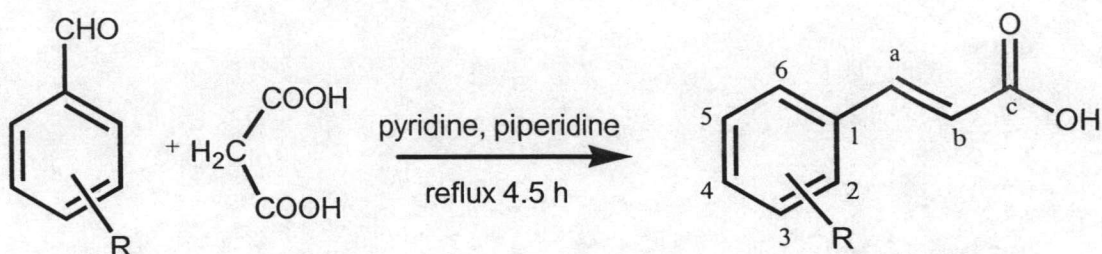


## CHAPTER III

### RESULT AND DISCUSSION

#### 3.1 Synthesis and UV Absorption Properties of Substituted *trans*-Cinnamic Acids

In this research, ten substituted *trans*-cinnamic acids were synthesized (table 3.1) using Knoevengel-Doebner condensation between one mole equivalent of malonic acid and one mole equivalent of substituted benzaldehyde. The general equation is shown below:



The structures of all synthesized compounds were well characterized using various spectroscopic techniques including <sup>1</sup>H, <sup>13</sup>C-NMR, IR (see section 3.6).

Experiments were done to obtain UV absorption properties ( $\lambda_{\max}$  and  $\epsilon$ ) of these *trans*-cinnamic acids. As shown in table 3.1 and figure 3.1, all ten substituted *trans*-cinnamic acids showed UVB filtering properties. Interestingly, 2,4,5-trimethoxycinnamic acid (**C8**) has two absorption bands;  $\lambda_{\max}$  of 287 nm ( $\epsilon = 11,723 \text{ M}^{-1}\text{cm}^{-1}$ ) and 336 nm ( $\epsilon = 12,832 \text{ M}^{-1}\text{cm}^{-1}$ ), which correspond to the UVB and UVA region respectively. The UVA filtering property can also be found in 2,5-trimethoxycinnamic acid (**C4**). Molar absorption coefficients ( $\epsilon$ ) of all ten acids were not far from the value of the *p*-methoxycinnamic acid (**C1**) (ester of **C1** is a commonly used UVB filter in various applications). In the case of the **C8** which is a novel UVA and UVB screening compound, the  $\epsilon$  value of both regions were more than ten thousand, indicating effectiveness of UV-screening in both regions.

Table 3.1 UV Absorption spectral data of substituted *trans*-cinnamic acids in methanol.

Cpds	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	$\lambda_{\max}$ (nm)	$\epsilon$ (M <sup>-1</sup> cm <sup>-1</sup> )
C1	H	H	OCH <sub>3</sub>	H	H	288	19696
C2	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	H	278	19527
C3	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H	H	274 312	10277 8818
C4	OCH <sub>3</sub>	H	H	OCH <sub>3</sub>	H	272 339	10869 4467
C5	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	287 311	13149 12443
C6	H	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H	281	15479
C7	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	281	16756
C8	OCH <sub>3</sub>	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	282 336	11723 12832
C9	H	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	292	13908
C10	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H	OCH <sub>3</sub>	310	19414

