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2-Ethylhexyl-2,4,5-trimethoxycinnamate and di-(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate as novel UVA filters

Thitinun Monhaphol, Bo Albinsson and Supason Pattanaargson Wanichwecharungruang

Abstract

A series of 2-ethylhexylmethoxy substituted cinnamates and benzalmalonates have been synthesized and characterized. 2-Ethylhexyl-2,4,5-trimethoxycinnamate (**E8**) and di-(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate (**B8**) show UVA absorption with high molar absorption coefficients (12000–14000 $cm^{-1}\,M^{-1}$ at 350 nm). **E8** undergoes *trans* to *cis* photoisomerization under UVA exposure causing the decrease in UV absorption efficiency. **E8** is more photostable than butyl methoxydibenzoylmethane (BMDBM). For example, 41.64 $J\,cm^{-2}$ UVA irradiation produces 20 ± 2% and 25 ± 2% loss in UV absorption for **E8** and BMDBM, respectively. Similar irradiation produces no change in the UV absorption of **B8**. Both the oily liquid **E8** and the yellow solid **B8** can be dissolved in various organic solvents, ranging from methanol to hexane, various silicone fluids and 2-ethylhexyl-4-trimethoxycinnamate (EHMC, a widely used UVB filter). A liquid broadband filter comprising **B8** and EHMC shows excellent photostability in both UVB and UVA regions.

Introduction

The adverse health effects of long-term exposure to UV radiation, such as direct cellular damage and immunosuppression, have been well realized, leading to photoageing, mutations and photocarcinogenesis. UVB radiation (280–320 nm) catalyses the formation of cyclobutane dipyrimidine dimers (CPDs) via a direct excitation pathway, which results in gene mutations (Ziegler et al 1993). Because UVA radiation (320–400 nm) is not directly absorbed by DNA, it had been considered to produce less harm than UVB. However, there is evidence that this radiation is involved in immunosuppression (Poon et al 2005), photoageing (Krutmann 2000) and DNA damages (Agar et al 2004). Recently, data have indicated that in human skin, UVA produces not only the oxidative DNA damage through 8-oxo-7,8-dihydro-2′-deoxyguanosine, but the radiation also induces CPD formation, probably via a triplet energy transfer photosensitization mechanism (Courdavault et al 2004). Interestingly, UVA also produces thymine cyclobutane dimers, which are less efficiently repaired than other CPDs produced upon UVB radiation (Courdavault et al 2005).

As a result, sunscreens have become the primary means to minimize those possible damaging effects and other kinds of photosensitivity and phototoxicity on human skin (Vielhaber et al 2006). Among the many efficient and stable UVB filters on the market, esters of 4-methoxycinnamic acid are among the most popularly used compounds in various cosmetic formulations. The most widely used derivative in this group is 2-ethylhexyl-4-methoxycinnamate (EHMC), which possesses a high molar absorption coefficient (ε), approximately 22000–24000 $M^{-1}\,cm^{-1}$ (at 309 nm), and causes only a few allergic reactions in human skin (Schauder & Ippen 1997). In contrast to the organic UVB filters, the choice of organic UVA filters is quite limited. Popular UVA screening compounds include benzophenones and dibenzoylmethanes. However, benzophenones are broad-spectrum filters with solvent-dependent absorption bands, low absorbing potential in UVA region and high transdermal absorption (Jiang et al 1999; Sarveiya et al 2004). Dibenzoylmethanes have a disadvantage of photoinstability caused by their reactive chemical structures (Bonda &

Sensor Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

Thitinun Monhaphol, Supason Pattanaargson Wanichwecharungruang

Department of Chemical and Biological Engineering/Physical Chemistry, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden

Bo Albinsson

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Correspondence: S. Pattanaargson Wanichwecharungruang, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand. E-mail: psupason@chula.ac.th

Marinelli 1999; Wahlberg et al 1999). In addition, the compound is not compatible with EHMC (Sayre 2005).

Other organic UVA filters include terephthalydenedicamphor sulfonic acid (Séite et al 1998) (Mexoryl SX), 2,4-bis{[4-(2-ethyl-hexyloxy)-2-hydroxyl]-phenyl}-6-(4-methoxyphenyl)-(1,3,5)-triazin (Tinosorb S) (Luther et al 1996), a triazine derivative and bis[6-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)]-phenol (Tinosorb-M) (Luther et al 1996), a benzotriazole derivative.

Inorganic pigments such as titanium dioxide and zinc oxide have recently been widely used as broad-spectrum filters in sunscreen formulations. Photocatalytic properties and the dermal uptake properties of microfine titanium dioxide have been controversially discussed (Sayes et al 2006). Positive (Kertész et al 2005; Tan et al 1996) and negative (Gamer et al 2006) detections of TiO_2 in human and porcine viable skin layers have been demonstrated.

Combinations of organic and inorganic filters have recently been introduced, e.g. inorganic beads encapsulated with organic filters (Lapidot et al 2003) and inorganic particulates grafted with organic chromophores (Walenzyk et al 2005). As a result, there is still a need for photostable organic UVA filtering chromophore to be used in the development of these hybrid UV filters.

An efficient organic UVA filter should not only provide screening in the UVA region but also be compatible with various UVB filters. The compound should be non-toxic and photostable and its physico-chemical properties, such as solubility, melting point and boiling point, must ease the formulation of various products. In this paper we have described the syntheses of some UVA filters: 2-ethylhexyl-2,4,5-trimethoxycinnamate, di-(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate and some related derivatives. Studies of their UV absorption properties and photostability are also presented.

