

# Synthesis of substituted 6, 7-dimethoxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one and their Apoptosis study

Ranjini.P<sup>1</sup>, Chaitramallu.M<sup>1</sup>, shilpa<sup>2</sup>.Devaraju kesagodu<sup>1\*</sup> prof .Y.B.Basavaraju.

Department of Chemistry, Yuvaraja's College, University of Mysore, Mysore, Department of biotechnology, Maharani Science College for Woman, Mysore, Department of Biotechnology, JSS College, Ooty road Mysore, Department of Studies in Chemistry, Manasagangotri Mysore, Karnataka, India.

[Passion49432020@gmail.com](mailto:Passion49432020@gmail.com), ranjinip21@gmail.com

## Abstract:

The field of biomedical analogs of podophyllotoxin is more in its infancy, but the explosion of interest in these molecules as inherently active therapeutic agents is increasing. There is growing interest in the design and synthesis of novel biocompatible analogs. The series of novel substituted podophyllotoxin derivatives were synthesized under mild conditions with satisfactory yield. Auto dock server analysis of the these analogs structure showed that the compounds were shown to interact with the proteins involved in Bcl-xL mediated pathway with inhibition constant of 0.000149 and 0.000584 for 5G and 5C respectively The recorded results of lipid peroxidation with treated samples were increasing with increasing concentration.

Keywords: [Podophyllotoxin](#), tetralone docking study, ETA cell Tryphan blue assay, Auto dock,.

## INTRODUCTION:

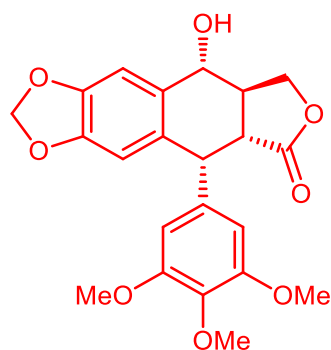
Podophyllotoxins are important natural products in the armamentarium of antineoplastic and antiviral agents. The biological assessment of podophyllotoxin (1) was followed by discovery of its mode of action and culminated in the synthesis of the anticancer drugs etoposide (2) and teniposide (3). The long journey from podophyllotoxin to (2 and 3) illustrates the fascinating development of clinically useful anticancer drugs from natural product prototypes through chemical modification. It is particularly distinctive that structural variation of podophyllotoxin caused a radical change in the mechanism of action. Today, several new analogs have emerged as potential drugs for several diseases.

Podophyllotoxin (1), a naturally occurring aryltetralin lignan, holds a unique position among natural products have been known for approximately 1000 years from its first application in folk medicines to its most recent developments in antitumor agents.<sup>1-8</sup> due to its remarkable biological activity and extensive use in traditional medicine, Podophyllotoxin(1) has remained an important starting point in the development of new therapeutic agents.

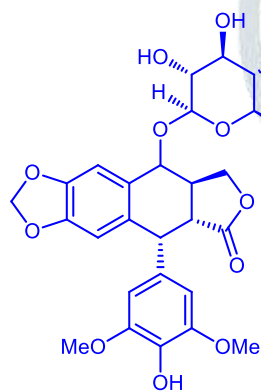
The podophyllotoxin are included in a wide variety of cancer chemotherapy protocols. Due to these biological activities, lignans, and especially cyclo lignans, have been the objective of numerous studies focused to prepare better and safer anticancer drugs. It is well accepted from structure-activity studies in this field that the trans-lactones are more potent as antineoplastics than the cis-lactones. Not only the configuration of the D ring is an important factor for high cytotoxic activity, but also a quasi-axial arrangement of the E ring is necessary. On this basis, studies on lignans have been addressed to modify the

lactone moiety and prepare analogs with heteroatoms at different positions of the cyclolignan skeleton. Our group has been working during the last few years on chemical synthesis of podophyllotoxin and analogs and we have prepared a large number of cyclolignan derivatives some of which display potent antiviral, immunosuppressive and cytotoxic activities. We have reported several new cytotoxic agents with nitrogen atoms, we are preparing mainly new compounds by modifications of the A and E cyclolignan-rings. This work describes new method of synthesis for substituted tetraline by Grignard reaction.

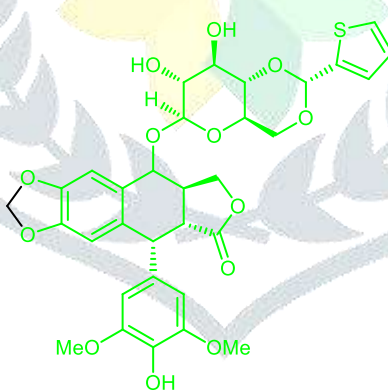
## Chemistry



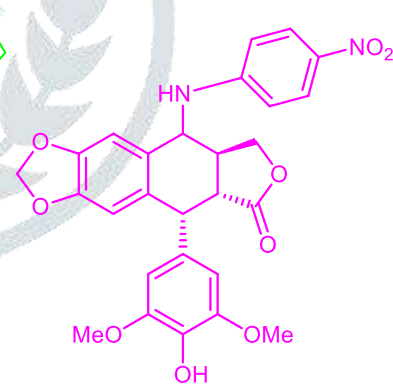
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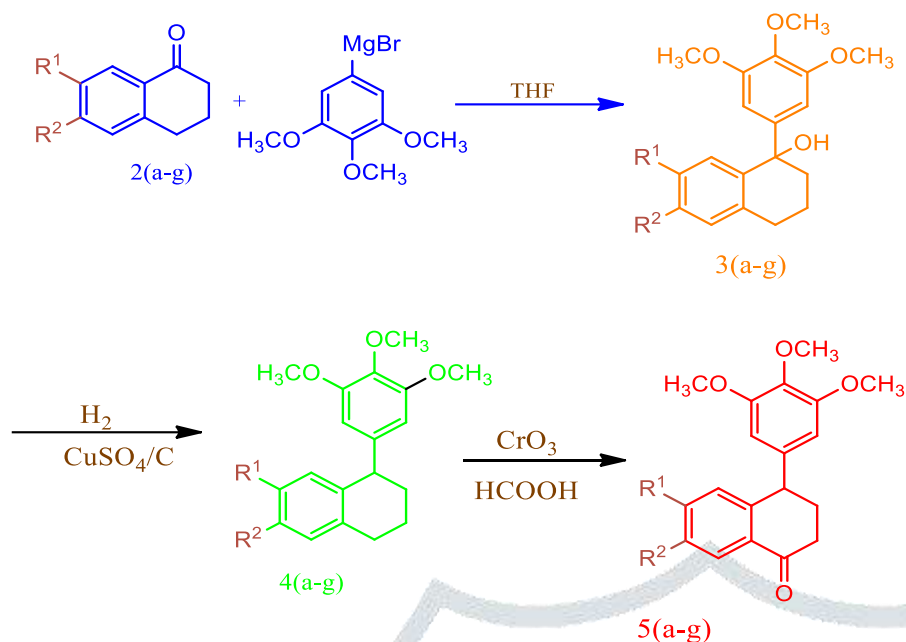
Etoposide 9



Tenoposide 10



GL-331 11



**Scheme 1:** protocol for the synthesis of aryl tertalone 1(a-g)

Where	R <sup>1</sup>	R <sup>2</sup>
<b>a</b>	OCH <sub>3</sub>	OCH <sub>3</sub>
<b>b</b>	H	OH
<b>c</b>	H	CH <sub>3</sub>
<b>d</b>	H	Cl
<b>e</b>	H	H
<b>f</b>	H	OCH <sub>3</sub>
<b>g</b>	H	NH <sub>2</sub>

### General procedure for the synthesis of 6, 7-substituted 4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5a-g)

A Grignard reaction was used to prepare 3(a-g). An oven dried three-necked flask outfitted with a reflux condenser, dropping funnel and magnetic stirrer. Approximately 1/4<sup>th</sup> of an aliquot of 1-bromo-3, 4, 5-trimethoxybenzene (10mmol) in 5ml of anhydrous THF was added to a mixture of magnesium turnings (10mmol) in 2.5ml of anhydrous THF with a small piece of iodine. As soon as the reaction mixture becomes colorless the remaining 1-bromo-3, 4, 5-trimethoxybenzene solution was added drop wise to the solution under a mild condition, stirring was then continued for 1 hour at 60<sup>0</sup>C temperature. A solution of 3, 4, 5-trimethoxyphenyl magnesium bromide (10mmol) was added slowly to the 6, 7-substituted-3, 4-dihydronaphthalen-1(2H)-one, (8.35mmol) 2.5 ml anhydrous THF solution at 60<sup>0</sup>C. After complete addition, the solution was allowed to stir at room temperature for another 20 minutes, a saturated ammonium chloride

solution (10ml) was added to hydrolyze the adduct at 0°C and the mixture was stirred for 10 minutes. The two layers were separated and the aqueous layer was extracted with ether (10ml in three portions), the combined organic layer was washed with brine solution and dried over MgSO<sub>4</sub> and filtered. The filtrate was concentrated in vacuum and the residue was purified by column chromatography gave 93.67% yield.