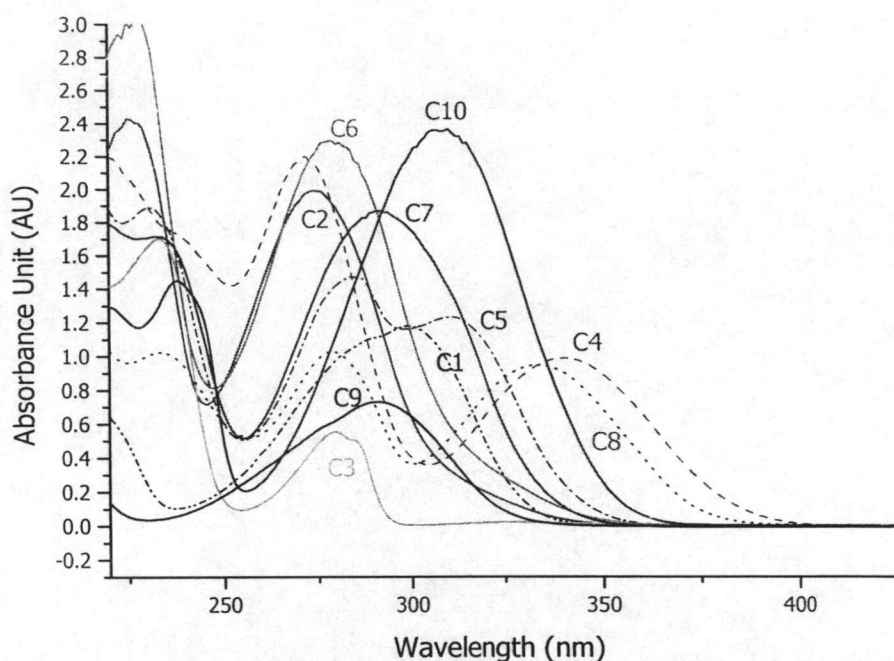


Figure 3.1 UV spectrum of ten substituted *trans*-cinnamic acids

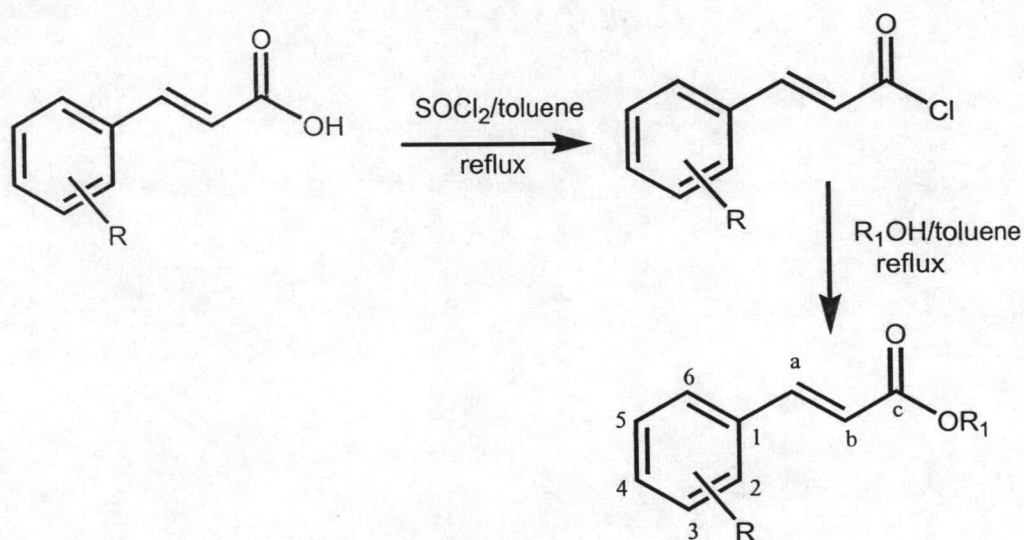
As can be seen in table 3.1, there is no relationship between number and position of methoxy groups and UV absorption properties ( $\lambda_{\text{max}}$  and  $\epsilon$ ) of the compounds. Usually one may expect to see higher  $\epsilon$  values in compounds with more electron-releasing groups attached to the aromatic ring. The results obtained, however, show no systematic correlation. The speculation on this is that the substituent groups at different position may influence planarity of the molecule. In other words, the molar absorption coefficient of the molecule is directly affected by both the electron density of the delocalizing system and also the planarity of the molecule (ease of delocalization).

### 3.2 Synthesis and UV Absorption Properties of *trans*-Cinnamate Esters

As can be seen in table 3.1, C8 is the only cinnamic acid that has both UVA and UVB screening properties. It was, therefore, subjected to farther esterification. Since C1 is the parent acid of the popular UVB filter, octyl-*p*-methoxycinnamate, it was also subjected to further esterification. Since the objective of this research is not only finding a novel UVA/B filtering agent, but also to improve the photoisomerization problem of the commonly used cinnamate sunscreen, cinnamic acid with this potential was, therefore,

subjected to further esterification. In this case, the compound with two methoxy groups ortho to the cis-trans isomerizable double bond is the **C10**. It might be possible that methoxy groups may cause some steric inhibition to the cis-trans isomerization process. Moreover, the molar absorption coefficient of **C10** is close to that of **C1**. Although the  $\lambda_{\max}$  of **C10** is about 10 nm higher than that of **C1** but the absorption profile (fig 3.1) is still acceptable for protecting the later half of UVB and the first half of UVA. In addition, the absorption range of **C10** combining with absorption range of **C8** give total protection of both UVA and UVB.

Three *trans*-cinnamate esters were, therefore, synthesized by esterification using one mole equivalent of *trans*-cinnamic acid changing to active carbonyl, acid chloride, and one mole equivalent of alcohol, 2-ethylhexyl alcohol. The general equation is shown below:



The absorption spectral properties ( $\lambda_{\max}$  and  $\epsilon$  values) of the three *trans*-cinnamate esters in methanol are reported in table 3.2 and their UV absorption profiles are shown in fig 3.2.

When measured in methanol all three esters showed small bathochromic shifts (8-22 nm) of the UV absorption when compared to their carboxylic acid precursors. This bathochromic shift can be explained from the fact that carboxylic ester is less polar than

its parent carboxylic acid, therefore, polar solvent can stabilize the parent carboxylic acid making the gap between the ground state and the excited state of the acid wider.<sup>11</sup>

Stabilization of ground state ester from polar solvent is unlikely compared to the stabilization of the ester's excited state, as a result, the gap between the ground state and excited state of the ester is smaller than that of the parent acid. The molar extinction coefficients of the three esters, **E1**, **E8** and **E10** are not very far from each other and are quite high, indicating effectiveness in UV screening.

