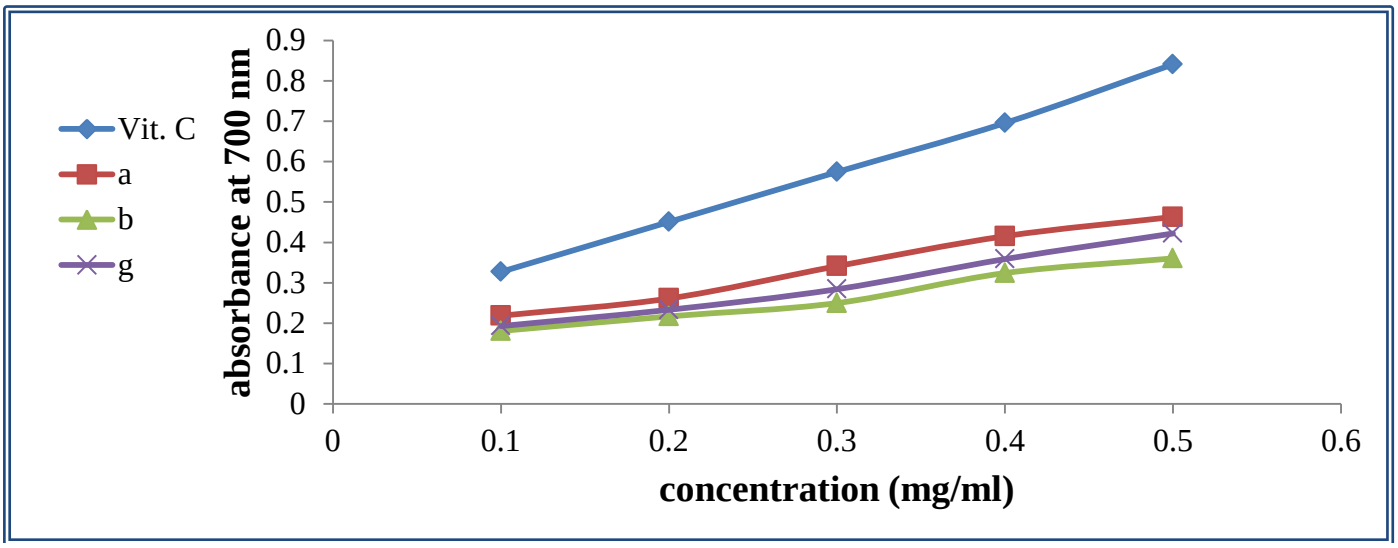


Figure 2: Reducing power of (a, b, g)

Anticancer Profiles

The anticancer potential of the developed compounds was assessed in terms of inhibition rate of cell growth (The percentage cytotoxicity) on MCF-7 cancer cell lines Table (5). Ethyl-4-(2-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1,2,3, 4-tetrahyd- ropyrimidine-5-carboxylate (a) on MCF-7 at varying concentrations (6.25, 12.5, 25, 50 and 100 µg/ml was determined and given in Figures (3) and Picture (1). From Figure (3), ethyl 4- (2-hydroxy-3-methoxyphenyl)-6-methyl-2- oxo-1, 2, 3, 4- tetrahydropyrimidine-5-carboxylate(a) had viability of 18, 26, 38, 55 and 70 % at 6.25, 12.5, 25, 50 and 100 µg/ml respectively, Thus, MCF-7 cells showed low viability on treatment with 5a which indicated good anticancer activities of this compound. Therefore, it may be concluded that these compounds follow different mechanisms and interact differently with different cellular targets.

Table 5: inhibition rate of cell growth for (a)

Comp.	Concentration µg/ml	Cytotoxicity %
a	6.25	18
	12.5	26
	25	38
	50	55
	100	70

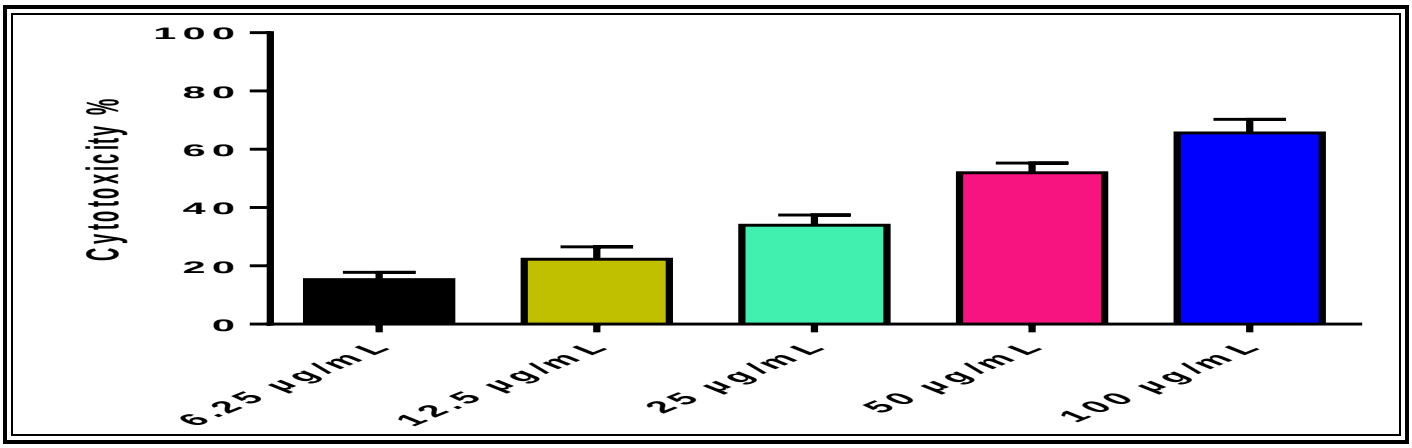
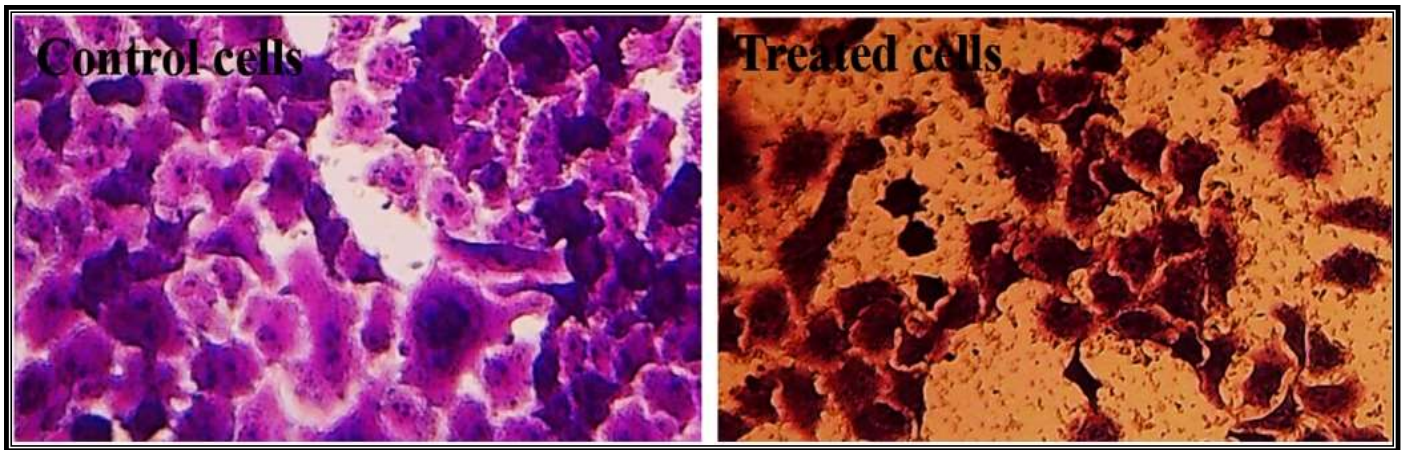


Figure 3: Cytotoxic effect of ethyl 4-(2-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (a) on MCF-7 cell line



Picture 1: Cytotoxic effect of ethyl 4-(2-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (a) at a concentration on of 100 µg/mL on MCF-7 cell line

Conclusion

Dihydropyrimidine-2(1H)-ones/thiones derivatives were synthesized and structurally characterized. The prepared Ethyl-4-(2-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahyd- ropyrimidine-5-carboxylate(a) showed cytotoxic activity against MCF-7 cell lines revealing good activities. As well, a number of compounds revealed good effectiveness as antioxidant and could be believed as valuable templates for further investigations to get more potent agents.

Acknowledgments

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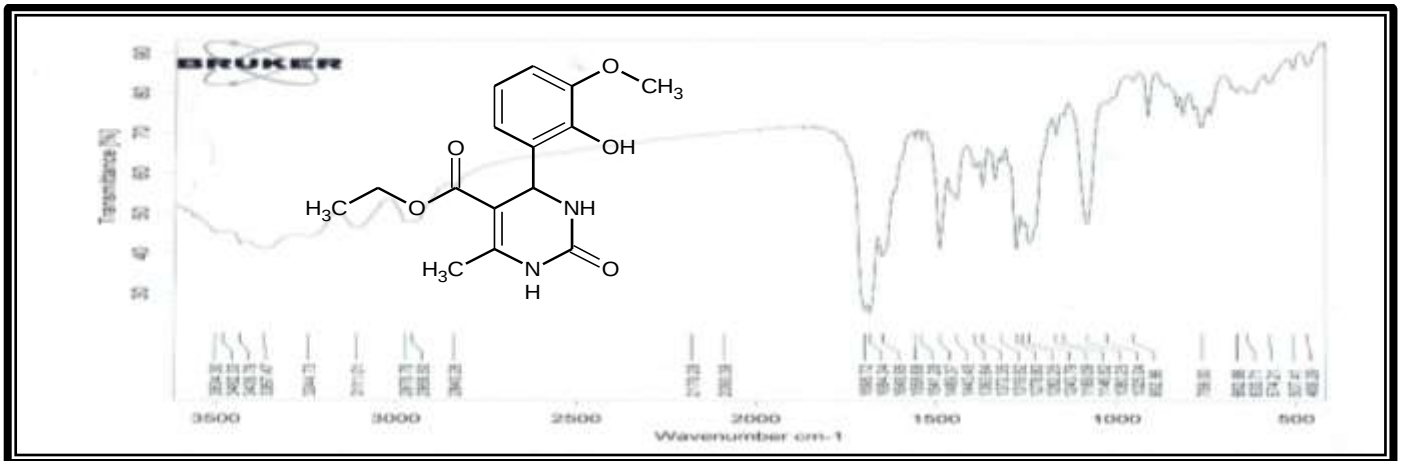


Figure: FT- IR spectrum of ethyl-4-(2-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4tetrahydropyrimidine-5-carboxylate. A

