Contents lists available at GrowingScience

Current Chemistry Letters

homepage: www.GrowingScience.com

Synthesis and photoprotective properties of new salicylic and vanillic acid derivatives

Alicja Wodnicka* **and Elżbieta Huzar**

West Pomeranian University of Technology, Szczecin, Faculty of Chemical Technology and Engineering, Institute of Organic Chemical Technology, Al. Piastów 42, 71-065 Szczecin, Poland

C H R O N I C L E

Article history:
Received January 2, 2017
Received in revised form
March 1, 2017
Accepted March 21, 2017
Available online
March 21, 2017

Keywords:
Phenolic acids derivatives
Salicylates
Vanillates
Photoprotection
Sun protection factor (SPF)

A B S T R A C T

A simple one-step procedure for synthesis of new derivatives of phenolic acids was developed. As the starting materials salicylic acid, vanillic acid and alkyl haloalkanoates were applied. The reactions were carried out in *N*,*N*-dimethylformamide (DMF) in the presence of anhydrous potassium carbonate. Conditions for regioselective synthesis of target compounds were established. The esters **3a-h** were obtained in great yields and were characterized by MS, ^{1}H and ^{13}C NMR spectra. Their photoprotective activity was evaluated *in vitro* by spectrophotometric method. The study revealed that the tested compounds are moderate UVB absorbers, but could be used to augment the effect of other photoprotective agents. Their SPF values were in the range from 3.63 to 4.26 for salicylates **3a-d** and from 3.03 to 3.51 for vanillates.

1. Introduction

People outdoors are exposed to solar radiation which consists of 56% of infrared light photons, 39% of visible light, and 5% of UV light.[1] Despite the fact that UV radiation is a minority stream of light that reaches the Earth's surface, it can cause the harmful effects on the human body. Excessive exposure to ultraviolet radiation is a major factor causing the skin cancer development.[2,3]

The solar UV radiation can be subdivided into three ranges: UVA (320-400 nm), UVB (280-320 nm), and UVC (190-280 nm).[2] UVC radiation does not reach the earth's surface because its rays are blocked in the stratosphere by the ozone layer, so it is not of biological relevance.[4] Unlike both UVA and UVB rays pass through the atmosphere and are able to cause biological effects.[5]

The ultraviolet light can induce various changes in the skin, both acute and chronic. Acute effects include vitamin D synthesis and pigmentation, which are positive and desirable by people during relaxation in the sun. On the other hand the excess sun exposure can lead to inflammation and immunosuppression.[5-7] Moreover, acute UV irradiation, even during a single exposure induces damage

* Corresponding author.
E-mail address: alicja.wodnicka@zut.edu.pl (A. Wodnicka)

doi: 10.5267/j.ccl.2017.3.002

of DNA.[7] Cutaneous malignant melanoma – the most lethal of the skin cancers, is frequently related with sporadic acute burning exposure to sunlight.[2,8] Non-melanoma skin cancers are initiated in the majority of cases by long-term chronic sunlight exposure.[2] Ultraviolet B causes direct photochemical damage of DNA leading to the creation of mutations. In contrast, ultraviolet A acts indirectly generating of reactive oxygen species.[2,9,10] Long-term sunlight exposure is also associated with the chronic effects such as the premature cutaneous aging.[6,9,11]

The risk of the potential problems connected with the exposure to ultraviolet light can by limited by wearing protective clothes or avoidance of sun exposure, especially during the time when disease-inducing solar radiation is relatively intense (from 11 am to 4 pm).[2,6] Unfortunately, this solution seems to be unacceptable in the global society, so the use of topical sunscreens is the only way to prevent cellular damage in the skin or reduce it to a minimum.[2,6,9]

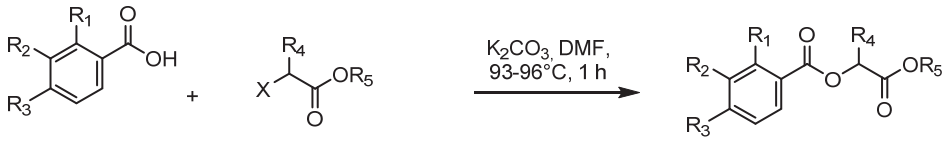
Photoprotective agents used in sunscreens can be classified into organic (chemical) and inorganic (physical) on the basis of their mechanism of action.[12] Organic agents absorb UV radiation with excitation to a higher energy state and returning to the stable state release insignificant amount of heat or emit fluorescent radiation.[2,12,13] They include *p*-aminobenzoic acid (PABA) and its derivatives, cinnamates, salicylates, camphors, benzophenones, among others.[1,2,6,10,12-14] Inorganic agents, primarily titanium dioxide and zinc oxide, reflect and scatter UV radiation increasing the optical pathway of the photons in the sunscreen formulation leading to higher efficiency of the organic photoprotective agents.[2,12,13] In order to provide a high level of sun protection against a wide range of the UV spectrum multiple compounds are usually incorporated into sunscreening cosmetics – no single photoprotective agent can provide a high sun protection factor[6,13] at the level required by consumers and regulatory agencies, e.g. FDA in the USA or the Commission of the European Communities in the EU. For this reason the list of new photoprotective substances is developed and different combinations of sunscreens have become the subject of producers' interests.[6,13]

Bearing the above in mind, the aim of this work was the synthesis of phenolic acids derivatives as the new photoprotective compounds. According to literature, sunscreen chemicals are generally aromatic compounds containing an electron-acceptor group conjugated with an electron-releasing group in *ortho* or *para* position.[15,16] Our research work describes the synthesis of salicylates with free hydroxyl group in position *ortho* and vanillates with hydroxyl group in position *para*.