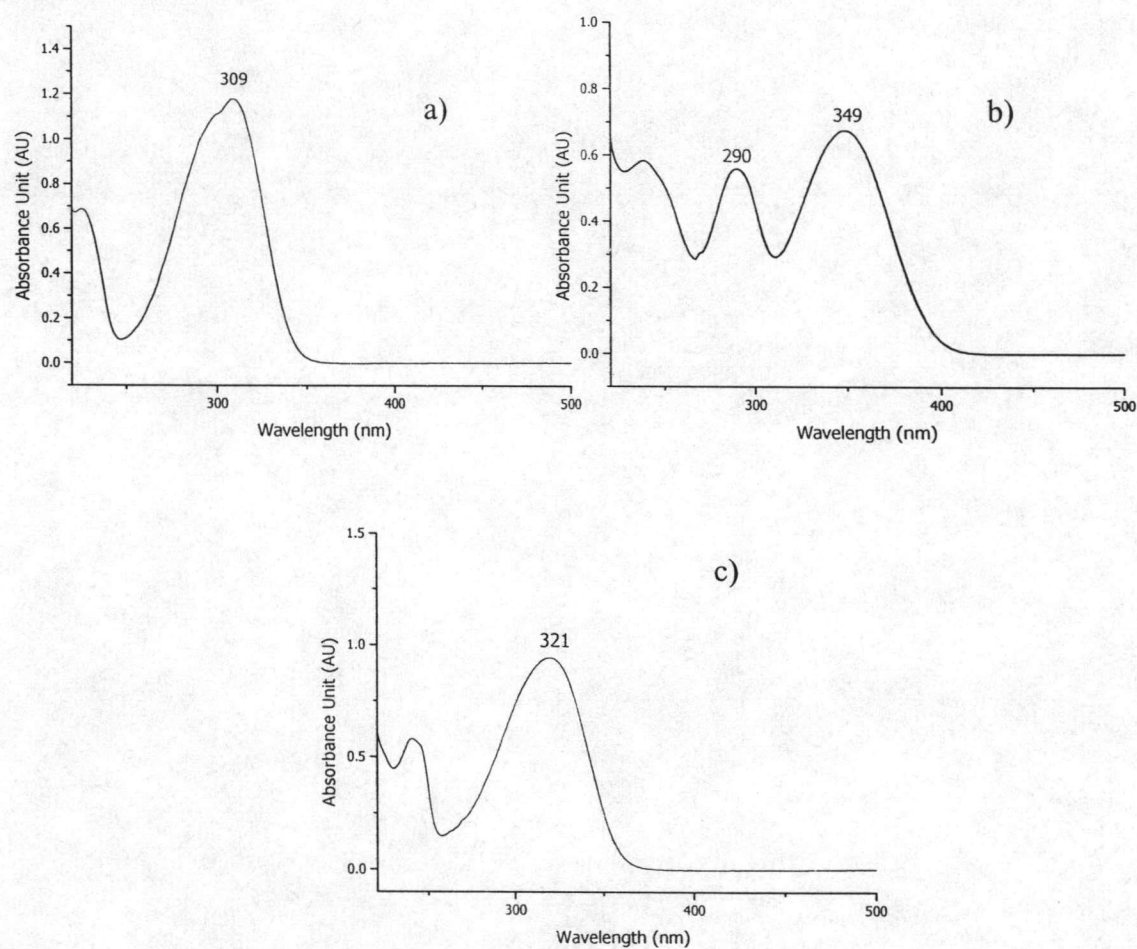


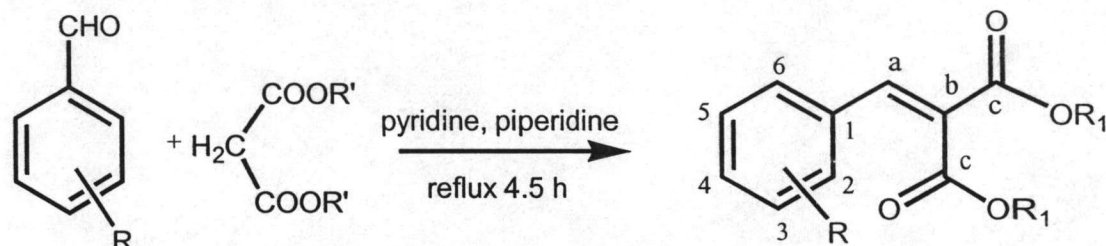
Figure 3.2 UV spectrum of a) 2-ethylhexyl-*p*-methoxycinnamate (**E1**), b) 2-ethylhexyl-2,4,5-trimethoxycinnamate (**E8**) and c) 2-ethylhexyl-2,4,6-trimethoxycinnamate (**E10**)

Table 3.2 UV Absorption data of substituted *trans*-cinnamate esters in methanol.

Cpds	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	R <sub>1</sub>	$\lambda_{\max}$ (nm)	$\epsilon$ (m <sup>-1</sup> cm <sup>-1</sup> )
<b>E1</b>	H	H	OCH <sub>3</sub>	H	H	CH <sub>2</sub> CH(CH <sub>2</sub> CH <sub>3</sub> )C <sub>4</sub> H <sub>9</sub>	309	18896
<b>E8</b>	OCH <sub>3</sub>	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	CH <sub>2</sub> CH(CH <sub>2</sub> CH <sub>3</sub> )C <sub>4</sub> H <sub>9</sub>	290 349	12367 14150
<b>E10</b>	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H	OCH <sub>3</sub>	CH <sub>2</sub> CH(CH <sub>2</sub> CH <sub>3</sub> )C <sub>4</sub> H <sub>9</sub>	321	22427