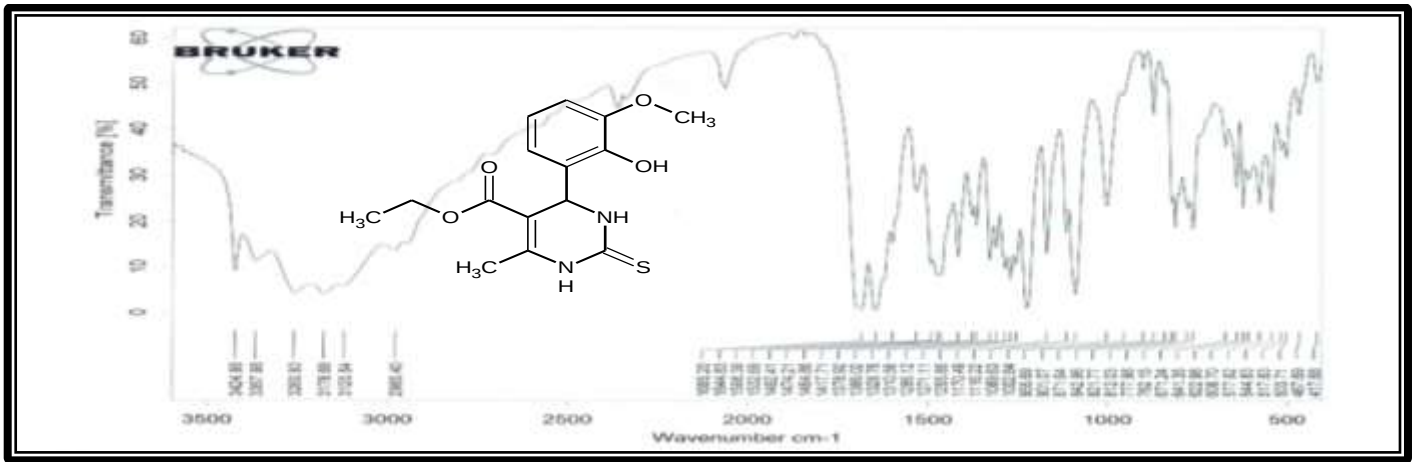


Figure: FT- IR spectrum of ethyl 4-(2-hydroxy-3-methoxyphenyl)-6-methyl-2-thioxo-1, 2, 3, 4tetrahydropyrimidine-5-carboxylate. B

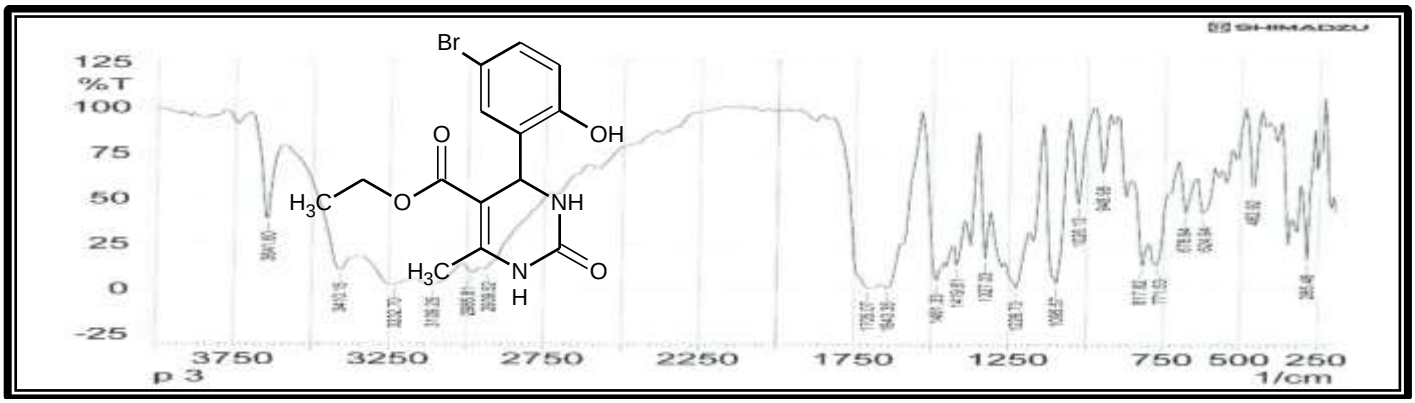


Figure: FT- IR spectrum of ethyl 4-(5-bromo-2-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate. C

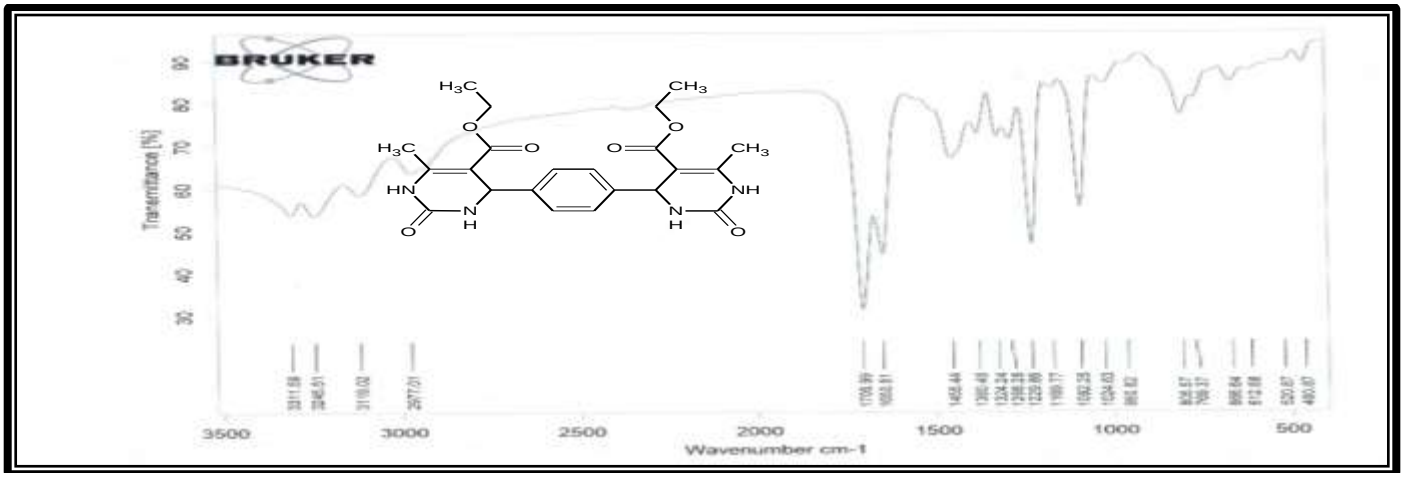


Figure : FT- IR spectrum of 4, 4'-benzene-1, 4-diylbis (ethyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate). D

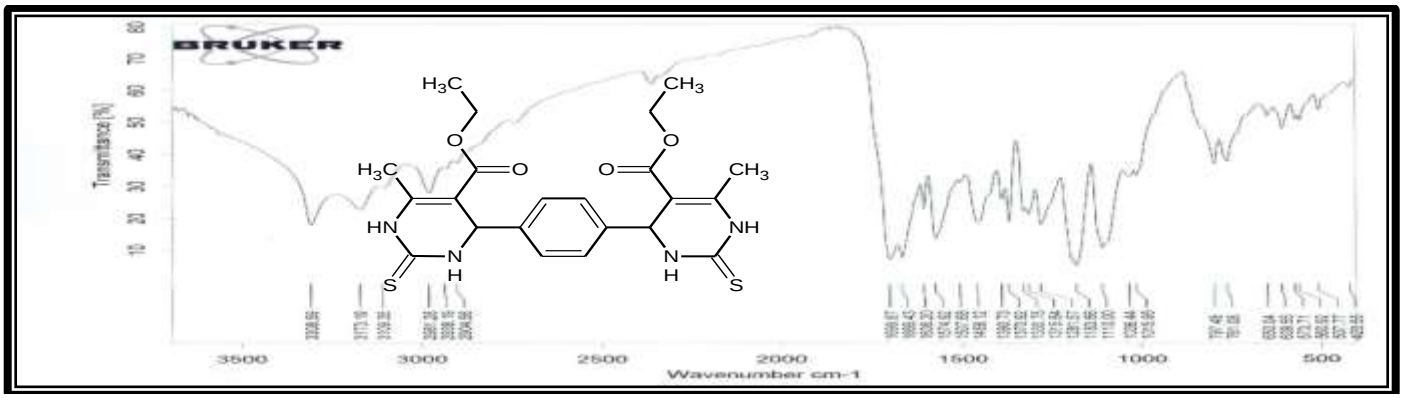


Figure : FT- IR spectrum of 4,4'-benzene-1,4-diylbis(ethyl-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate). E

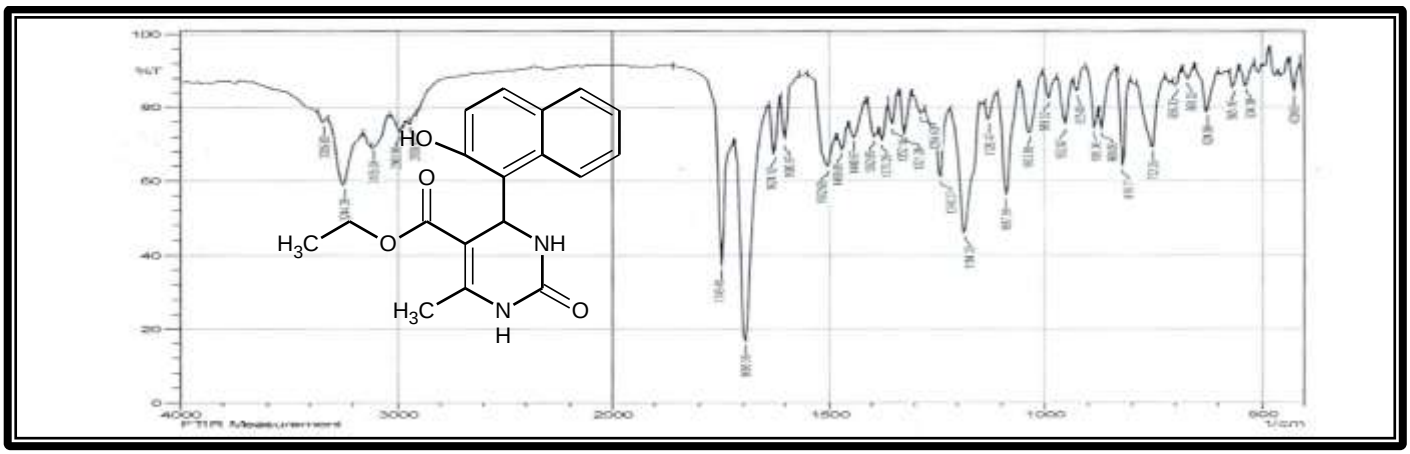


Figure : FT- IR spectrum of ethyl 4-(2-hydroxynaphthalen-1-yl)-6-methyl-2-oxo-1, 2, 3, 4tetrahydropyrimidine-5-carboxylate. F

