CHAPTER II

THEORY AND EXPERIMENTAL METHODS

2.1 Optical Spectroscopy

2.1.1 Steady-state measurement

The experimental technique used for photophysical study of the cinnamates in this work is optical spectroscopy. Both absorption and fluorescence measurement have been used in this project.

The initiating activation of photoreaction is provided by the absorption of light, in the UV, visible and near IR. An electron from an initially occupied state is excited to an unoccupied higher energy state. There are two excited electronic states derived from the electronic orbital configuration produced by light absorption. In one state, the two electron spins are paired and this is termed the singlet state. In the other state the two electron spins are unpaired and it is termed the triplet state. Consequently, returning to the ground state from the singlet excited state is spin-allowed and rapid with an emission of a photon whereas a returning from the triplet excited state is spin forbidden and slow. The processes that occur between the absorption and emission of light are illustrated by a Jablonski diagram (Figure 2.1).[33]

The ground, first, and second singlet excited electronic states are denoted S_0, S_1 and S_2, respectively. In each of these electronic states the fluorophores can exist in a number of vibrational energy levels, donated by 0, 1, 2, etc. The transitions between states are depicted as vertical lines to illustrate the instantaneous nature of light absorption. Transition occurs in less than 10^{-15} s, a time too short for significant displacement of nuclei. This is called the Frank-Condon principle.

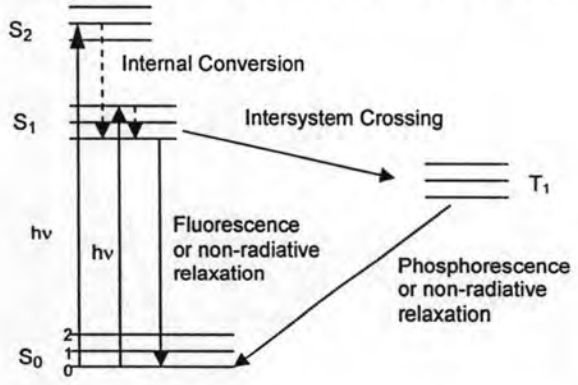


Figure 2.1 Jablonski energy diagram.

Molecules in the S_1 state can also undergo a spin conversion to the first triplet state, T_1. Emission from T_1 is a process termed phosphorescence and is generally shifted to longer wavelengths (lower energy) relative to fluorescence. Conversion of S_1 to T_1 is called intersystem crossing (ISC).

The fluorescence lifetime (τ) and quantum yield (ϕ_f) are perhaps the most important characteristics of a fluorophore. The meaning of the quantum yield and lifetime is best represented by a simplified Jablonski diagram, The fluorescence quantum yield is the ratio of the number of photon emitted to the number absorbed. The processes are governed by the fluorescence rate constants, k_f, and the non-radiative rate constant, k_{nr}, e.g. internal conversion (IC) and ISC. The fraction of fluorophores which decay through emission determines the fluorescence quantum yield, and is given by equation 2.1.

$$\phi_f = k_f / (k_f + k_{nr}) \qquad (2.1)$$

The lifetime of the excited state is defined by the average time the molecule spends in the excited state prior to return to the ground state. The fluorescence lifetime is defined by equation 2.2.

$$\tau = 1 / (k_f + k_{nr}) \qquad (2.2)$$

2.1.2 Time-Resolved Fluorescence

For time-resolved fluorescence measurements, a sample containing the fluorophore is excited with a very short pulse of light. The result is an initial population (n_0) of fluorophores in the excited state. The excited state population decays with a rate k_f+k_{nr}, given by equation 2.3.

$$dn(t)/dt = -(k_f + k_{nr})\, n\,(t) \qquad (2.3)$$

where n(t) is the number of excited molecules at time *t* following excitation, k_f is the fluorescence rate constant, and k_{nr} is the non-radiative decay rate.

Emission is a random event, and each excited fluorophore has the same probability of emitting in a given period of time. This results in an exponential decay of the excited state population (equation 2.4).

$$n(t) = n_0 \exp(-t/\tau) \qquad (2.4)$$

In general, the inverse of the lifetime is the sum of the rates which depopulate the excited state.

In this work, the technique used for time-resolved fluorescence measurement is called Time Correlated Single Photon Counting (TCSPC).[34] A short laser pulse initially excites the sample and triggers a build-up of a voltage ramp that is increasing with time and stopped when the first emitted photon from the sample is detected and its magnitude is correlated to a certain time. Therefore, repeating the procedure thousands of times, a histogram of emitted photons as a function of the time can be attained. The more counted photons, the more accurate the results.

2.1.3 Solvent Effects

Upon excitation, the fluorophore is assumed to be initially in the Franck-Condon state. Solvent relaxation proceeds with a rate k_s. If this rate is much slower than the decay rate, then one expects to observe the emission spectrum of the unrelaxed state. If solvent relaxation is much faster than the emission rate, then emission from the relaxed state will be observed. This is illustrated in Figure 2.2.

Typically, the fluorophore has a larger dipole moment in the excited state (μ_e) than in the ground state (μ_g). Following excitation, the solvent dipoles can reorient or relax around μ_e which lowers the energy of excited state. Therefore, as the solvent polarity is increased, the emission from a fluorophore is typically at lower energies or longer wavelengths. In other word, the interactions between the solvent and fluorophore affect the energy difference between the ground state and the excited state. This solvent polarity induced shift can be used to calculate the dipole moment of the excited state using the Lippert-Mataga equation (equation 2.5). Approximately this energy difference is a function of the refractive index (n) and dielectric constant (ε) of the solvent and is described by equation 2.5.