### 3.3 Synthesis and UV Absorption Properties of Benzalmalonate Derivatives

As mentioned in the introduction that *cis-trans* isomerization of OMC is responsible for the decrease in UV absorption efficiency (decrease  $\epsilon$  value) when the compound is exposed to UV light. To solve this problem, the dialkyl benzalmalonates were synthesized. The synthesis was done through Knoevengel-Doebner condensation between one mole equivalent of dialkyl malonate and one mole equivalent of substituted benzaldehyde. The general equation is shown below:



Two benzaldehydes, i.e., 2,4,5-trimethoxybenzaldehyde (precursor of **C8**) and *p*-methoxybenzaldehyde (precursor of **C1**), were chosen for benzalmalonate synthesis. Firstly, 2,4,5-trimethoxybenzaldehyde was condensed with diethylmalonate yielding **BM8-1** (yellow powder, soluble in polar solvent, slightly soluble in less polar solvent). Since **BM8-1** is soluble in only polar solvents, less polar benzalmalonate was prepared from 2,4,5-trimethoxybenzaldehyde and di-(2-ethylhexyl)-malonate. The product, di-(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate (**BM8-2**), is a yellow oil, which was excellently soluble in both polar and non polar solvents such as methanol and hexanes, respectively. In addition, the steric effect on UV absorption properties of two bulky alkyl

chains in **BM8-2** was evaluated by compared with dihexyl-2,4,5-trimethoxybenzalmalonate (**BM8-3**). **BM8-3** was prepared by the same procedure as **BM8-2** except that dihexylmalonate was used in place of di-(2-ethylhexyl)-malonate. As the three benzalmalonates, **BM8-1**, **BM8-2** and **BM8-3**, were prepared from 2,4,5-trimethoxybenzaldehyde, three more benzalmalonates derivative (dialkyl-*p*-methoxybenzalmalonate) were also prepared from *p*-methoxybenzaldehydes using the same three benzalmalonates (see table 3.3).

All benzalmalonate derivatives displayed spectra similar to their *trans*-cinnamate esters (fig 3.3). However,  $\lambda_{\max}$  of all benzalmalonate derivatives is about +2 to +10 nm higher than that of their parent esters. This bathochromic shift is a result of the second ester moiety which extends conjugation of the molecule.

Table 3.3 UV Absorption spectral data of benzalmalonate esters in methanol.

Cpds.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	$\lambda_{\max}$ (nm)	$\epsilon$ (M <sup>-1</sup> cm <sup>-1</sup> )
<b>BM1-1</b>	CH <sub>2</sub> CH <sub>3</sub>	H	H	OCH <sub>3</sub>	H	H	313	20736
<b>BM1-2</b>	CH <sub>2</sub> CH(CH <sub>2</sub> CH <sub>3</sub> )C <sub>4</sub> H <sub>9</sub>	H	H	OCH <sub>3</sub>	H	H	313	17698
<b>BM1-3</b>	(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub>	H	H	OCH <sub>3</sub>	H	H	313	18489
<b>BM8-1</b>	CH <sub>2</sub> CH <sub>3</sub>	OCH <sub>3</sub>	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	292 359	10606 14786
<b>BM8-2</b>	CH <sub>2</sub> CH(CH <sub>2</sub> CH <sub>3</sub> )C <sub>4</sub> H <sub>9</sub>	OCH <sub>3</sub>	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	292 359	11185 13843
<b>BM8-3</b>	(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub>	OCH <sub>3</sub>	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	292 359	10820 12896