Materials and Methods

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). All ^{1}H- and ^{13}C-NMR 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). UV spectra were obtained with the aid of an HP 8453 UV/VIS spectrophotometer (Agilent Technologies, CA) using a 1-cm quartz cell.

Solvents used in syntheses and spectroscopic techniques were reagent or analytical grades purchased from Labscan (Bangkok, Thailand). Solvents used in column chromatography were distilled from commercial-grade solvents before use. 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 EHMC and butyl methoxydibenzoylmethane (BMDBM) were a kind gift from Merck Co. Ltd (Bangkok, Thailand).

Preparation of *trans*-substituted cinnamic acid

Syntheses were done according to the method of Koo et al (1944) and Allen & Spangler (1943). In short, malonic acid (0.02 mol) and substituted benzaldehyde (0.015 mol) were dissolved in 5 mL pyridine and 0.15 mL of piperidine was added. The reaction mixture was refluxed at 70°C for 5 h. After the mixture had been cooled, 5 mL of conc. HCl and 40 mL of cold water were added. The solid was separated by suction filtration, washed with cold water and recrystallized with ethanol.

Preparation of 2-ethylhexyl-*trans*-substituted cinnamate

Syntheses were done according to the method of Womack & McWhirter (1943). *trans*-Substituted cinnamic acid (0.01 mol) was dissolved in dichloromethane. Oxalylchloride (0.015 mol) was then added slowly under N_2. The reaction mixture was stirred at room temperature for 1.5 h. Residue oxalylchoride was removed by rotary evaporator before 2-ethylhexanol (0.01 mol) was added. The mixture was stirred overnight at room temperature. The residue obtained after solvent evaporation was washed with 10% $NaHCO_3$. Crude reaction was purified by silica gel column eluting with hexane–ethylacetate to give 2-ethylhexyl-*trans*-substituted cinnamate.

2-Ethylhexyl-2-methoxycinnamate (**E1**)
Colourless oil (89%), ^{1}H NMR ($CDCl_3$) δ 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 (**E2**)
Colourless oil (72%), ^{1}H NMR ($CDCl_3$) δ 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 (**E3**)
Colourless 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 (**E4**)
Colourless oil (46%), ^1H NMR ($CDCl_3$) δ 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$\mathbf{H}_2$), 3.80, 3.78 (s, 2 × 3H, OC$\mathbf{H}_3$), 1.60–0.83 (m, 15H, -$C_7\mathbf{H}_{15}$); ^{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-2,5-dimethoxycinnamate (**E5**)
Colourless oil (33%), ^1H NMR ($CDCl_3$) δ 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$\mathbf{H}_2$), 3.86, 3.81 (s, 2 × 3H, -OC$\mathbf{H}_3$), 1.45–0.90 (m, 15H, -$C_7\mathbf{H}_{15}$); ^{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 (**E6**)
Colourless oil (79%), ^1H NMR ($CDCl_3$) δ 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$\mathbf{H}_2$), 3.84, 3.83 (s, 2 × 3H, OC$\mathbf{H}_3$), 1.59–0.82 (m, 15H, -$C_7\mathbf{H}_{15}$); ^{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 (**E7**)
Pale yellow oil (75%), ^1H NMR ($CDCl_3$) δ 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$\mathbf{H}_2$), 3.84, 3.81, 3.8 (s, 3 × 3H, OC$\mathbf{H}_3$), 1.57–0.83 (m, 15H, -$C_7\mathbf{H}_{15}$); ^{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 (**E8**)
Yellow oil (70%), ^1H 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$\mathbf{H}_2$), 3.95–3.82 (s, 9H, 3 × -OC$\mathbf{H}_3$) and 1.63–0.87 (m, 15H, -$C_7\mathbf{H}_{15}$); ^{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 (**E9**)
Pale yellow solid (65%), m.p. 64–65°C, ^1H 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$\mathbf{H}_3$), 1.63–0.86 (m, 15H, -$C_7\mathbf{H}_{15}$); ^{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).

Preparation of dialkyl-2,4,5-trimethoxybenzalmalonate

Syntheses were done according to the method of Koo (1944) and Allen & Spangler (1943). In short, malonate ester (0.02 mol) was dissolved in 5 mL pyridine and 2,4,5-trimethoxy benzaldehyde (0.02 mol) and 0.15 mL piperidine were added. The mixture was refluxed for 4.5 h at 70–75°C. After the mixture had been cooled, the solution was then washed with 2 × 10 mL water, 2 × 10 mL 1M hydrochloric acid and 10 mL saturated sodium bicarbonate. The organic solution was dried with anhydrous sodium sulfate.

Di-(2-ethylhexyl)-2′,4′,5′-trimethoxybenzalmalonate (**B8**)
Pale yellow solid (64%), m.p. 42–43°C, ^1H NMR ($CDCl_3$) δ (ppm): 8.03 (s, 1H, Ar-C**H** =), 6.95 (s, 1H, Ar-**H**), 6.45 (s, 1H, Ar-**H**), 4.13–4.12 and 4.09–4.08 (m, 4H, 2 × -OC$\mathbf{H}_2$-), 3.89, 3.82 and 3.76 (s, 9H, 3 × -OC$\mathbf{H}_3$), 1.38–0.75 (m, 30H, 2 × -$C_7\mathbf{H}_{15}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 167.8, 164.7 (2 × -**C**OOR), 154.0, 152.4, 142.9, 123.4, 111.5 and 96.4 (aromatic carbons), 137.1 (Ar-**C**H =), 113.5 (= **C**(COOR$_1$)$_2$), 56.3, 56.2 and 55.9 (-O**C**H$_3$), 68.1, 67.4, 38.7, 38.5, 30.3, 30.2, 28.8, 28.7, 23.7, 23.5, 22.9, 22.8, 14.0, 13.9, 10.9 and 10.8 (alkyl carbons). IR (neat, cm^{-1}): 2950, 2862, 1715, 1602, 1512, 1461, 1250, 1211 and 1130; Mw 506.43 (m/z).