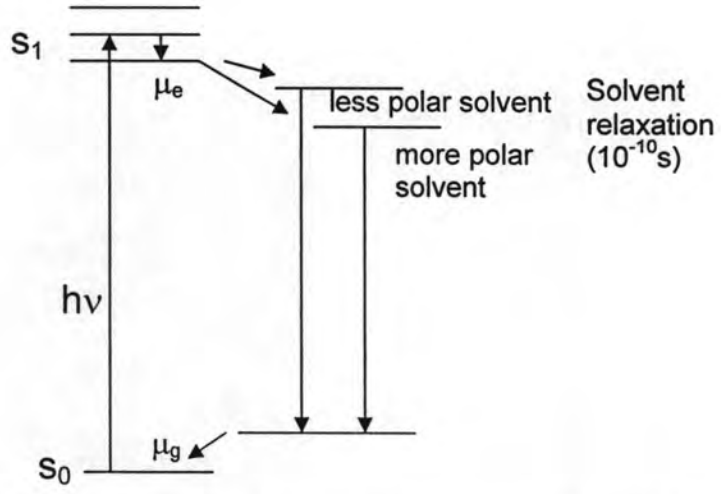


Figure 2.2 Jablonski diagram for fluorescence with solvent relaxation.

$$hc\tilde{\nu}_f = \frac{hc\tilde{\nu}_f^{vac} - 2\mu_e(\mu_e-\mu_g)\cdot\Delta f}{a_o^3} \qquad (2.5)$$

where $\tilde{\nu}_f$ and $\tilde{\nu}_f^{vac}$ are the spectral position of the solvent equilibrated fluorescence maxima and the value extrapolated to gas phase conditions, respectively; μ_e and μ_g are the dipole moments of the ground and excited states, and Δf is the Lippert solvent polarity parameter which describes the bulk solvent polarity by equation (2.6).

$$\Delta f = \frac{\varepsilon-1}{2\varepsilon+1} - \frac{1}{2}\frac{n^2-1}{2n^2+1} \qquad (2.6)$$

2.2. Experimental Methods

2.2.1 Absorption Spectroscopy

The absorption spectra measured in this project have been made at 200-500 nm on a Cary 4B UV-Vis spectrophotometer (Varian, Palo Alto, US). A new baseline has been recorded every day and the sample cell used was a 10 mm quartz cuvette.

Molar extinction coefficients (ε) were calculated by accurately weighing the sample and dissolving with solvent in a measuring flask of appropriate size. Serial dilutions were carried out to produce an optically dilute sample on which the absorption spectrum was recorded. The absorption coefficient was then calculated from Beer-Lambert law (equation 2.7).[35]

$$A = \varepsilon c l \qquad (2.7)$$

where A is the absorbance at a given wavelength, ε is the molar absorption coefficient in $M^{-1}cm^{-1}$, c is the molar concentration and l is the optical path length of the cell in cm.

2.2.2 Steady-state Fluorescence

The emission and excitation spectra have been measured on a Spex Fluorolog 3 (HORIBA Jobin Yvon Inc, Edison, US) equipped with a Xenon lamp. Spectra have been recorded with excitation wavelengths chosen to the maximum peak in the absorption spectrum and the spectral bandwidth for the emission has been varied according to the optical density of these peaks in order to get a good signal-to-noise ratio. The absorbance of the sample was less than 0.1 at the excitation wavelength in order to prevent inner filter effects.

Determination of the fluorescence quantum yield is accomplished by comparison of wavelength integrated intensity of the sample to that of the reference which in this work was quinine sulfate. The quantum yield of the sample is calculated using equation 8.[33]

$$\phi_S = \phi_R \frac{I_S \, OD_R \, n^2_S}{I_R \, ODs \, n^2_R} \qquad (2.8)$$

Where ϕ_R represents the fluorescence quantum yield of the reference, I is the integrated area under the respective fluorescence spectra, OD is the respective absorbances and n is the refractive indexes of the solvents used. The subscripts S and R refer to the sample and reference, respectively.

2.2.3 Time-Resolved Fluorescence

The experimental arrangement used for the time-resolved fluorescence measurements is schematically illustrated in Figure 2.3. The excitation pulse was provided by a Tsunami Ti:Sapphire laser (Spectra-Physics) which was pumped by a millennia Pro X laser (Spectra-Physics). To get the desired excitation wavelength the mode-locked laser pulses has been used from the Tsunami. The fundamental wavelength used (870 nm) was frequency tripled to 290 nm. The detector was a water

cooled micro-channel plate photo multiplier tube (MCP) from Hamamatsu (R3809U-50). The signal was digitalized using a multi-channel analyzer with 4096 channels (Edinburgh Analytical Instruments) and more than 10 000 counts were recorded in the top channel for each decay, in order to get good statistics. The decay was fitted to exponential expressions using the computer program FluoFit Pro version 4.

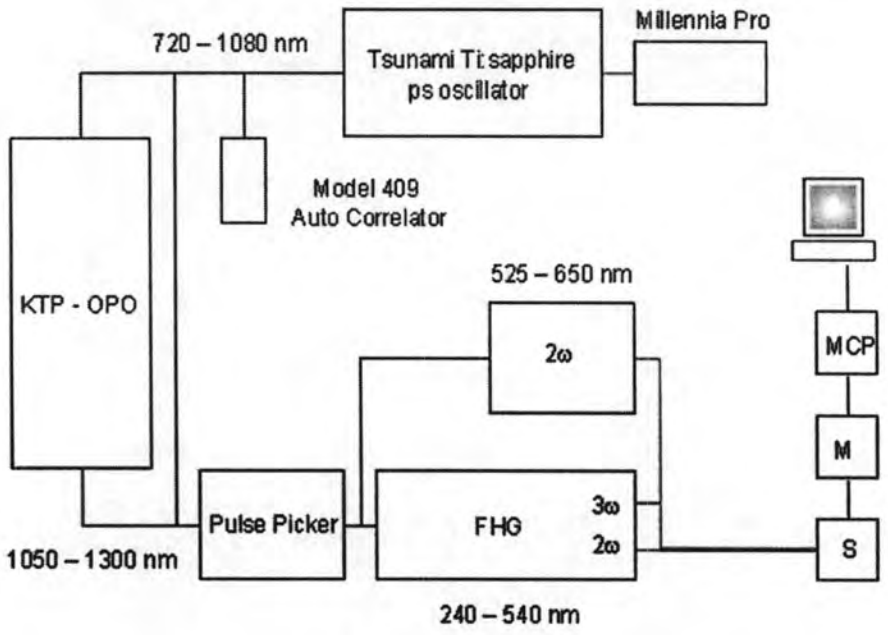


Figure 2.3 Set-up for Time Correlated Photon Counting (TCSPC); S= sample, M= monochromator, MCP = micro channel photomultiplier.

2.2.4 Temperature Studies

For the temperature dependent absorption and emission studies, a liquid nitrogen cooled cryostat (Oxford Instruments) was used. Any dissolved oxygen was removed by repeated freeze pump thaw cycles prior to measurements to prevent any unwanted quenching reactions by oxygen.