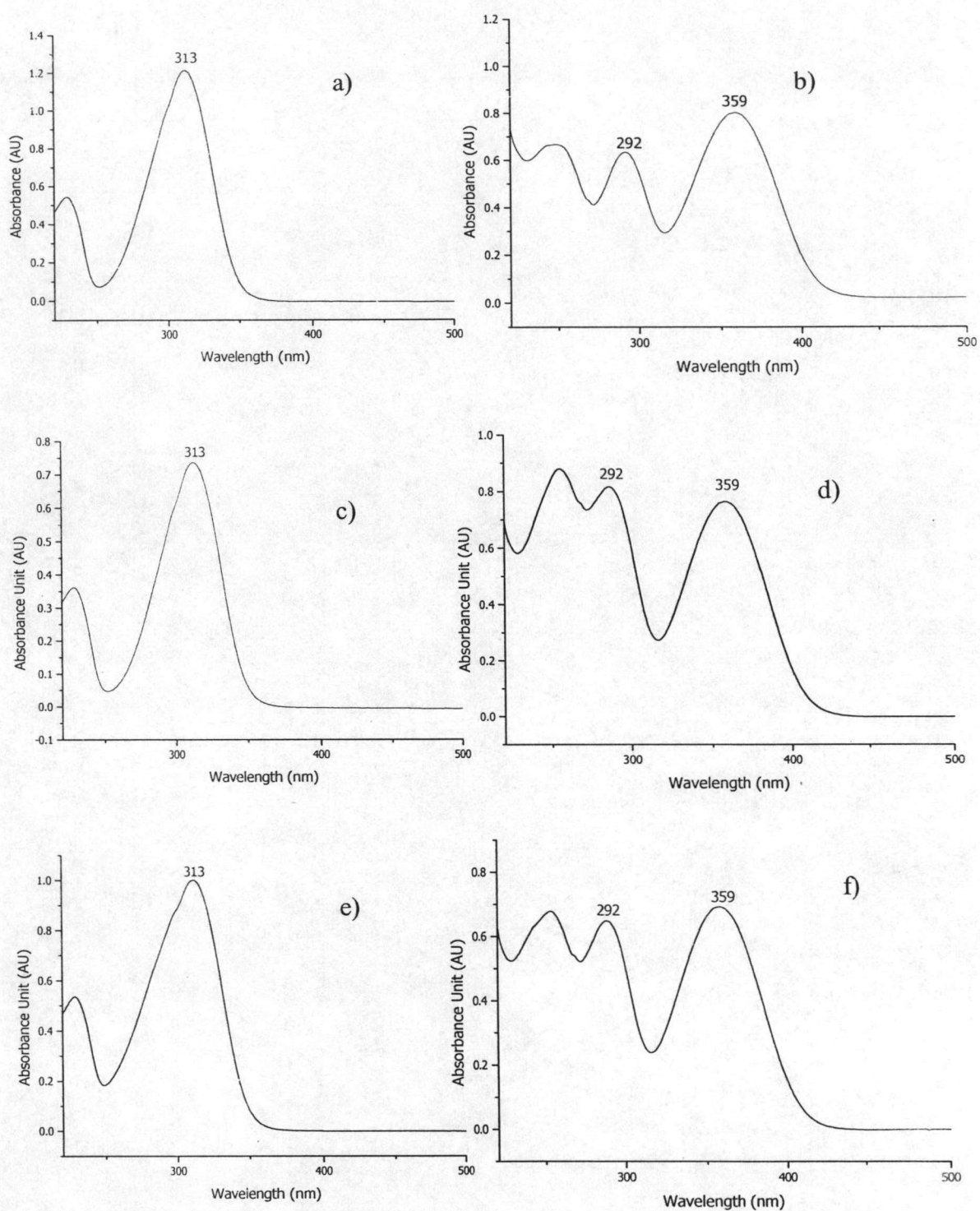


Figure 3.3 UV spectrum of a) **BM1-1**, b) **BM8-1**, c) **BM1-2**, d) **BM8-2**, e) **BM1-3** and f) **BM 8-3**



As can be seen in table 3.3, the chain length and branch of alkyl group affect neither the absorption wavelengths nor the  $\epsilon$  value. This is because alkyl chain is not a chromophore part of the molecule.

### 3.4 Photostability Test

Nine synthesized UV-filters, **E1**, **E8**, **E10**, **BM1-1**, **BM1-2**, **BM1-3**, **BM8-1**, **BM8-2** and **BM8-3**, were subjected to photostability test. The tests were done in methanol (results shown in fig 3.4) and hexane (result shown in fig 3.5) to compare the photostability of the test compounds in mediums of different polarity.

As can be seen from fig 3.4 and fig 3.5, all the diester-compounds (**BM1-1**, **BM1-2**, **BM1-3**, **BM8-1**, **BM8-2** and **BM8-3**) are photostable. The UVB absorption efficiency of the monoester compounds (**E1**, **E8** and **E10**) decreased as the compounds were exposed to UV-light. This decrease in UVB absorption was more pronounced in methanol than that in hexanes. The results agree with the previous studies<sup>11</sup> which have shown that the lost in UV absorption efficiency was due to trans to cis isomerization. This explains very well the photostability of all the diester compounds. We, therefore, have achieved the goal of solving the photounstable problem of cinnamate derivatives due to photoisomerization.

When comparing the monoester compounds, i.e., **E1**, **E8** and **E10**, it can be seen to that **E10** is the least photostable compound both in hexanes and in methanol. This may result from the di-ortho substitution on the benzene ring. As depicted in fig 3.6 in the ground state configuration, the di-ortho structure causes interaction between methoxy group and the hydrogen on the double bond next to benzene ring. As a result, the structure of the 90° twisted excited state is favorable. This should also result in this is true with the higher value of  $\epsilon$  of **E10** more formation of excited molecule (see table 3.2). This excited state structure, therefore, gives higher probability of cis-trans isomerization.

In opposite manner, **E8** is the most photostable among the three monoester compounds. Explanation on this is depicted in fig 3.7. Since there is a methoxy group on only one side, therefore, in order to minimize the interaction between hydrogen on the double bond next to the benzene ring and that ortho methoxy group, the excited state structures would not have the hydrogen fully perpendicular to the original plane. This

result is smaller probability of cis-trans isomerization. Moreover, such interaction causes less formation of excited molecule and this is confirmed by the lower value of  $\epsilon$  for **E8**.