2. Results and Discussion

*2.1. Synthesis of salicylic and vanillic acid esters (**3a-h**)*

The new salicylic and vanillic acid derivatives (**3a-h**) were obtained by reaction of particular acids with alkyl haloalkanoates in alkaline conditions (Scheme 1). Phenolic acids have two functional groups – carboxyl and hydroxyl, so different products could be formed. The initial aim was to establish parameters favourable to obtain esters with free hydroxyl group. For this purpose vanillic acid (**1b**) was treated with methyl 2-bromobutanoate (**2b**) at equimolar ratio (Scheme 2). The reactions were carried out in *N*,*N*-dimethylformamide (DMF) – an aprotic polar solvent in the presence of anhydrous potassium carbonate at 93-96 °C. The advantageous conditions of synthesis were determined by a single-factor experimental method. The reaction time and **1b**:K_2CO_3 molar ratio were changed. The selectivity of reaction and yields of target compound **3f** are presented in Table 1.



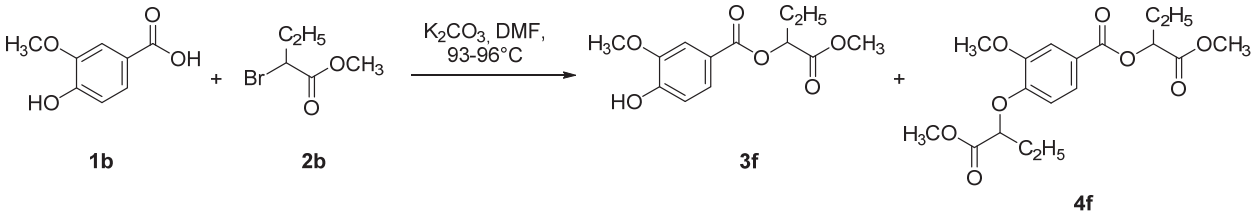
1a,b **2a-d** **3a-h**

1a: R_1=OH, R_2=H, R_3=H
1b: R_1=H, R_2=OCH_3, R_3=OH

2a: R_4=H, R_5=C_2H_5, X=Cl
2b: R_4=C_2H_5, R_5=CH_3, X=Br
2b: R_4=*n*-C_3H_7, R_5=CH_3, X=Br
2b: R_4=*n*-C_4H_9, R_5=CH_3, X=Br

3a: R_1=OH, R_2=H, R_3=H, R_4=H, R_5=C_2H_5 (96%)
3b: R_1=OH, R_2=H, R_3=H, R_4=C_2H_5, R_5=CH_3 (95%)
3c: R_1=OH, R_2=H, R_3=H, R_4=*n*-C_3H_7, R_5=CH_3 (92%)
3d: R_1=OH, R_2=H, R_3=H, R_4=*n*-C_4H_9, R_5=CH_3 (89%)
3e: R_1=H, R_2=OCH_3, R_3=OH, R_4=H, R_5=C_2H_5 (91%)
3f: R_1=H, R_2=OCH_3, R_3=OH, R_4=C_2H_5, R_5=CH_3 (97%)
3g: R_1=H, R_2=OCH_3, R_3=OH, R_4=*n*-C_3H_7, R_5=CH_3 (93%)
3h: R_1=H, R_2=OCH_3, R_3=OH, R_4=*n*-C_4H_9, R_5=CH_3 (96%)

Scheme 1. Synthesis of compounds **3a-h**



1b **2b** **3f** **4f**

Scheme 2. Formation of 1-methoxy-1-oxobut-2-yl vanillate (**3f**) and by-product 4f by the reaction of vanillic acid (**1b**) with methyl 2-bromobutanoate (**2b**)

Table 1. The reaction of vanillic acid (**1b**) with methyl 2-bromobutanoate (**2b**) in DMF

Entry	Molar ratio **1b**:K_2CO_3	Reaction time (h)	Ratio of **3f** to **4f** [1]	Yield[2] (%) **3f**
1	1.0:1.0	0.5	91/9	69
2	1.0:1.0	1.0	85/15	67
3	1.0:1.0	1.5	84/16	68
4	1.0:0.5	0.5	100/0	74
5	**1.0:0.5**	**1.0**	**100/0**	**97**
6	1.0:0.5	1.5	100/0	97

[1] estimated from GC-MS chromatogram
[2] isolated yield

Influence of potassium carbonate on both selectivity and yield was observed. The molar ratio of the vanillic acid to potassium carbonate of 1.0:1.0 in the entries 1-3 led to formation of 1-methoxy-1-oxobut-2-yl vanillate (**3f**) in yield of 67-69%. Under these conditions compound **4f** was also formed as a by-product. Reduction of the amount of potassium carbonate in the entries 4-6 resulted in an increase selectivity of the process. At the molar ratio of reactants of 1:0.5 (**1b**:K_2CO_3) the pure product **3f** was obtained. It was observed that the reaction time is an important factor that affects the final product yield. Conducting process for 30 min the yield of 74% was achieved. The rise of process efficiency to 97% was observed when longer reaction time was applied. Comparing the results from trials 5 and 6 it can be stated that reaction time 1 hour should be preferred from an economic point of view.

The established conditions were applied to synthesis of esters **3a-h**. The target products were obtained in excellent yields (89-97%). Salicylates **3b-d** and vanillates **3e-h** are new compounds. Their structures were confirmed by ^{1}H NMR, ^{13}C NMR and MS spectra. Compound **3a** was already described in the literature and was obtained by the insertion reaction of ethyl diazoacetate to salicylic acid in fluorinated alcohols.[17]

According to the literature,[18-22] synthesis of phenolic acid esters usually includes three steps: protection of hydroxyl group, esterification and removal of the protecting group. As the blocking agents benzyl halides or acetic anhydride are often applied to protect hydroxyl group.[22] They are removed after the esterification process by hydrogenolysis or hydrolysis, respectively. Unfortunately, application of protecting groups is inconvenient in industrial production. Multiple-step syntheses raise the costs of the production and are unfriendly for environment due to the need for additional materials and energy. In contrast, the proposed method is one-stage and occurs in good yields.