2.2.5 Theoretical methods

Quantum mechanical calculations were performed by the semi-empirical methods PM3 (ground state optimization) and INDO/S (excited state energies and transition moments) as implemented in the Hyperchem 5.1 program.

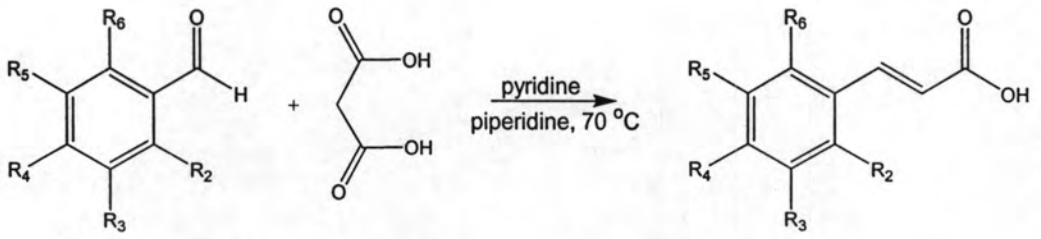
2.3. Materials

Cinnamate derivatives were synthesized by procedures below. All benzaldehydes, pyridine, 2-ethylhexanol, 1-hexanol and malonic acid were purchased from Fluka Chemical Company (Buchs, Switzerland). Piperidine was purchased from Sigma (Sigma Chemical Co., Steinheirg, Germany). Standard OMC was a kind gift from Merck Co. Ltd. (Bangkok, Thailand). Column chromatography was performed in silica gel (Merck Kieselgel 60 G) (Merck KgaA, Darmstadt, Germany). Melting points were determined with an Electrothermal 9100 melting point apparatus (American Instrument Exchange, Inc., MA, USA). The ^{1}H- and ^{13}C-MNR spectra were obtained in deuterated chloroform ($CDCl_3$) on a Varian Mercury NMR spectrometer which operated at 400.00 MHz for ^{1}H and 100.00 MHz for ^{13}C nuclei (Varian Company, CA, USA). For UV irradiation during the photostability experiments, broadband UVA (320-400 nm) was generated by an F24T12/BL/HO (PUVA) lamp (National Biological Corporation, Twinsburg, Ohio, USA) and broadband UVB (280-320 nm) was generated by an FSX24T12/UVB/HO lamp (National Biological Corporation, Twinsburg, Ohio, USA). UV Irradiance was measured using UVA-400C and UVB-500C power meters (National Biological Corporation, Twinsburg, Ohio, USA). Solvents used for spectroscopic measurements were spectroscopic grade. Methanol, tetrahydrofuran (THF) and iso-propanol were purchased from Labscan (Dublin, Ireland). Hexane was perchased from Baker chemicals (Devernter, Holland), acetonitrile from Merck (Damstad,Germany). Dichloromethan (DCM) was perchased from Fluka (Germany). Ethanol was perchased from Salveco chemical AB, (Sweden), diethyl ether (Riedel-Detlaen, Germany) and dimethyl sulfoxide (DMSO) (Scharlau chemie SA, Barcelona, Spain).

2.4. Synthesis of Cinnamates

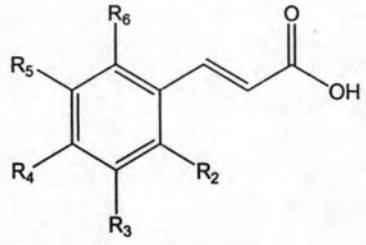
2.4.1 Synthesis of substituted-*trans*-cinnamic acids.[36]

The *trans*-cinnamic acids were synthesized using Knoevenagel-Doebner condensation between benzaldehyde and malonic acid as equation below (scheme 2.1).



Scheme 2.1 Synthetic pathway of substituted-*trans*-cinnamic acid

All cinnamic acid were purified by column chromatography and characterized by NMR and IR (see in appendix A). Their structures are shown in Figure 2.4.



No.	R_2	R_3	R_4	R_5	R_6
1A	OCH_3	H	H	H	H
2A	H	OCH_3	H	H	H
3A[a]	H	H	OCH_3	H	H
4A	OCH_3	OCH_3	H	H	H
5A	OCH_3	H	OCH_3	H	H
6A	OCH_3	H	H	OCH_3	H
7A	H	OCH_3	OCH_3	H	H
8A	OCH_3	OCH_3	OCH_3	H	H
9A[a]	OCH_3	H	OCH_3	OCH_3	H
10A[a]	OCH_3	H	OCH_3	H	OCH_3

[a]these three cinnamic acids have been synthesized in previous work.[22]

Figure 2.4 Structure and nomenclature of the synthesized substituted cinnamic acids.

2-methoxycinnamic acid (**1A**): White solid (98%), IR (KBr, cm^{-1}) 3007-2844 (CO**OH**), 2595, 1685, 1619, 1428, 1330 and 1245; ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.12-8.08 (d, *J*=15.6 Hz, 1H, Ar-C**H**=), 7.55-7.53 (d, *J*=7.8 Hz, 1H, Ar-**H**), 7.39, 7.38,

7.36 (t, 1H, Ar-**H**), 7.00, 6.98, 6.96 (t, 1H, Ar-**H**), 6.94, 6.92 (d, J=8.6 1H, Ar-**H**), 6.57-6.54 (d, *J*=16.4 Hz, 1H, =C**H**-COOH) and 3.90 (s, 3H, OC**H**$_3$); ^{13}C-NMR (DMSO-d_6) δ (ppm): 168.8 (-**C**OOH), 158.8 (Ar-**C**H=), 139.1, 132.2, 128.9, 122.4, 121.2, 119.7 (aromatic carbons), 112.2 (=**C**H-COOH) and 56.1 (O**C**H$_3$).

