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







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RESEARCH ARTICLE

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Straightforward sustainable synthesis of novel non-endocrine disruptive bio-based organic UV-B filters with antimicrobial activity

Matthieu M. Mention ^{a*}, Cédric Peyrot ^{a*}, Blandine Godon ^a, Jimmy Alarcán ^b, Fanny Brunissen ^a, Marina Grimaldi ^c, Patrick Balaguer ^c, Albert Braeuning ^b and Florent Allais ^a

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ABSTRACT

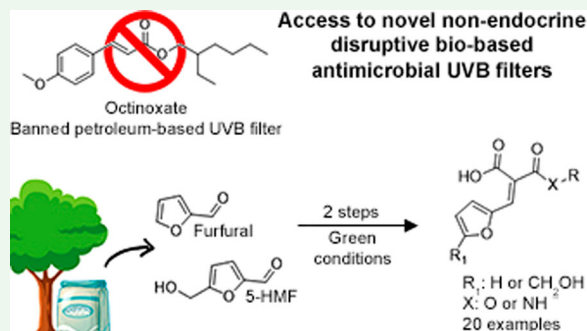
With an increasing demand for safe and natural products from both industries and consumers, paired with the recent ban of decried molecules (i.e. octinoxate, avobenzone or octocrylene) due to their high negative impact on humans and the environment (i.e. endocrine disruption, coral bleaching), safe bio-based alternatives are a necessary and promising surrogate to substitute current commercialized petroleum-based UV filters. In this context, a class of bio-based molecules, displaying interesting UV-B filtering properties and great photostability were developed from furfural and 5-hydroxymethylfurfural (HMF), using the Knoevenagel condensation with a set of green conditions to minimize the impact on environment. Furthermore, those furfural- and HMF-based molecules demonstrated antimicrobial properties as secondary activity, highly sought by industries. Some furan derivatives being recognized to exhibit toxicological risks, *in silico* and *in vitro* assays were conducted and demonstrated the absence of endocrine disruption activity for these new molecules.

ARTICLE HISTORY

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Furfural; HMF; Knoevenagel condensation; UV filters; antimicrobial




Introduction

While crucial for life on Earth, significant exposure to solar light can have negative effect on human skin (1). It has been widely shown that UV-B (280–320 nm) and UV-A (320–400 nm) radiations penetrate most of the skin barriers, leading to the formation of Reactive Oxygen Species (ROS) (2), which in turn, induce oxidative stress and damage such as skin photoaging, inflammations, lipid membrane alteration or DNA mutation (3–6). In order to protect oneself against those radiations, a wide range of sunscreens is available, containing either mineral (i.e. TiO₂, ZnO) or organic filters (i.e.

octinoxate, avobenzone or octocrylene). Those petroleum-based organic filters are increasingly criticized due to their negative impact on both humans and the environment (i.e. endocrine disruption, coral bleaching, toxic degradation products) (7–9). In response to this issue, some countries have started to ban such filters, for instance, Hawaii issued a bill against the use and sale of avobenzone- or octinoxate-containing sunscreens from January 1st, 2021 (10). Consequently, offering a bio-based alternative, with reduced environmental and public health risks, is essential to protect humans against those harmful radiations while reducing or eliminating the negative impact of such UV filters.

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Conjugated and aromatic compounds (i.e. phenolic and furfural derivatives) are known to absorb wavelengths in the ultraviolet spectrum (200–400 nm) (11,12). Furfural is a renewable bio-based chemical produced from a variety of agricultural byproducts (i.e. wheat bran, oat, corncobs or sawdust) (13), and has a wide range of uses, from solvent to intermediates in pharmaceuticals, monomers or even aroma (13,14). Lignin-derived phenols (*p*-hydroxybenzaldehydes) were recently used as starting materials to engineer new organic UV filters with great potential, their coverage was divided between UV-B and UV-A regions (15–17). Replacing phenols by furan rings – that offers a hypsochromic shift in absorbance – would allow to focus on a full coverage of UV-B, leading to a better fit to substitute decried organic petroleum-based organic filters.

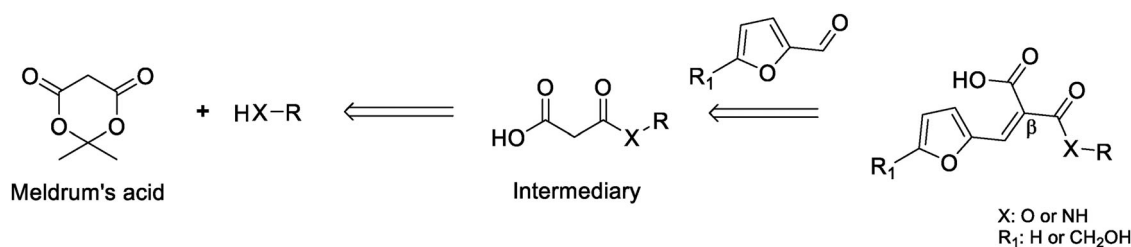
In regard to the aforementioned considerations, we focused our efforts on the synthesis of several furfural and HMF derivatives through a two-step synthesis (Scheme 1) including a Knoevenagel condensation based on a set of green conditions to minimize its environmental impact: use of ethanol and L-proline as solvent and catalyst, respectively, moderate heating and short reaction time. Under such conditions, we were able to synthesize a wide range of furfural and HMF derivatives exhibiting both an extended conjugation through their backbone and high steric hindrance on the β position (Scheme 1), two factors that have been shown as of critical importance to obtain anti-UV properties (11,18,19). The investigation of their absorbance properties and their photostability, benchmarked against that of octinoxate, proved them as potential sustainable alternatives. Moreover, furfural being known to exhibit antimicrobial activities (20), the synthesized compounds were tested against different strains and benchmarked against a paraben and phenoxyethanol, commonly found in cosmetic formulations. Assessing physico-chemical and biological properties allowed the identification of the most competitive compounds among all that have been synthesized. Finally, *in silico* and *in vitro* endocrine disruption assays were conducted to determine potential health risks associated to those new molecules.