2.2. Photoprotective properties

The obtained derivatives of salicylic and vanillic acids were screened for photoprotective activity. This property is specified by the index called as the sun protection factor (SPF). The sun protection factor is expressed as the ratio of the minimum dose of UV radiation causes erythema on skin protected by a sunscreen product to the minimum dose of UV radiation causes erythema on the same unprotected skin.[2]

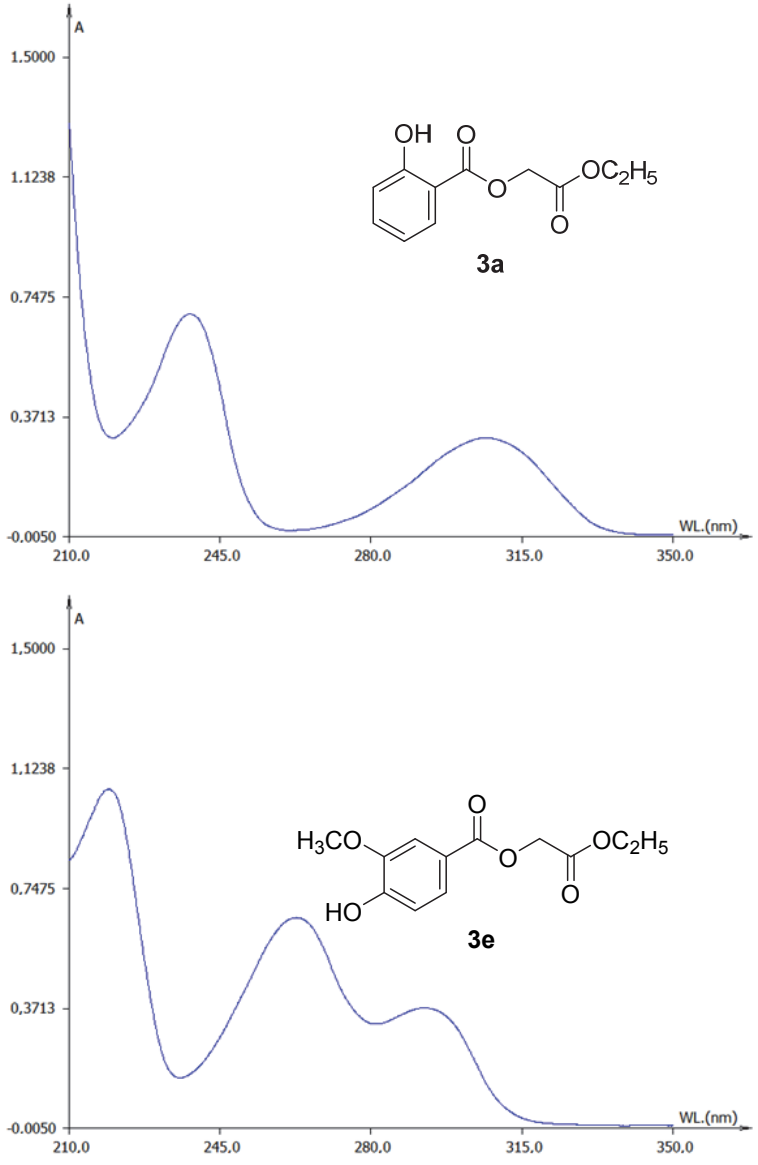


Fig. 1. The exemplary UV spectra of the obtained esters **3a** and **3e**

This parameter could be also tested *in vitro*. In our work spectrophotometric method was applied. For this purpose the obtained compounds were dissolved in ethyl alcohol and UV spectra in the range from 210 to 350 nm were recorded. Exemplary spectra of compounds **3a** and **3e** are presented in Fig. 1.

The tested substances absorb energy in the range of UVB and UVC. The salicylic acid derivatives (**3a-d**) spectra showed the maxima of absorption at 238 and 308 nm wavelength. The band at 308 nm is characteristic for aromatic carboxylic acids substituted with hydroxyl group in *ortho* position. The hydroxyl group in position *para* in compounds **3e-h** resulted in the presence of absorption band at 263 nm. Moreover, vanillic acid contains methoxy group, so in the spectra of **3e-h** the band at 293 nm was observed.

The photoprotective properties of the obtained compounds against UVB radiation were determined by the method developed by Mansur *et al.*[23] using homomenthyl salicylate (homosalate) as a standard. This method assumes that a sunscreen formulation containing 8% homosalate presents a SPF value of 4. The obtained values for compounds **3a-h** are presented in Fig. 2. The photoprotective properties of salicylates **3a-d** were similar to homosalate. The SPF values for these compounds were in the range from 3.63 to 4.26. It was observed that the length of alkyl chain (R_4) have influence on the photoprotective properties of salicylates **3a-d** – SPF values decreased with the increase of alkyl chain length. In contrast, for vanillates (**3e-h**) this relationship was reversed. Furthermore, this group of esters were less effective against UVB radiation – their SPF values were in the range from 3.03 to 3.51. However these compounds can be also used as the ingredients of photoprotective agents mixture in the sunscreen formulations. The advantage of the obtained vanillic acid esters is their flavor – mild and pleasant with spicy, floral and honey characteristics.