3-methoxycinnamic acid (**2A**): White solid (91%), IR (KBr, cm^{-1}) 2965-2833 (CO**OH**), 2564, 1685, 1630, 1424, 1311 and 1241. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.66-7.58 (d, *J*=16.1 Hz, 1H, Ar-C**H**=), 7.35, 7.29, 7.26 (t, 2H, Ar-**H**), 7.25-7.21 (d, J= 7.8 Hz, 2H, Ar-**H**), 6.97-6.87 (d, *J*=8.7 Hz, 2H, Ar-**H**), 6.58-6.52 (d, *J*=16.4 Hz, 1H, =C**H**-COOH) and 3.79 (s, 3H, OC**H**$_3$); ^{13}C-NMR (DMSO-d_6) δ (ppm): 168.1 (-**C**OOH), 160.0, 144.3, 130.4, 121.2, 120.0, 113.3 (aromatic carbons), 136.1 (Ar-**C**H=), 116.7 (=**C**H-COOH) and 55.6 (O**C**H$_3$).

4-methoxycinnamic acid (**3A**): White solid (54%), m.p. 171-173°C (lit.[27] 172-175 °C), IR (KBr, cm^{-1}) 2588-2287, 2935, 2542, 1685, 1623, 1600, 1510, 1444, 1312, 1254, 1219 and 1171; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.76-7.68 (d, *J*=16.1 Hz, 1H, Ar-C**H**=), 7.51-7.47 (d, *J*=8.9 Hz, 2H, Ar-**H**), 6.93-6.88 (d, *J*=8.7 Hz, 2H, Ar-**H**), 6.34-6.26 (d, *J*=15.8 Hz, 1H, =C**H**-COOH) and 3.79 (s, 3H, OC**H**$_3$); ^{13}C-NMR (DMSO-d_6) δ (ppm): 167.8 (-**C**OOH), 160.0, 129.9 (2×1C), 126.8, 144.3 (2×1C) (aromatic carbons), 143.7 (Ar-**C**H=), 116.4 (=**C**H-COOH) and 55.3 (O**C**H$_3$).

2,3-methoxycinnamic acid (**4A**): white solid (71%), IR (KBr, cm^{-1}) 2945-2829 (CO**OH**), 2583, 1684, 1625, 1482, 1435 and 1264. ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.13-8.09 (d, *J*=16.4 Hz, 1H, Ar-C**H**=), 7.19-7.17 (d, *J*=7.8 Hz, 1H, Ar-**H**), 7.10, 7.08, 7.06 (t, 1H, Ar-**H**), 6.98-6.96 (d, *J*=7.8 Hz, 1H, Ar-**H**), 6.53-6.49 (d, *J*=16.4 Hz, 1H, =C**H**-COOH) and 3.88 (s, 6H, 2xOC**H**$_3$); ^{13}C-NMR (DMSO-d_6) δ (ppm): 168.1 (-**C**OOH), 138.5 (Ar-**C**H=), 153.2, 148.0, 128.2, 124.8, 120.7, 119.3 (aromatic carbons), 115.0 (=**C**H-COOH) and 61.2, 56.2 (2xO**C**H$_3$).

2,4-methoxycinnamic acid (**5A**): white solid (85%), IR (KBr, cm^{-1}) 2995-2836 (CO**OH**, stretching), 1676, 1598, 1505, 1459, 1318 and 1209. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.78-7.73 (d, *J*=16.4 Hz, 1H, Ar-C**H**=), 7.60-7.58 (d, *J*=7.8 Hz, 1H, Ar-**H**), 6.68 (s, 1H, Ar-**H**), 6.58-6.56 (d, *J*=7.8 Hz, 1H, Ar-**H**), 6.38-6.33 (d, *J*=16.4 Hz, 1H, =C**H**-COOH) and 3.85, 3.50, (s, 2x3H, 2xOC**H**$_3$); ^{13}C-NMR (DMSO-d_6) δ (ppm): 168.7 (-**C**OOH), 139.2 (Ar-**C**H=), 162.2, 159.7, 139.2, 130.4, 116.7, 106.5, 98.8 (aromatic carbons), 115.8 (=**C**H-COOH) and 55.1, 55.9 (2xO**C**H$_3$).

2,5-methoxycinnamic acid (**6A**): yellow solid (88%), IR (KBr, cm^{-1}) 2961-2833 (CO**OH**), 2599, 1685, 1631, 1494, 1428 and 1265. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.74-7.70 (d, *J*=15.6 Hz, 1H, Ar-C**H**=), 7.14-7.12 (d, *J*=8.6 Hz, 1H, Ar-**H**), 7.07 (s, 1H, Ar-**H**), 6.33-6.29 (d, *J*=15.6 Hz, 1H, =C**H**-COOH) and 3.91 (s, 6H, 2xOC**H**$_3$); ^{13}C-NMR (DMSO-d_6) δ (ppm): 168.9 (-**C**OOH), 138.9 (Ar-**C**H=), 153.9, 153.0, 123.9, 120.0, 118.0., 113.4 (aromatic carbons), 113.0 (=**C**H-COOH) and 56.5, 56.0 (2xO**C**H$_3$).

3,4-methoxycinnamic acid (**7A**): white solid (97%), IR (KBr, cm^{-1}) 2961-2833 (CO**OH**), 2599, 1685, 1631, 1494, 1428 and 1265. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.74-7.70 (d, *J*=15.6 Hz, 1H, Ar-C**H**=), 7.14-7.12 (d, *J*=8.6 Hz, 1H, Ar-**H**), 7.07 (s, 1H, Ar-**H**), 6.33-6.29 (d, *J*=15.6 Hz, 1H, =C**H**-COOH) and 3.91 (s, 6H, 2xOC**H**$_3$); ^{13}C-NMR (DMSO-d_6) δ (ppm): 168.3 (-**C**OOH), 144.6 (Ar-**C**H=), 151.2, 149.2, 127.5, 123.1, 111.9., 110.7 (aromatic carbons), 117.1 (=**C**H-COOH) and 56.0, 55.9 (2xO**C**H$_3$).

2,3,4-trimethoxycinnamic acid (**8A**): White mirror-like needle crystal (55%), m.p. 168-170°C, IR (KBr, cm^{-1}) 3603-3343, 2974, 2939, 2827, 1693, 1619, 1584, 1495, 1460, 1266 and 1099; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.01-7.93 (d, *J*=16.11 Hz, 1H, Ar-C**H**=), 7.30-7.26 (d, *J*=8.85 Hz, 1H, Ar-**H**), 6.71-6.67 (d, *J*=8.80 Hz, 1H, Ar-**H**), 6.46-6.38 (d, *J*=16.06 Hz, 1H, =C**H**-COOH) and 3.90, 3.88, 3.86 (s, 9H, 3×OC**H**$_3$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 173.1 (-**C**OOH), 155.9, 153.5, 142.3, 123.7, 121.1, 107.6 (aromatic carbons), 141.8 (Ar-**C**H=), 116.1 (=**C**H-COOH), 61.5, 60.9 and 56.02 (3×O**C**H$_3$).