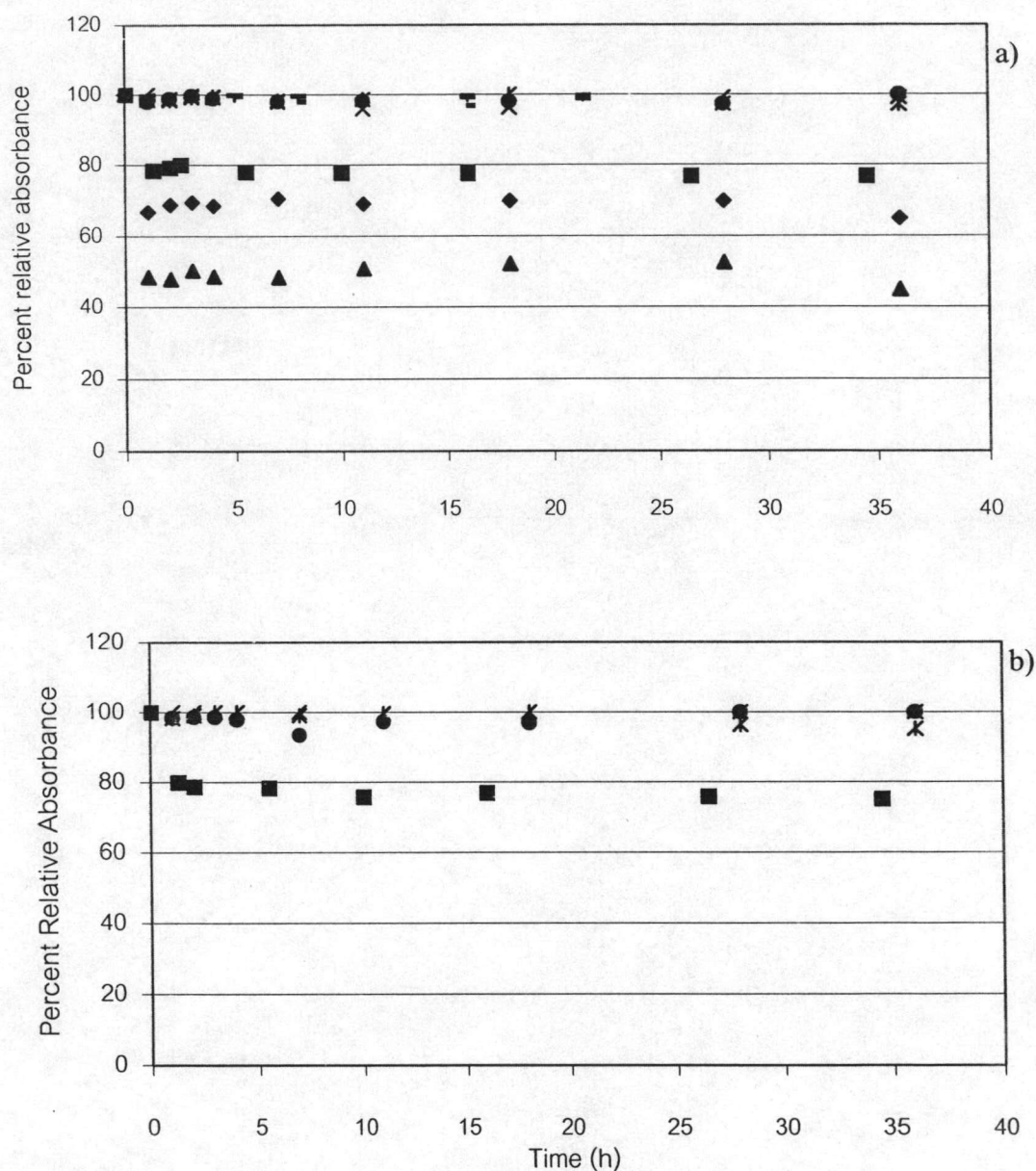


Figure 3.4 Photo-equilibrium of synthesized UV-filters in methanol; a) in UVB region and b) in UVA region;  $\blacklozenge$  E1,  $\blacksquare$  E8,  $\blacktriangle$  E10,  $\times$  BM8-1,  $*$  BM8-2,  $\bullet$  BM8-3,  $+$  BM1-1,  $-$  BM1-2 and  $-$  BM1-3