6, 7-substituted-1- (3, 4, 5-trimethoxyphenyl)-1, 2, 3, 4-tetrahydronaphthalen-1-ol **3(a-g)** (0.01 mole) was subjected to hydrogenation over 10% CuSO<sub>4</sub>-C in ethanol-formic acid (35:1.25 v/v) mixture. The catalyst was filtered off and the filtrate was distilled to remove ethanol and the residue was extracted with ether. The ether extract was washed with water and dried to give the crude product which was purified by column chromatography over silica gel using petroleum ether to give a product in 86.90%.

To a stirred solution of compound 6, 7-Substituted-1-(3, 4, 5-trimethoxyphenyl)-1, 2, 3, 4-tetrahydronaphthalene (0.01mole) **4(a-g)** in formic acid (14ml) was added with stirring at 0°C followed by chromium (VI) oxide (0.01mole) in water was added. The reaction mixture was stirred at 0-5°C for 7hr. After completion of the reaction, it was decomposed by pouring the reaction mixture into ice-water and extracted with ether. The ether extract was washed with water, sodium carbonate solution and again with water, dried over magnesium sulfate and recrystallized using ethanol to give a dark brown gummy solid in around 85.18%.

### **6, 7-dimethoxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5a)**

It was prepared by the oxidation of 8-(3, 4, 5-trimethoxyphenyl)-5, 6, 7, 8-tetrahydronaphthalen-2-ol (0.01mmole) **4a** with chromium (VI) oxide (0.01mmole) in acetic acid to give a brown semi solid in 82.18%.

IR: 1697-1699 (C=O), 3009-3128(Ar-CH);

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δppm 1.89–2.78 (4 H, m, CH<sub>2</sub>), 3.94 (15 H, s, OCH<sub>3</sub>), 4.43 (1 H, t, CH), 6.67(2 H, dd, Ar-H), 7.12-7.59 (2 H, dd, Ar-H);

$^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$ ppm 31.4( $\text{C}_3$ ), 37.4( $\text{C}_2$ ), 45.6( $\text{C}_4$ ), 56.7( $\text{C}-\text{C}_6$ ,  $\text{C}_7$ ,  $4^3$ ,  $4^5\text{-OCH}_3$ ), 60.5( $\text{C-4}^4\text{-OCH}_3$ ), 106.7( $\text{C-4}^2,4^6$ ), 109.2( $\text{C}_5$ ), 110.5( $\text{C}_8$ ), 127.3( $\text{C}_9$ ), 133.8( $\text{C}_{10}$ ), 136.7( $\text{C-4}^4$ ), 137.3( $\text{C-4}^1$ ), 147.5( $\text{C}_7$ ), 153.4( $\text{C-4}^3$ ,  $4^5$ ), 154.9( $\text{C}_6$ ), 198.0( $\text{C}_1$ );

MS,  $m/z$ : 372.25 ( $M^+$ ), 373.41( $M^++1$ );

Anal. Calcd. For  $\text{C}_{21}\text{H}_{24}\text{O}_6$ : C, 67.73; H, 6.50 O, 25.78 Found: C, 67.75; H, 6.51 O, 25.79 %.

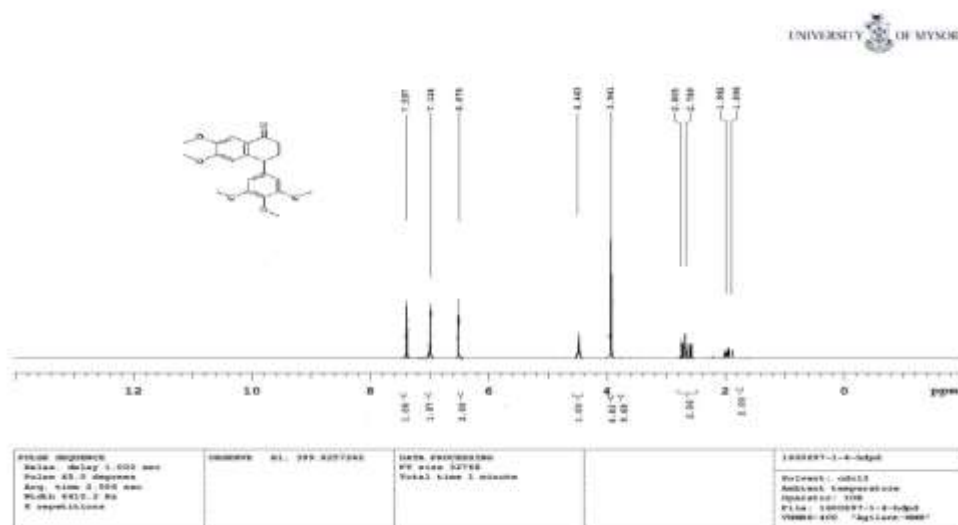


Figure 1:  $^1\text{H}$  NMR spectra of 6, 7-dimethoxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5a)

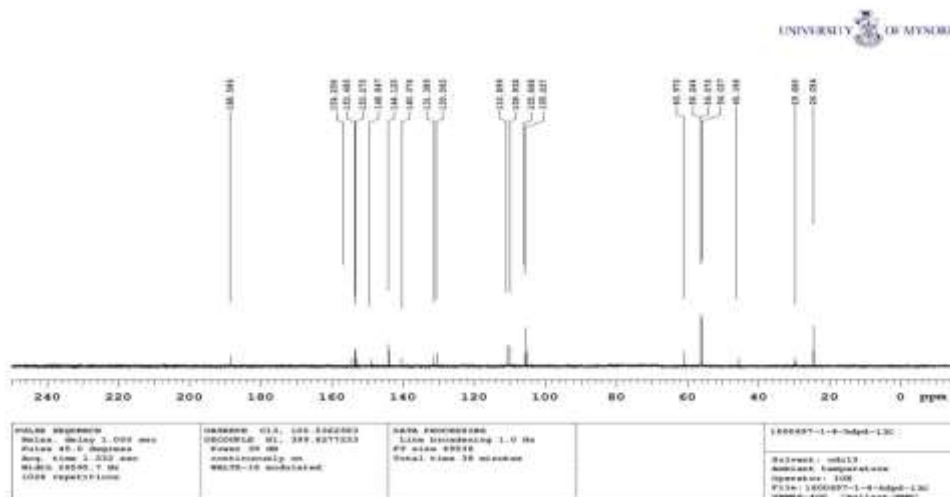
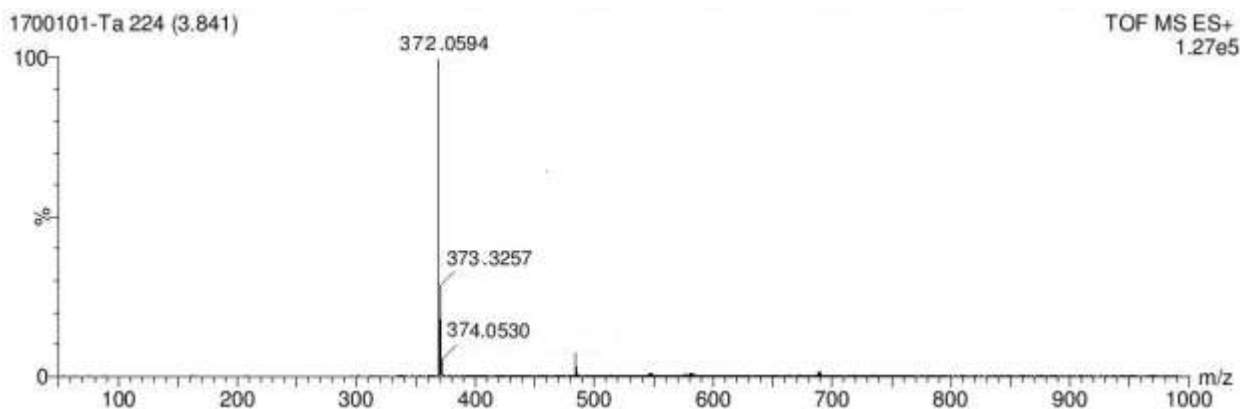


Figure 2:  $^{13}\text{C}$  NMR spectra of 6, 7-dimethoxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5a)



**Figure 3: mass spectra of 6, 7-dimethoxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5a)**

### **6-hydroxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5b)**

It was prepared by the oxidation of 8-(3, 4, 5-trimethoxyphenyl)-5, 6, 7, 8-tetrahydronaphthalen-2-ol (0.01mmole) **4b** with chromium (VI) oxide (0.01mmole) in acetic acid to give a brown semi solid in 82.18%.