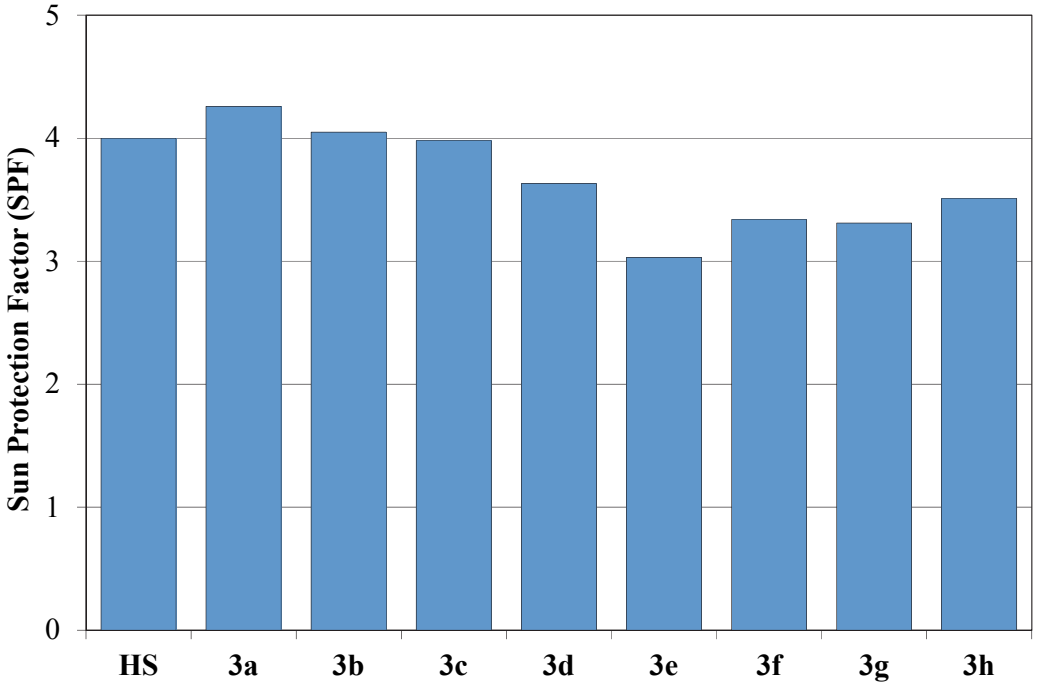


Fig. 2. The SPF values determined for compounds **3a-h** (the mean values of three determinations)

3. Conclusions

We have reported an efficient method for the preparation of salicylic and vanillic acid derivatives. Conditions for selective synthesis of 1-alkoxy-1-oxoalkan-2-yl salicylates (**3a-d**) and 1-alkoxy-1-

oxoalkan-2-yl vanillates (**3e-h**) in the one-step procedure were established. As the most favourable the time of reaction of 1 hour and the molar ratio of phenolic acid to potassium carbonate 1.0:0.5 were recognized. The target products were obtained in excellent yields (89-97%).

The photoprotective properties of the obtained compounds **3a-h** were tested *in vitro* using spectrophotometric method. Compound **3a** was described in the literature[17] but it was never considered as a photoprotective agent. The SPF values of salicylates **3a-d** were in the range from 3.63 to 4.26. Vanillates **3e-h** were less effective against UVB radiation – their SPF values were in the range from 3.03 to 3.51. Both salicylates and vanillates are moderate UVB absorbers, but they could be used to augment the effect of other UVB absorbers.

4. Experimental

4.1. Material and Methods

Vanillic and salicylic acids (≥99% purity) were purchased from Alfa Aesar. Ethyl chloroacetate (99% purity) was purchased from Aldrich and methyl 2-bromoalkanoates i.e. methyl 2-bromobutanoate, 2-bromopentanoate and 2-bromohexanoate were obtained according to the method described by Reinheckel.[24] All methyl 2-bromoalkanoates (**2b**-**2d**) were obtained as colourless oils. The yields and boiling points of 2-bromoesters were correspondingly: 93% and 49-51 °C/11 mmHg (Lit.[25] 52-54 °C/11 mmHg) for methyl 2-bromobutanoate **(2b)**; 95% and 59-63 °C/10 mmHg (Lit.[25] 71 °C/13 mmHg) for methyl 2-bromopentanoate (**2c**); 93% and 88-90 °C/14 mmHg (Lit.[25] 92-94 °C/15 mmHg) for methyl 2-bromohexanoate (**2d**). The others reagents and solvents were purchased in commercially available grade purity (>98%). *N*,*N*-Dimethylformamide (DMF) was dried over 4A molecular sieves. All other reagents and solvents were used without purification.

For determination of sun protection factor (SPF) homomenthyl salicylate (homosalate) – certified reference material purchased from Fluka and ethanol for spectroscopy were used.

The GC-MS analysis of the obtained products diluted with acetone was carried out using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 Network Mass Selective Detector (MSD). The separation was effected using a HP-5MSI capillary column with bonded (5% phenyl)methylpolysiloxane stationary phase (30 m × 0.25 mm I.D., 0.25 μm film thickness). The GC oven temperature was programmed: initial temperature 60 °C (hold for 3 min); ramp rate 10 °C/min; final temperature 300 °C (hold for 10 min). Helium was used as a carrier gas at a constant flow rate of 1.2 mL/min. The mass selective detector was working in electron impact mode (70 eV). The mass spectra were scanned in the range from 50 to 500 m/z.

^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as an internal standard on a TM Bruker DPX 400 spectrometer (400 MHz).

Melting points were determined using a Boetius apparatus and are uncorrected.

UV spectra of the obtained compounds were recorded on a GBC UV/VIS 916 double beam spectrophotometer using 1 cm quartz cuvettes, and ethanol as a blank.

