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# Novel synthesis of 1,2-diaza-1,3-dienes with potential biological activity from cinnamic acids and diazonium salts of anilines†

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Cinnamic acids are an important class of phenolic compounds, which have many beneficial effects on human health but are also interesting synthetic intermediates thanks to the presence of several reactive sites. While studying the reactivity of cinnamic acids with diazonium salts from aromatic amines, an unexpected reactivity has been discovered, leading to the formation of 1,2-diaza-1,3-dienes instead of traditional diazo-coupling products. The new compounds have been fully characterized by mono and bidimensional NMR spectroscopy and mass spectrometry. Preliminary studies on the biological activity of the compounds have been carried out testing both their antibacterial and antitumor activity, leading to promising results.

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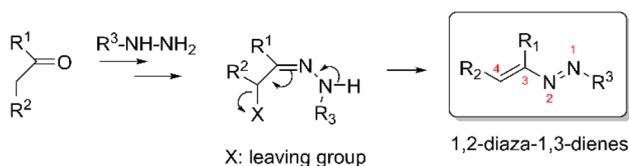
## 1 Introduction

According to the literature, 1,2-diaza-1,3-dienes (DDs) can be considered as hydrazine derivatives of carbonyl compounds, since they are usually prepared by 1,4-elimination of a good leaving group from a hydrazone moiety (Scheme 1).<sup>1</sup> The leaving group can be either present in the starting carbonyl compound or it can be introduced on the hydrazone intermediate,<sup>1,2</sup> but other methods of synthesis can be found in the literature, although they are related to specific reactions and have limited applications.<sup>2</sup> As an example, the treatment of  $\alpha,\beta$ -epoxyketones with hydrazines, initially producing the corresponding  $\alpha,\beta$ -

epoxyhydrazones, then gives  $\alpha$ -hydroxyazoalkenes by opening of the epoxy ring.<sup>2</sup>

The chemical properties of DDs are related to the conjugated heterodiene system and to the type of substituents present.<sup>3</sup> The characteristic of this heterodiene system is represented by the electrophilic property of C-4, promoted by the electron-withdrawing effect of the azo group, leading the 1,2-diaza-1,3-dienes to be good Michael acceptors through a regioselective nucleophilic attack at the terminal carbon atom in the 4-position.<sup>1</sup> Depending on the nucleophile used, which can be either a carbon atom or a hetero atom such as oxygen, nitrogen, sulfur, selenium and phosphorus, highly functionalized hydrazones can be formed, which represent the starting point for many reactions involved in the formation of heterocycle systems.<sup>1</sup> The presence of substituents on both carbon and nitrogen atoms is another factor that influences both the reactivity and stability of the system.<sup>3</sup> In fact, the presence of electron withdrawing groups on one or both of the terminal carbon and nitrogen atoms, improves the stability and enhances the electrophilic character of the diazadienes, favoring the regioselective nucleophilic attack in 4-position but also making them ideal substrates for the “inverse-electron demand” Diels–Alder reaction.<sup>1,3</sup> In general, aryl groups favor the stability but not the reactivity, due to the absence of functionalities which are useful for further reactions, whereas alkyl groups or terminal C=C bond do not favor stability, leading to collateral reactions.<sup>3</sup>

Cinnamic acids are a class of compounds with a phenyl-propanoid skeleton (C6–C3), which are largely present in the plant kingdom as free compounds or in a conjugated form.<sup>4</sup> Coffee beans, cocoa, tea, apples, brassica vegetables, grapes, citrus, pears are particularly rich of cinnamic acids, which are also present as ester conjugates with quinic acid, to give the



Scheme 1 General mechanism of DDs synthesis.