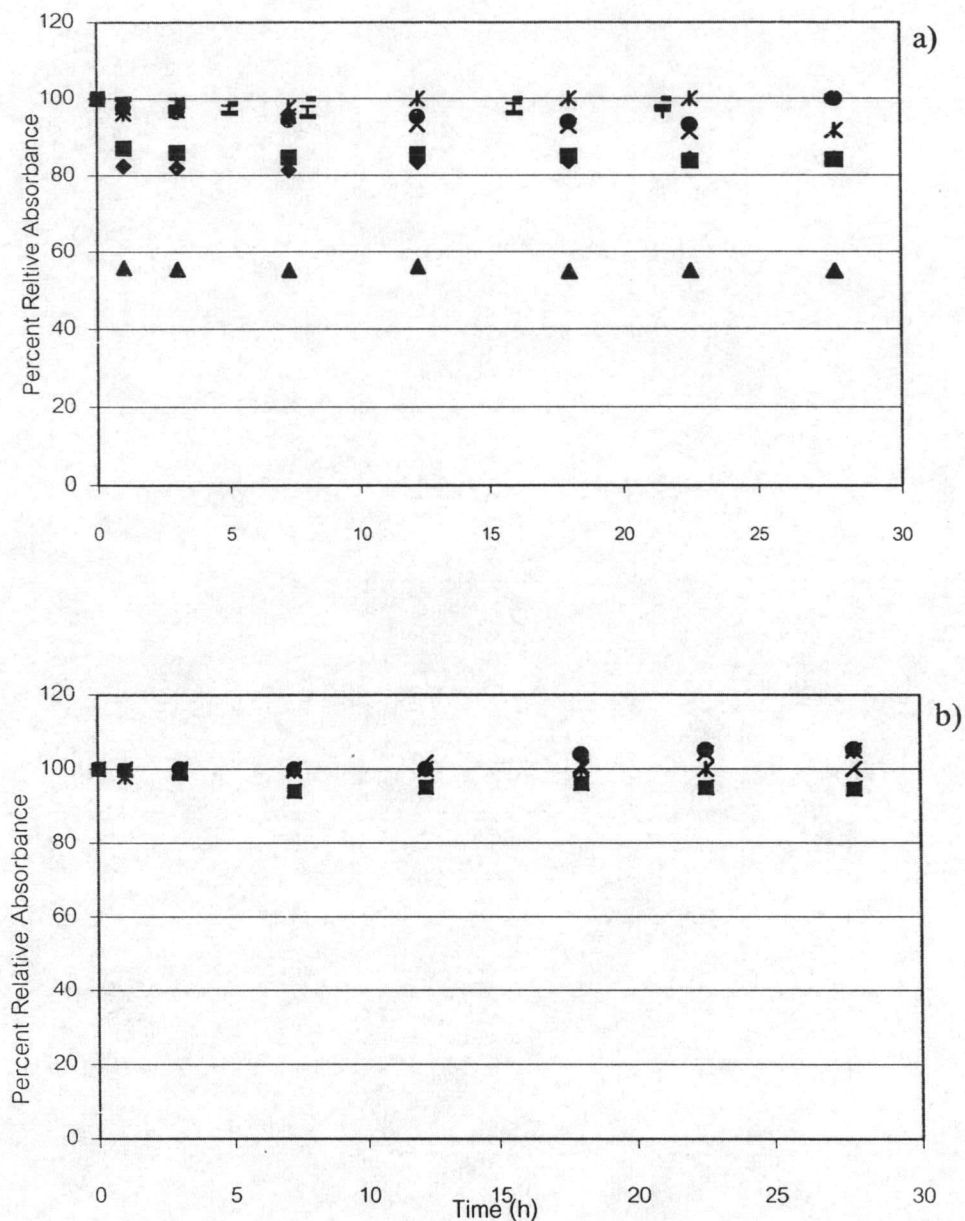


Figure 3.5 Photo-equilibrium of synthesized UV-filters in hexanes a) UVB region and b) UVA region;  $\blacklozenge$  E1,  $\blacksquare$  E8,  $\blacktriangle$  E10,  $\times$  BM8-1,  $*$  BM8-2,  $\bullet$  BM8-3,  $+$  BM1-1,  $-$  BM1-2 and  $-$  BM1-3

Solvents also affect the photostability of UV-filters as can be seen in fig 3.4 and 3.5. This agrees with the previous results given by Louise E.<sup>11</sup> The photostability of UV-filters increased when they were in the less polar solvent. This can be explained

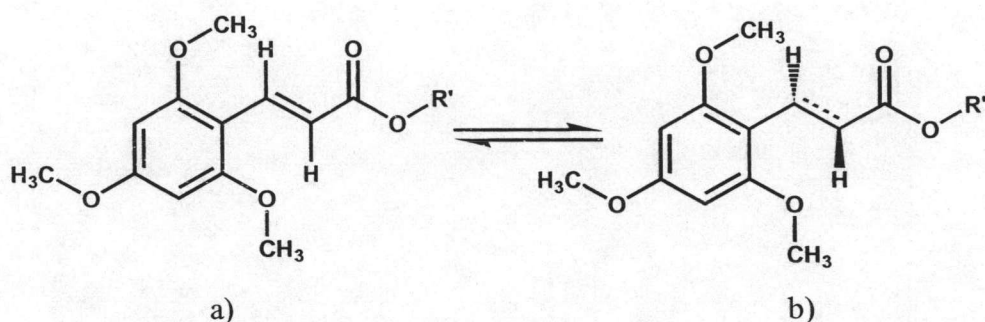


Figure 3.6 Proposed structures of (a) ground state and (b) excited state of **E10**

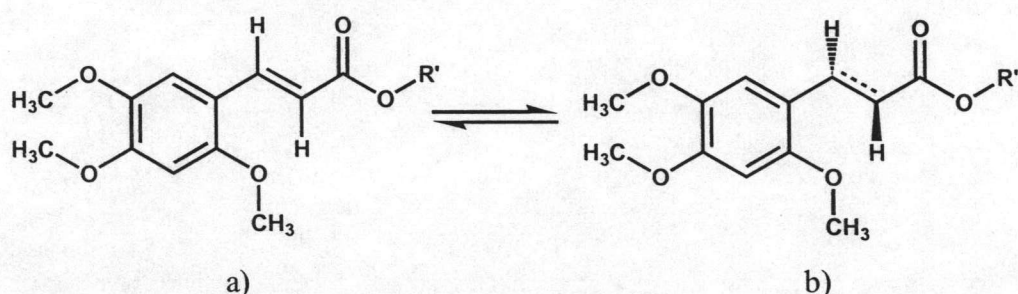


Figure 3.7 Proposed structures of (a) ground state and (b) excited state of **E8**

from the fact that *cis*-configuration of cinnamate esters are more polar than *trans*-configuration, therefore, *cis*-configuration is stabilized by more polar solvent such as methanol. On the other hand, less polar solvent such as hexanes can stabilize the *trans*-cinnamate ester better than the *cis*-configuration.

### 3.5 Irritation Test

Patch and Photopatch test<sup>a</sup> were selected as irritation tests for the synthetic UV-filters. In this study, four synthetic UV-filters were picked for testing. OMC was used as a standard UV-filter while pure white petrolatum was used as a control.