Materials and methods

Chemical synthesis

Aniline, furfural, heptan-1-ol, *tert*-butanol, choline chloride, *o*-cresol, nystatin, itraconazole and L-proline were purchased from Sigma Aldrich (St. Louis, MO, USA). Malonic acid, 2-ethylhexanol, *n*-butylamine, phenoxyethanol and 2,4-pentanedione were purchased from TCI (Tokyo, Japan). Organics 5-hydroxymethylfurfural was purchased from Carbosynth (Berkshire, UK). *Escherichia coli* (LMG 2092), *Bacillus subtilis* (LMG 7135) and *Candida albicans* (ATCC 22972) were purchased from BCCM (Belgium). Chloramphenicol, paraben and all solvents were purchased from Fisher Scientific (Hampton, NH, USA) and used as received. All chemicals were used directly without purification.

Chromatographic purifications were accomplished using a flash-prep LC system puriFlash[®] 4100 from Interchim (Montluçon, France) with prepacked silica column (30 μ m, Interchim PF-Si30-HP), dual wavelength collection (λ = 254/320 nm) in cyclohexane/ethyl acetate eluent. ¹H NMR spectra were recorded on a Bruker Fourier 300 (300 MHz) (Bruker, Billerica, MA, USA) and were calibrated with residual DMSO-*d*₆ protons signals at δ 2.50 ppm. Data are reported as follows: chemical shift (δ ppm), multiplicity, integration, coupling constant (Hz) and assignment. ¹³C NMR spectra were recorded on a Bruker Fourier 300 (75 MHz) (Bruker, Billerica, MA, USA) and were calibrated with residual DMSO-*d*₆ signal at δ 39.52 ppm. Data are reported as follows: chemical shift (δ ppm) and assignment. ¹H and ¹³C spectra are available in the ESI. All NMR assignments were made using COSY, HMBC and HSQC spectra. IR spectra were recorded on an Agilent Cary 630 FTIR Spectrometer (Wilmington, DE, USA) and are reported by frequency of absorption (cm⁻¹). Melting points were recorded on a Mettler Toledo MP50 Melting Point System (Greifensee, Switzerland) with ME-18552 sample tubes. HRMS were performed on an Agilent 1290 system (Wilmington, DE, USA), equipped with a PDA UV detector, and a 6545 Q-TOF mass spectrometer with a JetStream ESI probe operating at atmospheric pressure as the source.



Scheme 1. Retrosynthetic pathway for furfural and HMF derivatives synthesis.

Intermediary synthesis

All intermediaries were synthesized according to a previously described procedure (21).

General procedure for the Knoevenagel condensation of monoesters

The corresponding monoester intermediary (1 mmol), furfural or 5-hydroxymethylfurfural (1 mmol, 1 eq) and L-proline (0.1 mmol, 0.1 eq) were mixed in ethanol (1.3 mL). The reaction mixture was heated at 60°C and stirred for 4 h. After cooling at r.t., ethanol was evaporated under reduced pressure. Depending on purity, the crude product was purified either by precipitation or by flash chromatography. Characterizations for all Knoevenagel product are available in the ESI.

UV analysis and loss of absorbance

UV-Vis spectra were recorded from an ethanol solution containing the studied compound at 10 $\mu\text{mol.L}^{-1}$ placed in a 1 cm quartz cuvette and are reported in wavelength (nm). Loss of Absorbance was obtained by irradiating the solution for 1 h into a Rayonet® RPR-200 ($\lambda = 300 \text{ nm}$, $P = 8.32 \text{ W/m}^2$, stirring, $T = 35^\circ\text{C}$) from SNE Ultraviolet Co (Brandford, CT, USA) using 14 RPR-3000A lamps. Loss of absorbance was calculated in percentage at the λ_{max} by comparing absorbance at $t = 1 \text{ h}$ with the initial one.

Antibacterial assays

Overnight culture of *Escherichia coli*, *Candida albicans* or *Bacillus subtilis* were diluted (with Lysogeny Broth for bacteria and Potato Dextrose Broth for yeast) to $\text{OD}_{600} = 1$ and placed on agar-agar plates. Compounds of interest were solubilized in a 40% ethanol solution at a final concentration of 2.5, 1.25, 0.625, 0.31, 0.156, 0.078, 0.04 and 0.02%w. Disks were placed on the sowed agar-agar plate, soaked with 30 μL of solution containing the compound of interest and incubated at 30°C. Controls were used with ampicillin or chloramphenicol, nystatin or itraconazole and 40% ethanol. The minimal inhibitory concentrations (MICs) were determined by the presence of an inhibition circle around the soaked disk.