Diethyl-2′,4′,5′-trimethoxybenzalmalonate
Pale yellow solid (74%), m.p. 98–99°C, ^1H NMR ($CDCl_3$) δ (ppm): 8.04 (s, 1H, Ar-C**H** =), 6.98 (s, Ar-**H**, 1H), 6.46 (s, Ar-**H**, 1H), 4.34–4.21 (q, 4H, 2 × -OC$\mathbf{H}_2$-), 3.91, 3.86 and 3.78 (s, 9H, 3 × –OC$\mathbf{H}_3$), 1.39–4.19 (t, 6H, 2 × -C$\mathbf{H}_3$); ^{13}C NMR ($CDCl_3$) δ (ppm): 173.4, 171.8 (2 × -**C**OOR), 162.2, 156.5, 156.3, 142.5, 131.0 and 130.8 (aromatic carbons), 132.6 (Ar-**C**H=), 115.7 (=**C**(COOR$_1$)$_2$), 75.6, 75.5 and 75.3 (-O**C**H$_3$), 80.7, 80.6, 33.5 and 33.3 (alkyl carbons). IR (KBr, cm^{-1}); 2974, 2928, 2846, 1704, 1600, 1518, 1460, 1413, 1250, 1207, 1121 and 1021; Mw 338 (m/z).

Dihexyl-2′,4′,5′-trimethoxybenzalmalonate
Pale yellow solid (73%), m.p. 62–63°C, ^1H-NMR ($CDCl_3$) δ (ppm): 8.05 (s, 1H, Ar-C**H** =), 6.97 (s, 1H, Ar-**H**), 6.46 (s, 1H, Ar-**H**), 4.20–4.12 (t, 4H, 2 × -OC$\mathbf{H}_2$-), 3.91, 3.85 and 3.78 (s, 9H, 3 × -OC$\mathbf{H}_3$), 1.66–0.81 (m, 22H, 2 × -$C_5\mathbf{H}_{11}$); ^{13}C NMR ($CDCl_3$) δ (ppm): 176.6, 173.6 (2 × -**C**OOR), 163.0, 161.5, 151.8, 145.9, 122.2, 120.4 (aromatic carbons), 132.1

(Ar-**C**H=), 105.3 (=**C**(COOR$_1$)$_2$), 74.5, 74.2 and 65.1 (-O**C**H$_3$), 40.3, 37.5, 37.2, 34.5, 31.5, 31.4, 22.9 (alkyl carbons); IR (KBr, cm^{-1}): 2955, 2928, 2854, 1712, 1607, 1518, 1464, 1238, 1200 and 1029; Mw 450 (m/z).

Molar absorption coefficient determination

Molar absorption coefficient (ε) was obtained from the slope of the graph between absorbance (A) and concentration (c) (Beer's law, equation 1). A sample was accurately weighed and dissolved in a selected solvent using a measuring flask of appropriate size. The solution was then diluted into at least five serial optically dilute concentrations (giving absorbance of less than 1). The experiment was done in triplicate and a slope of the graph was obtained by a least square fit. The absorbance, A, is defined by Lambert Beer's law (equation 1):

$$A = \varepsilon bc \quad (1)$$

where ε is the molar extinction coefficient, b is the cuvette path length (1 cm) and c is the concentration.

Photostability test

The photostability of each UV filter was tested in methanol and hexane. The freshly prepared solution (0.0536 mM) was divided into two parts. One part was kept covered with foil at room temperature (dark sample), while the other was irradiated with 0.70 mW cm^{-2} broadband UVB (280–320 nm) generated by an FSX24T12/UVB/HO lamp (National Biological Corporation, Twinsburg, OH) and 11.5 mW cm^{-2} broadband UVA (320–400 nm) generated by an F24T12/BL/HO (PUVA) lamp (National Biological Corporation, Twinsburg, OH) at room temperature (irradiated sample) (see Figure 1 for spectral outputs of the lamps). UVA and UVB irradiances were measured using UVA-400C (detected irradiation at 315–400 nm) and UVB-500C (detected irradiation at 280–320 nm) power meters (National Biological Corporation, Twinsburg, OH), respectively. Experiment at various exposures was carried out by exposing a sample to such radiation for various time lengths. For example, 60 min of such irradiation gave the exposure of 41.64 J cm^{-2} UVA and 2.52 J cm^{-2} UVB. Then UV absorption profiles of both the dark and the irradiated samples were analysed on a UV/VIS spectrometer. Photostability of each compound was expressed as percent relative absorbance at the maximum absorption wavelength of the compound. Percent relative absorbance was calculated as follows:

$$\% \text{ relative absorbance} = 100 \times (\text{absorbance after t min of irradiation})/(\text{absorbance of the unirradiated sample}) \quad (2)$$

All experiments were done in triplicate. A Mann–Whitney *U*-test was used to indicate if the photostability of different compounds were significantly different. An average value from the three data points was reported with its standard deviation.

