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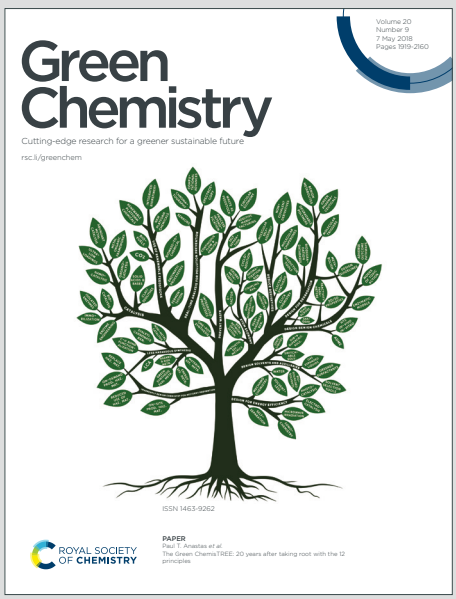

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ARTICLE

Expeditious and sustainable two-step synthesis of sinapoyl-L-malate and analogues: towards non-endocrine disruptive bio-based and water-soluble bioactive compounds

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Faced with the increasing demand from both the cosmetic industries and consumers for bio-based, safe and natural skin products, sinapoyl-L-malate, widely described for its UV protection in plants, appears to be an excellent alternative to substitute chemical filters in sunscreens. Unfortunately, the only synthetic routes described in the literature were not only tedious but also exhibit a strong negative environmental impact, thus seriously limiting the industrialization and commercialization of sinapoyl-L-malate. Herein, a shorter and greener synthetic pathway involving Meldrum's acid opening with unprotected naturally occurring hydroxy-acids and its subsequent Knoevenagel-Doebner condensation with biomass-derived *p*-hydroxybenzaldehydes was designed and developed. This two-step procedure, whom sustainability has been assessed using green metrics (atom economy (AE), process atom economy (PAE), E-factor and LCA), is a great alternative to the already reported procedures and allows the access to sinapoyl-L-malate and several analogs in average to good yield. The study of the anti-UV properties, stability against UV radiation, radical scavenging and antimicrobial activities of the targets revealed attractive properties as photostable UV filters, antioxidants and preservatives. Moreover, the water solubility brought by the free carboxylic acids facilitates the incorporation of these molecules in cosmetic formulations. Finally, their innocuousness toward endocrine disruption was demonstrated.

Introduction

Naturally occurring *p*-hydroxycinnamic acids and their derivatives - such as *p*-coumaric acid, ferulic acid, caffeic acid, sinapic acid, sinapine - represent an abundant and renewable source of phenolic compounds, rich in structural diversity with a wide range of high added value applications[1-5] . For instance, *p*-hydroxycinnamic acids can be used for the preparation of new bio-based and renewable polymers or as additives to provide additional properties to the different polymers (antioxidant, shape memory)[6-8]. In the form of dimers, they can be used as non-endocrine disruptive bisphenol A substitutes[9, 10]. More often, despite their very valuable biological properties, the use of phenolic compounds in the medical or cosmetic sectors is limited due to their very low solubility in water. In Nature, *p*-hydroxycinnamic acid derivatives such as sinapoyl-L-malate or sinapine are readily soluble in water thanks to their free carboxylic acids and ammonium moieties. Sinapoyl-L-malate, well-known for its UV filter properties in plants, has been the subject of multiple studies in order to understand its mechanism of action and identify, by structural analogy, components able to protect just as much from UV irradiation[11-14]. Nevertheless, being present in very small quantities in plants, and its extraction being too complex and economically irrelevant, the use of sinapoyl-L-malate at the industrial scale required the implementation of synthetic pathways. Until now, only two similar multi-step methodologies based on Steglich esterification have been reported in the literature for the synthesis of sinapoyl-L-malate and derivatives[15, 16]. Although they provide the target compounds in average yields, these synthetic methods are tedious to perform, require protection/deprotection sequences and activation using excess amount of coupling agent and toxic solvents, thus generating much waste to dispose of. In 2009, Allais *et al.*[15] described sinapoyl-L-malate production in 30% overall yield using a 6-step Steglich-based procedure. Not only the latter requires at least 26 days to be performed (excluding reaction processing time) due to the first extremely long copper-catalyzed synthesis of *tert*-butyl isourea, but also the latter was used in excess to prepare di-*tert*-butyl-L-malate, thus producing a stoichiometric amount of urea to be disposed of (Figure 1A). Moreover, for the subsequent coupling of sinapic acid with di-*tert*-butyl-L-malate, still through Steglich esterification, the phenol moiety had to be protected via acetylation to prevent its esterification (side reaction).