In silico endocrine toxicity

Endocrine toxicity properties of the furfural and HMF derivatives were investigated using the VEGA platform. <https://www.vegahub.eu/portfolio-item/vega-qsar/> The term 'endocrine toxicity' as used in this in

silico study encompasses five models: one estimation of receptor binding affinity (Estrogen Receptor (ER) Relative Binding Affinity model IRFMN) and four predictions of receptor-mediated effects (ER-mediated effect IRFMN/CERAPP, Androgen Receptor (AR)-mediated effect IRFMN/COMPARA, Thyroid Receptor α (TR α) effect NRMEA, and Thyroid Receptor β (TR β) effect NRMEA). For each model, a qualitative prediction (yes/no) and information about the reliability of the prediction (low, moderate, high) are provided.

In vitro endocrine toxicity

To characterize the ER α , AR, TR α and TR β activities, we used already established HELN hER α , U2OS hAR, HG5LN rTR α and HG5LN rTR β reporter cell lines (22–24). Briefly, HELN hER α cells were obtained by stably expression of human ER α in HELN (HeLa ERE-luciferase) cells whereas to characterize the AR activity, U2OS cells were stably co-transfected with a androgen responsive gene, ARE(Rad9)-collagenase-Luc-hygromycin, and a human AR expressing plasmid. Finally, HG5LN rTR α and HG5LN rTR β cells were obtained by stably expression of individual ligand binding domains of rat TR α and TR β fused to GAL4 DNA binding domain in HG5LN (HeLa GAL4REx5 – luciferase) cells. HELN ER α cells were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) without phenol red and 1 g/L glucose and supplemented with 5% stripped fetal bovine serum, 100 units/mL of penicillin, 100 $\mu\text{g/mL}$ of streptomycin, 0.5 $\mu\text{g/mL}$ puromycin and 1 mg/mL geneticin in a 5% CO $_2$ humidified atmosphere at 37°C. HG5LN rTR α and rTR β cells were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) containing phenol red and 1 g/L glucose and supplemented with 5% fetal bovine serum, 100 units/mL of penicillin, 100 $\mu\text{g/mL}$ of streptomycin, 0.5 $\mu\text{g/mL}$ puromycin and 1 mg/mL geneticin in a 5% CO $_2$ humidified atmosphere at 37°C. U2OS hAR cells were cultured in the same medium supplemented with hygromycin 0.25 mg/mL.

To measure endocrine activity, reporter cell lines were seeded at a density of 25,000 cells per well in 96-well white opaque tissue culture plates (Greiner CellStar) in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) without phenol red and 1 g/L glucose and supplemented with 5% stripped fetal bovine serum, 100 units/mL of penicillin, 100 $\mu\text{g/mL}$ of streptomycin (test medium). Compounds to be tested were added 24 h later, and cells were incubated at 37°C for 16 h. At the end of the incubation period, culture medium was replaced with test medium containing 0.3 mM luciferin. Luciferase activity was measured for

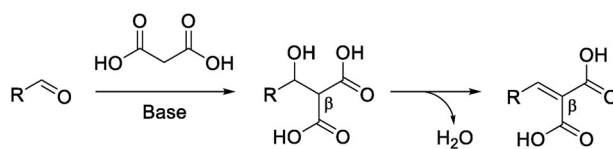
2 s in intact living cells using a MicroBeta Wallac luminometer (PerkinElmer). Tests were performed in quadruplicate in at least three independent experiments. Data were expressed as % of the maximal activity obtained with estradiol (E2) 10 nM (for ER α), methyltrienolone (R1181) 10 nM (for AR) and triiodothyronine (T3) 100 nM (for rTRs). Agonistic activities of the different receptors were tested in presence of increasing concentrations of chemicals (0.3–10 μ M). Antagonistic assays were performed using a concentration approximately 80% of maximal luciferase activity. The antagonistic activity of the chemicals (tested at 0.3–10 μ M) were determined by co-incubation with E2 0.1 nM (for hER α), R1181 0.3 nM (for hAR), T3 1 and 3 nM (for rTR α and rTR β , respectively).

Results

Synthesis

As described in previous studies, the steric hinderance on the β -position, in addition to an extended conjugation throughout the molecules, are necessary to confer good anti-UV properties to organic UV filters. However, beyond the level of absorbance and wavelength coverage, stability is also a critical parameter as molecules need to be active for a certain time in order to fully protect against harmful radiations. α,β -unsaturated esters can undergo a wide range of reactions on their C=C double bond, such as Michael reaction (25) or cycloaddition (26). A way to limit these reactions is to use bulky ester moieties in order to limit the access of potential reagents to the reactive double bond. However, Pallabi et al. (27) recently demonstrated that, even with high hindrance induced by the ester moiety, such molecules can easily undergo [2 + 2] cycloaddition under UV irradiation, reducing their conjugation and consequently, their level of UV protection through time. To avoid this, several new *p*-hydroxycinnamic derivatives were synthesized by introducing a second substituent on the β position (19), providing increased stability in result of the higher steric hinderance around the double bond. The Knoevenagel condensation, which consists in two key steps in the presence of a carbonyl group and an active hydrogen component, offers an easy access to such structures with a second substituents on the β position.