Each irradiated sample solution was then dried by rotary evaporation at 35–40°C in the dark (foil covering) before being subjected to ^{1}H NMR analysis.

Photostability tests of the two mixtures (in methanol), i. e. **E8** (3.45×10^{-5} M) + EHMC (9.02×10^{-6} M) and **B8** (8.69×10^{-5} M) + EHMC (8.81×10^{-6} M), were also carried out as described above. The experiments were done in triplicate and a Mann–Whitney *U*-test was used to indicate if the photostability of irradiated and unirradiated mixture were significantly different.

Results and Discussion

Synthesis, UV absorption and photostability of 2-ethylhexyl-*trans*-substituted cinnamates

The *trans*-cinnamic acids were successfully synthesized using Knoevenagel-Doebner condensation between benzaldehyde and malonic acid. *trans*-Cinnamates were then prepared by esterification between *trans*-cinnamoyl chloride, which was prepared in-situ from cinnamic acids and oxalylchloride, and 2-ethylhexanol (Figure 2). Structures and UV absorption properties of the synthesized compounds are shown in Table 1 and Figure 3. Among the nine cinnamate derivatives, **E5** and **E8** showed maximum absorption at the mid UVA (~350 nm) (Figure 3). Molar absorption coefficient values indicated that **E8** ($\varepsilon_{348\,nm} = 14200\,M^{-1}\,cm^{-1}$) was twice as efficient in absorbing the mid UVA radiation as **E5** ($\varepsilon_{352\,nm} = 6700\,M^{-1}\,cm^{-1}$). Analyses of the absorbances obtained from 41.64 J cm^{-2} UVA and 2.52 J cm^{-2} UVB irradiated-**E8** and -**E5** (the last data groups of the x axis of Figure 4) by a Mann–Whitney *U*-test indicate

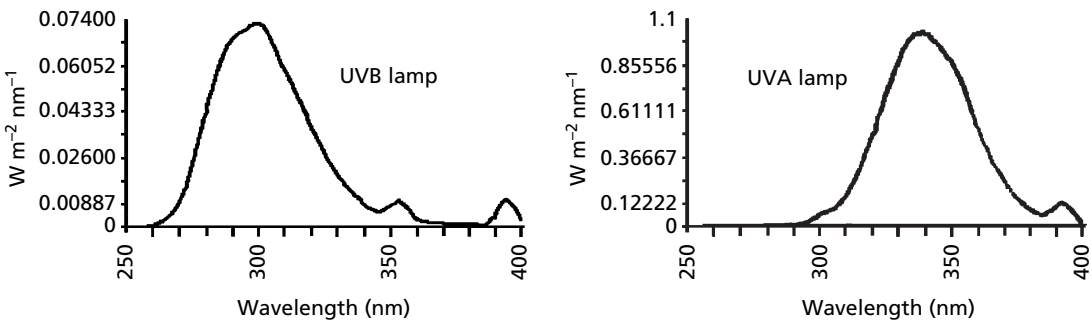


Figure 1 Irradiance of the UVB and UVA lamps used in the experiment.

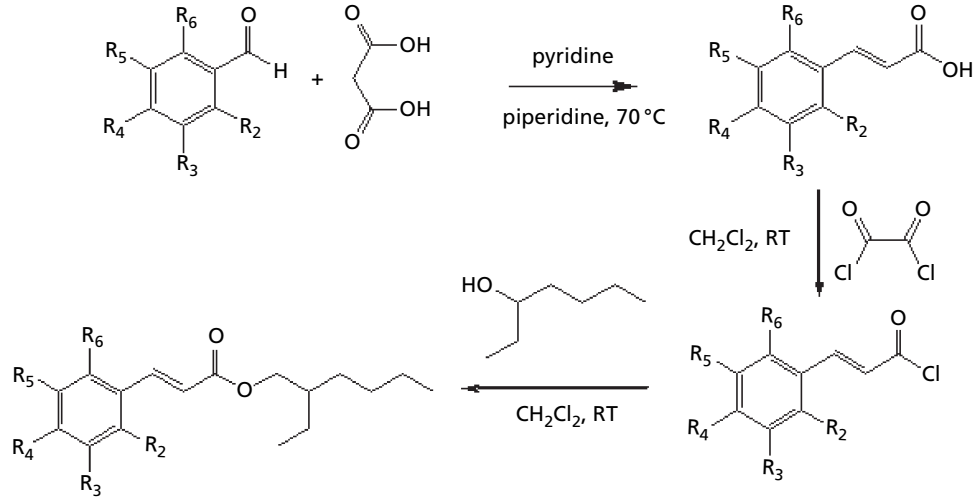
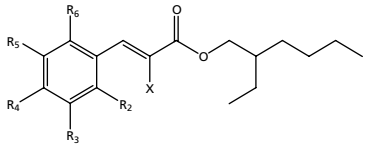


Figure 2 Synthetic pathway of 2-ethylhexyl-*trans*-cinnamates.