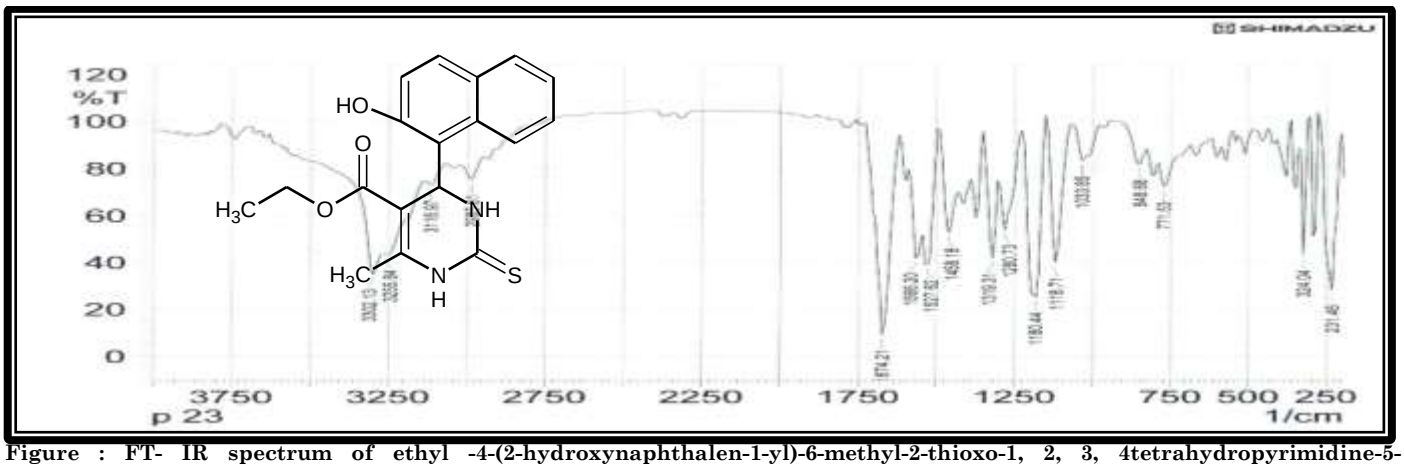


Figure : FT- IR spectrum of ethyl -4-(2-hydroxynaphthalen-1-yl)-6-methyl-2-thioxo-1, 2, 3, 4tetrahydropyrimidine-5-carboxylate. G

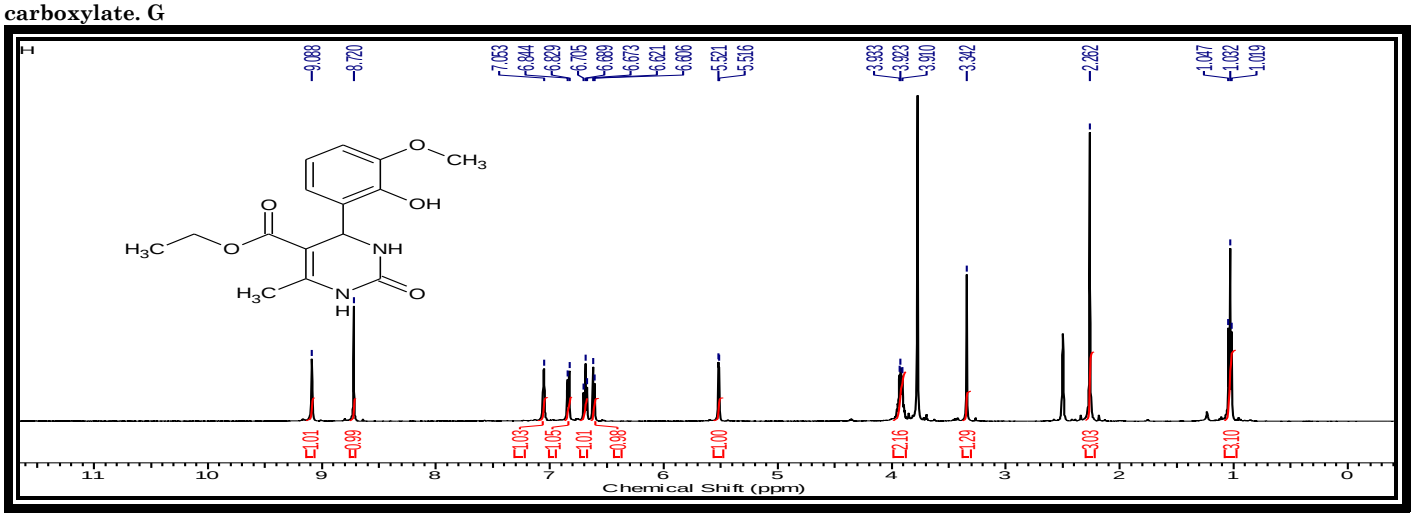


Figure :^{1}H-NMR spectrum of ethyl 4-(2-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4tetrahydropyrimidine-5-carboxylate. A

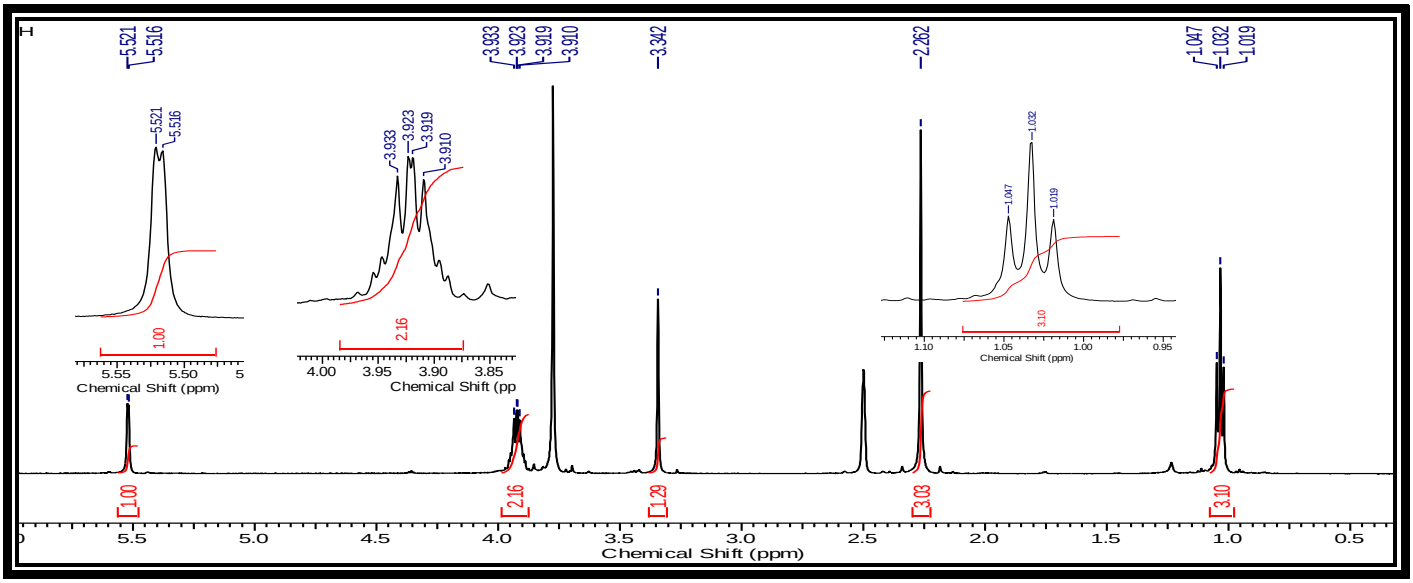


Figure : 1 H-NMR spectrum of expanded spectrum signal for aliphatic protonsethyl 4-(2-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate. A

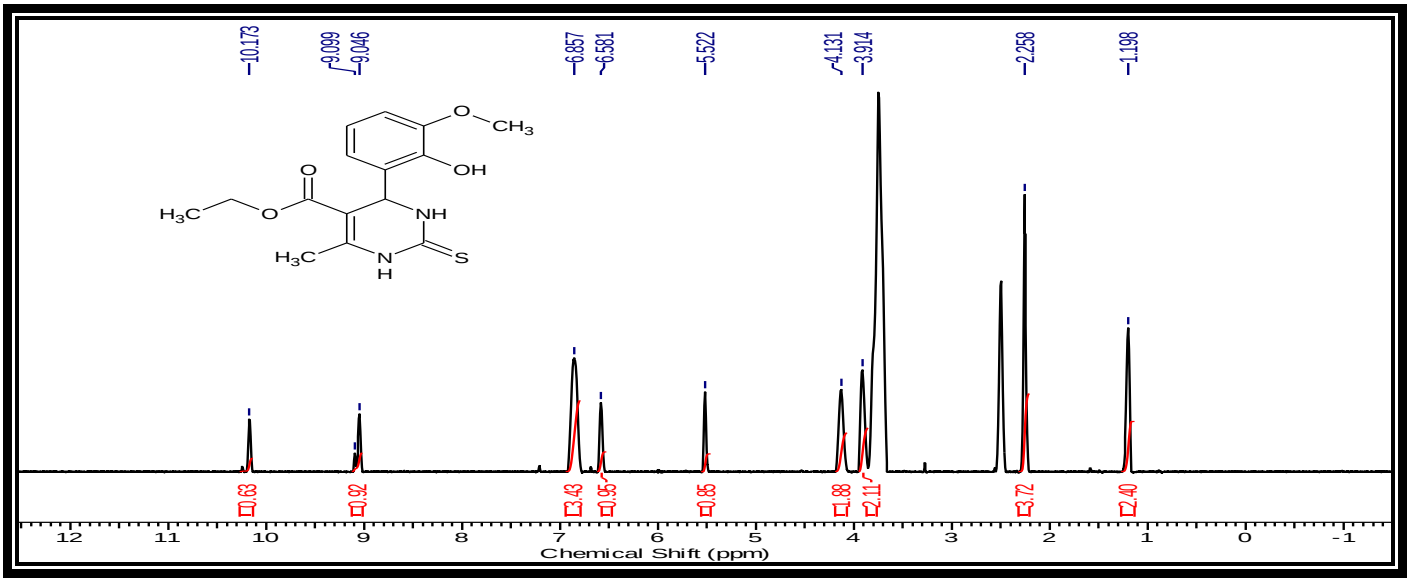


Figure :[1]H-NMR spectrum of ethyl 4-(2-hydroxy-3-methoxyphenyl)-6-methyl-2-thioxo-1, 2, 3, 4tetrahydropyrimidine-5-carboxylate. B

