

## ANTIOXIDANT ACTIVITIES OF AMINO CHROMENE DERIVATIVES WITH ADD ALKYL TAIL: SYNTHESIS AND CHARACTERIZATION

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### Abstract

The reaction between substituted 4-hydroxy benzaldehyde, active methylene compounds and resorcinol yield amino chromene derivatives. Structures of these were established upon the basis of IR, <sup>1</sup>HNMR, <sup>13</sup>CNMR, and MASS data. In vitro, antioxidants activities of these compounds against super oxide anion radical, nitric oxide radical, DPPH radical and hydrogen peroxide were evaluated and compared with standard natural antioxidants ascorbic acid

**Key words:** chromenes, imines, amines, antioxidants

### Introduction

Multicomponent reactions (MCRS) are reactions where numerous reactants involved in single synthetic operation and give new compounds.<sup>1</sup> This type of reactions avoids purification process and often wide variety of complex molecule in a single step, inturn it is very useful for saving solvent and reagents. Among many heterocyclic compounds, chromenes are very important due to its biological activity such as antioxidants,<sup>2</sup> anticancer, anti-microbial,<sup>3</sup> anti-inflammatory,<sup>4</sup> anti-HIV,<sup>5</sup> and anti-tumor,<sup>6</sup> alzimer disease<sup>7</sup> antihypotensive<sup>8</sup> and antileishmanial.<sup>9</sup> There are many reports shown that synthesis of different chromene derivatives and its applications (Figure 1).<sup>1, 10, 11</sup> A Knoevenagel condensation is the reaction between salicylaldehyde with active methylene compounds followed by intramolecular cyclisation to give imino derivatives<sup>11</sup>. As per reports, different products are obtained by control of a solvent, ratio of reagents and temperature<sup>3</sup> etc., Due to importance of these chromene derivatives, numerous green approaches have been developed under distinct conditions like thermal heating, microwave, ultrasonic, electrochemical, infrared, and solvent free conditions. We could not find many reports on variation of an alkyl side chain

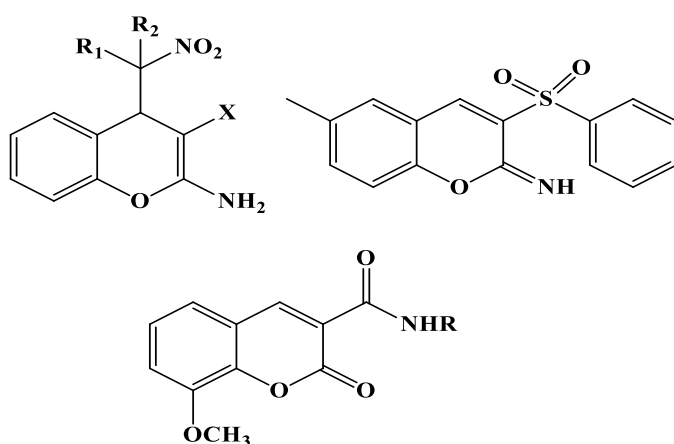


Figure 1. Examples for different chromene derivatives

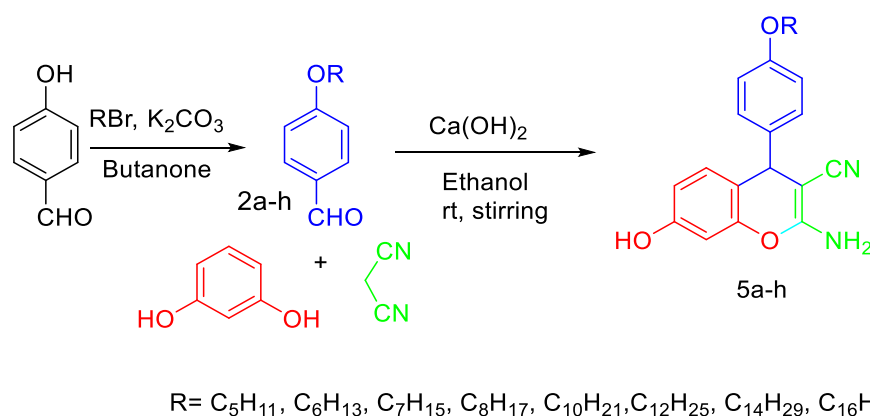
to see the effect on antioxidant properties of chromene derivatives. So we are motivated to synthesis imino and amino chromenes by taking alkylated aldehyde and malonitrile. Currently, many investigations are going on