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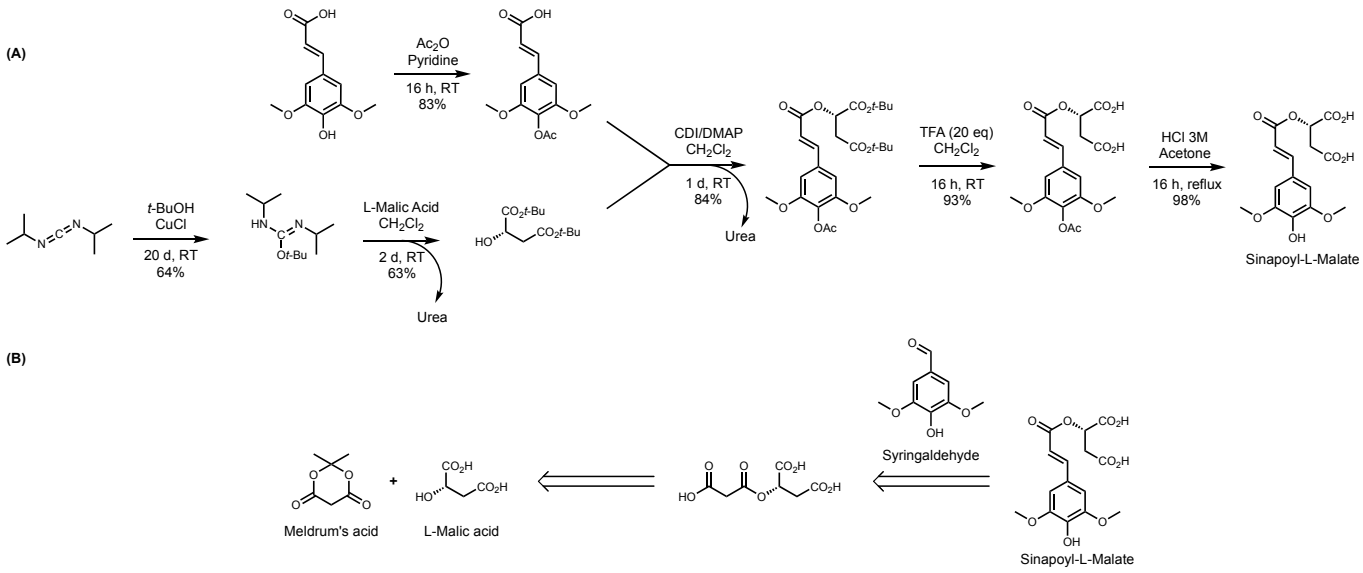

Figure 1: (A) Synthetic pathway published by Allais *et al.*[15]; (B) retrosynthetic pathway proposed herein for sinapoyl-L-malate production.

tert-Butanol and acetate protecting groups were then removed in a two-step sequence involving trifluoroacetic acid (TFA) used in large excess (20 eq), then HCl in acetone. Besides the solvents used for the reactions, several liters of solvents were also necessary (cyclohexane, ethyl acetate, dichloromethane, hexane, pyridine, water) for the five column purifications required to purify every single chemical intermediate. More recently, a patent described a very similar strategy, still based on Steglich coupling[16]. The main differences were (1) the protection of malic acid with *iso*-propanol instead of *tert*-butanol, (2) the *in situ* acetylation of the free phenol of sinapic acid which reduced the synthetic pathway by one step, and (3) the low selective H_2SO_4-mediated cleavage of the protecting groups that also promote the partial hydrolysis of sinapoyl-L-malate into sinapic acid and L-malic acid, thus explaining the very low yield (7%) despite the presence of only one column purification. In this context, with respect to the 12 principles of Green Chemistry, we sought to offer a shorter and greener synthetic pathway to limit the quantities of solvents while avoiding protection/deprotection sequences and the use of coupling agents and catalysts. For this, we have designed and optimized an expeditious two-step procedure towards sinapoyl-L-malate involving Meldrum's acid opening with unprotected naturally occurring L-malic acid and subsequent Knoevenagel-Doebner condensation of the resulting malonate monoester with unprotected biomass-derived *p*-hydroxybenzaldehydes (Figure 1B). Using just one chromatography column, sinapoyl-L-malate and sinapic acid, the reaction by-product which is also a value-added building block, were readily separated and recovered in good yield. This synthesis was then benchmarked against the two previous methods. Several green metrics were evaluated for each process. Unfortunately, the lack of details regarding the patent did not provide enough information to calculate the data[16]. However, we were able to compare the process reported by Allais *et al* and the one described in this study[15]. All these indicators confirmed the significant improvement brought by this novel sinapoyl-L-malate synthetic process (Figure 1B). This methodology has then been extended to different sinapoyl malate analogues starting from other renewable organic acids. Finally, the library of compounds was screened on different targets of biological activities, notably those of interest to cosmetics (*i.e.*, anti-UV, antioxidant, and antibacterial).

Results and Discussion

Synthetic pathway

Contrary to what was already described in the literature to obtain *p*-hydroxycinnamic esters (*i.e.*, esterification of *p*-hydroxycinnamic acids), we chose a different strategy and decided to carry out the opening of Meldrum's acid with malic acid to form the corresponding monoester, and then submit the latter to a Knoevenagel-Doebner condensation with syringaldehyde to access sinapoyl-L-malate (Figure 2). Although it was well-known that the Knoevenagel-Doebner reaction does not require the protection of the phenol,[1,4] the opening of Meldrum's acid in presence of free acid moieties had never been reported in the literature and remained to be explored.