As depicted in Scheme 2, the Knoevenagel condensation results in α,β -unsaturated carboxylic acids that can, under specific conditions, undergo a decarboxylation (i.e. Doebner modification). Fortunately, this side reaction can be avoided by carefully controlling the reaction conditions. For instance, short reaction time



Scheme 2. General steps for the Knoevenagel condensation.

and low temperature both play a favorable role to prevent this decarboxylation. To limit such decarboxylation, the Knoevenagel condensation of furfural/HMF and malonic acid derivatives was performed with the conditions recently described by Rioux et al. (19) to obtain *p*-hydroxycinnamic diacids, i.e. by using ethanol and L-proline as solvent and catalyst, respectively – with moderate heating and low reaction time. Thanks to this sustainable methodology, a library of 20 molecules based on furfural and HMF, presenting variations on the ester moiety was synthesized (Table 1). All the chemical structures were confirmed by ¹H- & ¹³C-NMR (spectra and NMR details are available in ESI pages S8–S27 and pages S2–S7, respectively) and HRMS analyses.

UV properties and photostability

In order to evaluate the UV properties of the synthesized furfural and HMF derivatives, their UV-Vis spectra were recorded and compared to that of octinoxate in regard of their maximum absorbance and wavelength coverage. Several phenolic-based alternatives for UV-B filters have already been studied (15,16,17,21) and, while they provide interesting results, their coverage does

Table 1. Furfural and HMF derivatives obtained through the Knoevenagel reaction.

N°	R ₁	R ₂	R ₃	Yield (%)
1	H	OH	OH	83
2	H	OH	OCH ₂ CH ₃	63
3	H	OH	O(CH ₂) ₆ CH ₃	72
4	H	OH	OCH ₂ CH(CH ₂ CH ₃)(CH ₂) ₃ CH ₃	73
5	H	OH	OCH(CH ₃) ₃	60
6	H	OH	[O(CH ₂) ₂ N(CH ₃) ₃] ⁺ , Cl ⁻	12
7	H	OH	OC ₆ H ₄ CH ₃	62
8	H	CH ₃	CH ₃	17
9	H	OH	NH(CH ₂) ₃ CH ₃	33
10	H	OH	NHC ₆ H ₆	10
11	CH ₂ OH	OH	OH	50
12	CH ₂ OH	OH	OCH ₂ CH ₃	35
13	CH ₂ OH	OH	O(CH ₂) ₆ CH ₃	34
14	CH ₂ OH	OH	OCH ₂ CH(CH ₂ CH ₃)(CH ₂) ₃ CH ₃	34
15	CH ₂ OH	OH	OCH(CH ₃) ₃	54
16	CH ₂ OH	OH	[O(CH ₂) ₂ N(CH ₃) ₃] ⁺ , Cl ⁻	11
17	CH ₂ OH	OH	OC ₆ H ₄ CH ₃	32
18	CH ₂ OH	CH ₃	CH ₃	37
19	CH ₂ OH	OH	NH(CH ₂) ₃ CH ₃	15
20	CH ₂ OH	OH	NHC ₆ H ₆	44

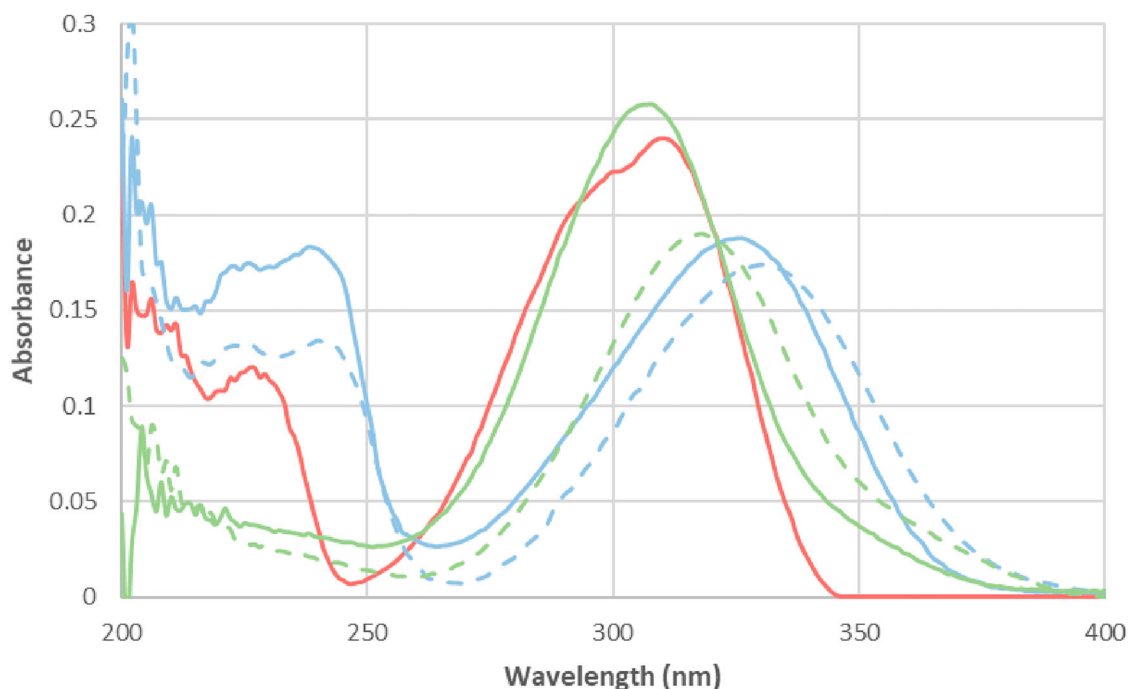


Figure 1. UV-vis spectra ($10 \mu\text{mol.L}^{-1}$ in ethanol) of octinoxate (red), sinapic acid (full blue), sinapic diacid (dotted blue), furfural diacid **1** (full green) and HMF diacid **13** (dotted green).