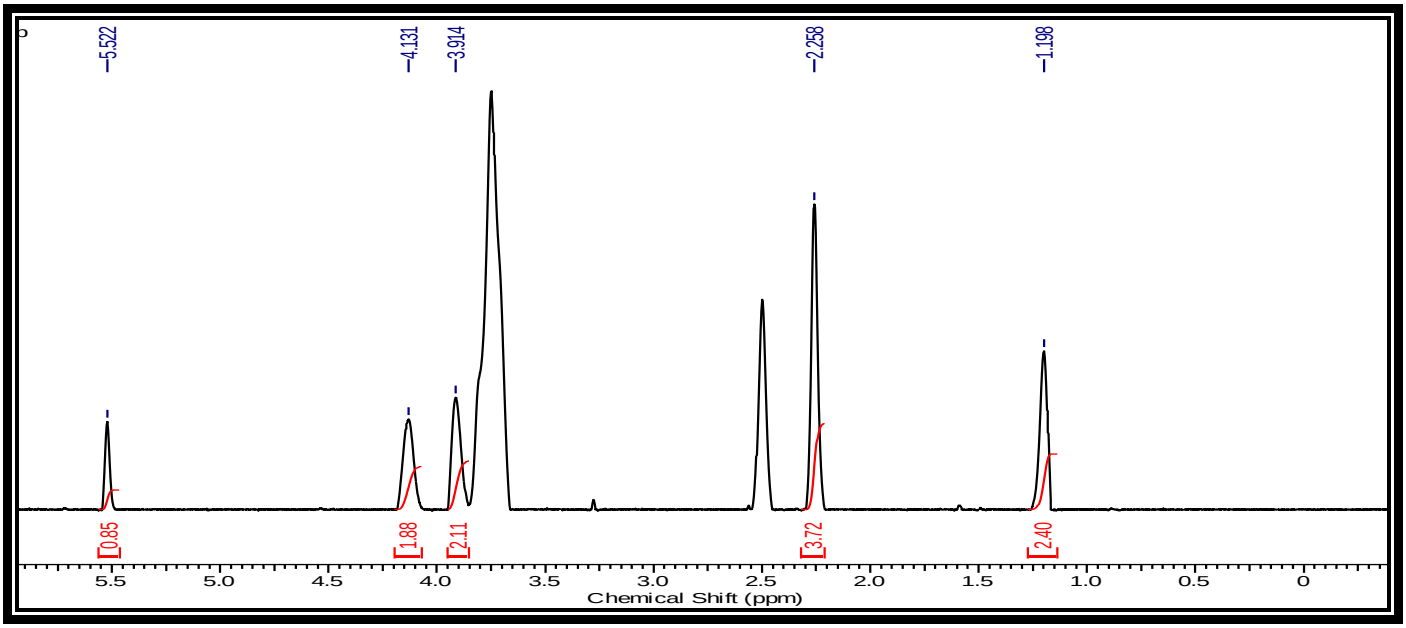


Figure : 1 H-NMR spectrum of expanded spectrum signal for aliphatic protons ethyl 4-(2-hydroxy-3-methoxyphenyl)-6-methyl-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate. B

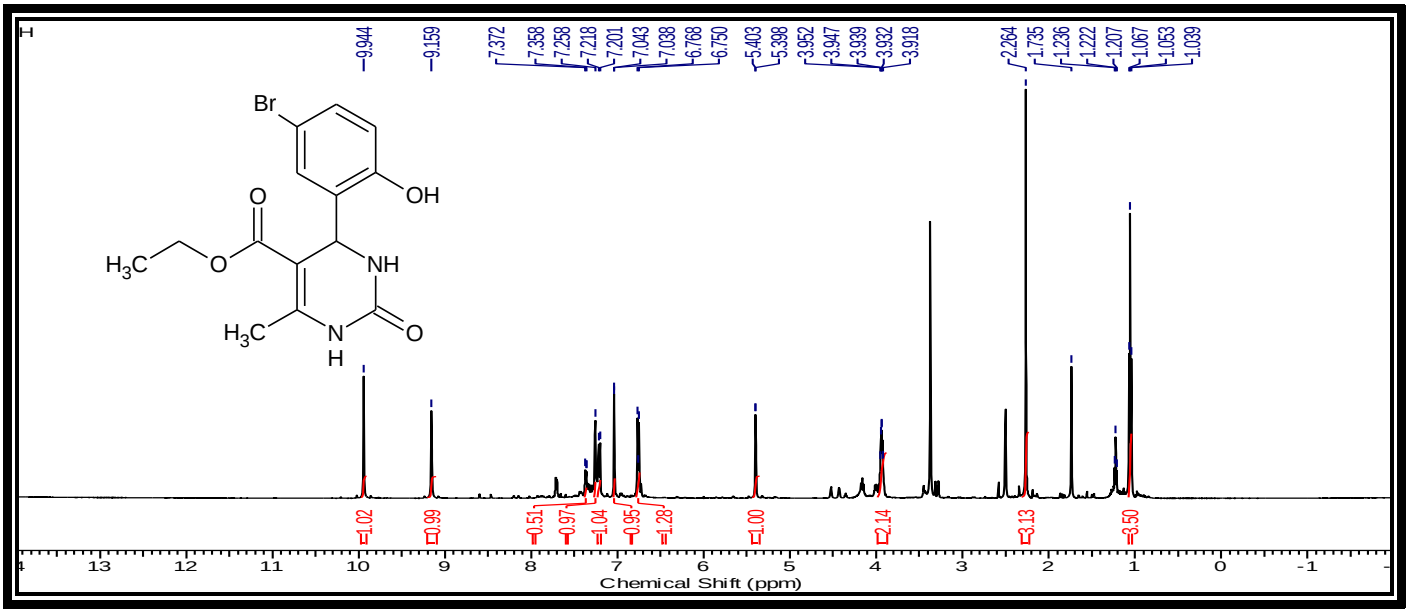


Figure :[1]H-NMR spectrum of ethyl 4-(5-bromo-2-hydroxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate. C

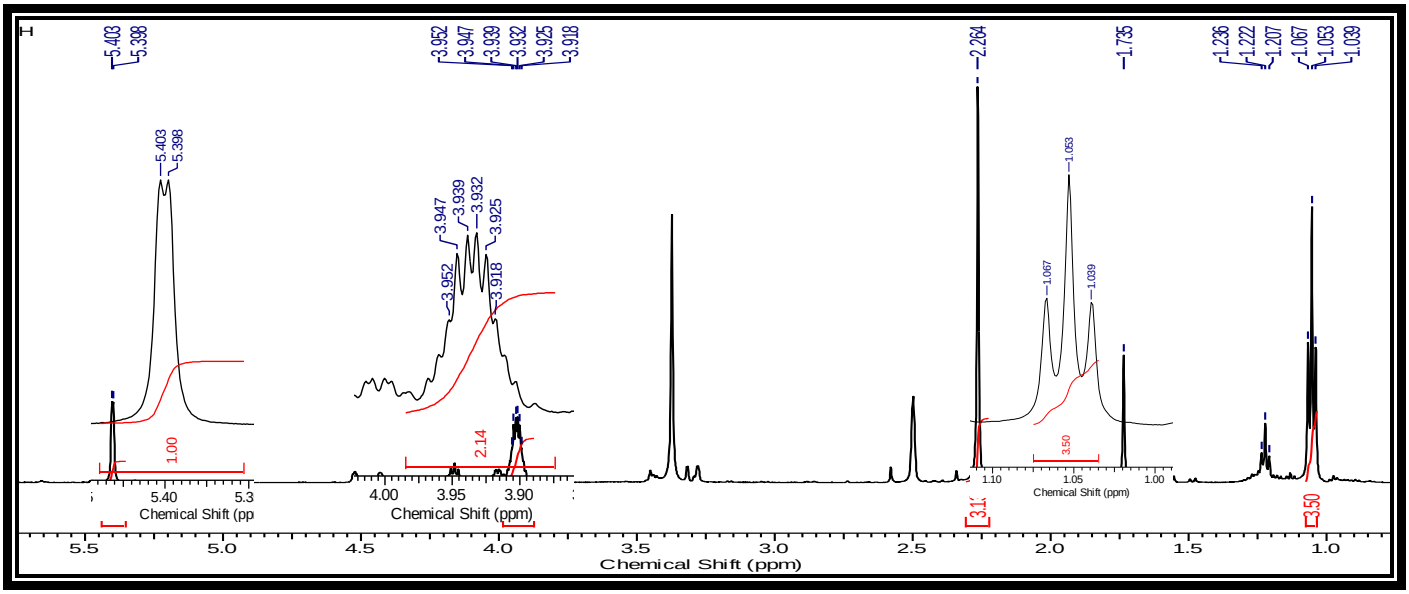


Figure : 1 H-NMR spectrum of expanded spectrum signal for aliphatic protons ethyl 4-(5-bromo-2-hydroxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate. C

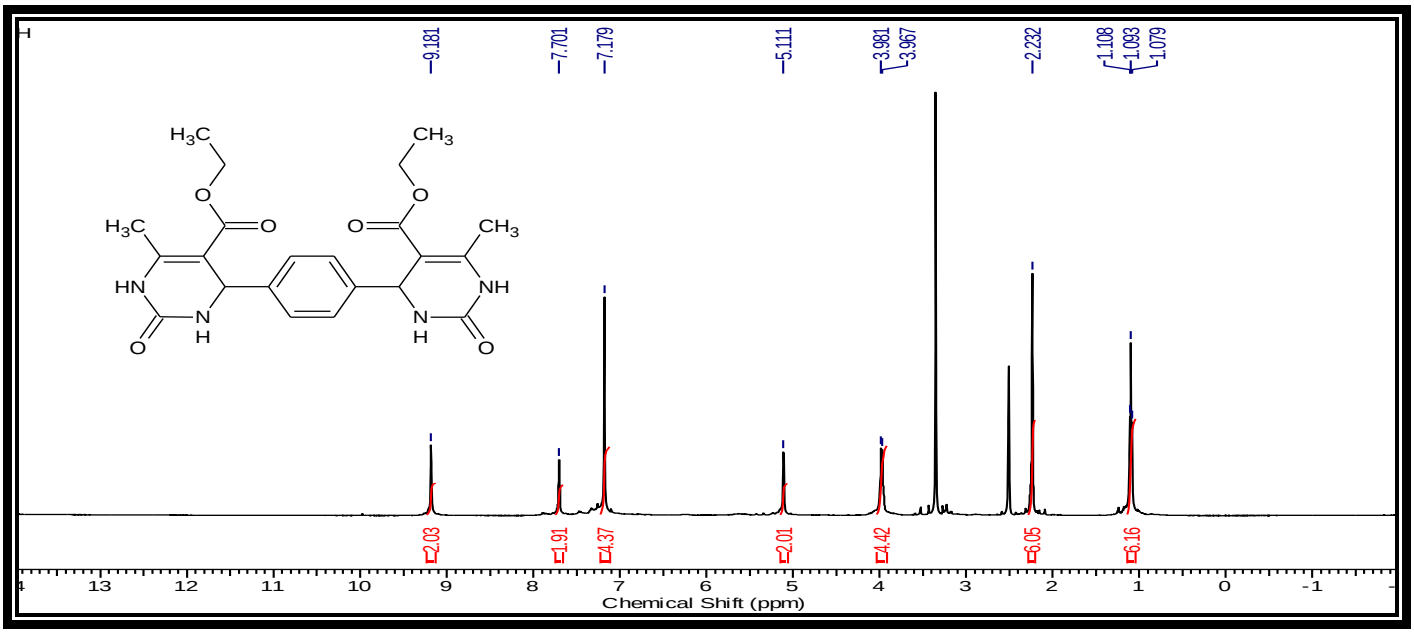


Figure :[1]H-NMR spectrum of 4, 4'-benzene-1, 4-diylbis (ethyl-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate). D