effect of free radicals in biological systems such as lipids, DNA and protein, also create many diseases like atherosclerosis, neurodegenerative disease, rheumatoid arthritis, age related disease, cancer initiation and tumor.<sup>12, 13, 14</sup> It is necessary to keep a proper level of natural antioxidant such as vitamin E, C and glutathione in a biological system in order to avoid serious health problems.<sup>15, 16, 17</sup> All these health problems are caused by action of free radical oxygen (ROS) and reactive nitrogen (RNS) species, commonly known as (RSs).<sup>18, 19</sup>

## Results and discusión

### Antioxidant activities of amino chromene derivative

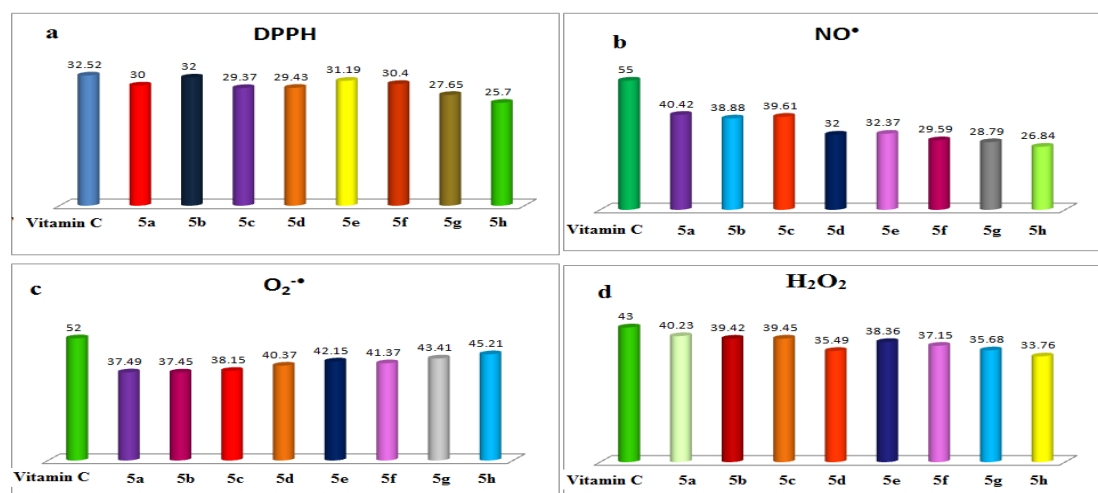
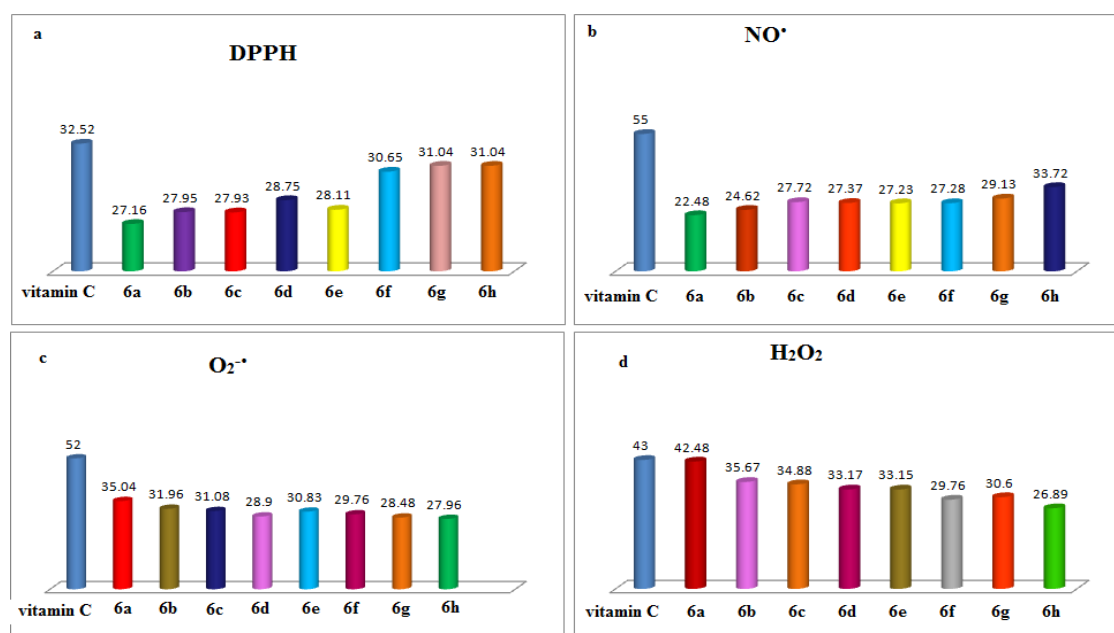
The free radical scavenging result 2-amino-7-hydroxy-4-(4-(alkyloxy)phenyl)-4H-chromene-3-carbonitrile was evaluated for all these derivatives **5a-h**. The free radical scavenging activity of chromene derivatives were evaluated through their ability to scavenge the free radicals like DPPH, NO<sup>•</sup>, O<sub>2</sub><sup>•-</sup>, H<sub>2</sub>O<sub>2</sub> using ascorbic acid as reference. The different concentrations of **5a-h** were prepared, and their antioxidant properties have been done by repeating the experiments for three times. All the molecules were showing best scavenging with four different radical. Among all these, 5h, 5g (DPPH), 5h, 5g (NO<sup>•</sup>), 5b, 5g (O<sub>2</sub><sup>•-</sup>), 5h, 5d (H<sub>2</sub>O<sub>2</sub>) having better free radical scavenging ability. The radical scavenging abilities have been shown in Table 1 and Figure 2.