IR: 1677-1710 (C=O), 3018-3139 (Ar-CH);

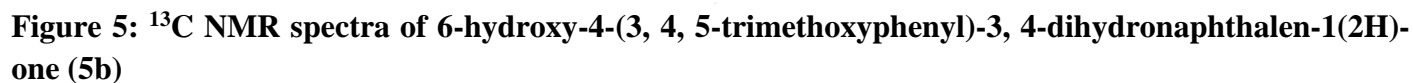
$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$ ppm 2.29–2.82 (4 H, m,  $\text{CH}_2$ ), 3.92 (9 H, s,  $\text{OCH}_3$ ), 3.98-4.02 (1 H, t, CH), 5.63(1H, s, OH), 7.21-7.98 (5 H, m, Ar-H);

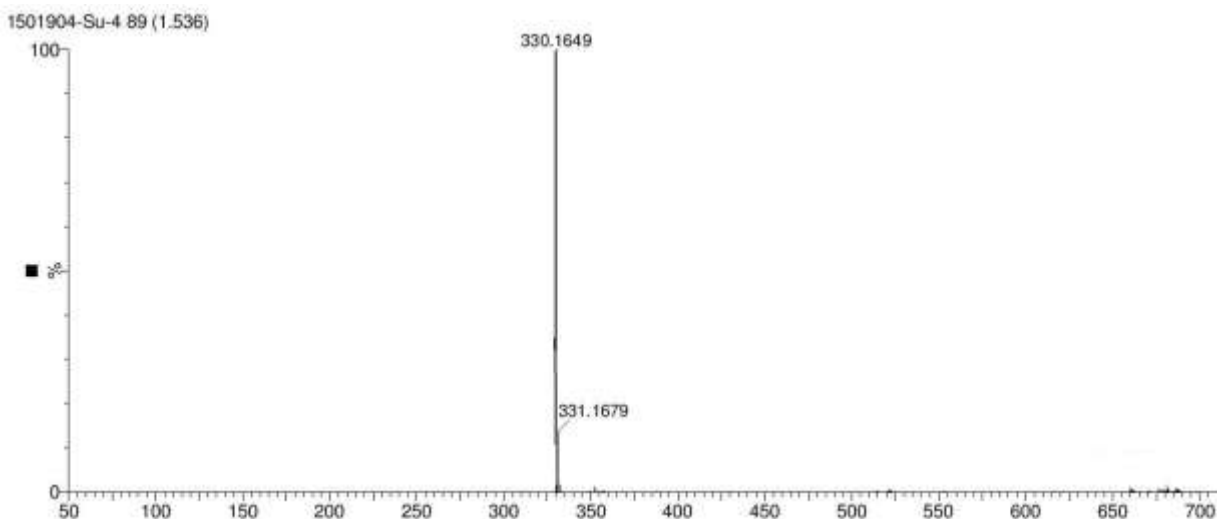
$^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$ ppm 31.2( $\text{C}_3$ ), 37.5( $\text{C}_2$ ), 45.9( $\text{C}_4$ ), 56.2( $\text{C}-4^3$ ,  $4^5\text{-OCH}_3$ ), 60.8( $\text{C}-4^4\text{-OCH}_3$ ), 106.6( $\text{C}-4^2$ ,  $4^6$ ), 113.3( $\text{C}_7$ ), 120.6( $\text{C}_5$ ), 126.6( $\text{C}_9$ ), 130.7( $\text{C}_8$ ), 136.7( $\text{C}-4^4$ ), 137.3( $\text{C}-4^1$ ), 141.9( $\text{C}_{10}$ ), 153.4( $\text{C}-4^3$ ,  $4^5$ ), 161.9( $\text{C}_6$ ), 198.4( $\text{C}_1$ );

MS,  $m/z$ : 330.16 ( $M^+$ ), 331.16( $M^++1$ );

Anal. Calcd. For  $\text{C}_{19}\text{H}_{20}\text{O}_5$ : C, 69.53; H, 6.15 O, 24.36 Found: C, 69.56; H, 6.14 O, 24.39 %.







**Figure 6: mass spectra of 6-hydroxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5b)**

#### **6-methyl-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5c)**

It was prepared by the oxidation of 7-methyl-1-(3, 4, 5-trimethoxyphenyl)-1, 2, 3, 4-tetrahydronaphthalene (0.01mmole) **4c** with chromium (VI) oxide (0.01mmole) in acetic acid to give brown gummy solid in 87.98%.

IR: 1695-1712 (C=O), 3025–3138 (Ar-CH);

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$ ppm 1.89–2.80 (4 H, m,  $\text{CH}_2$ ), 2.36 (3 H, s,  $\text{CH}_3$ ), 3.83(9H, s,  $\text{OCH}_3$ ), 4.23-4.44 (1 H, t, CH), 6.67–7.87 (4 H, m, Ar-H);

$^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$ ppm 21.6( $\text{CH}_3$ ), 31.3( $\text{C}_3$ ), 37.6( $\text{C}_2$ ), 45.5( $\text{C}_4$ ), 56.3( $\text{C}-4^3$ ,  $4^5\text{-OCH}_3$ ), 60.9( $\text{C}-4^4\text{-OCH}_3$ ), 106.7( $\text{C}-4^2, 4^6$ ), 125.2( $\text{C}_8$ ), 126.4( $\text{C}_7$ ), 128.0( $\text{C}_5$ ), 131.4( $\text{C}_9$ ), 136.7( $\text{C}-4^4$ ), 137.3( $\text{C}-4^1$ ), 140.4( $\text{C}_{10}$ ), 143.3( $\text{C}_6$ ), 153.4( $\text{C}-4^3$ ,  $4^5$ ), 198.1( $\text{C}_1$ );

MS,  $m/z$ : 326.15 ( $M^+$ ), 327.16( $M^++1$ );

Anal. Calcd. For  $\text{C}_{20}\text{H}_{22}\text{O}_4$ : C, 73.60; H, 6.79 O, 19.61 Found: C, 73.61; H, 6.75 O, 19.69 %.



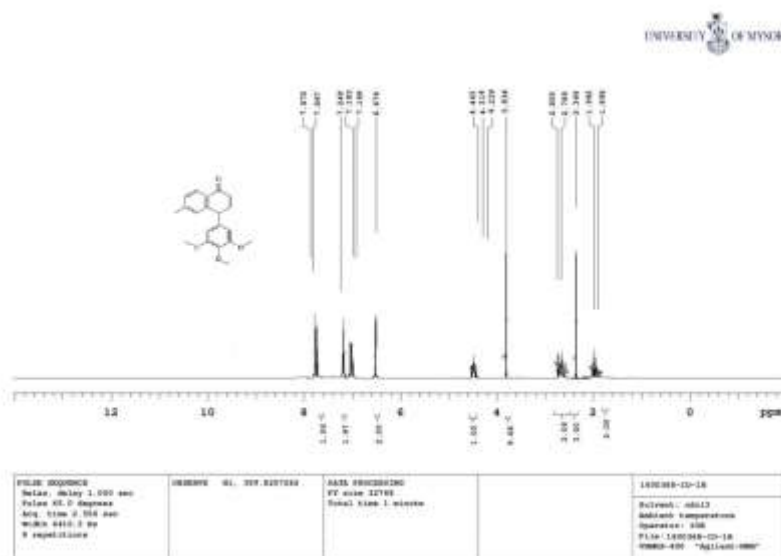


Figure 7:  $^1\text{H}$  NMR spectra of 6-methyl-4-(3,4,5-trimethoxyphenyl)-3,4-dihydronaphthalen-1(2H)-one (5c)