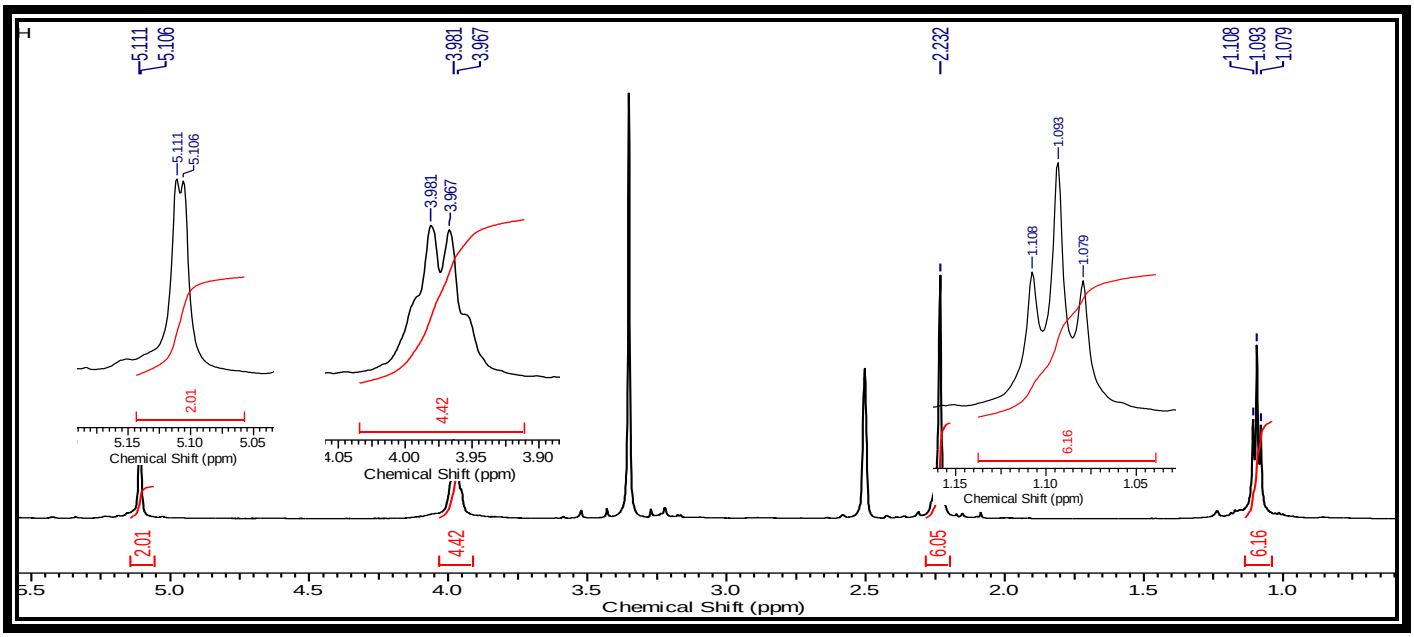


Figure: [1]H-NMR spectrum of expanded spectrum signal for aliphatic protons4, 4'-benzene-1,4-diylbis(ethyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyramidine-5-carboxylate). D

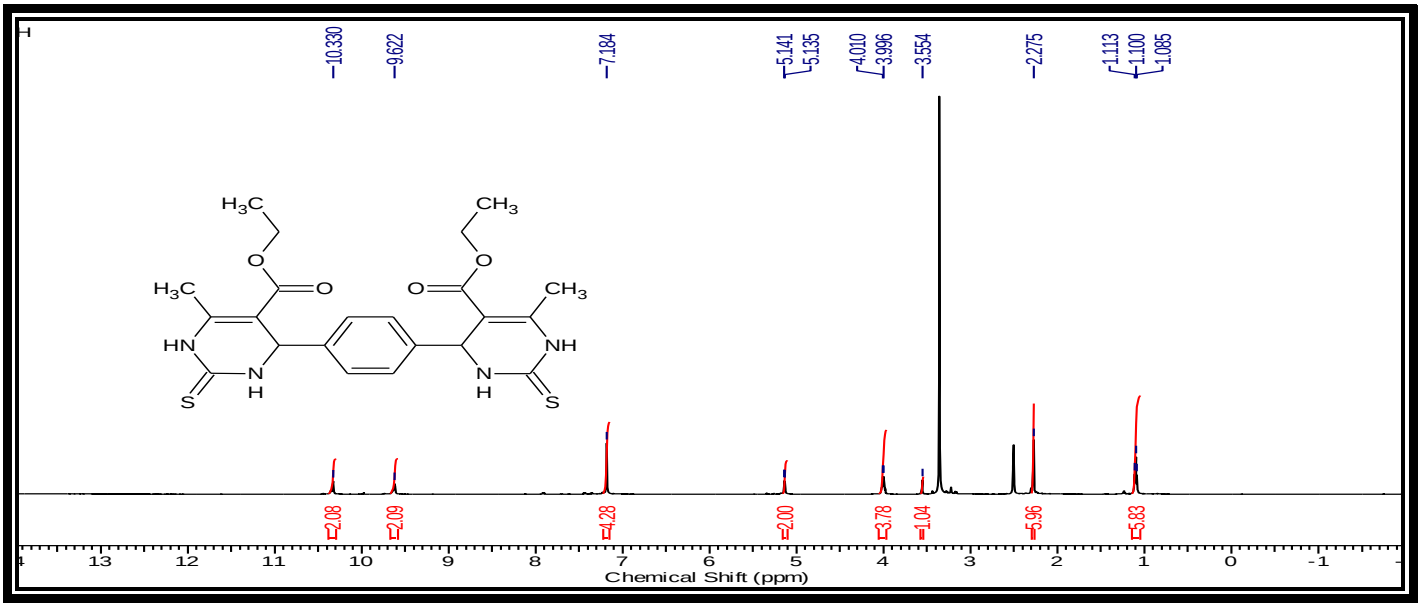


Figure: [1]H-NMR spectrum of 4, 4'-benzene-1,4-diylbis(ethyl-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate). E

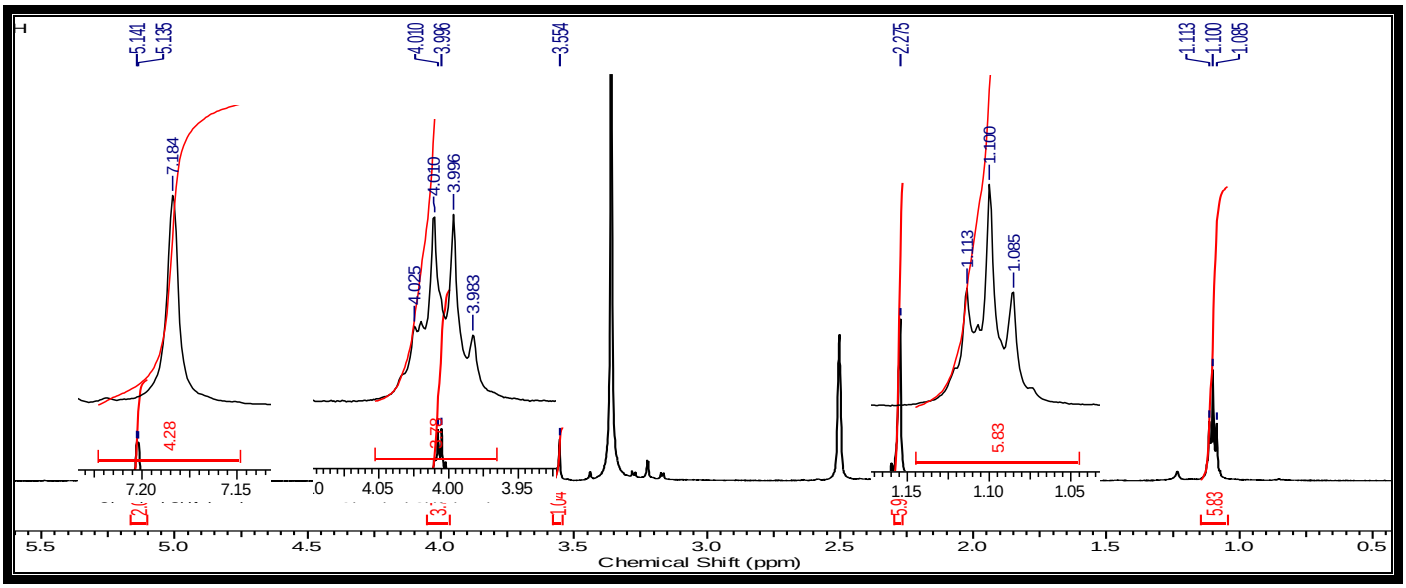


Figure : [1]H-NMR spectrum of expanded spectrum signal for aliphatic protons4, 4'-benzene-1, 4-diylbis(ethyl-6-methyl-2-thioxo-1,2,3,4-tetrahydropyri-midine-5-carboxylate). E