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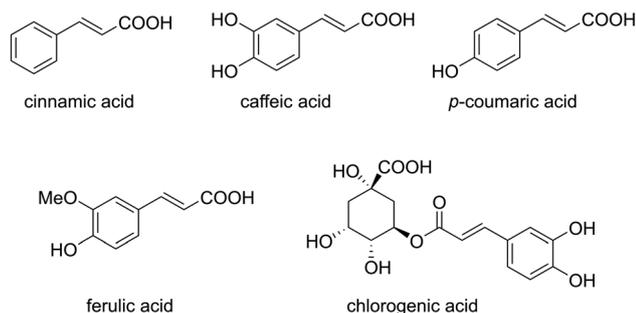
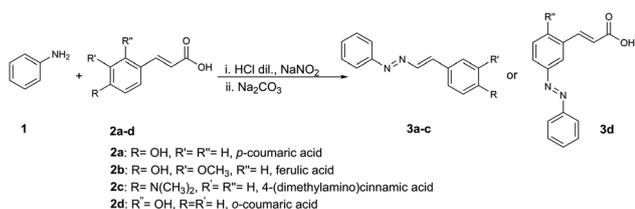


Fig. 1 Structure of the most representative cinnamic acids and chlorogenic acid.

class of compounds known as chlorogenic acids,<sup>4–9</sup> or esters with other acids, sugars or lipids, or forming amides with amino acids (Fig. 1).<sup>4,10</sup> They are also precursors of many natural products such as coumarins, lignans, isoflavonoids, flavonoids, stilbenes, aurones, anthocyanins, spermidines, and tannins,<sup>4</sup> but they can also be synthesized artificially through the Perkin reaction or a Knoevenagel condensation reaction from aromatic aldehydes.<sup>11,12</sup> In the last years, cinnamic acids received a lot of interest due to their biological activities, which include anti-cancer, antioxidant, antimicrobial, as well as applications in diabetes, tuberculosis, malaria and cardiovascular diseases.<sup>4,13</sup> They are also very interesting from the synthetic point of view thanks to the presence of multiple reactive sites, such as the polarized alkenyl moiety and the carboxylic group, being applied in total synthesis of some natural products.<sup>13</sup> Several reactions for these compounds have been reported in the literature, such as electrophilic addition, Michael addition and reactivity of the carboxylic acid moiety, but the most important one is probably the decarboxylative formation of C–C and C–heteroatom bonds.<sup>13</sup>

Due to our interest on different aspects of the chemistry of cinnamic acids, in the attempt of studying the reactivity of diazonium salts from aromatic amines with cinnamic acids, we have observed the unexpected formation of DDs (Scheme 2), thus unveiling a new reaction that could be exploited to develop novel synthetic pathways to these important compounds. The reaction described in the present work for formation of DDs is very advantageous since it takes place under mild reaction conditions, in an aqueous environment, without the need for any catalyst, and with very simple work-up procedures. We report here several examples of reactivity between diazonium salts and different substituted cinnamic acids and a reaction mechanism is proposed. Moreover, as the synthesized DDs



Scheme 2 Reactions of aniline with cinnamic acids.

have, in our opinion, potential biological activities due to the presence of the activated system which makes them reactive towards nucleophilic groups inside biomolecules, we have also carried out preliminary tests on the activities of the novel compounds on cancer cells and on bacteria.

## 2 Results and discussion

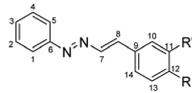
### 2.1 Structural elucidation

As a first attempt to study the reactivity in a diazo-coupling reaction of differently substituted cinnamic acids with diazonium salts from aromatic amines, the reaction between the diazonium salt of aniline **1**, obtained with sodium nitrite and diluted hydrochloric acid, with *p*-coumaric acid **2a** in sodium carbonate, in a stoichiometry of 1:2 respectively, was performed (Scheme 2). Immediately after addition of *p*-coumaric acid **2a** to the diazonium salt solution, the formation of a red precipitate has been observed, which has been isolated and characterized without the need of further purification. Spectroscopic analysis of the compound led to the assignment of the structure of 1,2-diaza-1,3-diene **3a** which was unexpectedly formed. In fact, the presence of the electron donating hydroxyl group in *para* position, with respect to the conjugated double bond of *p*-coumaric acid, was expected to lead the reaction towards a traditional diazo-coupling reaction with the diazonium salt of aniline.