not entirely fit that of octinoxate. As modification on the ester moiety proved to be of minor impact on the wavelength coverage (15), we first focused on the conjugation system, switching from phenols to furfural and HMF. Looking at the resulting UV-Vis spectra (Figure 1), modification of the conjugation system provided the desired hypsochromic shift. Both sinapic acid and sinapic diacid exhibited maximum absorption wavelength out of sync from the one of octinoxate (309 nm), at 325 and 330 nm, respectively, while the HMF based diacid (**11**) did not show a significant shift with a maximum absorption wavelength at 317 nm. The furfural derivative (**1**) offered a perfect fit to the octinoxate spectrum in the UV-B region, with similar level of absorbance and a maximum absorption wavelength at 308 nm. Once the modification of the conjugation system proved to be efficient to better fit the spectrum of octinoxate, emphasis was placed on the substitution on the ester moiety in an attempt to modulate the absorbance level.

Focusing on furfural derivatives (**1–12**) in Figure 2, the introduction of alkanes on the ester moiety (**2–5**) provided similar results than furfural diacid (**1**), the only difference being a small decrease in absorbance. Alkanes were quickly replaced by more complex substituents. The presence of a choline group (**6**) decreased further the level of absorbance, also shifting the spectrum back toward the UV-A region, while median results occurred with the use of a bulkier substituent

such as *o*-cresol (**7**) compared to the others. To go further, a ketone (**8**) and two amide derivatives (**9, 10**) were synthesized to identify whether the nature of the carbonyl function has a more significant impact on UV absorption than the previous modification performed on the ester moiety. Replacement of the two carbonyl functions by methyl ketones (**8**) led to a bathochromic shift and a small gain in absorbance compared to the initial diacid (**1**). Switching from ester to amide provided two different behaviors in regard to the chosen substituent. With an aliphatic chain (**9**), results were similar to the ones obtained with the esters, while the use of an aromatic amide (**10**) provided the highest bathochromic shift of all the furfural series (**2–12**), both with similar level of absorbance.

Contrary to the first series, HMF-based compounds (**11–20**) all exhibited similar or higher level of absorbance than their initial diacid (**11**), as presented in Figure 3. Very few of the synthesized molecules presented a bathochromic shift in addition to the one already caused by the HMF conjugation system. As already specified for the furfural derivatives, the use of choline as substituent (**16**) and a switch from ester to ketone (**18**) provided the same results in the HMF series. It is noteworthy to mention that the molecules bearing a phenylamide substituent (**20**), leading to the largest shift in wavelength coverage in its furfural version (**10**), did not induce any shift while associated with HMF. Beyond the individual impact of the