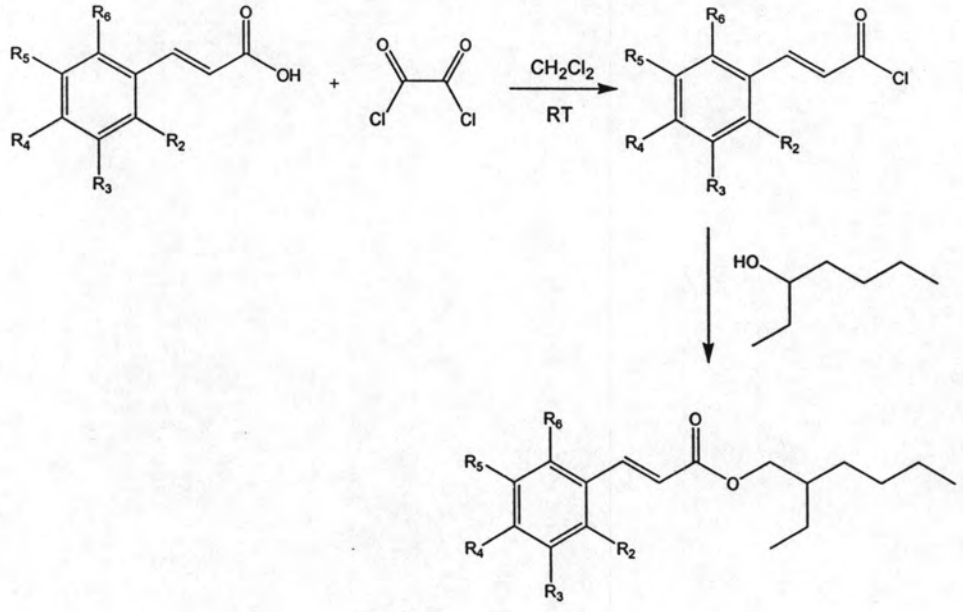
2,4,5-trimethoxycinnamic acid (**9A**): Yellow solid (88%), m.p. 165-167°C (lit.[28]164-166°C), IR (KBr, cm^{-1}) 3631-3335, 3009, 2935, 2823, 1685, 1600, 1514, 1464, 1433, 1401, 1299 and 1196; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.01-8.09 (d, *J*=16.0 Hz, 1H, Ar-C**H**=), 7.02, 6.85 (s, 2H, Ar-**H**), 6.41-6.32 (d, *J*=16.0 Hz, 1H, =C**H**-COOH), 3.92, 3.87 and 3.86 (s, 9H, 3×OC**H**$_3$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 173.3 (-**C**OOH), 154.2, 152.5, 143.2, 114.5, 110.9 and 96.67 (aromatic carbons), 141.9 (Ar-**C**H=), 114.6 (=**C**H-COOH), 56.4-56.1 (3×O**C**H$_3$).

2,4,6-trimethoxycinnamic acid (**10A**); Pale yellow solid (65%), m.p. 224-226°C, IR (KBr, cm^{-1}) 3623-3335, 3009, 2935, 2831, 1685, 1596, 1510, 1471, 1289, 1211 and 1025; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.89-7.81 (d, *J*=16.2 Hz, 1H, Ar-C**H**=), 6.58-6.50 (d, *J*=16.2 Hz, 1H, =C**H**-COOH), 6.27 (s, 2H, Ar-**H**), 3.43, 3.38 and

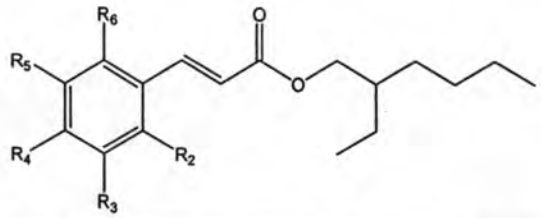
3.30 (s, 9H, 3×OC**H**$_3$); ^{13}C-NMR (DMSO-d_6) δ (ppm): 169.1 (-**C**OOH), 162.7, 160.7 (2×1C), 104.3, 90.82 (2×1C) (aromatic carbons), 134.74 (Ar-**C**H=), 117.43 (=**C**H-COOH), 65.4, 55.84 and 55.4 (3×O**C**H$_3$).

2.4.2 Synthesis of 2-ethylhexyl-*trans*-substituted cinnamates.

The *trans*-cinnamates were synthesized using esterification between *trans*-cinnamoyl chloride which prepared in situ from cinnamic acid and oxalylchloride react with 2-ethylhexanol (Scheme 2.2).[37] All the compounds were characterized by NMR and IR (see in appendix A). Their structures are shown in Figure 2.5.



Scheme 2.2 Synthetic pathway of the cinnamates



No.	R_2	R_3	R_4	R_5	R_6
1E	OCH_3	H	H	H	H
2E	H	OCH_3	H	H	H
3E	H	H	OCH_3	H	H
4E	OCH_3	OCH_3	H	H	H
5E	OCH_3	H	OCH_3	H	H
6E	OCH_3	H	H	OCH_3	H
7E	H	OCH_3	OCH_3	H	H
8E	OCH_3	OCH_3	OCH_3	H	H
9E	OCH_3	H	OCH_3	OCH_3	H
10E	OCH_3	H	OCH_3	H	OCH_3

Figure 2.5 Structure and nomenclature of the synthesized substituted cinnamates.

2-ethylhexyl-2-methoxycinnamate (**1E**), colorless oil (89%), ^{1}H-NMR ($CDCl_3$) δ (ppm) 7.94-7.90 (d, J=16.11 Hz, 1H, Ar-C**H**=), 7.45-7.43 (d, J =7.3 Hz, 1H, Ar-**H**), 7.29-7.25 (t, J=8.3, 8.3 Hz, 1H, Ar-**H**), 6.90-6.87 (t, 1H, J=7.3, 7.3 Hz Ar-**H**), 6.85-6.83 (d, J=7.8 Hz, 1H, Ar-**H**), 6.47-6.43 (d, J=16.1 Hz, 1H, , =C**H**-COOR), 4.05-4.04 (d, J=5.37 Hz, 2H, OC**H**$_2$), 3.81 (s, 3H, OC**H**$_3$), 1.57-0.83 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR; 167.78, 158.32, 139.98, 131.43, 128.88, 123.46, 120.69, 118.79, 111.12, 66.87, 55.47, 38.90, 30.52, 28.99, 23.89, 23.04, 14.11, 11.08.