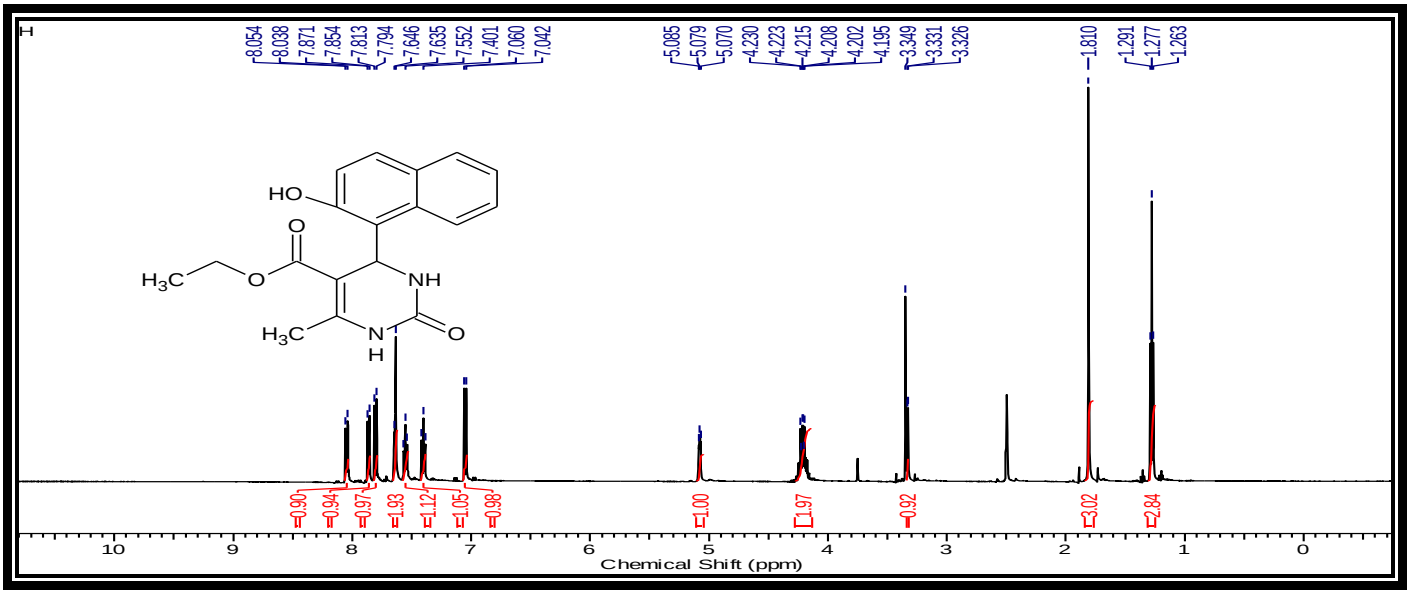


Figure :[1]H-NMR spectrum of ethyl 4-(2-hydroxynaphthalen-1-yl)-6-methyl-2-oxo-1, 2, 3, 4tetrahydropyrimidine-5-carboxylate. F

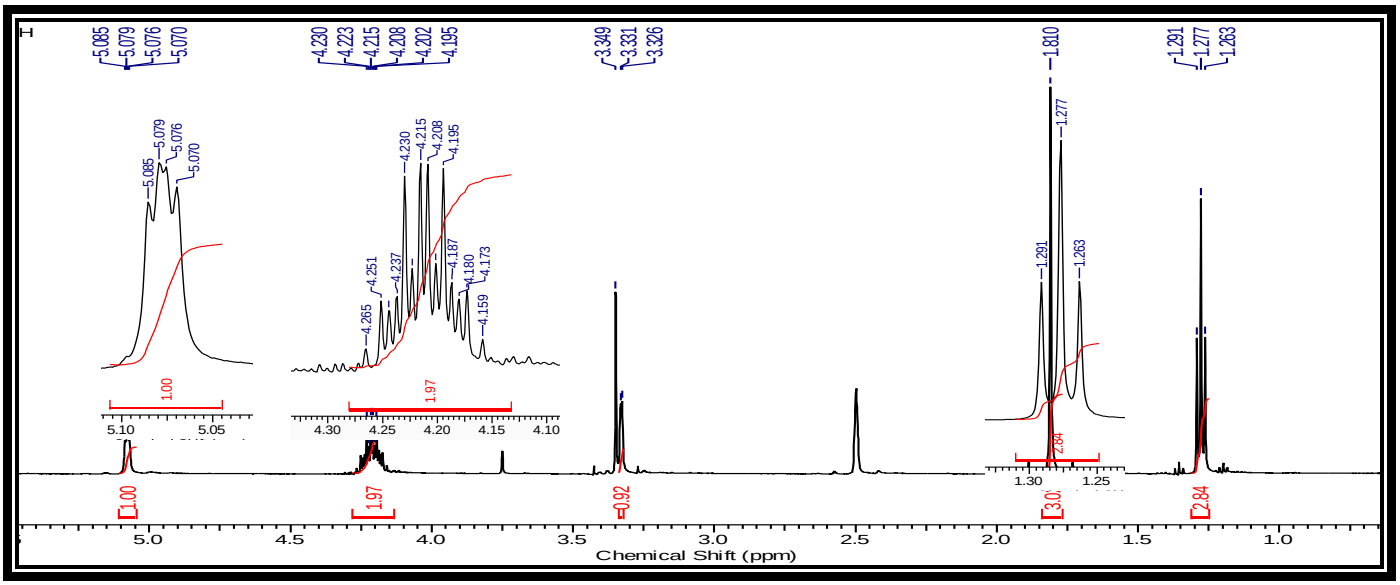


Figure : [1]H-NMR spectrum of expanded spectrum signal for aliphatic protonsethyl 4-(2-hydroxynaphthalen-1-yl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydrop- yrimidine-5-carboxylate. F

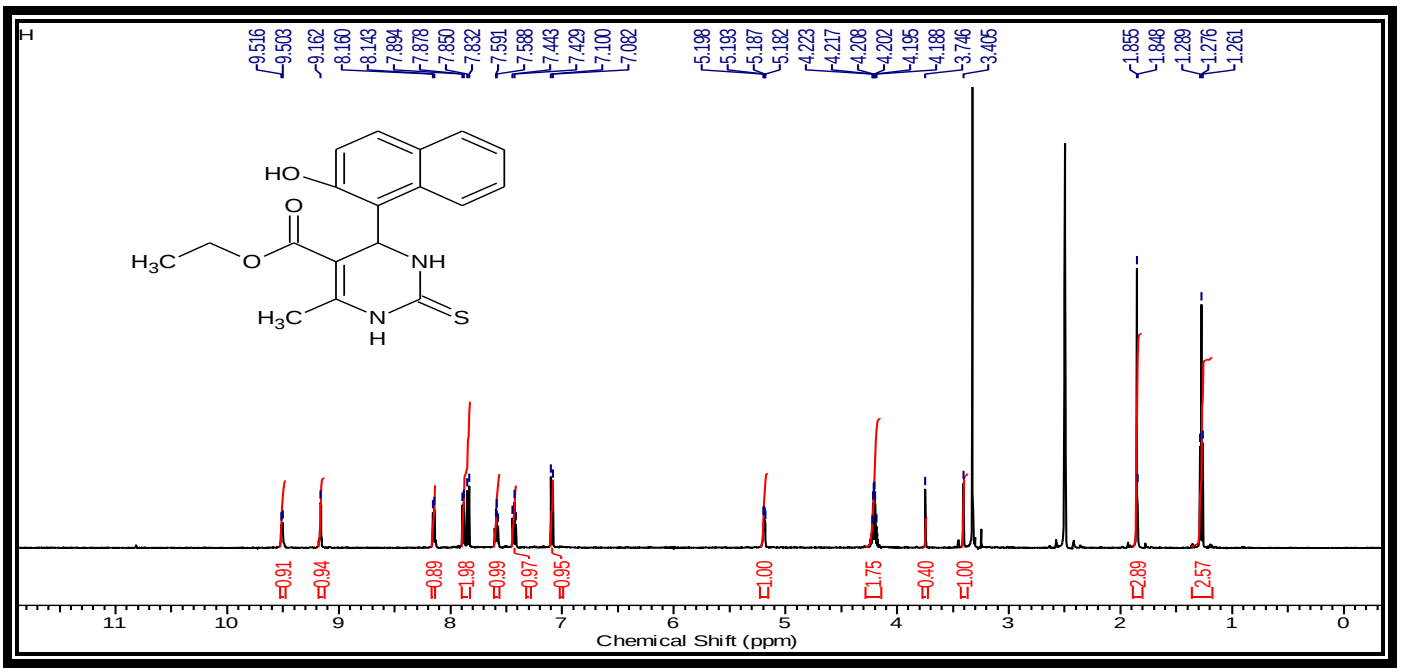


Figure: [1]H-NMR spectrum of ethyl -4-(2-hydroxynaphthalen-1-yl)-6-methyl-2-thioxo-1, 2, 3, 4 tetrahydropyrimidine-5-carboxylate. G

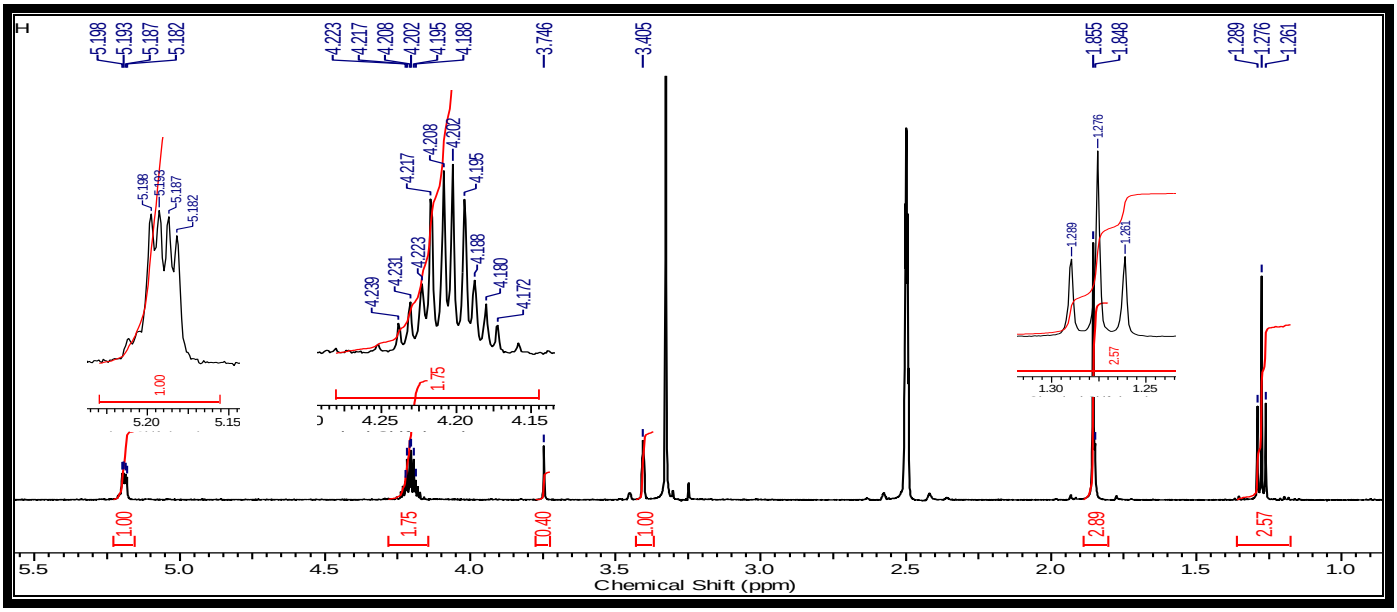


Figure : ^{1}H-NMR spectrum of expanded spectrum signal for aliphatic protonsethyl -4-(2-hydroxynaphthalen-1-yl)-6-methyl-2-thioxo-1,2,3,4tetrahydropyrimidine-5-carboxylate. G