4.2. Procedures of synthesis

2-Ethoxy-2-oxoethyl salicylate (3a): The mixture of salicylic acid (**1a**) (2.50 g, 18 mmol), ethyl chloroacetate (**2a**) (2.21 g, 18 mmol) and anhydrous K_2CO_3 (1.24 g, 0.9 mmol) in DMF (50 mL) was heated with stirring at 93-96 °C for 1 h. After pouring the reaction mixture into ice-water it was left for 24 h at 4 °C. The formed precipitate was filtered off, washed with water and dried in air. The crude solid was recrystallized from the mixture of methanol and water to afford a colourless solid (3.87 g,

96%), mp 34-35 °C. ^{1}H NMR (400 MHz; $CDCl_3$), δ (ppm): 10.42 (s, 1H, OH), 7.94 (dd, *J*=8.0, 1.7 Hz, 1H, Ar), 7.49 (ddd, *J*=8.7, 7.3, 1.7 Hz, 1H, Ar), 7.00 (dd, *J*=8.4, 0.8 Hz, 1H, Ar), 6.93-6.89 (m, 1H, Ar), 4.86 (s, 2H, CH_2), 4.27 (q, *J*=7.1 Hz, 2H, CH_2), 1.31 (t, *J*=7.1 Hz, 3H, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$), δ (ppm): 169.4 (C=O), 167.3 (C=O), 161.8 (C_{Ar}-2), 136.3 (C_{Ar}-4), 130.3 (C_{Ar}-6), 119.4 (C_{Ar}-5), 117.7 (C_{Ar}-3), 111.7 (C_{Ar}-1), 61.7 (CH_2), 61.2 (CH_2), 14.1 (CH_3); GC/MS (τ = 15.41 min.), MS: *m*/*z* (%) 224 (M^+, 24), 179 (5), 121 (52), 120 (100), 93 (13), 92 (38), 65 (23), 64 (7), 63 (8).

1-Methoxy-1-oxobut-2-yl salicylate (3b): Starting from salicylic acid (**1a**) (2.50 g 18 mmol), methyl 2-bromobutanoate **(2b**) (3.26 g, 18 mmol) and anhydrous K_2CO_3 (1.24 g, 9 mmol) in DMF (50 mL), the procedure described to prepare compound **3a** was followed to give **3b** as a colourless solid (4.07 g, 95%), mp 27°C. ^{1}H NMR (400 MHz; $CDCl_3$), δ (ppm): 10.49 (s, 1H, OH), 7.94 (dd, *J*=8.0, 1.8 Hz, 1H, Ar), 7.48 (ddd, *J*=8.8, 7.3, 1.9 Hz, 1H, Ar), 6.99 (dd, *J*=8.5, 0.9 Hz, 1H, Ar), 6.93-6.89 (m, 1H, Ar), 5.21 (dd, *J*=6.7, 5.5 Hz, 1H, CH), 3.78 (s, 3H, OCH_3), 2.10-1.99 (m, 2H, CH_2), 1.09 (t, *J*=7.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$), δ (ppm): 170.2 (C=O), 169.6 (C=O), 161.8 (C_{Ar}-2), 136.2 (C_{Ar}-4), 130.2 (C_{Ar}-6), 119.3 (C_{Ar}-5), 117.7 (C_{Ar}-3), 111.9 (C_{Ar}-1), 74.0 (CH), 52.4 (OCH_3), 24.6 (CH_2), 9.6 (CH_3); GC/MS (τ = 15.99 min.), MS: *m*/*z* (%) 238 (M^+, 92), 207 (9), 206 (19), 178 (15), 138 (20), 122 (14), 121 (97), 120 (100), 101 (6), 93 (26), 92 (51), 69 (7), 65 (34), 64 (8), 63 (8), 59 (19).

1-Methoxy-1-oxopent-2-yl salicylate (3c): The mixture of salicylic acid (**1a**) (2.50 g, 18 mmol), methyl 2-bromopentanoate **(2c**) (3.51 g, 18 mmol) and anhydrous K_2CO_3 (1.24 g, 9 mmol) in DMF (50 mL) was heated with stirring at 93-96 °C for 1 h. After the completion of the reaction the mixture was poured into ice-water. The product was extracted with methylene chloride. The obtained methylene chloride extract was washed with water and dried over anhydrous sodium sulfate for 30 min. Next, the solvent was evaporated under reduced pressure and the target product was obtained in a form of colourless oil (4.18 g, 92%). ^{1}H NMR (400 MHz; $CDCl_3$), δ (ppm): 10.49 (s, 1H, OH), 7.93 (dd, *J*=7.9, 1.8 Hz, 1H, Ar), 7.48 (ddd, *J*=8.8, 7.2, 1.7 Hz, 1H, Ar), 6.99 (dd, *J*=8.3, 1.1 Hz, 1H, Ar), 6.91 (ddd, *J*=8.2, 7.2, 1.1 Hz, 1H, Ar), 5.26 (dd, *J*=7.8, 5.0 Hz, 1H, CH), 3.78 (s, 3H, OCH_3), 2.04-1.93 (m, 2H, CH_2), 1.58-1.49 (m, 2H, CH_2), 1.00 (t, *J*=7.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$), δ (ppm): 170.4 (C=O), 169.6 (C=O), 161.8 (C_{Ar}-2), 136.2 (C_{Ar}-4), 130.2 (C_{Ar}-6), 119.3 (C_{Ar}-5), 117.7 (C_{Ar}-3), 111.9 (C_{Ar}-1), 72.8 (CH), 52.5 (OCH_3), 33.2 (CH_2), 18.6 (CH_2), 13.7 (CH_3); GC/MS (τ = 16.91 min.), MS: *m*/*z* (%) 252 (M^+, 90), 221 (7), 220 (12), 192 (14), 138 (33), 122 (13), 121 (96), 120 (100), 115 (16), 93 (24), 92 (37), 83 (12), 73 (11), 65 (30), 64 (7), 63 (6), 59 (11), 55 (17).