2-ethylhexyl-3-methoxycinnamate (**2E**); colorless oil (72%), ^{1}H-NMR ($CDCl_3$) δ (ppm) 7.66-7.62 (d, J=15.6 Hz, 1H, Ar-C**H**=), 7.31-7.27 (t, J =7.6 Hz, 1H, Ar-**H**), 7.12-7.11 (d, J=7.2 Hz, 1H, Ar-**H**), 7.04 (s, 1H, Ar-**H**), 6.93-6.92 (d, J=7.6 Hz, 1H, Ar-**H**), 6.45-6.41 (d, J=15.6 Hz, 1H, =C**H**-COOR), 4.13-4.11 (dd, J=2.4 Hz, 2H, OC**H**$_2$), 3.82 (s, 3H, OC**H**$_3$), 1.65-0.89 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR; 167.12, 159.90, 144.46, 135.83, 129.84, 120.75, 118.57, 116.08, 112.89, 66.95, 55.20, 38.88, 30.48, 28.98, 23.85, 23.02, 14.08, 11.03.

2-ethylhexyl-4-methoxycinnamate (**3E**): colorless oil (78%), ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.64-7.56 (d, *J*=16.0 Hz, 1H, Ar-C**H**=), 7.48-7.44 (d, *J*= 8.6 Hz, 2H, Ar-**H**),

6.90-6.89 (d, *J*= 8.8 HZ, 2H, Ar-**H**), 6.33-6.25 (d, *J*=16.0 Hz, 1H, =C**H**-COOR$_1$), 3.85 (s, 3H, OC**H**$_3$), 4.10-4.07 (d, *J*=5.7 Hz, 2H, -OC**H**$_2$) and 1.63-0.85 (m, 15H, -C$_7$**H**$_{15}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 167.6 (-**C**OOR), 161.3, 161.1, 129.7 (2×1C) and 114.3 (2×1C) (aromatic carbons), 144.1 (Ar-**C**H=), 115.8 (=**C**H-COOR$_1$), 66.8 (-O**C**H$_3$), 55.4, 38.9, 30.5, 28.9, 23.9, 23.0, 14.1 and 11.0 (alkyl carbons). IR (neat, cm^{-1}) 2959, 2928, 2875, 1743, 1710, 1635, 1607, 1507, 1460, 1312, 1254 and 1167; Mw 290 (m/z).

2-ethylhexyl-2,3-dimethoxycinnamate (**4E**); colorless oil (46%), ^{1}H-NMR ($CDCl_3$) δ (ppm) 7.95-7.91 (d, J=15.6 Hz, 1H, Ar-C**H**=), 7.10-7.01 (d, J =7.8 Hz, 1H, Ar-**H**), 7.00-6.97 (t, J=7.81, 7.32 Hz, 1H, Ar-**H**), 6.87-6.39 (d, J=8.3 Hz, 1H, Ar-**H**), 6.43-6.39 (d, J=16.1 Hz, 1H, =C**H**-COOR), 4.06-4.04 (d, J=5.9 Hz, 2H, OC**H**$_2$), 3.80, 3.78 (s, 2x3H, OC**H**$_3$), 1.60-0.83 (m, 15H, -C$_7$**H**$_{15}$); ^{13}C-NMR; 167.42, 153.15, 148.40, 139.22, 128.69, 124.20, 119.59, 119.14, 113.86, 67.00, 65.29, 61.33, 55.87, 41.96, 38.88, 30.54, 28.98, 23.93, 23.02, 14.13, 11.09.

2-ethylhexyl-2,4-dimethoxycinnamate (**5E**); pale yellow oil (72%), ^{1}H-NMR ($CDCl_3$) δ (ppm) 7.85-7.81 (d, J=16.1 Hz, 1H, Ar-C**H**=), 7.38-7.36 (d, J =8.3 Hz, 1H, Ar-**H**), 6.44-6.41 (d, J=8.3 Hz, 1H, Ar-**H**), 6.38 (s, 1H, Ar-**H**), 6.38-6.33 (d, J=16.6 Hz, 1H, =C**H**-COOR), 4.03-4.02 (d, J=5.9 Hz, 2H, OC**H**$_2$), 3.79, 3.76 (s, 2x3H, OC**H**$_3$), 1.38-0.83 (m, 15H, -C$_7$**H**$_{15}$); ^{13}C-NMR; 168.16, 162.67, 159.80, 139.89, 116.60, 116.10, 105.20, 98.36, 66.67, 55.44, 55.42, 38.91, 30.50, 28.98, 23.87, 23.03, 14.09, 11.05.

2-ethylhexyl-2,5-dimethoxycinnamate (**6E**); colorless oil (33%), ^{1}H-NMR ($CDCl_3$) δ (ppm) 8.02-7.98 (d, J=16.8 Hz, 1H, Ar-C**H**=), 7.09-7.07 (d, J =7.2 Hz, 1H), 6.92-6.91 (d, J=2.4 Hz, 1H, Ar-**H**), 6.88 (s, 1H, Ar-**H**), 6.54-6.50 (d, J=16.4 Hz, 1H, =C**H**-COOR), 4.15-4.14 (d, J=5.2 Hz, 2H, OC**H**$_2$), 3.86, 3.81 (s, 2x3H, -OC**H**$_3$), 1.45-0.90 (m, 15H, -C$_7$**H**$_{15}$); ^{13}C-NMR; 167.47, 149.15, 144.46, 127.42, 122.62, 115.93, 110.95, 109.50, 66.81, 65.14, 55.89, 55.82, 38.87, 30.48, 28.96, 23.81, 22.99, 14.10, 14.07, 11.02.