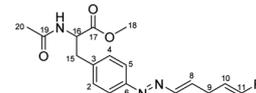
Integration of the <sup>1</sup>H-NMR signals (DMSO-*d*<sub>6</sub>) of the obtained product resulted in the assignment of 11 protons instead of the 10 expected in case of a diazo-coupling reaction, which is compatible with the formation of a diazadiene as **3a**. The assignments of <sup>1</sup>H-NMR and <sup>13</sup>C-NMR signals are reported in Tables 1 and 2. For compound **3a**, the two doublets at 7.64 ppm and 6.85 ppm (H-10/14 and H-11/13 in Table 1), each integrating for 2 protons with a coupling constant of 8.6 Hz, had the same pattern of the corresponding protons in the starting reagent *p*-coumaric acid **2a**, to demonstrate that the formation of a traditional diazo coupling product was not possible, since in that case there should be the loss of one proton replaced by the nitrogen atom of the diazonium salt, and this would completely change the multiplicity and integration of the signals. A completely different chemical shift was observed for the vinyl protons H-7 and H-8 (Table 1), one at 8.02 ppm and the other under the multiplet at 7.73 ppm, with a coupling constant of 13.7 Hz, whereas the coupling constant between these two protons in the starting cinnamic acid is of 16.0 Hz, and the two protons have chemical shifts of 7.48 ppm and 6.28 ppm. Diazadiene **3a** was formed as the only product with a sufficient purity so no further purification was needed.

<sup>13</sup>C-NMR (Table 2) and bidimensional HSQC and HMBC spectra (see ESI†) were registered to confirm the formation of the DD and fully characterize the new product. In particular, looking at the <sup>13</sup>C-NMR spectrum, it is important to highlight the absence of the carboxylic group of the starting reagent *p*-coumaric acid, and the presence of 10 carbon signals, three of which quaternary. All these data are in line with the hypothesized structure of a DD considering that in case of a diazo-coupling reaction, due to the loss of one proton on the

Table 1  $^1\text{H-NMR}$  of compounds **3a–c** and **5a–c** (400 MHz,  $\text{DMSO-d}_6$ ,  $\delta$  ppm, multiplicity,  $J$  in Hz)



**3a:** R = OH, R' = H  
**3b:** R = OH, R' = OCH<sub>3</sub>  
**3c:** R = N(CH<sub>3</sub>)<sub>2</sub>, R' = H



**5a:** R = OH, R' = H  
**5b:** R = OH, R' = OCH<sub>3</sub>  
**5c:** R = N(CH<sub>3</sub>)<sub>2</sub>, R' = H

	<b>3a</b>	<b>3b</b>	<b>3c</b>	<b>5a</b>	<b>5b</b>	<b>5c</b>
1 and 5	7.73, m	7.74, m	7.70, m	7.64, m	7.66, m	7.63, m
2 and 4	7.53, m	7.53, m	7.51, m	7.36, d (8.3)	7.37, d (8.4)	7.34, d (8.4)
3	7.47, m	7.47, m	7.43, m	—	—	—
7	8.02, d (13.7)	8.11, d (13.6)	8.02, d (13.6)	8.00, d (13.7)	8.09, d (13.7)	8.01, d (13.6)
8	7.73, m	7.70, d (13.6)	7.67, d (13.6)	7.64, m	7.66, m	7.63, m
10	7.64, d (8.6)	7.40, d (2.0)	7.61, d (8.9)	7.64, m	7.39, d (2.0)	7.63, m
11	6.85, d (8.6)	—	6.76, d (8.9)	6.84, d (8.7)	—	6.76, d (9.0)
13	6.85, d (8.6)	6.85, d (8.1)	6.76, d (8.9)	6.84, d (8.7)	6.84, d (8.2)	6.76, d (9.0)
14	7.64, d (8.6)	7.22, dd (8.1, 2.0)	7.61, d (8.9)	7.64, m	7.20, dd (8.2, 2.0)	7.63, m
OCH <sub>3</sub>	—	3.85, s	—	—	3.84, s	—
N(CH <sub>3</sub> ) <sub>2</sub>	—	—	3.00, s	—	—	3.00, s
15	—	—	—	3.08 and 2.95, dd (13.8, 5.6, 9.3)	3.09 and 2.95, dd (13.7, 5.6, 9.3)	3.07 and 2.94, dd (13.8, 5.6, 9.3)
16	—	—	—	4.51, ddd (9.3, 7.8, 5.6)	4.51, ddd (9.3, 7.8, 5.6)	4.50, ddd (9.3, 7.8, 5.6)
18	—	—	—	3.61, s	3.61, s	3.61, s
20	—	—	—	1.80, s	1.80, s	1.80, s
NH	—	—	—	8.38, d (7.8)	8.38, d (7.8)	8.37, d (7.8)