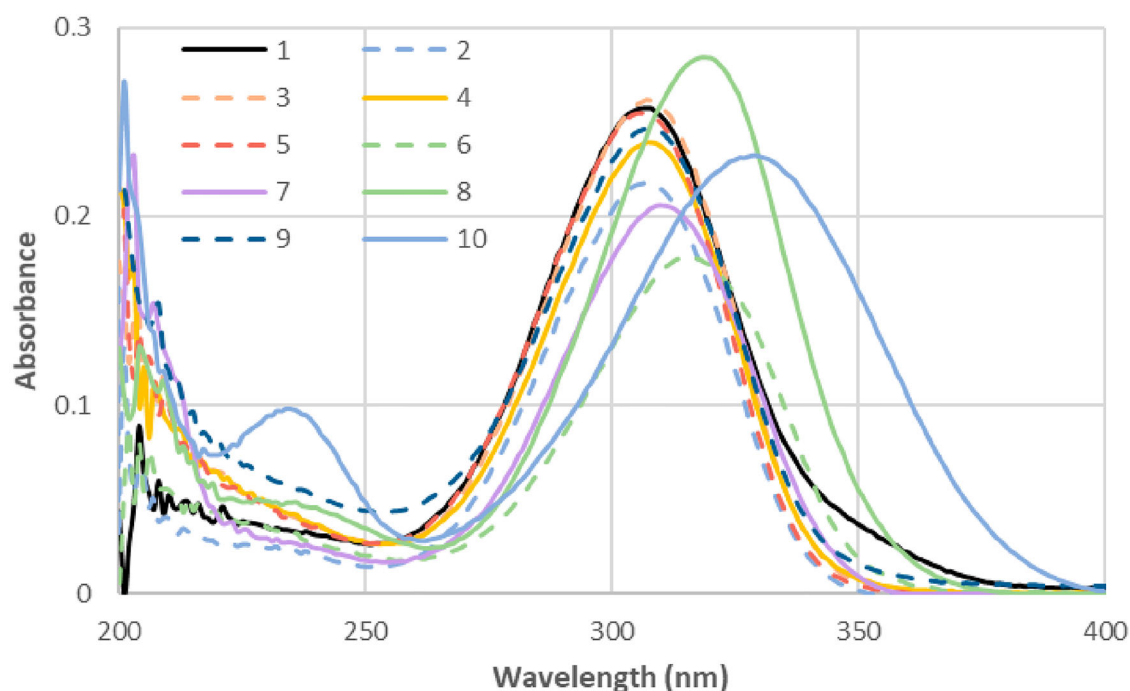


Figure 2. UV-vis spectra ($10 \mu\text{mol.L}^{-1}$ in ethanol) of the furfural series (**1–10**) with furfural diacid **1** (black), furfural ethyl acid **2** (dotted light blue), furfural heptyl acid **3** (dotted orange), furfural 2-ethylhexylacid **4** (yellow), furfural tert-butyl acid **5** (dotted red), furfural choline acid **6** (dotted green), furfural o-cresol acid **7** (purple), furfural diketone **8** (green), furfural butylamide acid **9** (dotted dark blue) and furfural benzylamide acid **10** (light blue).

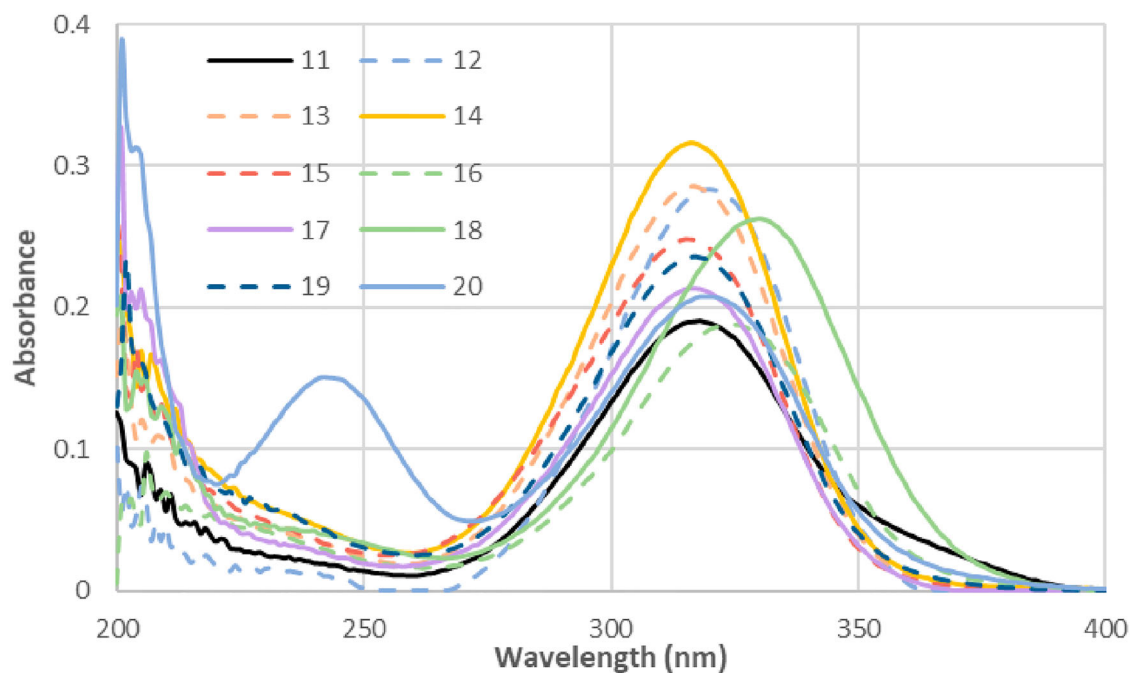


Figure 3. UV-vis spectra ($10 \mu\text{mol.L}^{-1}$ in ethanol) of the HMF series (**11–20**) with HMF diacid **11** (black), HMF ethyl acid **12** (dotted light blue), HMF heptyl acid **13** (dotted orange), HMF 2-ethylhexylacid **14** (yellow), HMF tert-butylacid **15** (dotted red), HMF choline acid **16** (dotted green), HMF o-cresol acid **17** (purple), HMF diketone **18** (green), HMF butylamide acid **19** (dotted dark blue) and HMF benzylamide acid **20** (light blue).

conjugation system and the carbonyl substitution on UV properties, their synergy can also have a significant impact on such properties. In addition to the

wavelength coverage and the absorbance intensity, synthesized molecules also need to show stability upon UV irradiation (i.e. loss of absorbance over time) to be