Table 1 UV Absorption properties of the synthesized cinnamates



E1–E9: X = H; B8: X=

Compound	R2	R3	R4	R5	R6	λmax (nm)	ε (M^{-1} cm^{-1})
E1	OCH3	H	H	H	H	276	18 100
						323	11 400
E2	H	OCH3	H	H	H	278	21 600
						314	7000
E3	H	H	OCH3	H	H	309	24 700
E4	OCH3	OCH3	H	H	H	283	16 300
E5	OCH3	H	H	OCH3	H	277	15 400
						352	6700
E6	H	OCH3	OCH3	H	H	294	8700
						322	15 400
E7	OCH3	OCH3	OCH3	H	H	307	18 800
E8	OCH3	H	OCH3	OCH3	H	290	12 400
						348	14 200
E9	OCH3	H	OCH3	H	OCH3	320	22 400
B8	OCH3	H	OCH3	OCH3	H	295	13 600
						358	15 700

that **E8** was significantly more photostable than **E5**. Although both **E6** and **E9** possessed high molar absorption coefficients at their UVA absorption maxima ($\varepsilon_{320\,nm}$ of 15 400 M^{-1} cm^{-1} for **E6** and $\varepsilon_{320\,nm}$ of 22 400 M^{-1} cm^{-1} for **E9**), their maxima were too close to the UVB region and their absorption efficiencies in the mid UVA (350 nm) were moderately low ($\varepsilon_{350\,nm}$ of ~5000 M^{-1} cm^{-1} for **E6** and $\varepsilon_{350\,nm}$ of ~7000 M^{-1} cm^{-1} for **E9**). In addition, **E9** was the least photostable (confirmed by a Mann–Whitney *U*-test) among nine synthesized cinnamates (Figure 4). In the case of **E1**, not only was its UVA absorption maxima too close to the UVB region but also its molar absorption coefficient in the mid UVA was too low ($\varepsilon_{323\,nm}$ = 8400 M^{-1} cm^{-1}, $\varepsilon_{350\,nm}$ = 2700 M^{-1} cm^{-1}) to be an interesting UVA filter. Furthermore, the compound was the second-least photostable among nine synthesized cinnamates. Comparing **E7** and **E3** (EHMC), the two compounds were effective UVB absorbers but **E3** had a larger molar absorption coefficient.

Among the 9 cinnamate derivatives, **E8** was not only the most photostable derivative but the compound also possessed a wide UVA absorption wavelength range with its maximum in the mid UVA (345–349 nm) region. In addition, **E8** also

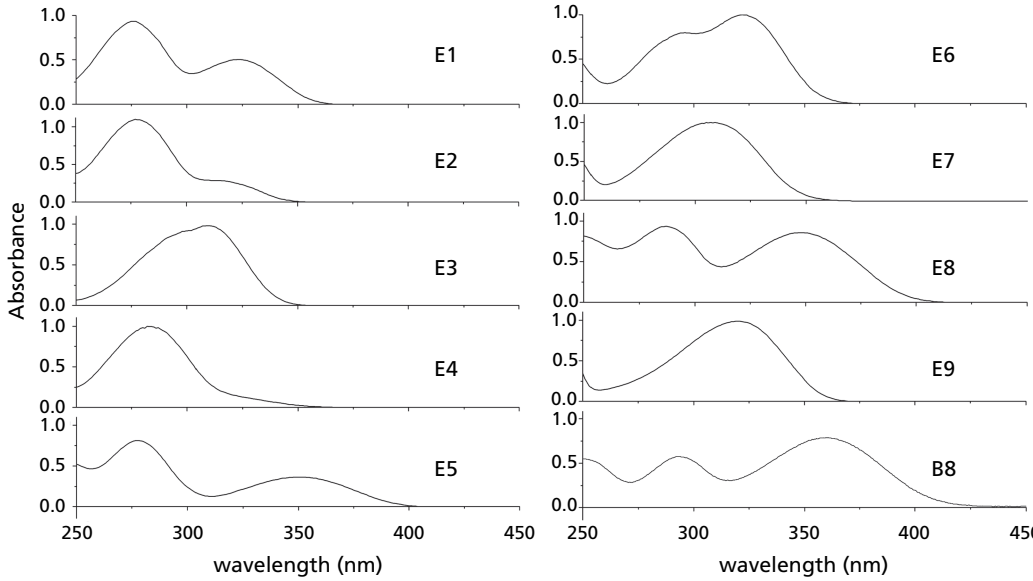


Figure 3 UV absorption spectra of 5×10^{-5} M solution (in methanol) of **E1**–**E9** and **B8**.

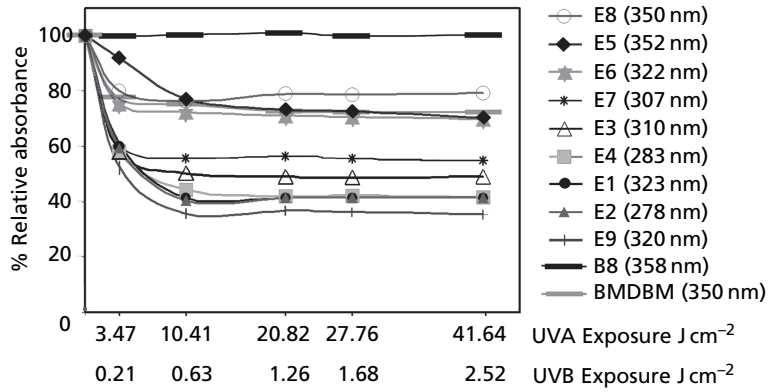


Figure 4 Photostability of 0.0536 mM **E1**–**E9**, **B8** and BMDBN (in methanol). The decrease in absorbance of each compound was monitored at its maximum absorption as indicated. UVB and UVA irradiation were done simultaneously to the sample solution in a quartz container with 1-cm pathlength. Each point is the average of three data points with s.d. of less than 2.89% relative absorbanc.

possessed attractive physico-chemical properties. The compound is a water-immiscible liquid at room temperature with a boiling point of 264°C. It is soluble in methanol, ethanol, ethylacetate, diethylether, tetrahydrofuran (THF), dichloromethane, hexane, isopropyl palmitate, various silicone fluids, 2-ethylhexyl-4-methoxycinnamate, etc.