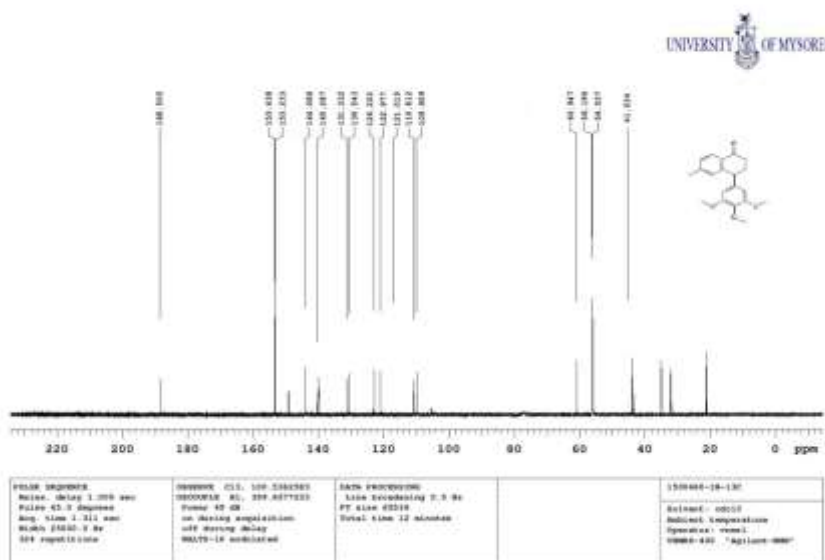
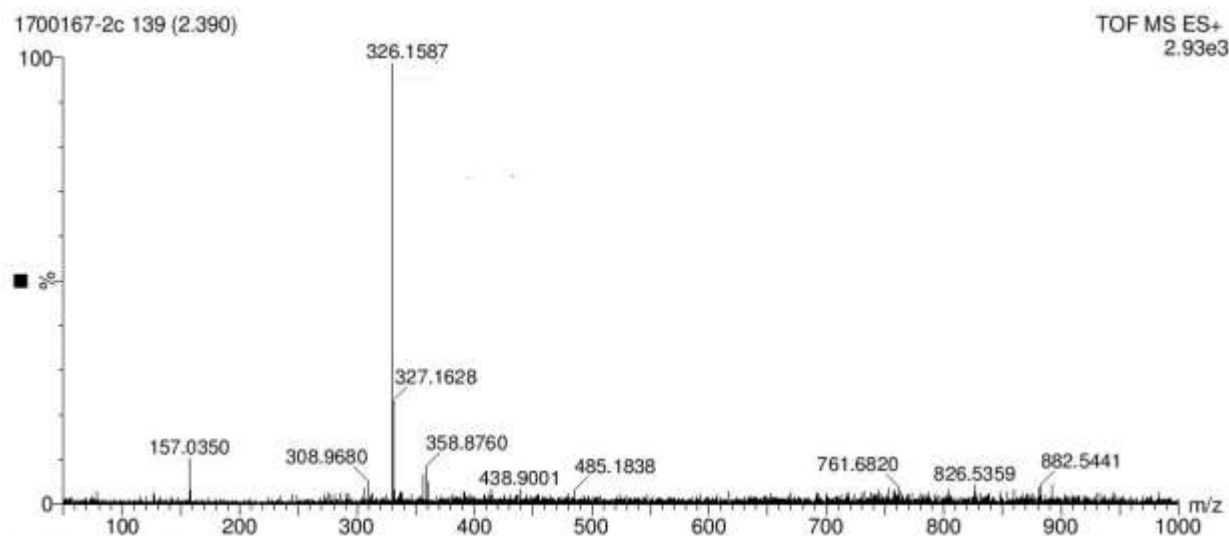


Figure 8:  $^{13}\text{C}$  NMR spectra of 6-methyl-4-(3,4,5-trimethoxyphenyl)-3,4-dihydronaphthalen-1(2H)-one (5c)



**Figure 9: mass spectra of 6-methyl-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5c)**

#### **6-chloro-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5d)**

It was prepared by the oxidation of 7-chloro-1-(3, 4, 5-trimethoxyphenyl)-1, 2, 3, 4-tetrahydronaphthalene (0.01mmole) **4d** with chromium (VI) oxide (0.01mmole) in acetic acid to give brown semi solid in 82.18%.

IR: 1685-1701 (C=O), 3020–3128 (Ar-CH);

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$ ppm 2.29- 2.82 (4 H, m,  $\text{CH}_2$ ), 3.90-3.92 (9H, s,  $\text{OCH}_3$ ), 4.06-4.09 (1 H, t, CH), 6.83–8.19 (5 H, m, Ar-H);

$^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$ ppm 31.6( $\text{C}_3$ ), 37.5( $\text{C}_2$ ), 45.1( $\text{C}_4$ ), 56.3( $\text{C}-4^3$ ,  $4^5\text{-OCH}_3$ ), 60.9( $\text{C}-4^4\text{-OCH}_3$ ), 106.9( $\text{C}-4^2, 4^6$ ), 126.2( $\text{C}_7$ ), 127.9( $\text{C}_5$ ), 130.7( $\text{C}_8$ ), 132.1( $\text{C}_9$ ), 136.4( $\text{C}-4^4$ ), 137.7( $\text{C}-4^1$ ), 139.3( $\text{C}_6$ ), 141.9( $\text{C}_{10}$ ), 153.4( $\text{C}-4^3$ ,  $4^5$ ), 195.8( $\text{C}_1$ );

MS,  $m/z$ : 347.27 ( $M^+$ ), 345.19( $M^++2$ );

Anal. Calcd. For  $\text{C}_{19}\text{H}_{19}\text{ClO}_4$ : C, 65.80; H, 5.52; Cl 10.22; O, 18.45 Found: C, 65.81; H, 5.55; Cl 10.20; O, 18.49 %.

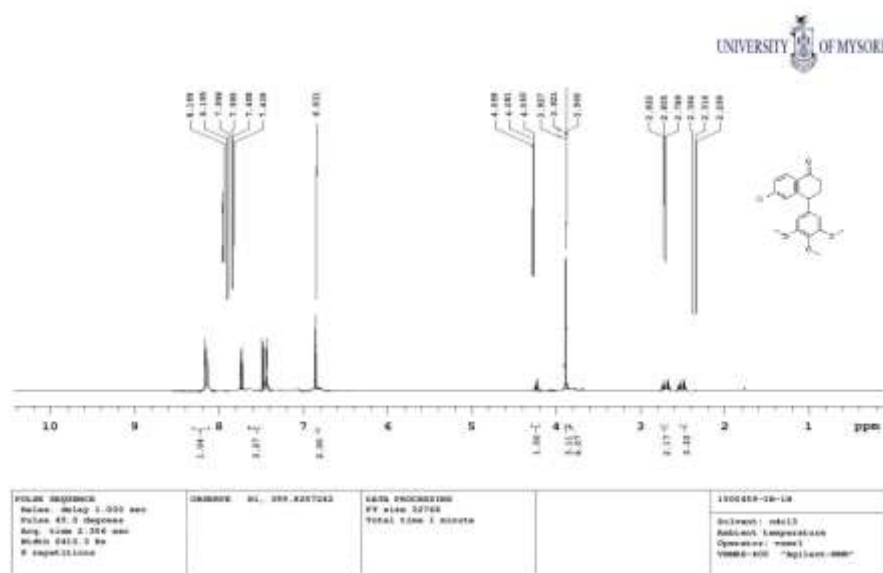


Figure 10:  $^1\text{H}$ NMR spectra of 6-chloro-4-(3,4,5-trimethoxyphenyl)-3,4-dihydronaphthalen-1(2H)-one (5d)