Table 3.4 summarizes the results of patch testing (PT) and photopatch testing (PTT) on ten volunteers.

<sup>a</sup> Patch Test (PT) is irritation testing at non-UV irradiation. Photopatch Test (PPT) is irritation testing at UV-irradiation.

Table 3.4 Results of patch test (PT) and photopatch test (PPT) of synthesized UV-filters on ten volunteers

Volunteers /Substance	- negative (non-allergic)				+ positive (allergic) <sup>a</sup>																	
	I		II		III		IV		V		VI		VII		VIII		IX		X			
	PT	PPT	PT	PPT	PT	PPT	PT	PPT	PT	PPT	PT	PPT	PT	PPT	PT	PPT	PT	PPT	PT	PPT		
OMC (standard)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+ <sup>b</sup>	-
Pure White	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Petrolatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>E2</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>BM8-1</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>BM8-2</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+ <sup>a</sup>	-
<b>BM8-3</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

<sup>a</sup> Level of allergic is divided into three levels; +1, +2 and +3 (+- is less than +1)

<sup>b</sup> Positive reaction (+-) shown after 48 hours applying

On patch testing, only one volunteer showed weak positive reaction to 2-ethylhexyl-*p*-methoxycinnamate (**E1**, OMC) and di-(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate (**BM8-2**) after 48 hours applying. However, nobody showed an erythema to these compounds, therefore, this result encourages further development of these compounds as UV-filters.

### 3.6 Spectroscopic Data of all synthesized compounds

The structures of all synthesized compounds were well characterized using various spectroscopic techniques including  $^1\text{H}$ ,  $^{13}\text{C}$ -NMR, IR and MS. Spectroscopic spectra of all compounds are shown in Appendix B.

#### 3.6.1 Infrared spectroscopy

All of *trans*-cinnamic acids display the characteristic of common functional groups in IR spectra. O-H stretching vibrations of acids are presented around 3623-3335  $\text{cm}^{-1}$  (br). The absorption band at 1693-1685  $\text{cm}^{-1}$  corresponds to the C=O stretching vibration of the acid. The C=C (next to aromatic ring) stretching vibration at 1623-1611  $\text{cm}^{-1}$  and C=C ring stretching at 1600-1444  $\text{cm}^{-1}$  are also detected. In the case of *trans*-cinnamate esters and dialkyl benzalmalonate, the C=O stretching vibrations are shown at 1743-1700  $\text{cm}^{-1}$ . The C-O stretching vibrations were detected at 1293-1254  $\text{cm}^{-1}$  and 1200-1172  $\text{cm}^{-1}$  for cinnamate esters and dialkyl benzalmalonate respectively.

#### 3.6.2 NMR spectroscopy

##### $^1\text{H}$ -NMR

For NMR spectroscopy,  $\text{CDCl}_3$  and  $\text{DMSO-}d_6$  were used as solvents for most substituted *trans*-cinnamic acids. The  $^1\text{H}$ -NMR spectra of *trans*-cinnamic acids generally show two double signals with each of 1H integration at 7.81-8.08 and 6.32-6.45 ppm ( $J=16.00$  Hz) which correspond to Ar-CH=CH-COOH (H-a and H-b). The coupling constant absolutely indicates *trans*-geometry in the molecule. Signals that were detected at 6.27-7.30 ppm and 3.35-3.92 ppm can be assigned for aromatic and methoxy protons respectively.

Similarly, the  $^1\text{H}$ -NMR spectra of *trans*-cinnamate esters, display almost the same spectra as those of *trans*-cinnamic acids, i.e., two double signals with each of 1H

integration at 8.11-7.56 and 6.75-6.25 ppm ( $J=16.30-15.97$  Hz) which correspond to Ar-CH=CH-COOR (H-a and H-b). The coupling constant also confirms the *trans*-geometry in the molecules. In the case of dialkyl benzalmalonate, the  $^1\text{H-NMR}$  spectra generally showed singlet signals at 8.05-7.63 ppm which correspond to Ar-CH=C(COOR)<sub>2</sub> (H-a).

### $^{13}\text{C}$ NMR

The  $^{13}\text{C-NMR}$  spectra of substituted *trans*-cinnamic acids generally display a signal belonging to carboxylic acid around 167.82-176.45 ppm. The signals of olefinic carbon (C-a and C-b) were detected in the range of 134.74-143.72 and 114.65-117.42 ppm respectively. The  $^{13}\text{C-NMR}$  spectra of cinnamate esters and dialkyl benzalmalonate esters generally exhibit signals of carbon connected to an oxygen atom (58.2-55.4 ppm) and alkyl carbon (80.7-40.3 ppm).

### 3.6.3 Mass Spectroscopy

The molecular weight of nine synthesized compounds were confirmed by MS data (see Appendix B). Table 3.6 summarizes molecular peak shown in mass spectrum of each compound.

Table 3.6 M/Z of nine synthetic compounds

Cpds	m/z
<b>C8</b>	238.00
<b>C10</b>	238.00
<b>E1</b>	290.00
<b>E8</b>	350.00
<b>E10</b>	350.00
<b>BM1-1</b>	278.99
<b>BM8-1</b>	338.00
<b>BM8-2</b>	506.43
<b>BM8-3</b>	450.00