Analyses of all 9 irradiated cinnamate (**E1**–**E9**) solutions by NMR spectroscopy revealed only the *trans*-to-*cis* photoisomerization. For example, NMR spectrum of the irradiated **E8** revealed the presence of only the *cis*-isomer (two couple signals at 7.21 and 7.18 ppm with J = 12.5 Hz and 5.92 and 5.88 ppm with J = 13.2 Hz, *cis*-C**H** = C**H**) in addition to the *trans*-isomer (Figure 5). No signal could be assigned to a cyclobutane dimer or other products. This result agrees with the findings of a study by Devanathan & Ramamurthy (1987) in which irradiation of dilute solution of cinnamates in benzene (10^{-3} to 10^{-4} M) resulted only in geometric isomerization. In addition, photostability study of dilute EHMC solution (3.4×10^{-3} M in methanol) also showed only *cis*-isomer (Pattanaargson & Limpong 2001). Pattanaargson et al (2004) reported that the lower absorption coefficient of the *cis*-isomer of EHMC (12600 M^{-1} cm^{-1} at 301 nm) compared with that of the *trans*-isomer (24000 M^{-1} cm^{-1} at 309 nm) was responsible for the loss in UV absorption efficiency of the irradiated EHMC solution (10^{-4} to 10^{-5} M). The decrease in absorbance of the nine irradiated samples, therefore, was probably correlated to the lower molar absorption coefficients of the *cis*-isomers.

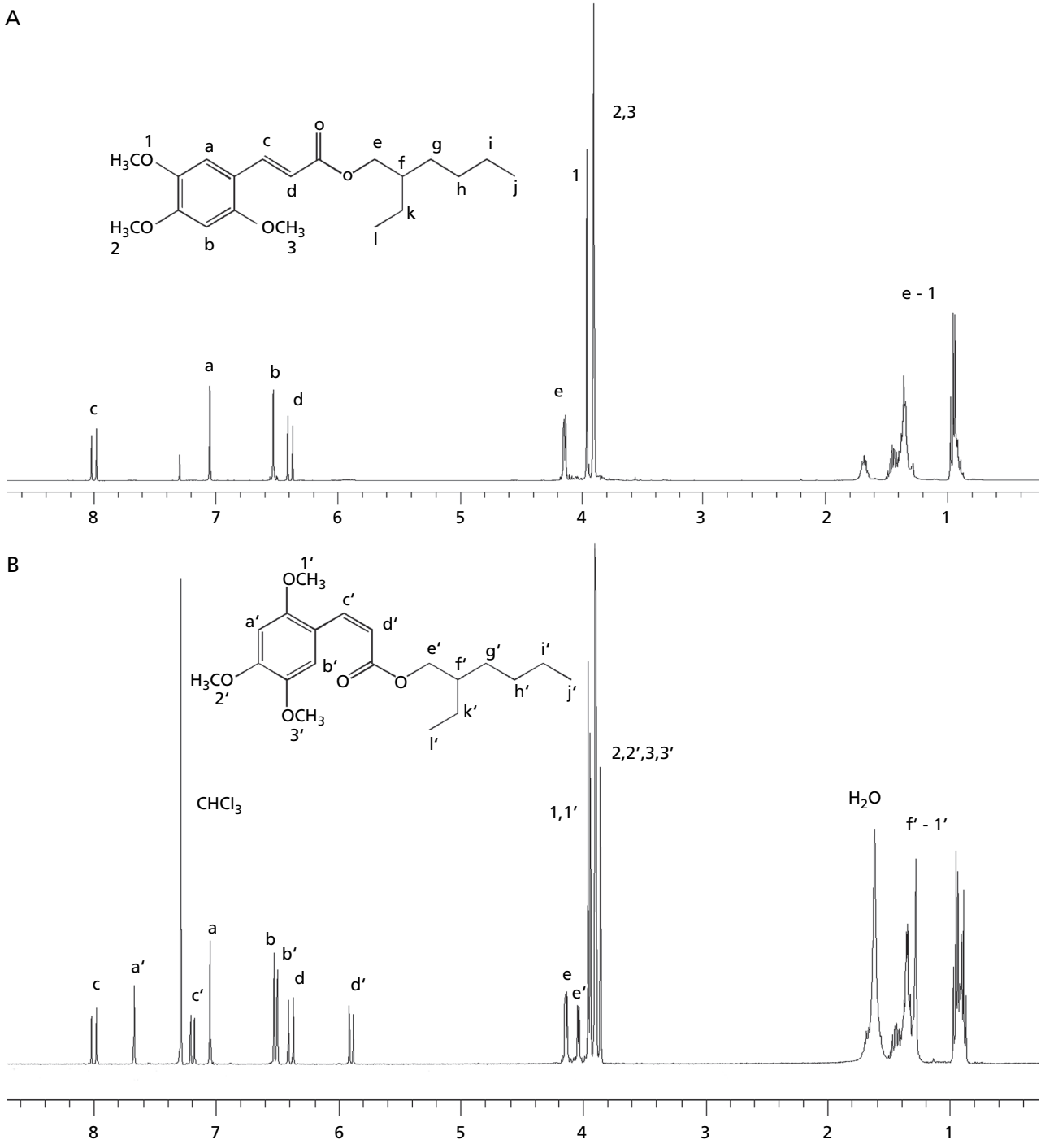


Figure 5 ^{1}H NMR spectra of unirradiated **E8** (A) and irradiated **E8** (B).