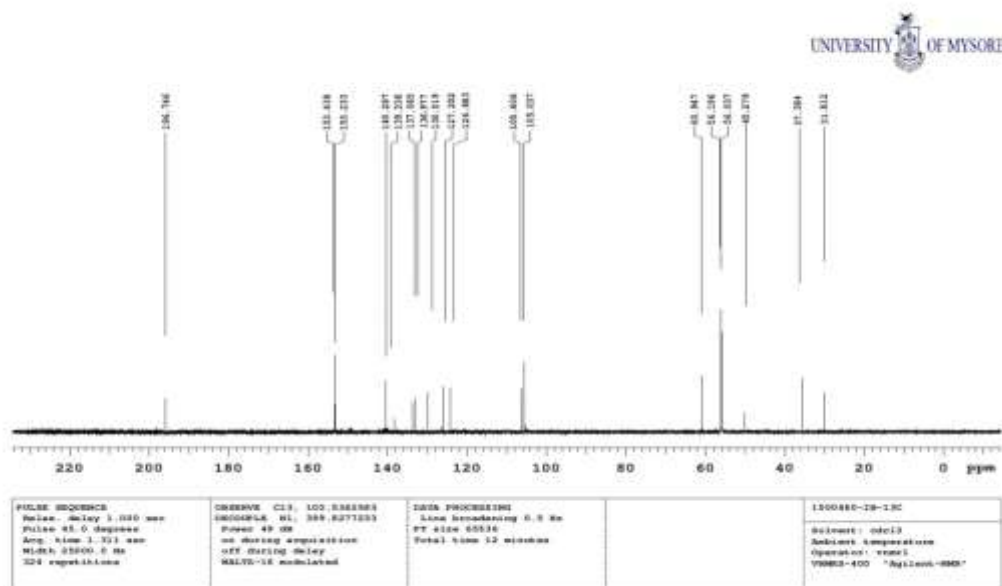
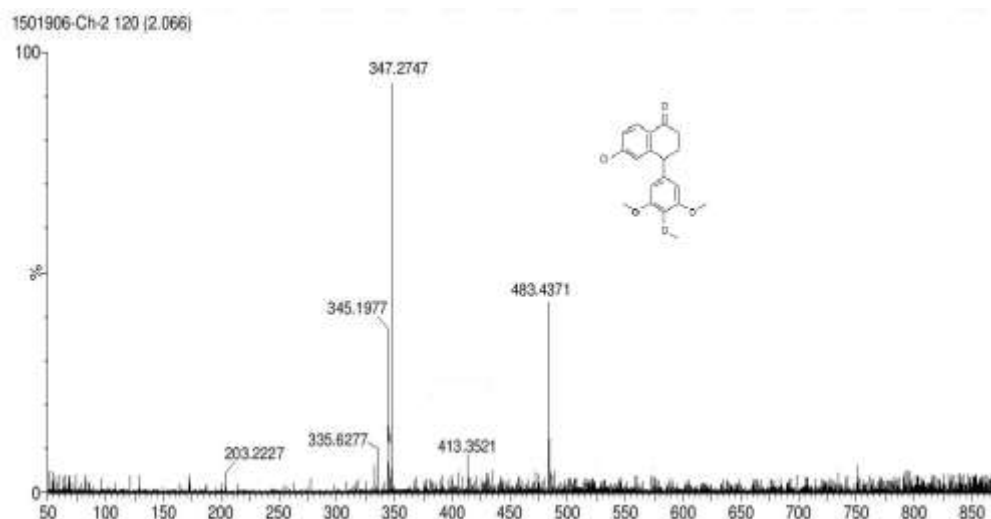


Figure 11:  $^{13}\text{C}$ NMR spectra of 6-chloro-4-(3,4,5-trimethoxyphenyl)-3,4-dihydronaphthalen-1(2H)-one (5d)



**Figure 12: mass spectra of 6-chloro-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5d)**

#### **4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5e)**

It was prepared by the oxidation of 1-(3, 4, 5-trimethoxyphenyl)-1, 2, 3, 4-tetrahydronaphthalene (0.01mmole) **4e** with chromium (VI) oxide (0.01mmole) in acetic acid to give brown semi solid in 82.98%.

IR: 1691-1698 (C=O), 3023–3125 (Ar-CH);

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$ ppm 2.62–2.70 (4 H, m,  $\text{CH}_2$ ), 3.80-3.91(9H, s,  $\text{OCH}_3$ ), 4.06-4.10 (1 H, t, CH), 6.52(2 H, dd, Ar-H), 7.33-7.83 (6 H, m, Ar-H);

$^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$ ppm 31.4( $\text{C}_3$ ), 37.6( $\text{C}_2$ ), 45.6( $\text{C}_4$ ), 56.5( $\text{C}-4^3$ ,  $4^5\text{-OCH}_3$ ), 60.8( $\text{C}-4^4\text{-OCH}_3$ ), 106.1( $\text{C}-4^2, 4^6$ ), 126.1( $\text{C}_7$ ), 128.1( $\text{C}_5, \text{C}_8$ ), 133.6( $\text{C}_6$ ), 134.0( $\text{C}_9$ ), 136.7( $\text{C}-4^4$ ), 137.3( $\text{C}-4^1$ ), 140.5( $\text{C}_{10}$ ), 153.4( $\text{C}-4^3$ ,  $4^5$ ), 198.0( $\text{C}_1$ );

MS,  $m/z$ : 312.1 ( $M^+$ ), 313.1 ( $M^++1$ );

Anal. Calcd. For  $\text{C}_{19}\text{H}_{20}\text{O}_4$ : C, 73.06; H, 6.45 O, 20.49. Found: C, 73.07; H, 6.43, O, 20.50%.

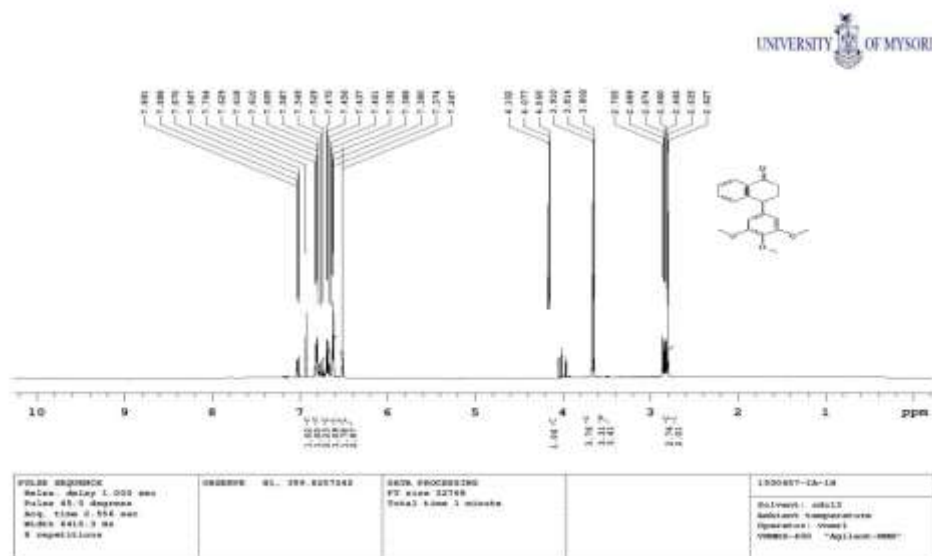


Figure 13:  $^1\text{H}$ NMR spectra of 4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5e)

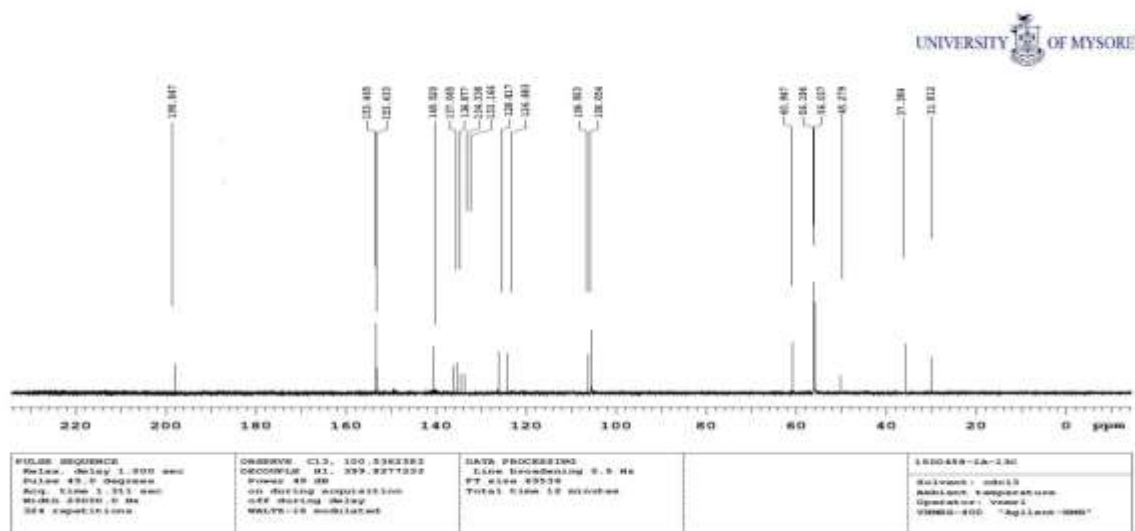
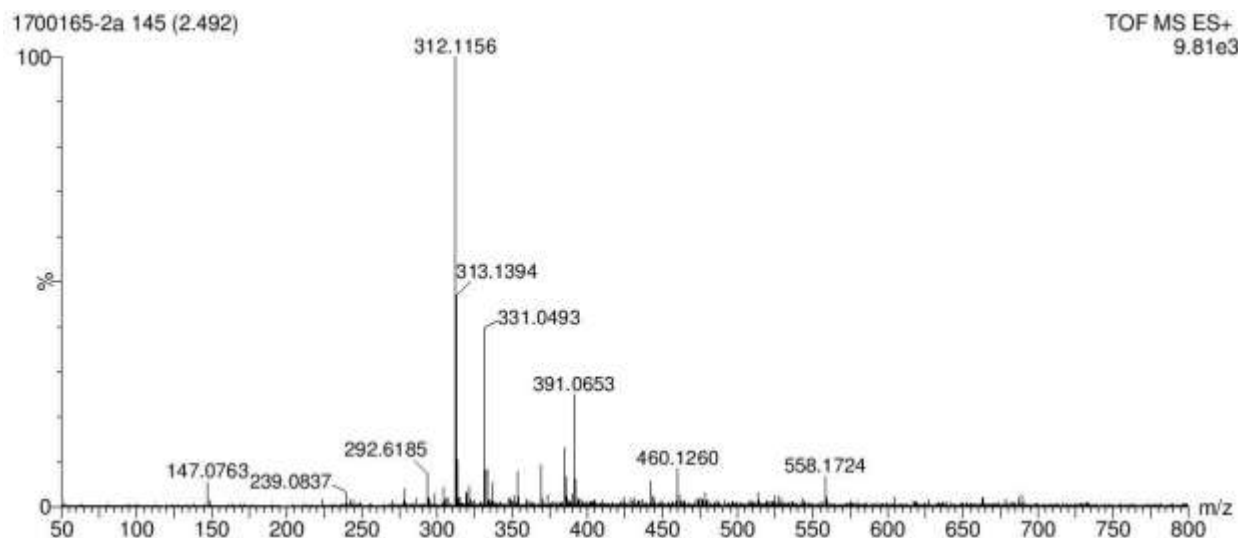