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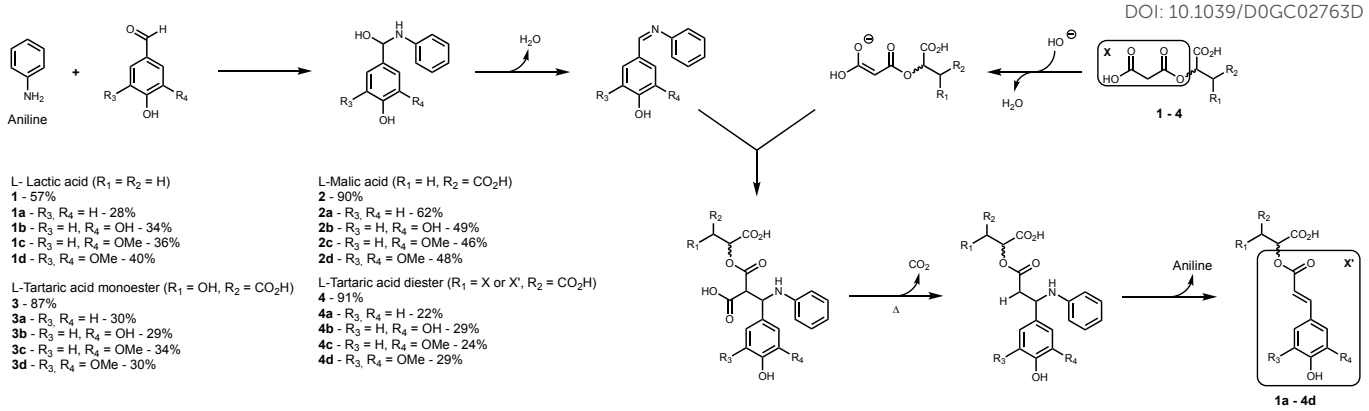

Figure 2: Formation of sinapoyl-L-malate and analogues (**1a**-**4d**) through Knoevenagel-Doebner condensation with various *p*-hydroxybenzaldehydes and malonate derivatives (**1**-**4**)

Table 1: Reaction conditions for the synthesis of malonate ester from Meldrum's (1 eq) and malic acid

Entry	L-Malic acid (eq)	T (°C)	t (h)	Solvent	Mono ester (%)*	Malonic acid (%)*
1	1	95	3	None	74	26
2	1	95	1	None	72	28
3	1.5	95	1	None	73	27
4	1	75	16	THF	90	10

* Obtained by ^{1}H NMR.

This reaction, which relies on the very specific reactivity of Meldrum's acid towards nucleophiles, was first performed without solvent at 95 °C at which Meldrum's acid melts and dissolves the nucleophile, and with strict stoichiometry[17-19] (Table 1, entry 1). Under these conditions, the presence of free acids promoted Meldrum's acid degradation into malonic acid. Indeed, after 3 hours of reaction, 74% of monoester and 26% of malonic acid, resulting from Meldrum's acid degradation, were observed by NMR. To avoid, or at least limit, this side-reaction, we modulated the equivalents of the different reactants, the reaction time and also used a solvent. Reducing reaction time did not affect the percentage of monoester as monoester was obtained in 72% conversion after 1 hour (Table 1, entry 2). An increase in malic acid equivalents did not significantly change the monoester conversion either (Table 1, entry 3). We then tested the reaction in THF at reflux to lower the reaction temperature and limit the thermally-induced degradation of Meldrum's acid. This resulted in a significant increase of the conversion in monoester (90%) and a concomitant decrease of the conversion in malonic acid (Table 1, entry 4). In a sustainability perspective, despite the lower conversion in monoester, it would be preferable to carry out this step without solvent. However, in order to obtain the best overall yield, conditions in Table 1 entry 4 were chosen as the optimal ones. After completion, the reaction mixture was not purified but directly subjected to the Knoevenagel-Doebner reaction after THF removal under *vacuo*. This method, initially developed and optimized with L-malic acid as nucleophile, has been extended to other organic acids (*i.e.*, L-lactic acid and L-tartaric acid) thus providing access to mono and di-esters analogues (Figure 2).

The Knoevenagel-Doebner reaction has been widely used and described in the literature to access various *p*-hydroxycinnamic acids in good yields[20-22]. Conventionally, it is carried out in the presence of pyridine and aniline despite their recognized and decried toxicity[23]. Several eco-friendlier alternatives have recently been described such as the use of ionic liquid to substitute pyridine[24-26], microwave activation to reduce the reaction time[21, 27] or the use of renewable amino-acid to replace aniline[28,29]. However, some of these new methods are difficult to upscale industrially. Recently, Peyrot *et al.* have proposed a more eco-responsible and industrially transposable access to hydroxycinnamic acids requiring ethanol as solvent and proline as catalyst[4]. We therefore tested the classic Knoevenagel-Doebner conditions (Table 2, entry 1) as well as that reported by Peyrot *et al.* (Table 2, entry 2). The classic Knoevenagel conditions (60 °C, 16 h) starting from syringaldehyde and the 90/10 monoester and malonic acid crude mixture from Table 1, Entry 4 led to sinapoyl-L-malate and sinapic acid in 66% and 17% conversions, respectively. In presence of ethanol and proline, the ratio was reversed, leading to sinapoyl-L-malate and sinapic acid in 16% and 35% conversions, respectively. The majority of the other by-products (49%) correspond to a total – or partial – esterification of L-malic acid leading to mono/di-ethyl malonate of sinapic acid, most likely due to the proline's acid moiety that catalyses the esterification. In order to avoid it, the Knoevenagel-Doebner reaction was carried out in DMF (Table 2, entry 3). Unfortunately, although it resulted in a decrease in the conversion of these by-products, the conversion in sinapoyl-L-malate was not improved, worst yet, that of sinapic acid was promoted. Despite its lesser eco-friendly nature compared to ethanol/proline, pyridine/aniline was selected as being the optimal conditions.