Scheme 1. Synthetic procedures for series **5** and **6**

Table I. Antioxidant activity of chromene derivatives (5a-h)

SN	Compound	IC <sub>50</sub> (μM)			
		DPPH	NO <sup>•</sup>	O <sub>2</sub> <sup>•-</sup>	H <sub>2</sub> O <sub>2</sub>
1	Vitamin C	32.52±0.11	55.00±0.12	52.00±0.56	43.00±0.65
2	5a	30.00±0.24	40.42±0.24	37.49±0.39	40.23±0.33
3	5b	32.00±0.09	38.88±0.36	37.45±0.28	39.42±0.24
4	5c	29.37±0.47	39.61±0.65	38.15±0.58	39.45±0.90
5	5d	29.43±0.38	32.00±0.77	40.37±0.34	35.49±0.60
6	5e	31.19±0.57	32.37±0.28	42.15±0.11	38.36±0.46
7	5f	30.40±0.27	29.59±0.24	41.37±0.30	37.15±0.52
8	5g	27.65±0.16	28.79±0.14	43.41±0.08	35.68±0.35
9	5h	25.70±0.17	26.84±0.21	45.21±0.44	33.76±0.08

Fig.2. Antioxidant activity of 5a-h in IC<sub>50</sub> valuesFig.3. Antioxidant activity of 6a-h in IC<sub>50</sub> values