Figure 14 :  $^{13}\text{C}$ NMR spectra of 4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5e)



**Figure 15 : mass spectra of 4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5e)**

#### **6-methoxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5f)**

It was prepared by the oxidation 7-methoxy-1-(3, 4, 5-trimethoxyphenyl)-1, 2, 3, 4-tetrahydronaphthalene (0.01mmole) **4f** with chromium (VI) oxide (0.01mmole) in acetic acid to give brown semi solid in 85.48%.

IR: 1687-1701 (C=O), 3018–3139 (Ar-CH);

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$ ppm 1.95–2.66 (4 H, t,  $\text{CH}_2$ ), 3.90(12H, s,  $\text{OCH}_3$ ), 4.04-4.12 (1 H, t, CH), 6.62(2 H, dd, Ar-H), 6.85 –8.28(3 H, m, Ar-H);

$^{13}\text{C}$  NMR( $\text{CDCl}_3$ ):  $\delta$ ppm 31.4( $\text{C}_3$ ), 37.4( $\text{C}_2$ ), 45.9( $\text{C}_4$ ), 55.8( $\text{C}_6\text{-OCH}_3$ ), 56.8( $\text{C-4}^3$ ,  $4^5\text{-OCH}_3$ ), 60.9( $\text{C-4}^4\text{-OCH}_3$ ), 106.7( $\text{C-4}^2$ ,  $4^6$ ), 104.6( $\text{C}_5$ ), 111.9( $\text{C}_7$ ), 126.5( $\text{C}_9$ ), 130.5( $\text{C}_8$ ), 136.7( $\text{C-4}^4$ ), 137.5( $\text{C-4}^1$ ), 141.5( $\text{C}_{10}$ ), 153.6( $\text{C-4}^3$ ,  $4^5$ ), 165.9( $\text{C}_6$ ), 198.0( $\text{C}_1$ );

MS,  $m/z$ : 342.15 ( $M^+$ ), 343.23( $M^++1$ );

Anal. Calcd. For  $\text{C}_{20}\text{H}_{22}\text{O}_5$ : C, 70.16; H, 6.49 O, 23.36 Found: C, 70.17; H, 6.45 O, 23.39 %.



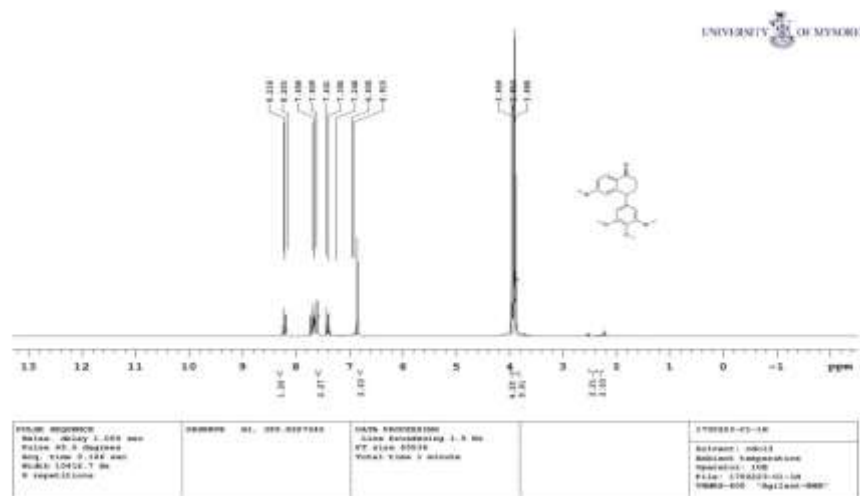


Figure 16 :  $^1\text{H}$ NMR spectra of 6-methoxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5f)

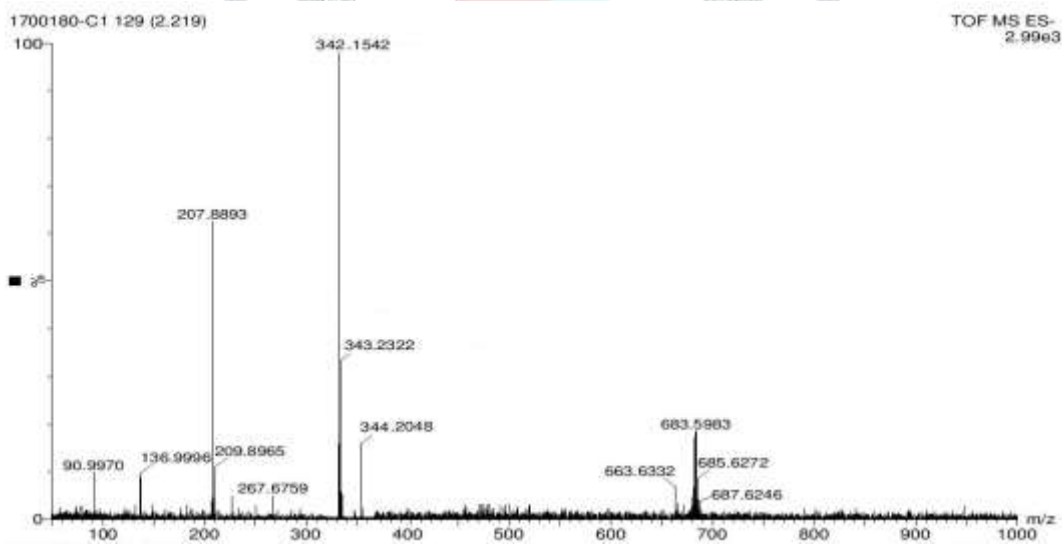


Figure 17: mass spectra of 6-methoxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5f)

**6-amino-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5g)**

It was prepared by the oxidation 7-amino-1-(3, 4, 5-trimethoxyphenyl)-1, 2, 3, 4-tetrahydronaphthalene (0.01mmole) **14g** with chromium (VI) oxide (0.01mmole) in acetic acid to give brown semi solid in 85.48%.