1-Methoxy-1-oxohex-2-yl salicylate (3d): Starting from salicylic acid (**1a**) (2.50 g, 18 mmol), methyl 2-bromohexanoate **(2b**) (3.26 g, 18 mmol) and anhydrous K_2CO_3 (1.24 g, 9 mmol) in DMF (50 mL), the procedure described to prepare compound **3c** was followed to give **3d** as a colourless oil (4.27 g, 89%). ^{1}H NMR (400 MHz; $CDCl_3$), δ (ppm): 10.49 (s, 1H, OH), 7.93 (dd, *J*=7.9, 1.8 Hz, 1H, Ar), 7.48 (ddd, J=8.7, 7.2, 1.8 Hz, 1H, Ar), 6.99 (dd, *J*=8.5, 1.1 Hz, 1H, Ar), 6.91 (ddd, *J*=8.1, 7.2, 1.1 Hz, 1H, Ar), 5.25 (dd, *J*=6.9, 5.8 Hz, 1H, CH), 3.78 (s, 3H, OCH_3), 2.03-1.97 (m, 2H, CH_2), 1.52-1.35 (m, 4H, 2 CH_2), 0.94 (t, *J*=7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$), δ (ppm): 170.4 (C=O), 169.6 (C=O), 161.8 (C_{Ar}-2), 136.2 (C_{Ar}-4), 130.2 (C_{Ar}-6), 119.3 (C_{Ar}-5), 117.7 (C_{Ar}-3), 111.9 (C_{Ar}-1), 73.1 (CH), 52.5 (OCH_3), 30.9 (CH_2), 27.3 (CH_2), 22.3 (CH_2), 13.9 (CH_3); GC/MS (τ = 17.85 min.), MS: *m*/*z* (%) 266 (M^+, 57), 138 (16), 129 (7), 122 (5), 121 (64), 120 (100), 97 (7), 93 (8), 92 (10), 69 (9), 65 (10).

2-Ethoxy-2-oxoethyl vanillate (3e): The mixture of vanillic acid (**1b**) (2.50 g, 14.9 mmol), ethyl chloroacetate (**2a**) (1.62 g, 14.9 mmol) and anhydrous K_2CO_3 (1.04 g, 7.5 mmol) in DMF (50 mL) was heated with stirring at 93-96 °C for 1 h. After the completion of the reaction the mixture was poured into ice-water. The product was extracted with methylene chloride. The obtained methylene chloride extract was washed with water and dried over anhydrous sodium sulfate for 30 min. Next, the solvent was evaporated under reduced pressure. The crude product was crystallized from the mixture of methanol and water to afford a white solid (3.46 g, 91%), mp. 73-76 ºC. ^{1}H NMR (400 MHz; $CDCl_3$), δ (ppm): 7.70 (dd, *J*=8.3, 1.9 Hz, 1H, Ar), 7.58 (d, *J*= 1.9 Hz, 1H, Ar), 6.95 (d, *J*=8.3 Hz, 1H, Ar), 6.18 (s, 1H, OH), 4.82 (s, 2H, CH_2), 4.26 (q, *J*=7.1 Hz, 2H, CH_2), 3.93 (s, 3H, OCH_3), 1.30 (t, *J*=7.1 Hz,

3H, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$), δ (ppm): 168.1 (C=O), 165.7 (C=O), 150.5 (C_{Ar}-4), 146.2 (C_{Ar}-3), 124.8 (C_{Ar}-1), 121.2 (C_{Ar}-6), 114.2 (C_{Ar}-2), 112.0 (C_{Ar}-5), 61.5 (CH_2), 61.1 (CH_2), 56.1 (O**C**H_3), 14.1 (CH_3); GC/MS (τ = 18.93 min.), MS: *m*/*z* (%) 254 (M^+, 32), 152 (9), 151 (100), 123 (12), 108 (9), 52 (8).

1-Methoxy-1-oxobut-2-yl vanillate (3f): Starting from acid **1b** (2.50 g 14.9 mmol), methyl 2-bromobutanoate **(2b**) (2.68 g, 14.9 mmol) and anhydrous K_2CO_3 (1.04 g, 7.5 mmol) in DMF (50 mL), the procedure described to prepare compound **3a** was followed to give **3f** as a white solid (3.86 g, 97%), mp 99-100 ºC. ^1H NMR (400 MHz; $CDCl_3$), δ (ppm): 7.70 (dd, *J*=8.4, 2.0 Hz, 1H, Ar), 7.56 (d, *J*= 1.9 Hz, 1H, Ar), 6.94 (d, *J*=8.4 Hz, 1H, Ar), 6.23 (s, 1H, OH), 5.17 (dd, *J*=7.0, 5.4 Hz, 1H, CH), 3.93 (s, 3H, OCH_3), 3.77 (s, 3H, $COOCH_3$), 1.98-2.05 (m, 2H, CH_2), 1.08 (t, *J*=7.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$), δ (ppm): 170.9 (C=O), 165.9 (C=O), 150.5 (C_{Ar}-4), 146.3 (C_{Ar}-3), 124.6 (C_{Ar}-1), 121.5 (C_{Ar}-6), 114.2 (C_{Ar}-2), 111.9 (C_{Ar}-5), 73.7 (CH), 56.1 (OCH_3), 52.3 (COO**C**H_3), 24.8 (CH_2), 9.7 (CH_3); GC/MS (τ = 19.30 min.), MS: *m*/*z* (%) 268 (M^+, 86), 169 (5), 168 (55), 153 (12), 152 (33), 151 (100), 123 (28), 122 (5), 108 (13), 80 (5), 77 (5), 65 (7), 59 (5), 52 (8).