Table 2: Reaction conditions for the Knoevenagel-Doebner reaction (reaction time = 16 h)

Entry	Solvent	Catalyst	T (°C)	SM (%)*	SA (%)*	Other (%)*
1	Pyridine	Aniline	60	66	17	17
2	Ethanol	Proline	95	16	35	49
3	DMF	Proline	95	16	67	17

* Obtained by ^{1}H NMR.

This two-step synthetic methodology was then implemented to various *p*-hydroxybenzaldehydes (vanillin, syringaldehyde, 3,4-dihydroxybenzaldehyde and *p*-hydroxybenzaldehyde) as well as different mono-/di-esters deriving from L-lactic and L-tartatric acids (Compounds **1**-**4**) to constitute a library of 16 compounds isolated in acceptable yields (Figure 4, **1a-d, 2a-d, 3a-d & 4a-d**). It is worth mentioning that the majority of the compounds have been synthesized up to this point by tedious synthesis routes leading to low yields[15,30,31] whereas some have just been identified in plant extracts[32,33]. To assess the sustainability of the synthetic pathway proposed herein, we used several indicators called green metrics[34], which evaluate the environmental impact of the process as well as its effectiveness[35]. We calculated the Atom Economy (AE) and the Process Atom Economy (PAE), to determine how much of starting material was directly incorporated to the final product. The Environmental Factor (E-Factor) allowed to determine the quantity of all inputs and by-products of the process to synthesize 1 g of targeted compound (*i.e.*, sinapoyl-L-malate)[36]. Details on the calculations are available in ESI. As presented in Table 3, the two-step synthesis described in this paper allowed a significant improvement of the Green Metrics compared to the process of Allais *et al*., with an increase for both the AE and the PAE, indicating that more of the starting material was directly transferred into the product. As for the E-Factor, the value was reduced 12-fold, the lower number of steps of our synthetic pathway compared to the one of Allais *et al*. resulted to a lesser use of inputs and a lower generation of by-products. All the green metrics calculated make it possible to confirm the considerable progress in terms of sustainability for the sinapoyl-L-malate and analogues production.

Table 3: Green metrics comparison between the synthetic pathway of Allais *et al.*[15] and the one proposed herein

Green Metrics	*Allais et al.*	*Peyrot et al.*
Atom Economy (%)	29.4	73.9
Process Atom Economy (%)	0.5	36
E-factor (g/g of product)	2809	230

In addition to the green metrics, the Life Cycle Assessment (LCA) makes possible to study in detail the environmental impact of each different reagents involved in the synthetic process[37]. The analysis was carried out for compound **2c** production, following a standard method described in the ISO 14040-44 norm. Technical and market information were obtained from experimental and bibliographic databases and then processed using openLCA software and EcoInvent 3.4 database. The environmental impacts were calculated with CML 2001 (from the University of Leiden)[38]. Four benchmarks were selected and the results are shown in Figure 3.

Regarding the indicators, while the ecotoxicity (human, aquatic and terrestrial) values were very low, the impact on climate was non-negligible as the production of 1 g of **2c** led to the release of 2.05 kg CO_2-eq. However, as seen in Figure 3, all the indicators were strongly impacted by the presence of malic acid in the process (parameters details are available in ESI). Indeed, currently, malic acid is mainly produced from fossil resources, in particular by chemical oxidation of *n*-butane[39]. Nevertheless, malic acid is a naturally occurring organic acid that can be biotechnologically produced[40].

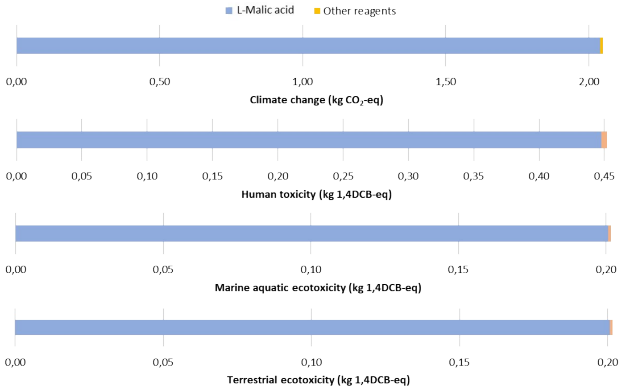

Figure 3: Environmental impact of the reagents for **2c** synthesis

Several approaches are already described in the literature, such as production by fermentation processes (bacteria and fungus) from renewable sugar.[41,42] The use of a bio-sourced malic acid would certainly make possible to provide an even more optimal process and would significantly reduce the CO_2 generated as well as the global toxicity. Although the synthetic steps have been largely optimized for the production of this type of compound, dependence on fossil resources still remains a limitation in terms of environmental impact reduction. Overall, this straightforward and sustainable two-step synthetic route leading to satisfactory yields opens the door to the use of these molecules in various fields such as cosmetic applications.

Cosmetic activities

While the main limitation of phenolic compounds in cosmetic or pharmaceutical applications remains their low solubility in water, the synthetic route presented above allows access to compounds with free carboxylic acid(s) and phenol(s), making them much more hydrophilic. This is particularly the case for malates and mono-tartrates where the presence of two acid groups topples the hydrophilic/hydrophobic balance in favour of solubility in water, allowing the evaluation of numerous biological properties such as the antioxidant power, the UV absorption and especially the antibacterial activity of the compounds.