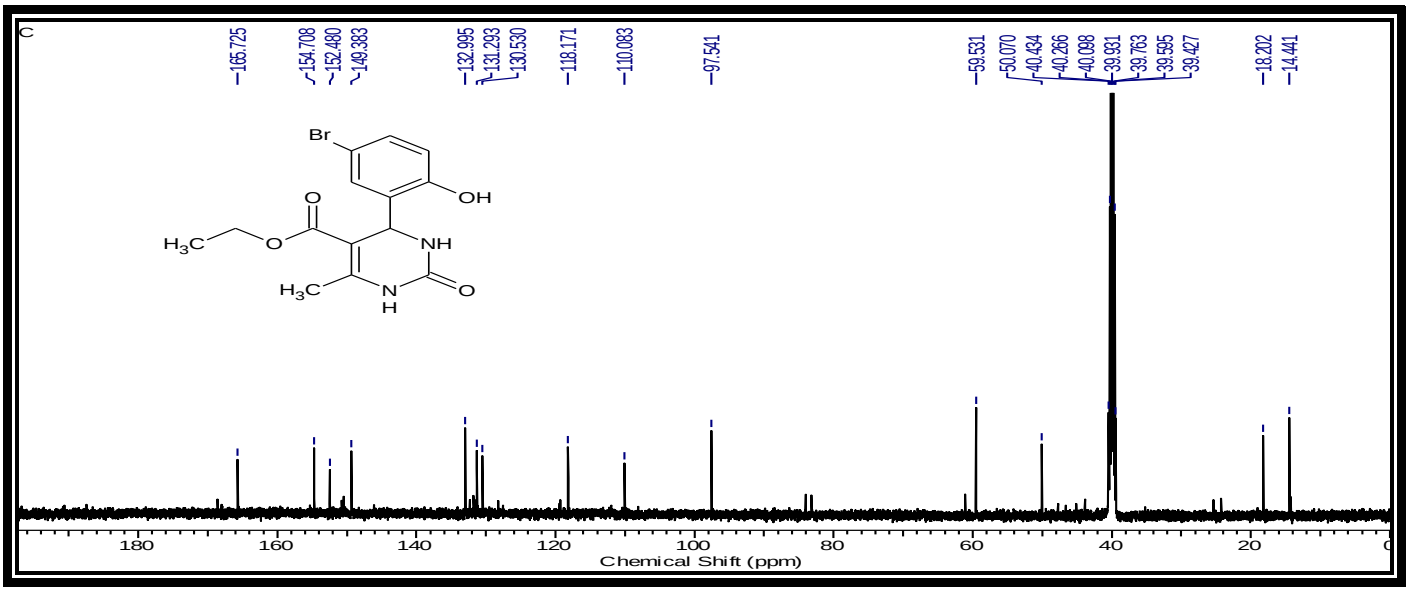


Figure : ^{13}C- NMR spectrum ofethyl 4-(5-bromo-2-hydroxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate. C

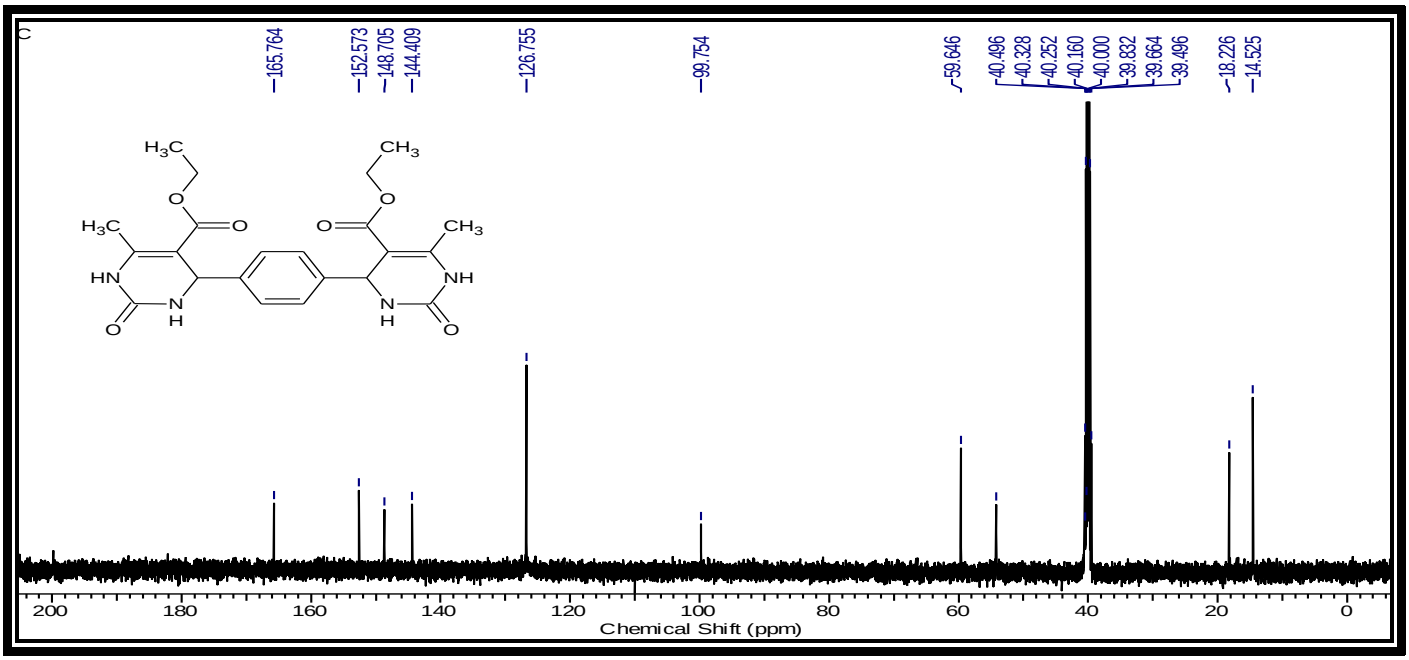


Figure : ^{13}C- NMR spectrum of 4, 4'-benzene-1, 4-diylbis (ethyl-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate). D

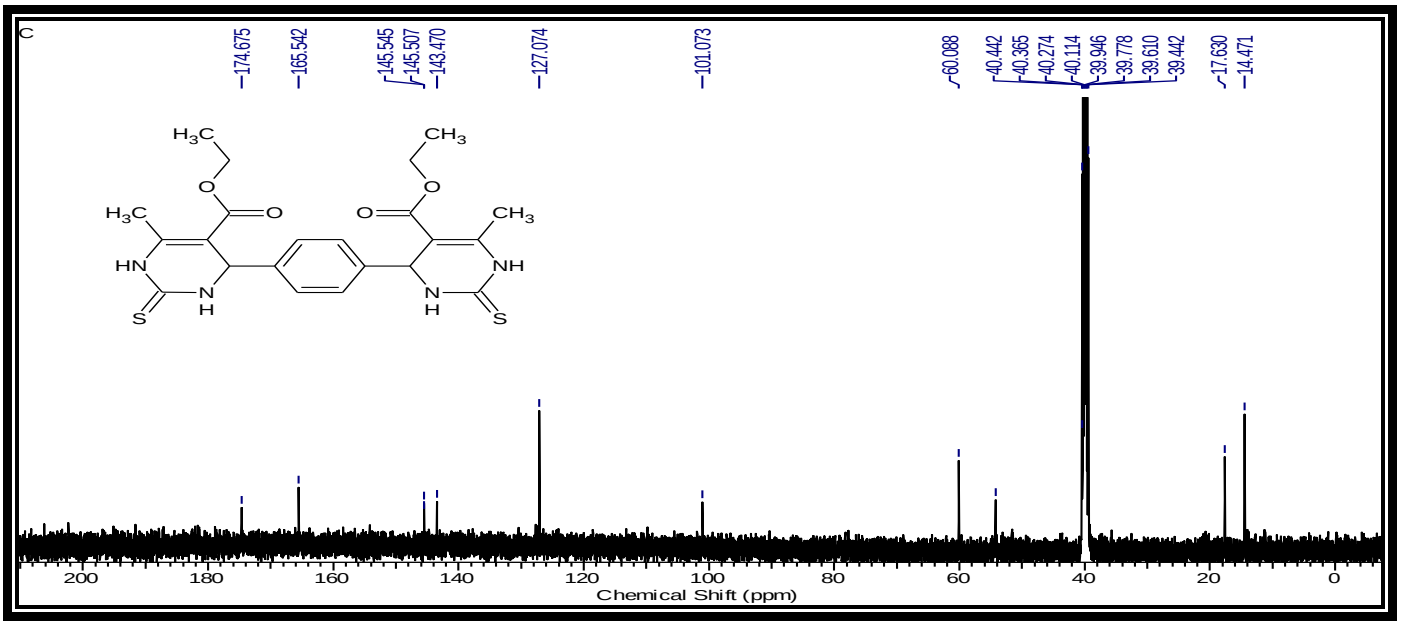


Figure : ^{13}C- NMR spectrum of4, 4'-benzene-1, 4-diylbis (ethyl-6-methyl-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate). E