## Experimental

<sup>1</sup>H NMR spectra were recorded using Bruker (300MHz) spectrometer. For the <sup>1</sup>H NMR spectra, the chemical shifts are reported in ppm relative to SiMe<sub>4</sub>(TMS) as an internal standard and coupling constants are presented in Hz. Infrared spectra were recorded on JASCO- FTIR spectrometer(4000-400cm<sup>-1</sup>); the spectral positions are given in wave numbers (cm<sup>-1</sup>). Electrospray ionization mass spectrometry (ESI-MS) analysis was performed in the negative ion mode on a liquid chromatography-ion trap mass spectrometer (LCQ Fleet, Thermo Fisher Instruments Limited, US). Spectra of these compounds were given in supplementary information. The DPPH radical scavenging activity of the compounds was measured according to the method of Blais.<sup>20</sup> The assay of nitric oxide (NO) scavenging activity is measured based on method reported.<sup>21</sup> The ability of the compounds to scavenge hydrogen peroxide was determined using the method available in literature.<sup>22</sup> The superoxide anion radical (O<sub>2</sub><sup>•-</sup>) scavenging assay was based on the capacity of the complexes to inhibit formazan formation by scavenging the superoxide radicals generated in the riboflavin-light-NBT system.<sup>23</sup>

**General procedure for the synthesis of 4-alkoxy benzaldehyde: 2a-h**

A mixture of 4-hydroxy benzaldehyde (10mmol, 1eq) 1-bromoalkane (15mmol, 1.5eq), anhydrous  $K_2CO_3$  (15mmol, 1.5eq) and butanone 20ml, the catalytic amount of KI was added to the mixture was refluxed for 4 hours. Reaction mixture was concentrated, poured into water and extracted with dichloromethane (DCM) (20ml x 2). The combined organic layer was washed with brine and over anhydrous  $Na_2SO_4$ . Evaporation of solvent furnished a brown colored mass which was purified by column chromatography on 60-120 mesh silica gel. Elution with a mixture of ethyl acetate–pet ether (1:9) furnished the pure light yellow oily liquid.<sup>24</sup>

**General procedure for the preparation of 4- add alkoxy benzoic acid: 3a-h**

The 4-alkoxy benzaldehyde (1g) was dissolved in butanone (20ml) and Jones reagent (1.7g  $CrO_3$ , 2 ml  $H_2SO_4$  and 6 ml  $H_2O$ ) was slowly added to this mixture and stirred for 1 hour. After 1 hour, to this mixture water was added slowly. The white precipitate was filtered; it was washed with water and recrystallized by ethanol give pure product.<sup>24</sup>

**General procedure for the preparation of 4-formyl-3-hydroxyphenyl-4-(alkoxy) benzoate: 4a-h**

A stirred solution of 4- alkoxy benzoic acid (1eq), 2, 4-dihydroxy benzaldehyde (1.1eq), N, N-Dicyclohexyl carbodiimide (DCC) (3eq) and catalytic amount of (DMAP) dimethyl amino pyridine in (DCM) dichloro methane solution was added at the room temperature, mixture was vacuum created and stirred for overnight under  $N_2$  atmosphere. The precipitate N, N-dicyclohexyl urea was filtered off. The filtrate was diluted with (20ml) DCM and washed with water and dried over anhydrous  $Na_2SO_4$ . Evaporate solvent by vacuum pump and purified by column chromatography 60-120 mesh silica gel. Elution with a mixture of (1:9) ethyl acetate–pet ether furnished the pure a product. The product was recrystallized from  $CH_2Cl_2$ -acetonitrile to obtain a white solid.<sup>24</sup>

**2-amino-7-hydroxy-4-(4-(alkoxy) phenyl)-4H-chromene-3-carbonitrile: 5a-h**

A mixture of resorcinol (1.0mmol), 2-(4-methoxybenzylidene), malononitrile (1.5 mmol), and  $Ca(OH)_2$ (1.0mmol) in 5mL of methanol was stirred at room temperature for 5 min. After completion of the reaction monitored by TLC, the crude was washed with ethyl acetate, dissolved with THF and filter to separate the catalyst. Solvent was removed from filtrate gave the pure product.