1. UV filters and stability

Sinapoyl-L-malate is a molecule that plants produce to protect themselves from the negative effects of UVs. Several studies have highlighted its action mechanism and structure-activity relationships, which allowed to determine the key structural features for a good protection, such as the α,β-unsaturated ester and the presence of a sterically hindered electron rich ester moiety, favouring a *cis-trans* isomerisation upon UV exposure[12-14]. However, until now, the lack of efficient and straightforward synthetic procedure to access this molecule prevented to produce and study sinapoyl-L-malate analogues.

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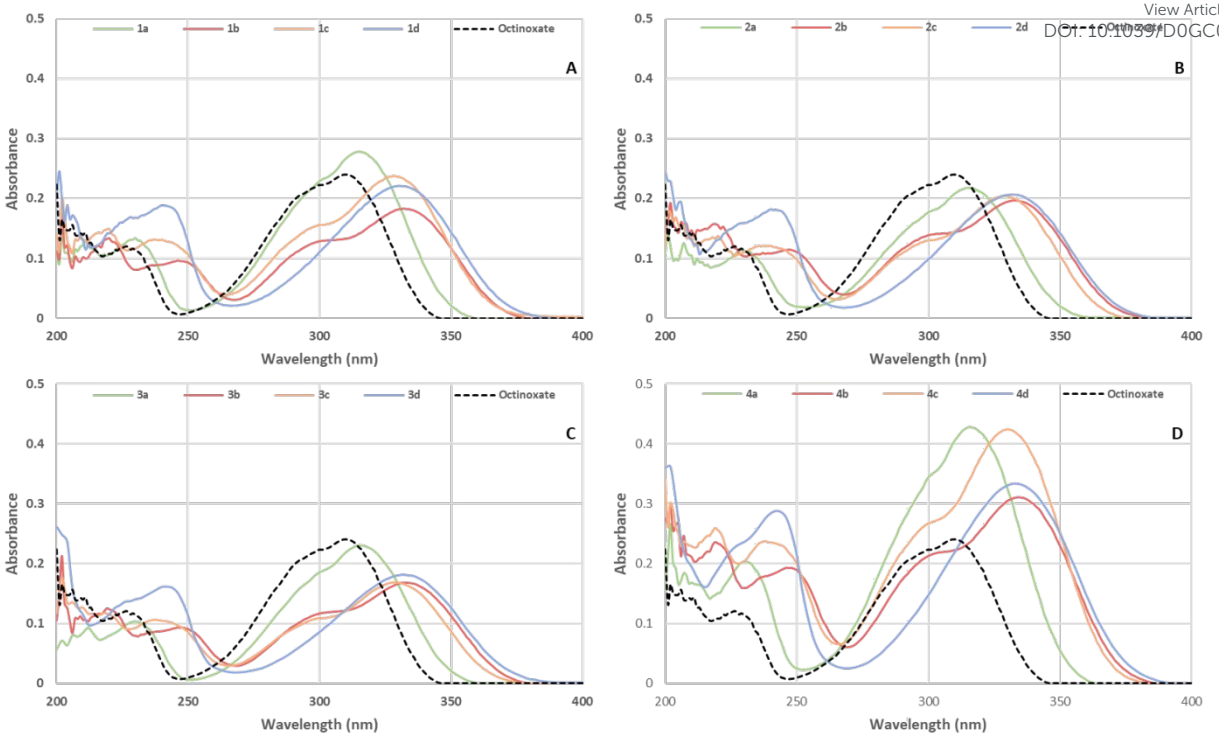

Figure 4: UV spectra (10 µM in ethanol) of synthesized p-hydroxycinnamic esters (A: lactate series, B: malate series, C: tartrate monomer series, and D: tartrate dimer series) compared with octinoxate

The library being now available, the absorbance spectra of each of the molecules in ethanol at 10 µM has been determined. The spectra obtained are grouped by series in Figure 4. The compounds having mainly absorbance properties in the UV-B region, we have chosen to benchmark them against octinoxate, one of the most common UV-B solar filter in sunscreens. When looking globally at sinapoyl-L-malate and its analogues (**1a-d**, coumaric structure provided an hypsochromic shift, allowing the corresponding compounds (*i.e.*, **1a**, **2a** & **3a**) to better fit the spectrum of octinoxate, which is also a *p*-hydroxycinnamic ester (*i.e.*, coumarate ester). Only coumaroyl-L-lactate **1a** provided an UV spectrum perfectly fitting that of octinoxate with a better absorbance compared to the others monophenols (*i.e.*, lactate, malate and tartrate monomer series). The use of L-tartaric acid allowed the obtention of dimers (**4a-d**), leading to an expected significant increase of absorbance levels (Figure 4D). While the wavelength coverage was not impacted by the introduction of a second phenol moiety, the increase of absorbance allowed the coumaric (**4a**), caffeic (**4b**) and ferulic (**4c**) based dimer to level with octinoxate in the UV-B region (280-315 nm) while covering part of the UV-A region (315-400 nm). The sinapic-based dimer (**4d**) displayed a similar profile only with a narrower coverage in the UV-B region. Overall, all four coumaric based compounds (**1a**, **2a**, **3a** and **4a**) stood out with a coverage area in line with octinoxate regardless of the ester moiety. Without exceeding that of octinoxate, the intensity of absorbance for most of the synthesized monomeric compounds were similar. The use of dimers allowed to significantly improve those properties, both in intensity and wavelength coverage. Although absorbance is of great importance, a good sun filter **2a-d** & **3a-d**), the nature of the ester moiety (*i.e.*, lactate, malate or tartrate) did not seem to significantly impact the UV properties, as all compounds showed similar wavelength coverage throughout the series with absorbance around 0.2, with **1a** being the only exception nearing a value of 0.3 (Figure 4A, B & C). While the presence of caffeic, ferulic or sinapic moieties resulted in a similar wavelength coverage, the use of a must also prove stable towards UV in order to ensure its effectiveness. The stability of each of the compounds was therefore evaluated. For this, a solution at 10 µM was irradiated under UV (λ = 300 nm, P = 8.32 W/m², T = 35 °C, 1 hour). The loss of absorbance (LoA) was obtained by comparing the resulting absorbance after one hour of irradiation and the initial one. The values are gathered in Figure 5.