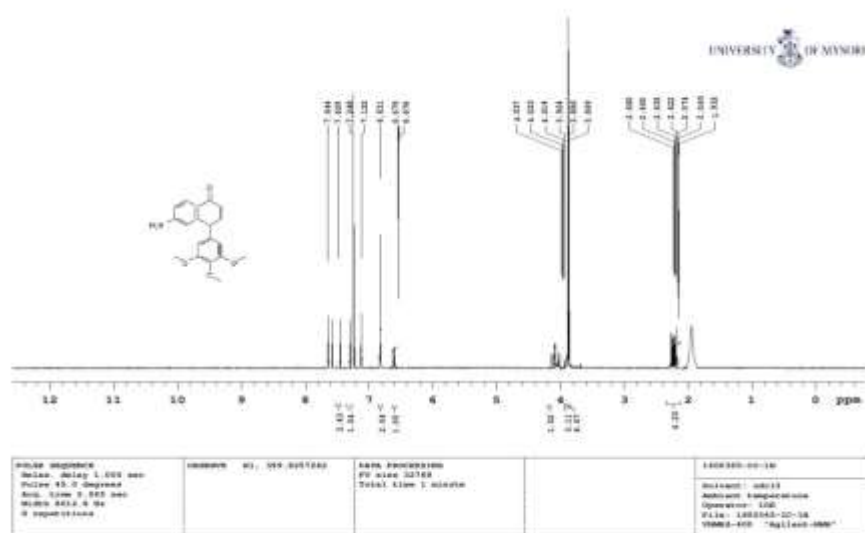
IR: 1687-1698(C=O), 3022–3139 (Ar-CH), 3350-3360(NH);

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δppm 1.95–2.66 (4 H, t, CH<sub>2</sub>), 3.91(9 H, s, OCH<sub>3</sub>), 4.01-4.06 (1 H, t, CH), 6.67-7.39(6 H, m, Ar-H);

<sup>13</sup>C NMR(CDCl<sub>3</sub>): δppm 31.4(C<sub>3</sub>), 37.4(C<sub>2</sub>), 45.9(C<sub>4</sub>), 56.1(C-4<sup>3</sup>, 4<sup>5</sup>-OCH<sub>3</sub>), 60.9(C-4<sup>4</sup>-OCH<sub>3</sub>), 106.7(C-4<sup>2</sup>, 4<sup>6</sup>), 111.6(C<sub>7</sub>), 115.1(C<sub>5</sub>), 124.0(C<sub>9</sub>), 130.1(C<sub>8</sub>), 136.7(C-4<sup>4</sup>), 137.3(C-4<sup>1</sup>), 141.3(C<sub>10</sub>), 153.3(C<sub>6</sub>), 153.4(C-4<sup>3</sup>, 4<sup>5</sup>), 198.3(C<sub>1</sub>);

MS,  $m/z$ : 327.00 ( $M^+$ ), 328.87( $M^++1$ );

Anal. Calcd. For C<sub>19</sub>H<sub>21</sub>NO<sub>4</sub>: C, 69.71; H, 6.47; O, 19.55 Found: C, 69.72; H, 6.46; O, 19.56 %.



**Figure 18 : <sup>1</sup>HNMR spectra of 6-amino-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5g)**

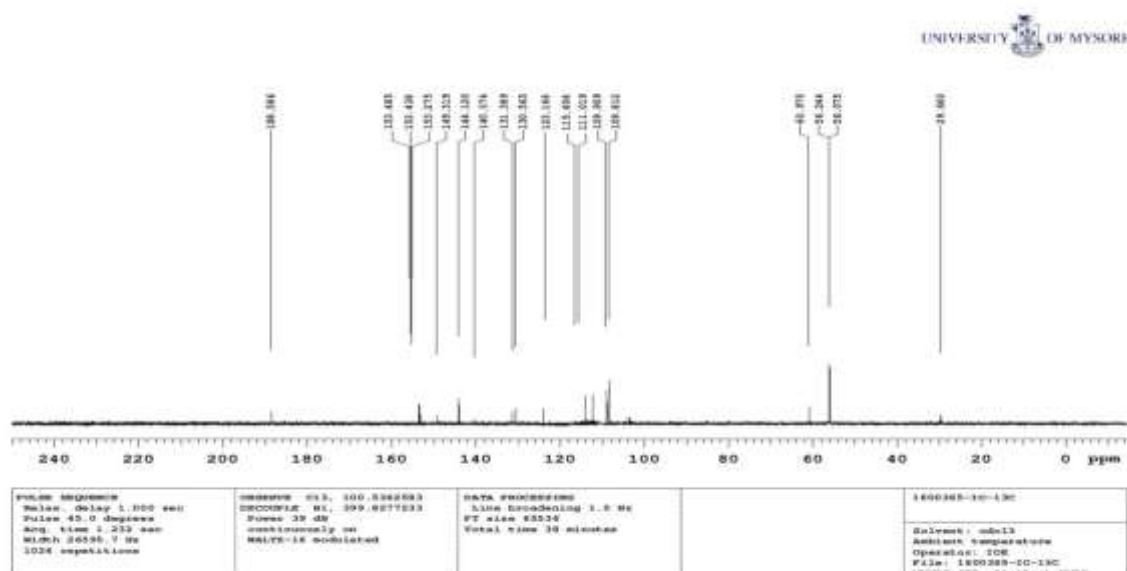


Figure 19 :  $^{13}\text{C}$ NMR spectra of 6-amino-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5g)

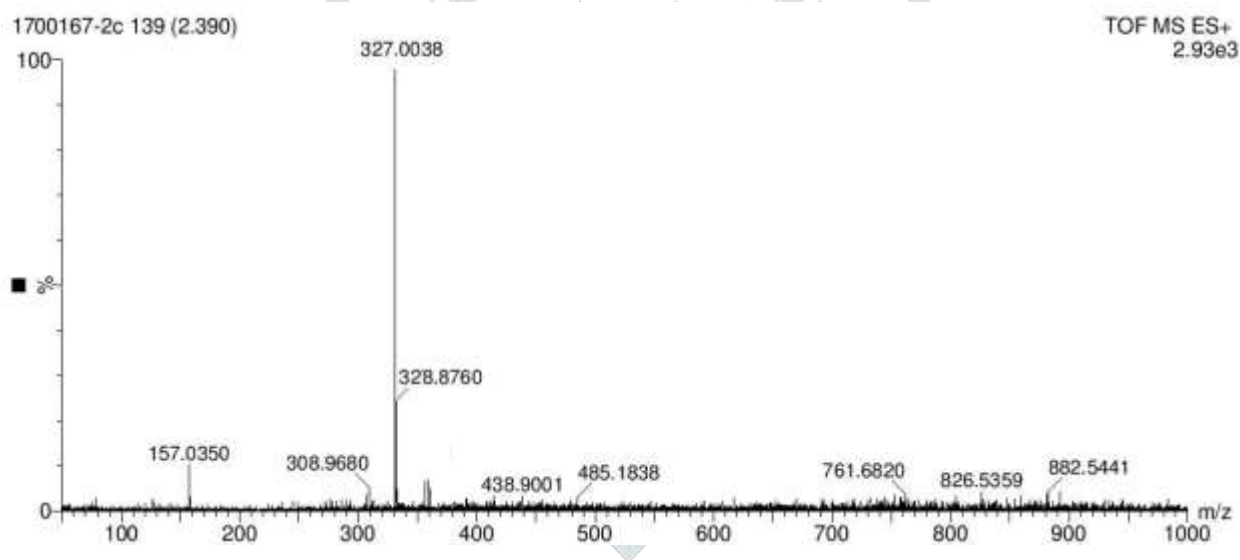
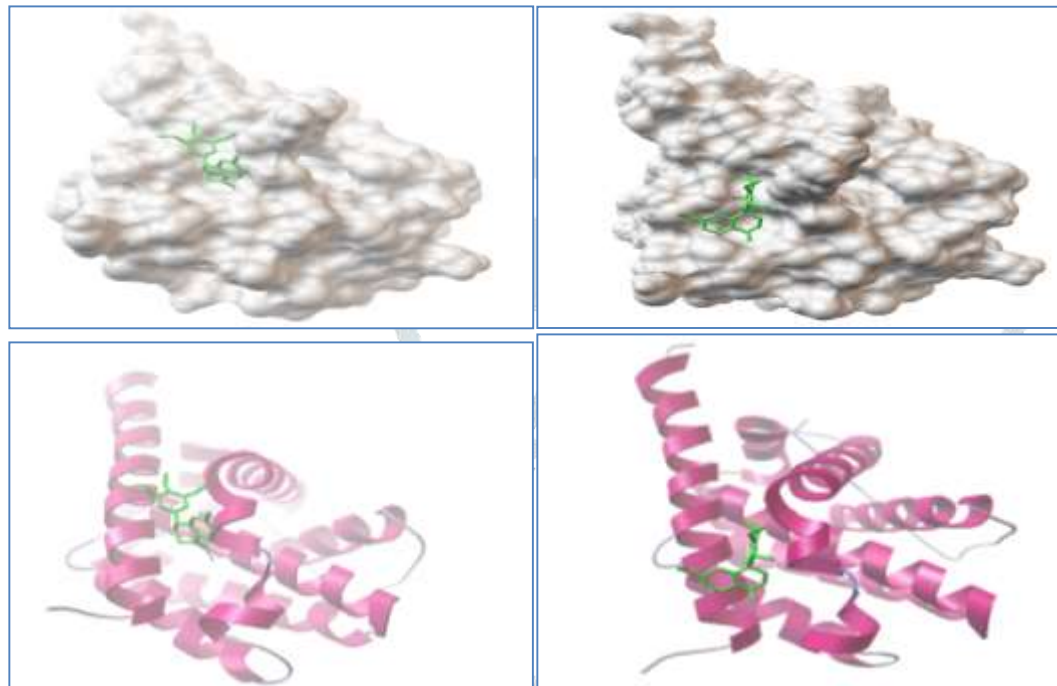


Figure 20: mass spectra of 6-amino-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (5g)

**Docking studies:** autodock server analysis of **5G** [6 amino 4(3, 4,5-trimethoxy phenyl tetralone)] and **5C** [6 methyl4-(3, 4, 5-trimethoxyphenyl) 3, 4 dihydronapfthalenelactone-1(2H) one] revealed that these synthesized compounds functions through Bcl-xL mediated pathway.