**2-amino-7-hydroxy-4-(4-(methoxy) phenyl)-4H-chromene-3-carbonitrile (5a)**

**Mp:** 192°C; **Y**=80%; white solid; **IR,  $\nu_{max}$  (KBr,  $cm^{-1}$ ):** 3479.70 ( $NH_2$ ), 3271.38(OH), 2931.90 Aliphatic (C-H), 2189.28 ( $C\equiv N$ ), 1641.48 ( $-C=C-$ );  **$^1H$  NMR ( 300 MHz,  $DMSO-d_6$ ):**  $\delta$ = 9.05(s, 1H, ArOH), 7.07(d,  $J$ =9.0Hz, 2H, ArH), 6.80(d,  $J$ =9.0Hz, 2H, ArH), 6.74(d,  $J$ =9.0Hz, 1H, ArH), 6.53-6.48(m, 2H, ArH), 5.16(s, 2H,  $NH_2$ ), 4.56(s, 1H, CH), 3.89(t,  $J$ =6.0Hz, 2H,  $OCH_2$ ), 1.75-1.70(m, 2H,  $CH_2$ ), 1.42-1.30(m, 8H,  $CH_2$ ), 0.88(t,  $J$ =6.0Hz, 3H,  $CH_3$ );  **$^{13}C$  NMR (75 MHz  $DMSO-d_6$ ):**159.5, 158.0, 157.0, 149.0, 137.4, 129.9, 128.7, 120.3, 114.5, 113.9, 112.8, 102.8, 67.9, 60.2, 31.4, 29.1, 25.5, 22.4, 13.8; **MS (EI):**  $m/z$ =351.22( $M^+$ ).

**2-amino-4-(4-(propyloxy) phenyl)-7-hydroxy-4H-chromene-3-carbonitrile (5b)**

**Mp:** 202°C; **Y**=82%; white solid; **IR,  $\nu_{max}$  (KBr,  $cm^{-1}$ ):** 3423.76 ( $NH_2$ ), 3292.60(OH), 2924.18 Aliphatic (C-H), 2189.28 ( $C\equiv N$ ), 1645.33 ( $-C=C-$ );  **$^1H$  NMR ( 300 MHz,  $DMSO-d_6$ ):**  $\delta$ = 9.00(s, 1H, ArOH), 7.07(d,  $J$ =9.0Hz, 2H, ArH), 6.80(d,  $J$ =9.0Hz, 2H, ArH), 6.74(d,  $J$ =9.0Hz, 1H, ArH), 6.53-6.48(m, 2H, ArH), 5.16(s, 2H,  $NH_2$ ), 4.56(s, 1H, CH), 3.89(t,  $J$ =6.0Hz, 2H,  $OCH_2$ ), 1.75-1.70 (m, 2H,  $CH_2$ ), 1.41-1.30(m, 6H,  $CH_2$ ), 0.88(t,  $J$ =6.0Hz, 3H,  $CH_3$ );  **$^{13}C$  NMR (75 MHz  $DMSO-d_6$ ):**159.5, 158.0, 157.1, 149.0, 137.4, 129.9, 128.7, 120.3, 114.5, 113.9, 112.8, 102.6, 67.9, 60.2, 31.4, 29.1, 25.6, 22.4, 13.8; **MS (EI):**  $m/z$ =365.21( $M^+$ ).