1-Methoxy-1-oxopent-2-yl vanillate (3g): Starting from vanillic acid (**1b**) (2.50 g, 14.9 mmol), methyl 2-bromopentanoate **(2c**) (2.91 g, 14.9 mmol) and anhydrous K_2CO_3 (1.04 g, 7.5 mmol) in DMF (50 mL), the procedure described to prepare compound **3e** was followed to give **3g** as a white solid (3.91 g, 93%), mp 47-49 ºC. ^1H NMR (400 MHz; $CDCl_3$), δ (ppm): 7.70 (dd, *J*=8.3, 1.9 Hz, 1H, Ar), 7.57 (d, *J*= 1.9 Hz, 1H, Ar), 6.95 (d, *J*=8.3 Hz, 1H, Ar), 6.06 (s, 1H, OH), 5.22 (dd, *J*=7.8, 5.1 Hz, 1H, CH), 3.95 (s, 3H, OCH_3), 3.77 (s, 3H, $COOCH_3$), 1.89-2.03 (m, 2H, CH_2), 1.48-1.58 (m, 2H, CH_2), 0.99 (t, *J*=7.4 Hz, 3H, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$), δ (ppm): 171.1 (C=O), 165.9 (C=O), 150.4 (C_{Ar}-4), 146.2 (C_{Ar}-3), 124.6 (C_{Ar}-1), 121.5 (C_{Ar}-6), 114.1 (C_{Ar}-2), 111.9 (C_{Ar}-5), 72.5 (CH), 56.1 (OCH_3), 52.3 (COO**C**H_3), 33.3 (CH_2), 18.6 (CH_2), 13.7 (CH_3); GC/MS (τ = 20.08 min.), MS: *m*/*z* (%) 282 (M^+, 58), 169 (6), 168 (67), 153 (10), 152 (23), 151 (100), 123 (18), 108 (9), 65 (5), 52 (5).

1-Methoxy-1-oxohex-2-yl vanillate (3h): Starting from vanillic acid (**1b**) (2.50 g, 14.9 mmol), methyl 2-bromohexanoate **(2b**) (2.70 g, 14.9 mmol) and anhydrous K_2CO_3 (1.04 g, 7.5 mmol) in DMF (50 mL), the procedure described to prepare compound **3c** was followed to give **3h** in a form of beige semi-solid (4.24 g, 96%). ^1H NMR (400 MHz; $CDCl_3$+DMSO-d6), δ (ppm): 7.65 (dd, *J*=8.3, 1.9 Hz, 1H, Ar), 7.55 (d, *J*=1.9 Hz, 1H, Ar), 6.94 (d, *J*=8.3 Hz, 1H, Ar), 5.17 (dd, 7.0, 5.4 Hz, 1H, CH), 3.92 (s, 3H, OCH_3), 3.91 (s, 1H, OH), 3.76 (s, 3H, $COOCH_3$), 1.97 (q, *J*=7.3 Hz, 2H, CH_2), 1.52-1.35 (m, 4H, $2CH_2$), 0.94 (t, *J*=7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$+DMSO-d6), δ (ppm): 171.1 (C=O), 166.0 (C=O), 151.3 (C_{Ar}-4), 147.0 (C_{Ar}-3), 124.3 (C_{Ar}-1), 120.8 (C_{Ar}-6), 114.7 (C_{Ar}-2), 112.4 (C_{Ar}-5), 72.5 (CH), 56.0 (OCH_3), 52.2 ($COOCH_3$), 30.9 (CH_2), 27.3 (CH_2), 22.2 (CH_2), 13.8 (CH_3); GC/MS (τ = 20.91 min.), MS: *m*/*z* (%) 296 (M^+, 61), 169 (9), 168 (84), 153 (12), 152 (28), 151 (100), 123 (23), 108 (11), 65 (6), 52 (5).

4.3. Determination of sun protection factor (SPF)

The photoprotective properties of the prepared compounds was tested *in vitro* based on spectrophotometric analysis of dilute solutions.[23] For this purpose the solutions of compounds **3a-h** and homomenthyl salicylate (homosalate) in ethanol were prepared. The concentration of these solutions was 16 µg/mL. The absorbance of samples in solution form was measured in wavelength range of 290 to 320nm, every 5 nm wavelength interval. The following equation was applied to calculate the SPF:

$$SPF = CF \times \sum_{\lambda=290nm}^{320nm} EE_\lambda \times I_\lambda \times A_\lambda$$

where: CF – correction factor, EE_λ – erythemogenic effect of radiation at wavelength λ, I_λ – intensity of solar light at wavelength λ, and A_λ – absorbance of the solution at wavelength λ.