2-ethylhexyl-3,4-dimethoxycinnamate (**7E**); colorless oil (79%), ^{1}H-NMR ($CDCl_3$) δ (ppm) 7.56-7.52 (d, J=15.6 Hz, 1H, Ar-C**H**=), 7.04-7.02 (d, J =8.3 Hz, 1H, Ar-**H**), 6.98 (s, 1H, Ar-**H**), 6.80-6.78 (d, J=8.3 Hz, 1H, Ar-**H**), 6.26-6.22 (d, J=16.1 Hz, 1H, =C**H**-COOR), 4.05-4.03 (dd, J=2.93, 3.42 Hz, 2H, OC**H**$_2$), 3.84, 3.83 (s, 2x3H, OC**H**$_3$), 1.59-0.82 (m, 15H, -C$_7$**H**$_{15}$); ^{13}C-NMR; 167.47, 149.15, 144.46, 127.42,

122.62, 115.93, 110.95, 109.50, 66.81, 65.14, 55.89, 38.87, 30.45, 28.96, 23.81, 22.99, 14.07, 11.02.

2-ethylhexyl-2,3,4-trimethoxycinnamate (**8E**); pale yellow oil (75%), ^{1}H-NMR ($CDCl_3$) δ (ppm) 8.21-7.78 (d, J=16.1 Hz, 1H, Ar-C**H**=), 7.21-7.19 (d, J =8.8 Hz, 1H, Ar-**H**), 6.22-6.60 (d, J=8.81H, Ar-**H**), 6.34-6.31 (d, J=16.1 Hz, 1H, =C**H**-COOR), 4.04-4.03 (d, J=2.9 Hz, 2H, OC**H**$_2$), 3.84, 3.81, 3.8 (s, 3x3H, OC**H**$_3$), 1.57-0.83 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR; 167.72, 155.45, 153.23, 142.33, 139.41, 123.06, 123.06, 121.52, 117.14, 107.59, 66.79, 61.40, 60.86, 56.01, 38.88, 30.52, 329.12, 23.90, 23.33, 14.06, 11.05

2-ethylhexyl-2,4,5-trimethoxycinnamate (**9E**): yellow oil (70%), ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.99-7.91 (d, *J*=16.1 Hz, 1H, Ar-C**H**=), 7.24, 6.48 (s, 2H), 6.38-6.30 (d, *J*=16.0 Hz, 1H, =C**H**-COOR), 4.10-4.07 (d, *J*= 5.8, 2H, -OC**H**$_2$), 3.95-3.82 (s, 9H, 3×-OC**H**$_3$) and 1.63-0.87 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 167.8 (-**C**OOR$_1$), 153.7, 151.9, 143.1, 114.9, 110.6 and 96.8 (aromatic carbons), 139.2 (Ar-**C**H=) and 115.7 (=**C**H-COOR$_1$), 56.3, 56.2 and 55.9 (3×-O**C**H$_3$), 66.6, 38.8, 30.4, 28.8, 23.8, 22.9, 13.9 and 10.9 (alkyl carbons). IR (neat, cm^{-1}) 2931, 1858, 2631, 1701, 1611, 1508, 1461, 1293 and 1161; Mw 350 (m/z)

2-ethylhexyl-2,4,6-trimethoxycinnamate (**10E**): pale yellow solid (65%), m.p. 64-65°C, ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.11-8.03 (d, *J*=16.2 Hz, 1H, Ar-C**H**=), 6.75-6.67 (d, *J*=16.2 Hz, 1H, =C**H**-COOR), 6.09 (s, 2H), 4.09-4.06 (d, *J*= 5.7, 2H), 3.85-3.80 (s, 9H, 3×-OC**H**$_3$), 1.63-0.86 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 180.5 (-**C**OOR$_1$), 154.8, 154.6, 109.8, 109.6, 85.8 (aromatic carbons), 136.9 (Ar-**C**H=) and 125.2 (=**C**H-COOR$_1$), 75.0 (2×1C), 74.7 (-O**C**H$_3$ carbons), 58.2, 49.83, 48.3, 43.2, 42.3, 33.4 and 30.4 (alkyl carbons). IR (KBr, cm^{-1}); 2951, 2924, 1685, 1603, 1561, 1460, 1266, 1207, 1153 and 1114; Mw 350 (m/z)

Cis-isomers of five selected cinnamates, **1E**, **2E**, **3E**, **9E** and **10E** were produced by irradiate *trans*-cinnamates by UV light and isolated by column chromatography using appropriate ration of hexane and ethylacetate as eluent. The ^{1}H, ^{13}NMR, IR and MS characterization of these cis-isomers show below

2-ethylhexyl-cis-2-methoxycinnamate (*cis*-**1E**), colorless oil, ^{1}H-NMR ($CDCl_3$) δ (ppm) 7.94-7.90 (d, J=16.11 Hz, 1H, Ar-C**H**=), 7.52-7.50 (d, J =7.8 Hz, 1H, Ar-**H**), 7.32, 7.30, 7.28 (t, 1H, Ar-**H**), 7.16-7.13 (d, *J*=12.5 Hz, 1H, Ar-C**H**=), 6.94, 6.92, 6.90 (t, 1H, Ar-**H**), 6.88-6.86 (d, J =7.8 Hz, 1H, Ar-**H**), 5.99-5.96 (d, J=12.5 Hz, 1H, =C**H**-COOR), 2.98-3.83 (dd, 2H, OC**H**$_2$), 3.83 (s, 3H, OC**H**$_3$), 1.25-0.80 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 159.0 (-**C**OOR$_1$), 140.5, 139.6, 131.5, 130.2, 121.2, 120.5, 111.5, 110.6 (Aromatic carbons), 66.6 (O**C**H$_3$), 55.8, 38.6, 30.3, 28.9, 23.6, 22.5, 14.3, 11.4 (alkyl carbons).

2-ethylhexyl-cis-3-methoxycinnamate (*cis*-**2E**); colorless oil, ^{1}H-NMR ($CDCl_3$) δ (ppm) 7.27-7.23 (m, Ar-**H**+$CHCl_3$), 7.21 (s, 1H, Ar-**H**), 7.10-7.08 (d, J=7.8 Hz, Ar-**H**), 6.93-6.90 (d, J =12.5 Hz, 1H, Ar-C**H**=), 6.88.-6.86 (d, J=6.2 Hz, 1H, Ar-**H**), 5.97-5.94 (d, J=12.5 Hz, 1H, =C**H**-COOR), 4.03-4.00 (dd, 2H, OC**H**$_2$), 3.81 (s, 3H, OC**H**$_3$), 1.29-0.82 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR; 166.8, 141.1, 136.9, 133.5, 129.2, 122.2, 120.3, 114.8, 114.6, 66.86, 55.21, 38.63, 30.31, 28.89, 24.1, 23.5, 14.0, 11.5.