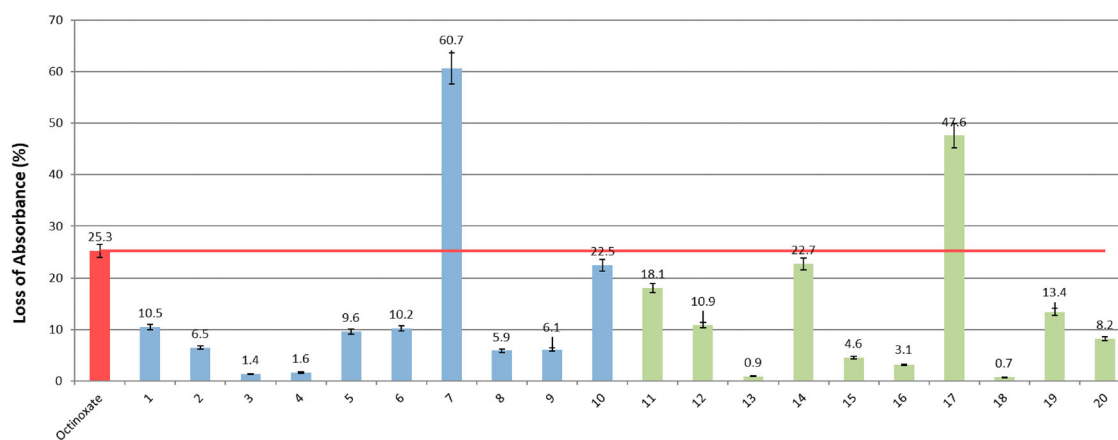


Figure 4. Loss of Absorbance (in percentage) of solutions ($C = 10 \mu\text{mol.L}^{-1}$) after UV irradiation ($\lambda = 300 \text{ nm}$, $P = 8.32 \text{ W/m}^2$, $T = 35^\circ\text{C}$, stirring) for 1 h: reference (red), furfural series (blue, **1–10**) and HMF series (green, **11–20**). Each value is presented with the mean \pm standard error.

considered as potential alternatives for current UV filters. To study the behavior of our molecules, solutions in ethanol ($C = 10 \mu\text{mol.L}^{-1}$) were irradiated for 1 h under UV light ($\lambda = 300 \text{ nm}$, $P = 8.32 \text{ W/m}^2$, stirring, $T = 35^\circ\text{C}$). The comparison of absorbance between $t = 0$ and $t = 1 \text{ h}$ provided the percentage of absorbance lost, which is presented in Figure 4 and in the ESI (pages S28–S29). In such conditions, octinoxate lost 25.3% of its initial absorbance, this value was set as the threshold to evaluate the photostability of furfural and HMF derivatives. At first glance, although furfural diacid (**1**) was more stable than HMF diacid (**11**), the HMF series (**11–20**) seemed to be more photostable in a general way. All molecules showed a lower loss of absorbance compared to that of octinoxate, except for the two compounds bearing *o*-cresol as substituent (**7** and **17**). Phenolic esters ($\text{R-CO}_2\text{-Ar}$) being known to be easily hydrolyzed (28), under such drastic conditions with continuous UV irradiations, one can assume the phenolic ester function is prone to photodegradation, leading to the high loss of absorbance ($> 45\%$). With the exception of four molecules (**7**, **10**, **14** and **17**), the functionalization of the initials diacids (**1**, **11**) to increase the steric hinderance surrounding the β position led to a lower loss of absorbance (i.e. better photostability). Just as for the wavelength coverage and the absorbance intensity, the synergy between the conjugation system and the carbonyl substitution also has a significant impact on the photostability. Looking at the amide derivatives (**9–10**, **19–20**), aliphatic ester (**9**) proved to be more stable than the phenolic one (**10**) when combined with the furfural ring, this result being inverted when switching to an HMF ring (**19**, **20**). The same trend could be observed with the derivatives bearing a *tert*-butyl (**5**, **15**) and a choline (**6**, **16**) substituent.

The above structure–activity relationships (SARs) confirmed the previous observations concerning anti-UV properties: conjugation induced by the aromatic ring and extended throughout the backbone of the molecule, coupled with steric hinderance around the β position, are key components to provide compounds with good potential as UV filters. Moreover, UV properties (i.e. wavelength coverage, absorbance intensity and photostability) can be fine-tuned by carefully manipulating the synergy between the conjugation system and the carbonyl substitution.

Antimicrobial activity

To prevent the development of pathogens, such as bacteria, leading to degradation of the sunscreens' organoleptic properties (e.g. aspect, sight, smell, touch), preservatives are an important part of cosmetic formulations. For a long time, parabens have been widely used in this role. However, faced with multiple criticisms over the years, their usage has largely diminished (29). In the same way, phenoxyethanol, though still widely used today, is being criticized for its potential health risks (30). Offering new healthy and renewable alternatives to such decried compounds is therefore essential. To assess the potential of the novel furfural- and HMF-derived molecules as preservatives, a pre-screening was carried out on three different strains: *Escherichia coli* (gram-negative), *Bacillus subtilis* (gram-positive) and *Candida albicans* (yeast). Minimum inhibitory concentration (MIC) were determined by using the diffusion method on agar-agar sowed with pure strains, a method allowing to assess the potential activity of hydrophobic molecules (Table 2; ESI pages S30–S34). From a general point of view, the conjugation system mostly

Table 2. MIC values of synthesized compounds (**1–20**) on *Escherichia coli*, *Candida albicans* and *Bacillus subtilis* compared to phenoxyethanol and paraben.

Compound	MIC (wt %)		
	<i>E. coli</i>	<i>C. albicans</i>	<i>B. subtilis</i>
Phenoxyethanol	2.5	2.5	1.25
Ethylparaben	1.25	1.25	2.5
1	1.25	– ^a	1.25
2	1.25	0.625	– ^a
3	0.42	0.830	0.11
4	0.156	0.078	0.31
5	1.25	0.625	1.25
6	– ^a	– ^a	– ^a
7	0.31	0.31	0.625
8	– ^a	– ^a	– ^a
9	– ^a	– ^a	0.31
10	0.31	– ^a	0.31
11	1.25	– ^a	1.25
12	1.25	– ^a	– ^a
13	0.31	0.625	0.156
14	0.625	0.625	0.625
15	1.25	– ^a	1.25
16	– ^a	– ^a	– ^a
17	0.625	0.31	0.31
18	– ^a	– ^a	– ^a
19	– ^a	– ^a	– ^a
20	0.31	– ^a	0.31