The values of $EE_\lambda \times I_\lambda$ are constants determined by Sayre et. al and known from the literature.[26,27] They are presented in Table 2. Correction factor was determined experimentally taking into account the value of SPF for homomenthyl salicylate is equal to 4.[23]

Table 2. Normalized values used for the calculation of SPF[26,27]

Wavelength λ (nm)	$E_\lambda \times I_\lambda$
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

References

1. González S., Fernández-Lorente M., and Gilaberte-Calzada Y. (**2008**) The latest on skin photoprotection. *Clin. Dermatol.*, 26 (6) 614-626.
2. Lautenschlager S., Wulf H. C., and Pittelko M. R. (**2007**) Photoprotection. *Lancet*, 370 (9586) 528-537.
3. Gilchrest B. A., Eller M. S., Geller A. C., and Yaar M. (**1999**) The pathogenesis of melanoma induced by ultraviolet radiation. *N. Engl. J. Med.*, 340 (17) 1341-1348.
4. Brenner M., and Hearing V. J. (**2008**) The protective role of melanin against UV damage in human skin. *Photochem. Photobiol*., 84 (3) 539-549.
5. Beissert S., and Schwarz T. (**1999**) Mechanisms involved in ultraviolet light-induced immunosuppression. *J. Invest. Dermatol. Symp. Proc.*, 4 (1) 61-64.
6. Petrazzouli M. (**2000**) Advances in sunscreens. *Curr. Probl. Dermatol.*, 12 (6) 287-290.
7. Matsumura Y., and Ananthaswamy H. N. (**2004**) Toxic effects of ultraviolet radiation on the skin. *Toxicol. Appl. Pharmacol.*, 195 (3) 298-308.
8. Wang S. Q., Setlow R., Berwick M., Polsky D., Marghoob A. A., Kopf A. W., and Bart R. S. (**2001**) Ultraviolet A and melanoma: a review. *J. Am. Acad. Dermatol.*, 44 (5) 837-846.
9. Koshy J. C., Safa E. Sharabi S. E., Jerkins D., Cox J., Cronin S. P., and Hollier L. H. Jr. (**2010**) Sunscreens: evolving aspects of sun protection. *J. Pediatr. Health Care*, 24 (5) 343-346.
10. Sambandan D. R., and Ratner D. (**2011**) Sunscreens: An overview and update. *J. Am. Acad. Dermatol.*, 64 (4) 748-758.
11. Wilson B. D., Moon S., and Armstrong F. (**2012**) Comprehensive review of ultraviolet radiation and the current status on sunscreens. *J. Clin. Aesthet. Dermatol.*, 5 (9) 18-23.
12. Klimová Z., Hojerová J., and Pažoureková S. (**2013**) Current problems in the use of organic UV filters to protect skin from excessive sun exposure. *Acta Chim. Slov.*, 6 (1) 82-88.
13. Latha M.S., Martis J., Shobha V., Sham Shinde R., Bangera S., Krishnankutty B., Bellary S., Varughese S., Rao P., and Kumar B. R. N. (**2013**) Sunscreening agents. *J. Clin. Aesthet. Dermatol.*, 6 (1) 16-26.
14. Kullavanijaya P., and. Lim H. W. (**2005**) Photoprotection. *J. Am. Acad, Dermatol.*, 52 (6) 937-958.
15. Wolf R., Wolf D., Morganti P., and Ruocco V. (**2001**) Sunscreens. *Clin. Dermatol.*, 19 (4) 452-459.
16. Shaath N. A. (**1987**) On the theory of ultraviolet absorption by sunscreen chemicals. *J. Soc. Cosmet. Chem.*, 38 (3) 193-207.
17. Dumitrescu L., Azzouzi-Zriba K., Bonnet-Delpon D., and Crousse B. (**2011**) Nonmetal catalyzed insertion reactions of diazocarbonyls to acid derivatives in fluorinated alcohols. *Org. Lett.*, 13 (4) 692-695.

18. Catel Y., Aladedunye F., and Przybylski R. (**2010**) Synthesis, radical scavenging activity, protection during storage, and frying by novel antioxidants. *J. Agric. Food Chem.*, 58 (20) 11081-11089.
19. Lamoral-Theys D., Pottier L., Kerff F., Dufrasne F., Proutière F., Wauthoz N., Neven P., Ingrassia L., Van Antwerpen P., Lefranc F., Gelbcke M., Pirotte B., Kraus J.-L., Nève J., Kornienko A., Kiss R., and Dubois J. (**2010**) Simple di- and trivanillates exhibit cytostatic properties toward cancer cells resistant to pro-apoptotic stimuli. *Bioorg. Med. Chem.*, 18 (11) 3823-3833.
20. Haddleton D. M., Sahota H. S., Taylor P. C., and Yeate S. G. (**1996**) Synthesis of polyester dendrimers. *J. Chem. Soc. Perkin Trans. 1*, 1996 (7) 649-656.
21. Torlopov M. A., Udoratina E. V., and Kuchin A. V. (**2013**) Synthesis of inulin esters of phenylcarboxylic acids. *Russ. J. Org. Chem.*, 49 (5) 702-706.
22. Spivey A. C., and Leese D. (**2002**) Synthetic methods. Part (III) Protecting groups. *Annu. Rep. Prog. Chem., Sect. B*, 98, 41-60.
23. Mansur J. S.; Breder M. N. R., Mansur M. C. A., Azulay R. D. (**1986**) Determinação do fator de proteção solar por espectrofotometria. *An. Bras. Dermatol.*, 61 (3) 121-124.
24. Reinheckel H. (**1960**) Über halogen- und stickstoffhaltige Derivate aliphatischer Carbonsäuren, I. Die indirekte α-Bromierung von Fettsäureestern. *Chem. Ber.*, 93 (10) 2222-2229.
25. Kwiecień H. (**1996**) Synthesis and properties of new 2-alkyl-1,4-benzoxazepine derivatives. Part I. Synthesis and cyclization of 2-phenoxyalkanoic acid derivatives. *Polish J. Chem.*, 70 733-741.
26. Dutra E. A., Oliveira D. A. G. C., Kedor-Hackmann E. R. M., and Santoro M. I. R. M. (**2004**) Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. *Braz. J. Pharm. Sci.*, 40 (3) 381-385.
27. Kaur C. D., and Saraf S. (**2010**) In vitro sun protection factor determination of herbal oils used in cosmetics. *Pharmacogn. Res.*, 2 (1) 22-25.