**2-amino-4-(4-(pentyloxy) phenyl)-7-hydroxy-4H-chromene-3-carbonitrile (5c)**

**Mp:** 168°C; **Y**=85%; white solid; **IR,  $\nu_{\max}$  (KBr,  $\text{cm}^{-1}$ ):** 3425.69 ( $\text{NH}_2$ ), 3221.23(OH), 2924.18 Aliphatic (C-H), 2187.35 ( $\text{C}\equiv\text{N}$ ), 1647.26 ( $-\text{C}=\text{C}-$ );  **$^1\text{H}$  NMR( 300 MHz,  $\text{DMSO}-d_6$ ):**  $\delta$ = 9.06(s, 1H, ArOH), 7.09(d,  $J$ =9.0Hz, 2H, ArH), 6.81(d,  $J$ =9.0Hz, 2H, ArH), 6.74(d,  $J$ =9.0Hz, 1H, ArH), 6.54-6.49(m, 2H, ArH), 5.28(s, 2H,  $\text{NH}_2$ ), 4.56(s, 1H, CH), 3.90(t,  $J$ =6.0Hz, 2H,  $\text{OCH}_2$ ), 1.76-1.69 (m, 2H,  $\text{CH}_2$ ), 1.42-1.25(m, 8H,  $\text{CH}_2$ ), 0.88(t,  $J$ =6.0Hz, 3H,  $\text{CH}_3$ );  **$^{13}\text{C}$  NMR (75 MHz  $\text{DMSO}-d_6$ ):**159.37, 157.7, 156.7, 148.7, 137.3, 129.6, 128.4, 120.4, 114.2, 113.6, 112.5, 102.5, 67.6, 59.2, 31.4, 28.9, 28.6, 25.6, 22.2, 13.8; **MS (EI):**  $m/z$ =378.35( $\text{M}^+$ ).

**2-amino-7-hydroxy-4-(4-(heptyloxy) phenyl)-4H-chromene-3-carbonitrile (5d)**

**Mp:** 162°C; **Y**=87%; white solid; **IR,  $\nu_{\max}$  (KBr,  $\text{cm}^{-1}$ ):** 3425.69 ( $\text{NH}_2$ ), 3286.81(OH), 2918.40 Aliphatic (C-H), 2189.28 ( $\text{C}\equiv\text{N}$ ), 1643.41 ( $-\text{C}=\text{C}-$ );  **$^1\text{H}$  NMR( 300 MHz,  $\text{DMSO}-d_6$ ):**  $\delta$ = 9.02(s, 1H, ArOH), 7.09(d,  $J$ =9.0Hz, 2H, ArH), 6.81(d,  $J$ =9.0Hz, 2H, ArH), 6.72(d,  $J$ =9.0Hz, 1H, ArH), 6.54-6.49(m, 2H, ArH), 5.17(s, 2H,  $\text{NH}_2$ ), 4.57(s, 1H, CH), 3.90(t,  $J$ =6.0Hz, 2H,  $\text{OCH}_2$ ), 1.79-1.70 (m, 2H,  $\text{CH}_2$ ), 1.43-1.29(m, 10H,  $\text{CH}_2$ ), 0.90(t,  $J$ =6.0Hz, 3H,  $\text{CH}_3$ );  **$^{13}\text{C}$  NMR (75 MHz  $\text{DMSO}-d_6$ ):**159.4, 157.7, 156.7, 148.8, 137.3, 129.7, 128.5, 120.4, 114.2, 113.6, 112.5, 102.6, 67.6, 59.4, 31.4, 29.0, 28.9, 25.7, 22.3, 13.8; **MS (EI):**  $m/z$ =393.32( $\text{M}^+$ ).