aromatic ring portion of the cinnamic acid, three more carbons should be observed, and there would be two more quaternary carbons. In this way it was possible to confirm that we were in front of a new reaction, never reported in literature before, leading to the formation of DD.

Structure of compound **3a** was also confirmed by accurate mass analysis, showing a peak at  $m/z$  225.1023  $[\text{M} + \text{H}]^+$  (theoretical 225.1022) and 247.0843  $[\text{M} + \text{Na}]^+$  (theoretical 247.0842) (see ESI<sup>†</sup>).

A second reaction was performed using ferulic acid **2b**, which differs from **2a** by the presence of a methoxy group in *ortho* position with respect to the OH. In this case the reaction has been tried using two different stoichiometric ratios, 1 : 2 as in the case of *p*-coumaric acid, and 1 : 1. In all the tested conditions, the formation of the corresponding DD **3b** has been observed, even if the presence of variable amounts of byproducts has been observed in some cases depending on the experimental conditions, which has made us think that the presence of one further electron donating group might increase the reactivity of the product in side reactions. Different work-up strategies have been evaluated, since it has been observed that in some cases precipitation of the final product did not occur, and extraction with organic solvent was necessary to recover all the product. The best condition resulted to be the 1 : 2 stoichiometry, extracting the aqueous solution with ethyl acetate, leading to a yield of 98% of the diazadiene **3b**, that resulted pure enough to be used without further purification. Also in this

case, the formation of diazadiene **3b** has been proved through  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  analysis which confirmed retention of the pattern of the original ferulic acid, without the loss of any aromatic proton of the starting cinnamic acid part. Structural assignment was also confirmed by mass spectrometry analysis since two peaks at 255.1120 and 277.0947  $m/z$  were observed corresponding to  $[\text{M} + \text{H}]^+$  and  $[\text{M} + \text{Na}]^+$  ions respectively.

The reaction between aniline **1** with 4-(dimethylamino) cinnamic acid **2c** using a 1 : 1 stoichiometry confirmed this type of reactivity giving compound **3c** as the only product with an 81% yield.  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  spectroscopy and mass spectrometry analyses led to the assignment of the diazadiene structure to compound **3c**.

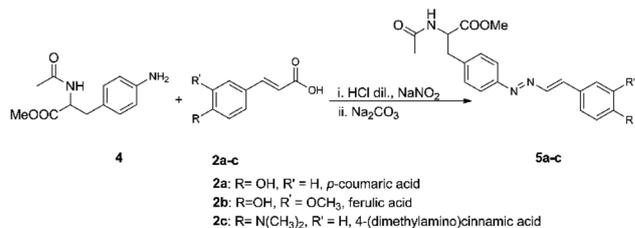
Changing the hydroxycinnamic acid from *p*-coumaric acid **2a** to *o*-coumaric acid **2d**, which has the same electronic effect, the reaction moved towards the formation of the diazo-coupling product **3d**. In this case, no precipitate formed, and the reaction mixture was extracted with ethyl acetate, thus obtaining an orange solid, which did not necessitate any further purification. From  $^1\text{H-NMR}$  analysis, it was possible to identify 10 protons from integration, instead of the 11 in case of formation of the diazadiene. The aromatic part of the cinnamic acid portion presents three signals, each integrating for 1H and the coupling constants observed are in accordance with a substitution at the *para* position with respect to the OH group of the *o*-coumaric acid (Scheme 3). The two protons of the double bond do not show relevant changes in the chemical shifts with respect to the starting reagent

**Table 2**  $^{13}\text{C}$ -NMR of compounds **3a–c** and **5a–c** (100 MHz or 126 MHz, DMSO- $d_6$ ,  $\delta$  ppm, multiplicity,  $J$  in Hz)