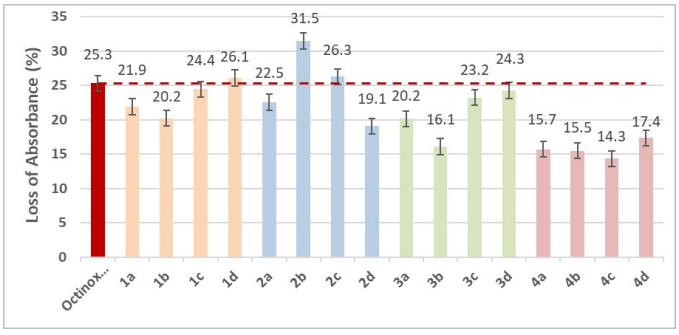

Figure 5: Loss of absorbance for synthesized compounds (orange: lactate series, blue: malate series, green: tartrate monomer series and red: tartrate dimer series) after 1 h of UV irradiations, compared to octinoxate (concentration = 10 µM in ethanol)

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Under these irradiation conditions, octinoxate showing a 25% LoA, the acceptable LoA limit was set at 25% in order to select the compounds that can compete, or even better, outperform octinoxate. Regarding the lactate series, the four molecules exhibited comparable LoA, really close to that of octinoxate with values between 20 and 26% (Figure 5, compound **1a-d**). The malate series was less homogeneous as compounds **2a** and **2d** were below the limit (22.5 and 19.1% LoA, respectively), the compound **2c** (26.3% LoA) flirted with the acceptable limit whereas **2b** was clearly more impacted by UV irradiation (31.5% LoA). The tartrate series was very satisfying as all of the compounds had a LoA lower than that of octinoxate. Values very close to 25% were observed for the tartrate monomer series (Figure 5, compound **3a-d**) while the dimers were clearly more stable with an average LoA around 15% (Figure 5, compounds **4a-d**). From a global point of view, the majority of the compounds of the library were at least comparable, or even more stable, than octinoxate in terms of stability. If one considers both the absorbance intensity and the stability, coumaric analogues matched with the reference in terms of coverage area and stability. However, the tartrate dimers series remained the most interesting as they have both greater absorbance than the reference, wider wavelength coverage, and a lower loss of absorbance. The UV properties of the synthesized molecules described in this study lead sinapoyl-L-malate analogues to be considered as potential candidates to replace the highly criticized octinoxate in cosmetic formulations.

2. Antioxidant activity

Present in the majority of cosmetic products, antioxidants allow both the stabilization of formulations and the protection of the skin against damages linked to free radical formation[43-45]. Reactive Oxygen Species (ROS) are formed by biochemical reactions, within the cell, under the action of UV, ozone or stress[46,47]. Their high reactivity induces irreversible cellular damages which can lead to skin aging acceleration, inflammation, oxidative stress or even carcinogenesis[48,49]. It is therefore essential to add antioxidant compounds to cosmetic formulations to prevent all of the aforementioned risks. For this, several antioxidants are conventionally used, such as BHA (butylated hydroxyanisol), BHT (butylated hydroxytoluene) or Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which are strongly criticized for their probable endocrine and carcinogenic effects[50-52]. The withdrawal of these compounds is imminent and pushes the cosmetic industries to rethink their formulations. The use of polyvalent molecules, which can be at the same time UV filter, antioxidants or even more, is therefore a major asset to limit the number of compounds present in the formulations while retaining biological properties. The antiradical properties of the molecules produced above were assessed and benchmarked against the EC_{50} values of the references aforementioned. The EC_{50} value (half-maximum effective concentration) was defined as the amount (in nmol) of antiradical compound required to reduce 50% of DPPH free radicals. Results are summarized in Figure 6. From a general point of view, independently of the series, and as expected, coumaric based molecules did not exhibit antioxidant activity. A global trend could be seen in the four series, with better activities for caffeic, then sinapic, and finally ferulic based molecules (caffeic > sinapic > ferulic >>> coumaric). The nature of the ester had little impact on the antioxidant activity with, however, better activities of the dimers (**4a-d**) mostly due to the presence of two free phenols on those molecules. All compounds presenting antiradical activities with a caffeic or sinapic core proved competitive with the references, while the use of a ferulic base allowed only to challenge the properties of BHT. Only compounds **4b**, **4c** and **4d** exhibited EC_{50} values lower than all of the references, which made them promising bio-based substitutes. In addition, as described above, they also possess significant UV filter properties as well as good photostability. The dual activity of these molecules makes them the compounds of choice for cosmetic formulations, especially sunscreen lotions.