It should be noted here that all photostability tests were done using artificially generated broadband UVB and broadband UVA lamps (see Figure 1 for spectral outputs of the lamps). A sample in the quartz container of 1 cm path length was receiving 0.70 mW cm^{-2} UVB and 11.5 mW cm^{-2} UVA, respectively. This UVB radiation was about 6–7 times more powerful than sunlight UVB. The used UVA radiation was about twice as powerful as sunlight UVA. Sunlight measurements carried out in March on a normal clear sky day around noon in Bangkok have recorded values of 0.10–0.13 mW cm^{-2} in UVB and 5.0–5.2 mW cm^{-2} in UVA.

Since it has been known that a solvent's polarity can affect the photostability of sunscreens (Bonda & Marinelli 1999; Pattanaargson et al 2004), photostability studies of **E8** and **E3**

(EHMC) were carried out in hexane (non-polar solvent) and methanol (polar solvent). It was found that upon irradiation, both cinnamates were more photostable in hexane than in methanol. This agrees with the previous result in which EHMC was shown to be more photostable in less polar solvents (Pattanaargson et al 2004).

Another important property of a good UV filter is that the maximum absorption wavelength and molar absorption coefficient of the compound should not be much perturbed when changing from one solvent to another. In the case of **E8**, the absorption wavelength and molar absorption coefficient were slightly affected by the type of solvent (Table 2). Changing from methanol to hexane caused a blue shift at the maximum absorption of 4 nm and decreased **E8**'s molar absorption coefficient at the UVA region of about 17.5%. This small sensitivity will still enable a rough prediction of absorption wavelengths when the compound is being used in cosmetic formulations. It also ensures that the absorption profile and UV screening efficiency would not change much when the product was applied onto skin where the UV filtering agent would encounter a new environment.

Synthesis, UV absorption and photostability of dialkyl-2,4,5-trimethoxybenzalmalonates

As mentioned earlier, NMR analysis has confirmed that a *cis–trans* isomerization of **E8** was responsible for the decrease in UV absorption efficiency when the compound was exposed to UV light. To solve this problem, three benzalmalonate derivatives were synthesized. The synthesis was done through Knoevengel-Doebner condensation between one mole equivalent of malonate (di-(2-ethylhexyl)-malonate (R′=2-ethylhexyl), diethyl-malonate (R′=ethyl) and dihexyl-malonate (R′=hexyl)) and one mole equivalent of 2,4,5-trimethoxybenzaldehyde. The general equation is represented in Figure 6.

Table 2 Molar absorption coefficients of **E3**, **E8** and **B8** in various solvents

Compound	ε ($M^{-1}cm^{-1}$)		
	MeOH	THF	Hexane
E8	14200 (349 nm)	12900 (348 nm)	11720 (345 nm)
	12400 (290 nm)	10500 (289 nm)	11700 (284 nm)
E3	24600 (310 nm)	17480 (309 nm)	18700 (303 nm)
B8	15700 (358 nm)	18800 (358 nm)	14900 (350 nm)
	13600 (295 nm)	14000 (293 nm)	13400 (289 nm)

The resulting di-(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate (**B8**) is pale yellow solid with a melting point of 42°C. The UV absorption profile of **B8** resembles that of **E8** (i.e., two absorption maxima corresponding to the UVB (292 nm, 10 100 $M^{-1}cm^{-1}$) and the UVA (360 nm, 14 500 $M^{-1}cm^{-1}$) regions (see Figure 3 and Table 1). However, λ_{max} in the UVA region of **B8** (360 nm) is about 10 nm longer than that of **E8** (350 nm). This bathochromic shift is a result of the second ester moiety, which extends the conjugation of the molecule. In methanol the absorption profile of **B8** tails to about 420 nm, causing a yellow colour (Figure 3). Since there is no possible *cis–trans* isomerization in the **B8** structure, the compound is very photostable (Figure 4). Mann–Whitney *U*-analyses indicate that **B8** is significantly more photostable than **E8** and that **E8** is significantly more photostable than BMDBM (Figure 4). Actually, the UVA absorption efficiency of **B8** is unaffected by UV irradiation. **B8** can be dissolved in methanol, ethanol, ethylacetate, diethylether, THF, dichloromethane, hexane, isopropyl palmitate, various silicone fluids, 2-ethylhexyl-4-methoxycinnamate, etc.

The maximum UV absorption wavelength and molar absorption coefficient of **B8** were slightly affected by solvent (Table 2). A blue shift at the maximum absorption (in UVA region) of 8 nm and only 5% decrease in molar absorption coefficient (in UVA region) were detected when changing from methanol to hexane.

Besides di-(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate (**B8**), diethyl-2,4,5-trimethoxybenzalmalonate and dihexyl-2,4,5-trimethoxybenzalmalonate were also synthesized. Both compounds had similar UV absorption properties to **B8**. Diethyl-2,4,5-trimethoxybenzalmalonate was insoluble in non-polar media, such as isopropyl palmitate or silicone fluids. Dihexyl-2,4,5-trimethoxybenzalmalonate, however, could be dissolved in both polar and non-polar solvents, including methanol, ethanol, THF, hexane, isopropyl palmitate, silicone fluids, etc. The melting points of diethyl-2,4,5-trimethoxycinnamate and dihexyl-2,4,5-trimethoxycinnamate were 99 and 62°C, respectively.