**2-amino-4-(4-(nonyloxy) phenyl)-7-hydroxy-4H-chromene-3-carbonitrile (5e)**

**Mp:** 182°C; **Y**=86%; white solid; **IR,  $\nu_{\max}$  (KBr,  $\text{cm}^{-1}$ ):** 3481.63 ( $\text{NH}_2$ ), 3271.38(OH), 2918.40 Aliphatic (C-H), 2191.21 ( $\text{C}\equiv\text{N}$ ), 1643.41 ( $-\text{C}=\text{C}-$ );  **$^1\text{H}$  NMR( 300 MHz,  $\text{DMSO}-d_6$ ):**  $\delta$ = 9.29(s, 1H, ArOH), 7.08(d,  $J$ =9.0Hz, 2H, ArH), 6.80(d,  $J$ =9.0Hz, 2H, ArH), 6.77(d,  $J$ =9.0Hz, 1H, ArH), 6.51-6.45(m, 2H, ArH), 5.93(s, 2H,  $\text{NH}_2$ ), 4.54(s, 1H, CH), 3.90(t,  $J$ =6.0Hz, 2H,  $\text{OCH}_2$ ), 1.83-1.69(m, 2H,  $\text{CH}_2$ ), 1.43-1.12 (m, 14H,  $\text{CH}_2$ ), 0.87(t,  $J$ =6.0Hz, 3H,  $\text{CH}_3$ );  **$^{13}\text{C}$  NMR (75 MHz  $\text{DMSO}-d_6$ ):**159.2, 157.0, 156.2, 148.2, 137.0, 128.9, 127.8, 119.0, 113.6, 113.0, 111.7, 101.8, 66.9, 57.7, 30.8, 28.6, 28.3, 28.3, 24.8, 21.6, 13.1; **MS (EI):**  $m/z$ =420.91( $\text{M}^+$ ).

**2-amino-4-(4-(undecyloxy) phenyl)-7-hydroxy-4H-chromene-3-carbonitrile (5f)**

**Mp:** 150°C; **Y**=87%; white solid; **IR,  $\nu_{\max}$  (KBr,  $\text{cm}^{-1}$ ):** 3481.63 ( $\text{NH}_2$ ), 3261.74(OH), 2918.40 Aliphatic (C-H), 2191.21 ( $\text{C}\equiv\text{N}$ ), 1643.41 ( $-\text{C}=\text{C}-$ );  **$^1\text{H}$  NMR( 300 MHz,  $\text{DMSO}-d_6$ ):**  $\delta$ = 9.19(s, 1H, ArOH), 7.59(d,  $J$ =9.0Hz, 2H, ArH), 7.09(d,  $J$ =9.0Hz, 2H, ArH), 6.81(d,  $J$ =9.0Hz, 1H, ArH), 6.53-6.48(m, 2H, ArH), 5.62(s, 2H,  $\text{NH}_2$ ), 4.56(s, 1H, CH), 3.90(t,  $J$ =6.0Hz, 2H,  $\text{OCH}_2$ ), 1.76-1.72(m, 2H,  $\text{CH}_2$ ), 1.43-1.14(m, 22H,  $\text{CH}_2$ ), 0.88(t,  $J$ =6.0Hz, 3H,  $\text{CH}_3$ );  **$^{13}\text{C}$  NMR (75 MHz  $\text{DMSO}-d_6$ ):**159.5, 157.7, 156.8, 148.8, 137.3, 129.7, 128.5, 120.4, 114.2, 113.67, 112.5, 102.4, 67.6, 59.4, 31.5, 29.3, 29.2, 29.1, 29.0, 25.7, 22.3, 13.8; **MS (EI):**  $m/z$ =447.40( $\text{M}^+$ ).