^aNo inhibition observed.

had an impact on the *Candida albicans* inhibition, furfural giving better results than HMF, while the best results on all three strains were obtained with aliphatic substituents on the ester moiety. The various substitutions performed on the synthesized compounds enabled a selective efficacy toward the different microorganisms tested, offering the possibility to precisely choose a molecule in relation to the strain of interest. From all the assessed structures, compounds (**3–5**), (**7**), (**13–14**) and (**17**) stand as potential substitutes for the two references, exhibiting antimicrobial activity against all three strains. MICs obtained for those molecules were at least similar, or even better, than that of phenoxyethanol and ethylparaben.

In silico endocrine toxicity

Regarding endocrine toxicity, the potential effects were predicted *in silico* regarding estrogen, androgen and

thyroid receptors for all synthesized molecules as a first screening, some furan derivatives being known for their potential toxicity toward human health (31). Full results can be found in ESI (page S34). All furfural and HMF derivatives (**1–20**) were predicted to exert no binding affinity for ER. Those results, however, were met with low reliability of the predictions and were therefore regarded as inconclusive. On the other hand, predictions on the ER-mediated effects showed no potential of the test compounds to exert effects, with good reliability for most of them. Predictions toward AR also showed no effects by the molecules, with moderate reliability for the vast majority of them. Besides, none of the molecules was predicted to exhibit effects on thyroid receptors, with good reliability for all predictions.

In vitro endocrine toxicity

In order to confirm the predictions obtained from the *in silico* study, some compounds were tested *in vitro* on the same receptors: ER α , AR, TR α and TR β . ER α is controlled by estrogenic hormone 17 β -estradiol (E2) while AR is regulated by natural androgens (i.e. testosterone and dihydrotestosterone) and TR plays critical roles in cardiac and skeletal muscles. To determine the interactions of the synthesized molecules with the different receptors, tests were performed in reporter cell lines in absence (agonist) or presence (antagonist) of the respective receptors' references (E2/ER α , R1881/AR and T3/TR α & TR β). Compounds (**3**) and (**5**) were chosen in regard of their good UV properties (absorbance and photostability) and antimicrobial activity against the three strains tested. To determine the effect of a modification on the aromatic ring, the HMF analogs (**13**) and (**15**) were also studied. Results for the interaction with ER α are presented in Figure 5. All data for other receptors are available in the ESI (pages S35-S36).

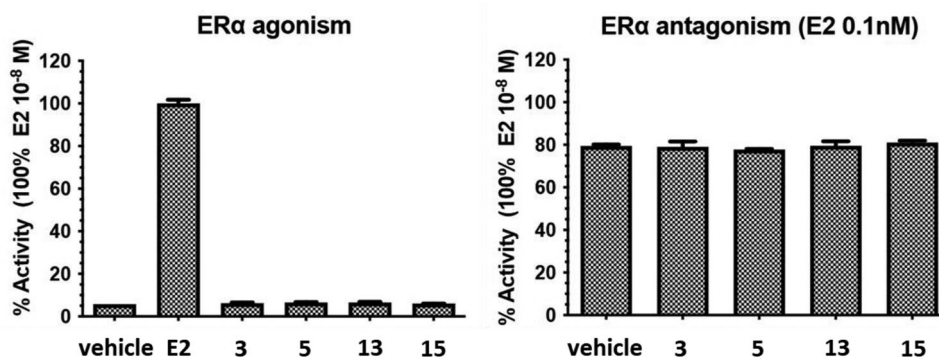


Figure 5. ER α activity for synthesized compounds (**3**), (**5**), (**13**) and (**15**), for agonist and antagonist interactions at 10^{-6} mol.L $^{-1}$.

Endocrine toxicity tests were performed from 3E-07 to 1E-05 M and revealed no agonistic nor antagonistic interactions between the furan derivatives and the selected receptors, confirming *in silico* study results in regard of TR α and TR β . Those experimental data allowed to lift the uncertainty concerning the other receptors (i.e. ER α and AR) as all four tested molecules proved to be innocuous. These preliminary results tend toward a harmlessness of furfural and HMF ester derivatives, leading to consider them as substitute for current decried preservatives and UV-filters still in the market.

Conclusions

The green synthesis of furfural and HMF derivatives, through a Knoevenagel condensation with sustainable conditions, led to new bio-based molecules displaying several properties. The study of their UV properties highlighted their capacity to act as a substitute for octinoxate (UV-B filter reference) with similar wavelength coverage and absorbance while displaying better overall photostability. Some of the synthesized molecules exhibited a secondary bioactivity as preservatives. A study of this activity against three different strains (gram positive, gram negative and yeast) showed that these molecules can be carefully designed to target a specific strain, some being active against all three microorganisms with lower MIC than the two references, phenoxyethanol and ethylparaben. Finally, *in silico* and *in vitro* testing for endocrine disruption were conducted to reveal the absence of interaction with the estrogen, androgen and thyroid receptors, making them promising substitutes to heavily criticized petroleum-based UV filters and preservatives.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Conflicts of interest

F.A., C.P., M.M.M. and B.G. have filed a patent based on the work described here (Patent application no FR2105932).

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