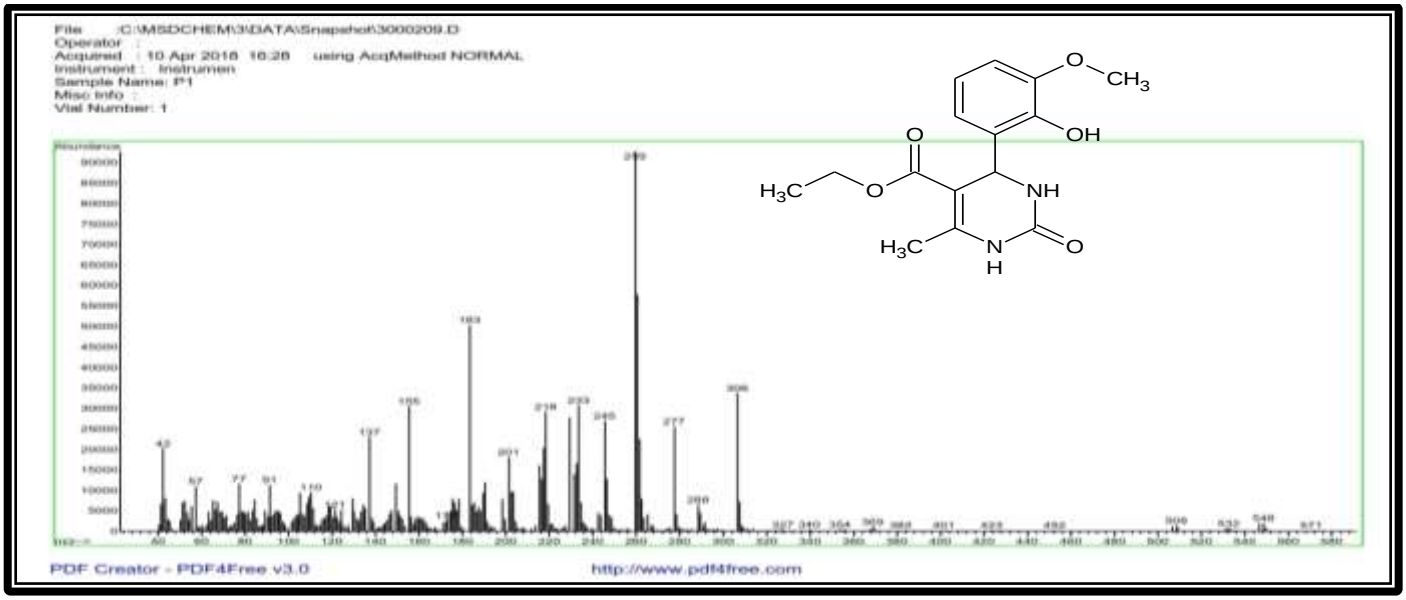


Figure : Mass spectrum of ethyl 4-(2-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate. A

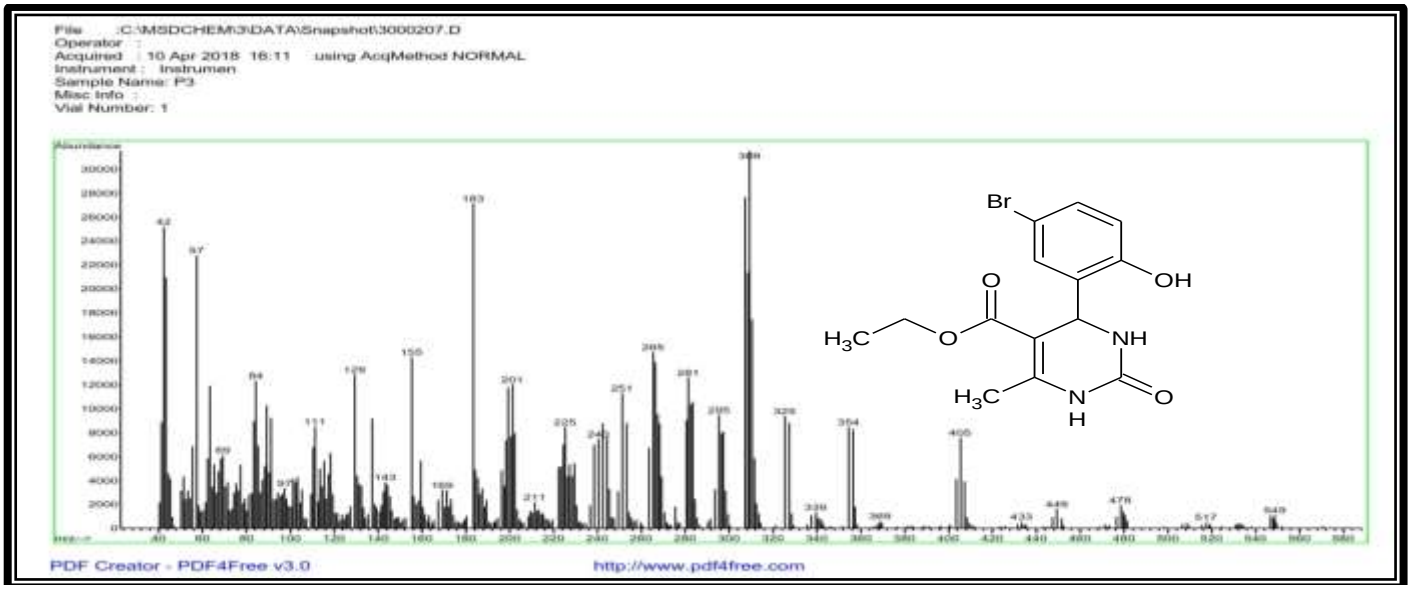
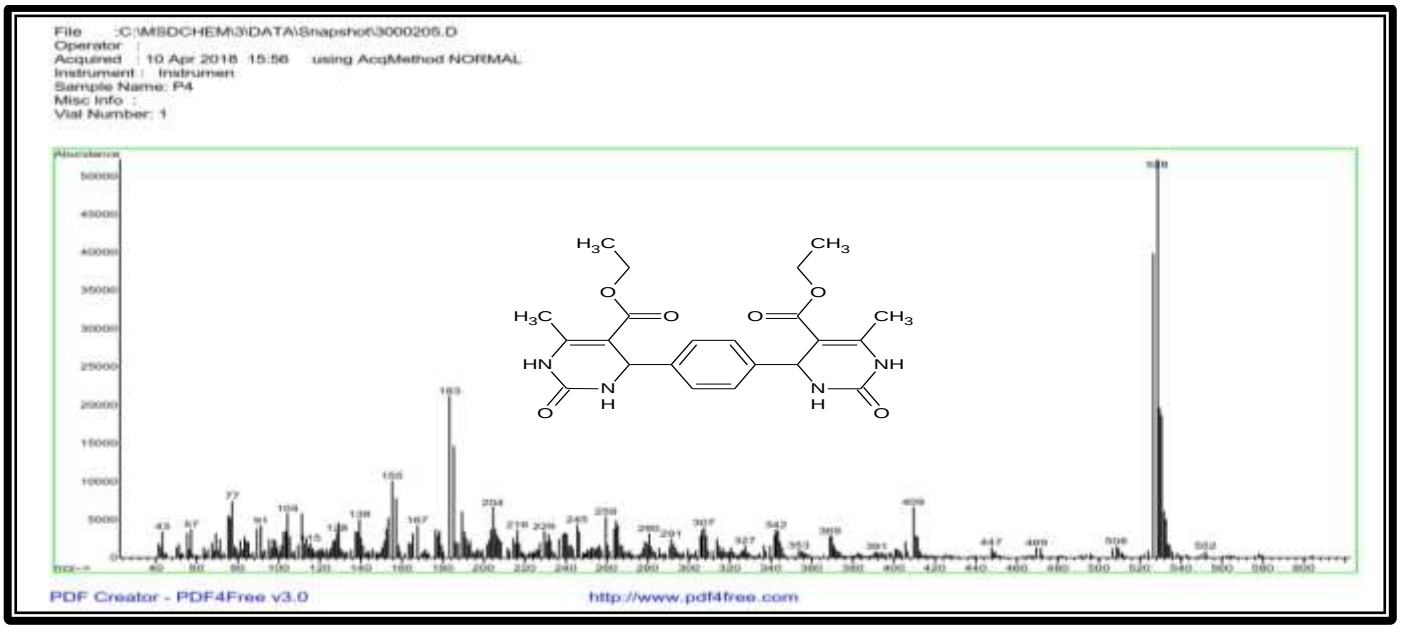


Figure: Mass spectrum of ethyl 4-(5-bromo-2-hydroxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate. C



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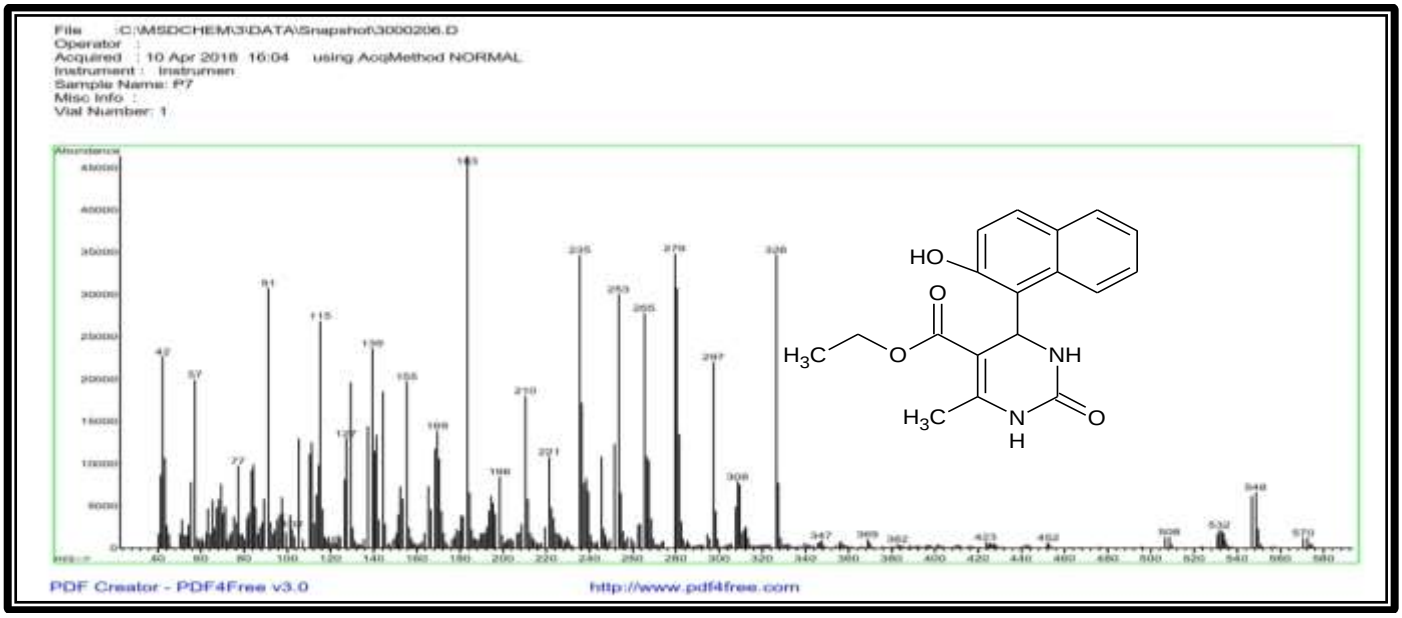

Figure : Mass spectrum of ethyl 4-(2-hydroxynaphthalen-1-yl)-6-methyl-2-oxo-1, 2, 3, 4tetrahydropyrimidine-5-carboxylate. F