**2-amino-4-(4-(tridecyloxy) phenyl)-7-hydroxy-4H-chromene-3-carbonitrile (5g)**

**Mp:** 143°C; **Y**=86%; white solid; **IR,  $\nu_{\max}$  (KBr,  $\text{cm}^{-1}$ ):** 3481.63 ( $\text{NH}_2$ ), 3265.59(OH), 2918.40 Aliphatic (C-H), 2191.21 ( $\text{C}\equiv\text{N}$ ), 1645.33 ( $-\text{C}=\text{C}-$ );  **$^1\text{H}$  NMR( 300 MHz,  $\text{DMSO}-d_6$ ):**  $\delta$ = 9.06(s, 1H, ArOH), 7.09(d,  $J$ =9.0Hz, 2H, ArH), 6.81(d,  $J$ =9.0Hz, 2H, ArH), 6.75(d,  $J$ =9.0Hz, 1H, ArH), 6.55-6.49(m, 2H, ArH), 5.35(s, 2H,  $\text{NH}_2$ ), 4.57(s, 1H, CH), 3.90(t,  $J$ =6.0Hz, 2H,  $\text{OCH}_2$ ), 1.77-1.72(m, 2H,  $\text{CH}_2$ ), 1.43-1.26(m, 26H,  $\text{CH}_2$ ), 0.88(t,  $J$ =6.0Hz, 3H,  $\text{CH}_3$ );  **$^{13}\text{C}$  NMR (75 MHz  $\text{DMSO}-d_6$ ):**158.9, 156.8, 156.0, 148.0, 136.8, 128.7, 127.5, 119.6, 113.3, 112.8, 111.5, 101.6, 66.7, 57.5, 30.6, 28.3, 28.1, 28.0, 24.8, 21.3, 12.9; **MS (EI):**  $m/z$ =476.83( $\text{M}^+$ ).

**2-amino-7-hydroxy-4-(4-(pentadecyloxy) phenyl)-4H-chromene-3-carbonitrile (5h)**

**Mp:** 126°C; **Y**=86%; white solid; **IR,  $\nu_{\max}$  (KBr,  $\text{cm}^{-1}$ ):** 3338.89 ( $\text{NH}_2$ ), 3221.23(OH), 2920.32 Aliphatic (C-H), 2218.21 ( $\text{C}\equiv\text{N}$ ), 1604.83 ( $-\text{C}=\text{C}-$ );  **$^1\text{H}$  NMR( 300 MHz,  $\text{DMSO}-d_6$ ):**  $\delta$ = 9.06(s, 1H, ArOH), 7.09(d,  $J$ =9.0Hz, 2H, ArH), 6.81(d,  $J$ =9.0Hz, 2H, ArH), 6.75(d,  $J$ =9.0Hz, 1H, ArH), 6.55-6.49(m, 2H, ArH), 5.34(s, 2H,  $\text{NH}_2$ ), 4.57(s, 1H, CH), 3.90(t,  $J$ =6.0Hz, 2H,  $\text{OCH}_2$ ), 1.77-1.72(m, 2H,  $\text{CH}_2$ ), 1.43-1.26(m, 26H,  $\text{CH}_2$ ), 0.88(t,  $J$ =6.0Hz, 3H,  $\text{CH}_3$ );  **$^{13}\text{C}$  NMR (75 MHz  $\text{DMSO}-d_6$ ):**158.2, 157.0, 156.2, 148.2, 137.0, 128.9, 127.8, 119.8, 113.6, 113.0, 111.7, 101.8, 66.9, 57.8, 30.8, 28.6, 28.4, 28.3, 28.2, 25.0, 21.6, 13.9; **MS (EI):**  $m/z$ =504.85( $\text{M}^+$ ).

## Conclusion

A series of 2-amino-7-hydroxy-4-(4-(alkyloxy) phenyl)-4H-chromene-3-carbonitrile and 3-cyano-2-imino-2H-chromen-7-yl 4-(alkyloxy) benzoate have been synthesized. The structures were confirmed by  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , FT-IR and mass spectroscopic techniques. The compounds exhibited excellent radical scavenging activities against super oxide anion radical, nitric oxide radical, DPPH radical and hydrogen peroxide. Among all the derivatives in series I 5h, 5g (DPPH), 5h, 5g ( $\text{NO}^\bullet$ ), 5a, 5b ( $\text{O}_2^\bullet$ ), 5h, 5d, ( $\text{H}_2\text{O}_2$ ) having better free radical scavenging ability. Based on the result, it is clear that these can be used as good antioxidant in the field of medicinal and food industry.

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