The low melting point of **B8** (42°C) will ease the use of this filter during formulation. Both **E8** and **B8** can be dissolved completely in EHMC. As a liquid UV filter can be used in many cosmetic formulations more easily than a solid filter, a liquid mixture of EHMC and **B8** could be prepared. The obtained mixture showed broad spectral absorption with excellent photostability and great solubility. In addition, combination between **E8** and **B8** also resulted in a liquid UVA filter with excellent solubility.

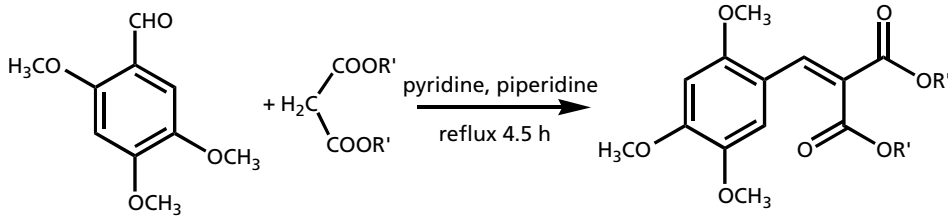


Figure 6 Synthesis of benzalmalonate derivatives.

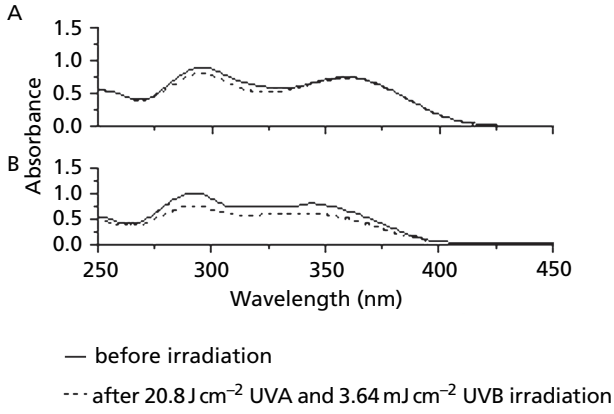


Figure 7 UV absorption spectra of the two mixtures; **B8** (8.69×10^{-5} M) + EHMC (8.81×10^{-6} M) (A) and **E8** (3.45×10^{-5} M) + EHMC (9.02×10^{-6} M) (B), before and after UV exposure (20.8 J cm^{-2} UVA and 1.26 J cm^{-2} UVB).

The **B8** + EHMC mixture was more photostable than **E8**+EHMC mixture (see Figure 7). A Mann–Whitney *U*-analysis indicated no significant difference in UV absorbance obtained from the unirradiated and the irradiated (20.8 J cm^{-2} UVA and 1.26 J cm^{-2} UVB) **B8** + EHMC mixture. The presence of **B8** could, therefore, help increase the stability of EHMC under UV exposure. This synergistic photostability of EHMC and **B8** therefore results in a very photostable liquid broadband filter. It should be noted that a small absorption around 400–420 nm of the mixture caused the liquid to develop a yellow colour. The **E8** + EHMC mixture was less yellow but after being exposed to 20.8 J cm^{-2} UVA and 1.26 J cm^{-2} UVB, there was a loss of about 20 ± 2% and 24 ± 2% in UVA and UVB absorption efficiencies, respectively. At this concentration range, EHMC alone usually loses about 50% of its UVB absorption efficiency after such exposure (see Figure 4). The improved photostability in the UVB region of **E8** + EHMC mixture, compared with EHMC alone, is probably a contribution of some UVB absorption property of **E8**.

For the safety issue, both **E8** and **B8** are now being subjected to acute oral toxicity study in rats. Skin irritation study of the two compounds using patch and photopatch tests in volunteers, together with a transdermal penetration study of the two compounds, are also underway.

Conclusion

This study has shown that organic UVA filters based on 2,4,5-trimethoxycinnamate (**E8**) and 2,4,5-trimethoxybenzalmalonate (**B8**) could be developed. The pale yellowish liquid **E8** possesses great photostability with mid UVA absorption maximum (348 nm, 14 150 M^{-1} cm^{-1}). The compound undergoes *trans* to *cis* photoisomerization only slightly upon the UV irradiation, therefore loss of UV absorption properties upon UV irradiation is small. This water-immiscible oil can be dissolved in various organic solvents ranging from methanol to hexane and various silicone fluids, making it very easy to use during formulation. The yellow solid di-(2-ethylhexyl)-2,4,5-trimethoxybenzalmalonate (**B8**) also shows mid UVA absorption maximum (360 nm, 14 500 M^{-1} cm^{-1}). **B8** cannot *cis–trans* isomerize, therefore its UV absorption efficiency is unaffected by UV exposure. The compound can be melted easily at 42°C into light yellow oil. The water-immiscible **B8** can be dissolved in various organic solvents, ranging from methanol to hexane, and various silicone fluids. In addition, the compound can be dissolved completely in both the commercial EHMC and **E8**, yielding liquid broad spectral filter and liquid UVA filter, respectively. The liquid mixture between **B8** + EHMC shows broad spectral UV absorption with excellent UVA and UVB photostability. A broad spectral filter comprising **E8** and EHMC shows better photostability in the UVB area than pure EHMC.

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