Compounds	Binding energy	Inhibition constant
<b>5G</b> [6 amino 4(3, 4,5-trimethoxy phenyl ) 3, 4 dihydronapfthalene-1(2H) one]	-5.22	0.000149
<b>5C</b> [6 methyl4-(3, 4, 5-trimethoxyphenyl) 3, 4 dihydronapfthalene-1(2H) one]	-4.68	0.000584

**5G****5C****Auto dock server analysis of 5G and 5C.**

## Biological activity

### Materials and methods

#### 1.Cell culture and maintenance

EAT (Ehrlich Ascites tumour) cells were procured from the National Centre for Cell Science, Pune, India. The cells were grown in suspension culture in Dulbecco's modified Eagle's medium (Life Technologies, USA) containing 10% foetal bovine serum (Sigma, USA) and antibiotics (100 U/ml penicillin and 100 µg /ml streptomycin) in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. For all experiments, Dulbecco's modified Eagle's Medium containing 5% foetal bovine serum was used.

## 2.2 Acridine orange/ethidium bromide staining methods

EAT cells grown in 12-well plates ( $5 \times 10^5$  cells/ well) were treated with and 6-methyl-4-(3, 4, 5-trimethoxyphenyl)-3, 4- dihydronaphthalen-1(2H)-one (**5c**) and 6-amino-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (**5g**) for 2 h. After washing once with phosphate-buffered saline, the cells were stained with 100  $\mu$ l of a mixture (1:1) of acridine orange ethidium bromide (4 $\mu$ g/ml) solutions. The cells were immediately washed once with phosphate-buffered saline and viewed under a Leica inverted fluorescent microscope (Leica DM 1000)

### Results:

#### Effects of extracts treatment in vitro on EAT cell morphology

EAT cells treated and control groups were dual stained with acridine orange and ethidium bromide and examined under a fluorescent microscope. No significant morphological changes were observed in the control cells, most of them appeared green with intact nuclei. However, the cells from the 6-methyl-4-(3, 4, 5-trimethoxyphenyl)-3, 4- dihydronaphthalen-1(2H)-one (**5C**) and 6-amino-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (**5G**) treated groups showed the early and late stages of apoptosis, manifested by the shrunken and crescent-shaped orange nuclei, membrane blebbing and apoptotic bodies containing fragmented nuclei. The percentage of apoptotic cells was determined by counting the number of apoptotic cells under the microscope in ten random fields in comparison to the vehicle treated control. Results, clearly show increased apoptosis induction by 69% and 54% by the 5c and 5g treatments, respectively. The present results revealed that 6-methyl-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (**5C**) and 6-amino-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (**5G**) compounds showed potent pro-apoptotic activity in the EAT tumor model.

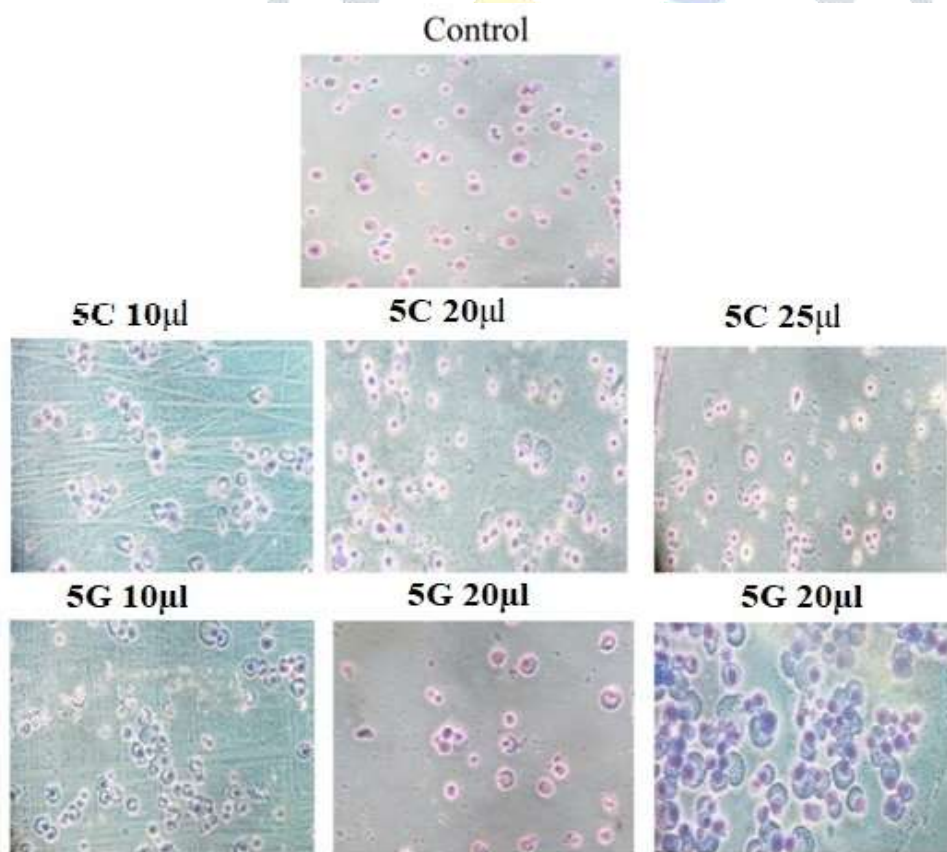
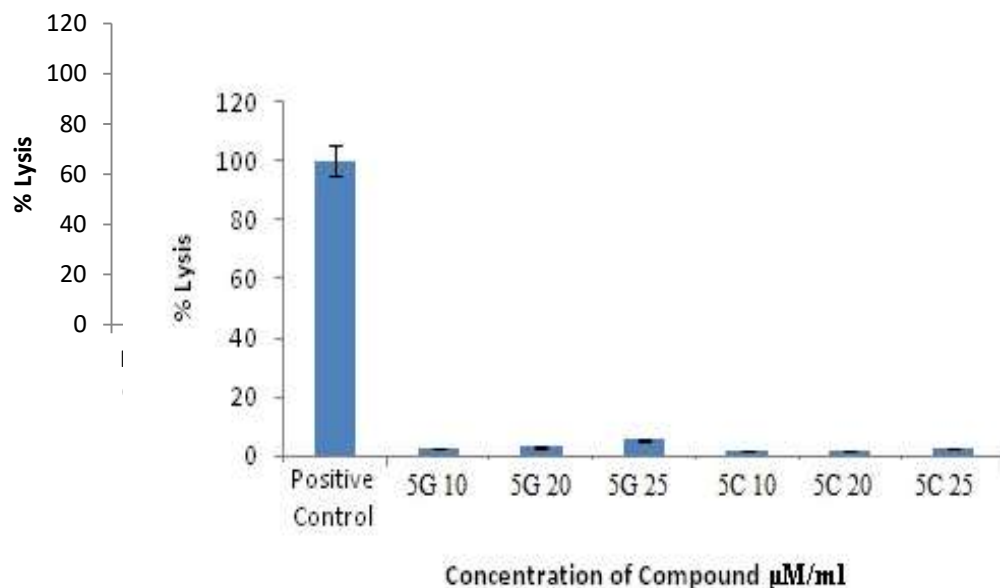


Figure 2. Morphological apoptotic changes in ETA cells. The cells were seeded for 24 h and then treated with



different concentrations. Cells morphological changes were studied using microscope. As observed, cell death morphologically depending on treatment concentration.



### ***In vitro* cytotoxicity of compounds assessed by Trypan blue dye exclusion assay**

The cytotoxic effects of the 5G and 5C were determined *in vitro* using EAT cells. The number of viable cells present in the treated cell suspension was counted by differentiating the live cells from the dead ones. A dose dependent decrease in viable cells was observed in treated cells. The percentage viability in cells treated with compounds 6-methyl-4-(3, 4, 5-trimethoxyphenyl)-3, 4- dihydronaphthalen-1(2H)-one (**5C**) and 6-amino-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one (**5G**) showed higher cytotoxicity of 53%, 46%, 24% and 76%, 65% and 38% compared to the control cells as shown in Fig.

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