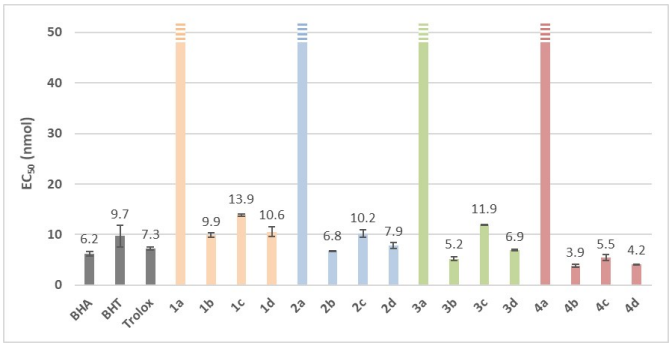

Figure 6: EC_{50} of synthesized compounds (orange: lactate series, blue: malate series, green: tartrate monomer series and red: tartrate dimer series) for DPPH (2,2-diphenyl-1-picrylhydrazyl) inhibition in ethanol.

3. Antibacterial activity

Preservatives are also part of the additives commonly used in cosmetic formulations to prevent the development of pathogens such as bacteria. For this, parabens have been used for a long time. Nevertheless, in recent years, their use has largely diminished in the face of multiple criticisms. Phenoxyethanol, still widely used today, tends to be more and more criticized in view of its probable multiple health risks. It is therefore essential to offer new healthy and renewable alternatives. In order to pre-screen the conservative activities of our molecules, we carried out a 24 hours *Escherichia coli* growth inhibition test in the presence of a concentration range for each water-soluble molecule. The growth of bacteria was monitored by absorbance spectroscopy and the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined. The MIC and MBC for each compound are reported in Table 4 and can be benchmarked against that of phenoxyethanol. Despite the presence of free acids in the compounds, some of them proved to be not soluble in the bacteria culture medium (LB broth). Malate and tartrate monomer series were tested, as well as compounds **1b** and **4b**, the caffeic moiety on the molecules

allowing a better water solubility than the others of their respective series.

Table 4: MIC and MBC values of synthesized compounds on *Escherichia coli* compared to phenoxyethanol.

Entry	MIC (% w)	MBC (% w)
Phenoxyethanol	0.31	0.63
1a	*	*
1b	0.31	0.63
1c	*	*
1d	*	*
2a	0.31	0.63
2b	0.31	0.63
2c	0.31	0.63
2d	0.63	1.25
3a	0.31	0.63
3b	0.31	0.63
3c	0.31	0.63
3d	0.31	0.63
4a	*	*
4b	0.31	0.63
4c	*	*
4d	*	*

*Not soluble at 6 or 3%w in LB Broth

Results were generally comparable. MIC values were found between 0.63 and 0.31%w and MBC values between 0.63 and 1.25%w, and competitive to those obtained for phenoxyethanol on *E. Coli*, which is currently used as 0.32%w in cosmetic formulation. These compounds are thus potential bio-based alternatives to phenoxyethanol, which complement the range of biological activities of the compounds presented here.

4. Endocrine disruption

Most of the aforementioned commercial references (octinoxate, BHT, parabens) are strongly criticized for their endocrine disrupting effects53,54. In order to replace these substances, one must offer healthy alternatives for human health. The absence of interaction between our analogues with androgen receptor (AR), pregnane X receptor (PXR) and estrogen receptor α (ERα) was assessed (details are available in ESI). AR is regulated by interaction with natural androgens like testosterone and dihydrotestosterone while ERα is controlled by the estrogenic hormone 17β-estradiol (E2). Finally, PXR takes part to the xenobiotic receptor family. Agonist and antagonist tests were performed in reporter cells lines respectively in absence or presence of respective receptors reference molecules (E2/ERα, R1881/AR and SR-12813/PXR). Based on previous work, modification on the p-hydroxycinnamic core (sinapic, caffeic, ferulic or coumaric) did not present any endocrine disrupting activity13,55,56. We have therefore chosen to look at the ester nature impact on the nuclear receptor interactions. We fixed the sinapic core and thus test the whole series with four different esters (1d, 2d, 3d, 4d). Previous UV properties were evaluated at 1E-05 M and endocrine tests were performed from 3E-07 to 3E-05 M, then we opted to look at the concentration needed to exhibit the previous UV properties (i.e., 1E-05 M). Results are presented in Figure 7.