2-ethylhexyl-cis-4-methoxycinnamate (*cis*-**3E**): colorless oil (78%), IR (neat, cm^{-1}) 2957, 2928, 2864, 1715, 1603, 1509, 1470, 1256 and 1163. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.76-7.73 (d, *J*=12.4 Hz, 1H, Ar-C**H**=), 6.94-6.92 (d, *J*= 8.6 Hz, 2H, 2xAr-**H**), 6.88-6.86 (d, *J*= 8.8 HZ, 2H, 2xAr-**H**), 5.93-5.88 (d, *J*=16.0 Hz, 1H, =C**H**-COOR$_1$), 4.15-4.09 (dd, 2H, -OC**H**$_2$) 3.84 (s, 3H, OC**H**$_3$), and 1.63-0.85 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 169.4 (-**C**OOR), 160.3, 127.5, 132.0 (2×1C) and 113.4 (2×1C) (aromatic carbons), 143.0 (Ar-**C**H=), 117.4 (=**C**H-COOR$_1$), 66.7 (-O**C**H$_3$), 55.3, 38.7, 38.7, 30.4, 28.9, 23.7, 23.0, 14.0 and 11.0 (alkyl carbons).

2-ethylhexyl-2,4,5-trimethoxycinnamate (*cis*-**9E**): yellow oil, ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.69 (s, 2H), 7.23-7.19 (d, *J*=12.5 Hz, 1H, Ar-C**H**=), 7.05 (s, 2H), 5.90-5.87 (d, *J*=12.48 Hz, 1H, =C**H**-COOR), 6.54-6.51 (d, 2H), 4.23-4.18 (dd, 2H, -OC**H**$_2$), 3.92-3.90 (s, 9H, 3×-OC**H**$_3$) and 1.39-0.82 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 166.9 (-**C**OOR$_1$), 152.8, 151.0, 142.3, 117.3, 115.6 and 96.4 (aromatic carbons), 139.0 (Ar-**C**H=) and 114.4 (=**C**H-COOR$_1$), 56.5, 56.4 and 56.0 (3×-O**C**H$_3$), 66.6, 38.7, 30.4, 28.9, 23.7, 22.9, 14.0 and 10.9 (alkyl carbons). IR (neat, cm^{-1}) 2945, 2922, 2860, 1712, 1606, 1509, 1462, 1318, 1213 and 1163; Mw 350 (m/z)

2-ethylhexyl-2,4,6-trimethoxycinnamate (*cis*-**10E**): pale yellow oil, IR (neat, cm^{-1}); 2949, 2864, 1715, 1602, 1459, 1411, 1334, 1272, 1206 and 1123; Mw 350

(m/z)^{1}H-NMR ($CDCl_3$) δ (ppm): 6.82-6.78 (d, *J*=12.2 Hz, 1H, Ar-C**H**=), 6.12 (s, 2H, Ar-H), 5.98-5.95 (d, *J*=12.2 Hz, 1H, =C**H**-COOR), 3.85-3.82 (dd, 2H, -OCH_2), 3.81, 3.78 (s, 9H, 3×-O**CH**$_3$), 1.25-0.78 (m, 15H, -C_7**H**$_{15}$); ^{13}C-NMR ($CDCl_3$) δ (ppm): 167.3 (-**C**OOR$_1$), 161.6, 132.9 (2x1C), 107.1, 90.3 (2×1C), 85.8 (aromatic carbons), 158.4 (Ar-**C**H=) and 121.5 (=**C**H-COOR$_1$), 55.5 (2x1C), 55.2 (-O**C**H$_3$ carbons), 66.3, 38.7, 30.4, 28.9, 23.6, 22.9, 14.0 and 11.0 (alkyl carbons).

2.5. Acute Oral Toxicity Test

The Forty Sprague Dawley (SD) rats (6 weeks old) of both sexes (National Laboratory Animal Centre (NLAC) Thailand) were kept in individual stainless steel cages for 1 week acclimatization. The animals were feed with commercial pellet diet (082 diet, NLAC) and tap water bottles as provided. The animals were maintained at 25 °C and 12:12 dark-light period. The animals were randomly divided into 5 groups of 5 animals of both sexes with a common ration of 1.0 as 50, 500, 5000 mg/kg body weight as well as a control group. 2-Ethylhexyl-2,4,5-trimethoxycinnamate (**9E**) was diluted to appropriated concentrations using the mineral oil while the solid **9B** was dispersed in Tween-20. The test compound was fed by oral administration and their clinical signs were observed at 1, 4, 24, 36, 48, 60, 72 and 84 hours after the administration.

On the first day before the administration and on the seventh day of the necropsy, blood was collected for hematology and clinical chemistry analyses. The measured hematological parameters included white blood cell count (WBC), red blood cell count (RBC), % hemoglobin (%Hb), % hematocrit (% Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin volume (MCH) and mean corpuscular hemoglobin concentration (MCHC) . The blood cells were manually count on hemocytometer (Life Science Dynamics of Arnaparn Co.Ltd, Bangkok, Thailand), the Hct value was measured by microhematocrit centrifuge method using microhematocrit centrifuge (Hettich Haematocrit, Germany) and the Hb value was measured by cyanomethemoglobin. Clinical blood chemistry parameters tested included blood urea nitrogen (BUN; diacetyl monoxime method (DAMO)), creatinine (Alkaline Pitrate – end point reaction method), alanine aminotransferese (ALT, SGPT; Reitman –Franekl method), aspartate aminotransferase (AST, SGOT; Reitman –Franekl method) and cholesterol (cholesterol oxidase; Chol-PAP method).

Spectroscopic works of all assays were done on spectrophotometer (Spectronic® 20 Genesys™ Spectronic Instrument, USA). The data were analyzed using Student's t-test and ANOVA test ($P<0.05$) by the SAS version 8.8 computer software.

The animals were sacrificed, the visceral organs were collected in 10% buffered formalin and subjected to histopathology evaluation. Liver, heart, lung, spleen and kidney samples were histologically processed and stained with hematoxylin and eosin and observed under a light microscope.