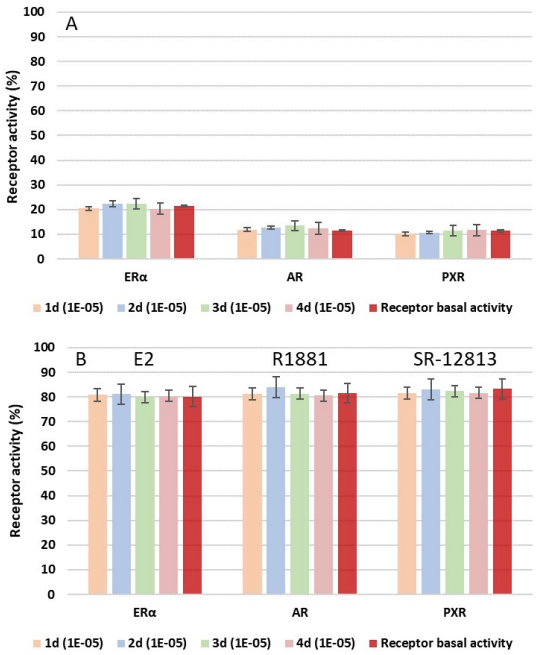

Figure 7: Receptor activities (%) of estrogen receptor α (ERα), androgen receptor (AR), and pregnane X receptor (PXR) for synthesized compounds (A: agonist and B: antagonist interactions) at 10^{-5} mol.L^{-1}.

Part A shows the agonist evaluation. The four molecules did not show agonistic potential on ERα, AR and PXR. The same approach was performed for antagonist study where the selected molecules were put in competition with the reference ligand for each receptor. Figure 7B shows specific recognition of the ligand with respect to its receptor despite the presence of the tested molecules, confirming the absence of antagonistic activities. These preliminary results confirmed the harmlessness of these new molecules, allowing to seriously consider them as substitute for current petro-sourced preservatives, UV filters, or antioxidant still on the market.

Conclusions

A new sustainable two-step synthetic pathway has been developed to access sinapoyl-L-malate and analogues without the need of protection/deprotection sequences nor coupling agents and metal-catalyst. An optimization of the Knoevenagel-Doebner conditions adapted to the free acid presence will be considered subsequently. However, compared to the two reported multistep procedures, this methodology allowed to increase the yield while drastically improving atom economy, process atom economy and E-factor. This synthetic route has been implemented on various naturally occurring mono- and diacids and biomass-derived *p*-hydroxybenzaldehydes to create a library of 16 *p*-hydroxycinnamate derivatives bearing free acids. These molecules, hardly studied until now due to their tedious access, have demonstrated a wide range of biological

activities. The UV filters properties observed for some molecules highlight their capacity to substitute octinoxate (UV-B filter reference) both in terms of absorbance and photostability. Some of them also exhibited promising antioxidant and antibacterial properties, making them polyvalent sustainable UV filters with secondary bioactivities (*i.e.*, preservative, radical scavenging). An in-depth study of the preservative activity on other strains (gram positive, fungus, yeast) will be the subject of a future publication. In addition, the absence of endocrine disruption activity for those molecules make them a promising alternative for a future substitution of the strongly criticized petroleum-based UV filters currently used.

Conflicts of interest

F.A., C.P., M.M.M and R.F. have filed a patent based on the work described here (Patent application n° FR 2001241).

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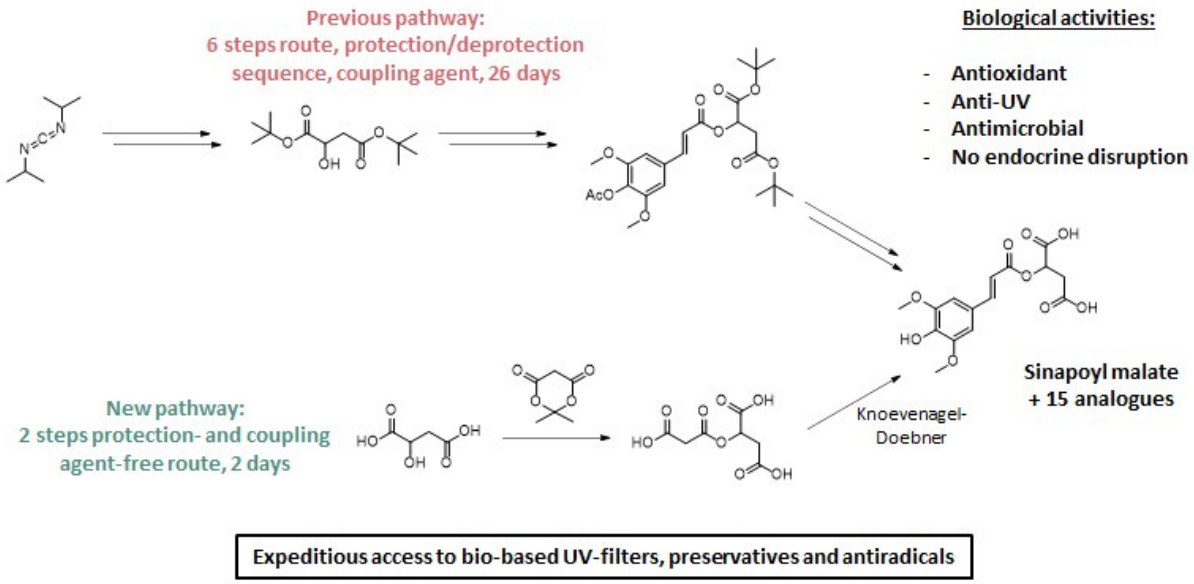

Straightforward and sustainable access to bioactive sinapoyl malate and analogues through Meldrum's acid opening with unprotected hydroxy-acid(s) and subsequent Knoevenagel-Doebner condensation with *p*-hydroxybenzaldehydes.