**3a:** R = OH, R' = H  
**3b:** R = OH, R' = OCH<sub>3</sub>  
**3c:** R = N(CH<sub>3</sub>)<sub>2</sub>, R' = H  
**5a:** R = OH, R' = H  
**5b:** R = OH, R' = OCH<sub>3</sub>  
**5c:** R = N(CH<sub>3</sub>)<sub>2</sub>, R' = H

Compound						
Position	3a	3b	3c	5a	5b	5c
1 and 5	122.02	121.99	121.82	121.91	121.92	121.80
2 and 4	129.39	129.35	129.28	130.04	130.07	130.05
3	130.49	130.43	129.90	140.05	140.05	139.50
6	152.46	152.48	152.64	151.23	151.26	151.54 or 151.48
7	143.94	144.29	142.59	143.94	144.30	142.66
8	143.49	143.71	144.01	143.06	143.33	143.71
9	125.63	126.19	122.00	125.64	126.21	122.09
10	130.31	111.09	129.97	130.22	111.04	130.00
11	116.05	148.05	111.98	116.01	148.07	112.07
12	159.70	149.18	151.49	159.63	149.17	151.54 or 151.48
13	116.05	115.79	111.98	116.01	115.80	112.07
14	130.31	123.21	129.97	130.22	123.19	130.00
OCH <sub>3</sub>	—	55.72	—	—	55.73	—
N(CH <sub>3</sub> ) <sub>2</sub>	—	—	39.5	—	—	39.52
15	—	—	—	36.53	36.55	36.58
16	—	—	—	53.37	53.39	53.49
17	—	—	—	172.05	172.07	172.14
18	—	—	—	51.89	51.91	51.96
19	—	—	—	169.37	169.40	169.55
20	—	—	—	22.24	22.26	22.30



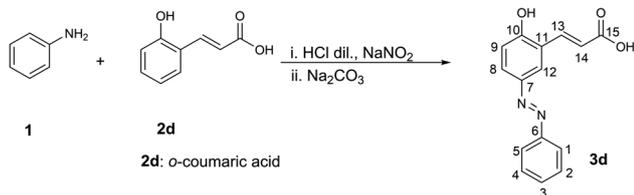
**Scheme 4** Reactions of *p*-amino-phenylalanine with cinnamic acids.

new diazadiene products (Scheme 4). We have chosen to test the reaction on this amino acid in the view of obtaining biomolecule-related DDs. Acetyl-*p*-amino-phenylalanine methyl ester has been used for the reaction thus having both the amino and the carboxy terminals of the amino acid protected. In all the three cases it was possible to obtain the corresponding diazadiene products **5a–c** in good yields, ranging between 71% and 96%, in a pure form and without the need for any purification. The structures have been confirmed through 1D and 2D-NMR spectra, as well as accurate mass spectrometry.

### 2.3 The mechanism

The effect of an electron-donating group at the *para* position of the cinnamic acids seems to suggest a bielectronic mechanism for the reaction. The hypothesis is that such group promotes the nucleophilic attack of the  $\alpha$  vinyl carbon towards the electrophilic diazonium salt. Then, decarboxylation of the intermediate leads to the reestablishing of aromaticity, thus obtaining the completely conjugated DD. The hypothesized mechanism is reported in Scheme 5.

This option is very different from that of other reactions involving diazonium salts and unsaturated compounds. One previously documented reaction, involving also cinnamic acid, is the Meerwein arylation reaction, originally discovered in 1939 as the copper-catalyzed addition of an aryl-diazonium salt with unsaturated compound, even if an intramolecular version of the reaction, the Pschorr reaction, was firstly reported in 1896.<sup>14</sup> The proposed mechanism is radical, and the reaction takes place better when the olefinic double bond is activated by an electron-withdrawing group, such as carbonyl, cyano or aryl.<sup>14,15</sup> The result is the addition of the aryl group from the diazonium salt to the carbon atom in  $\beta$  position with respect to the activating group.<sup>15</sup> Anyway, this reaction gave low yields, high catalyst loading and formation of side products.<sup>14</sup> Nevertheless, other options alternative to reduction of diazonium salts by copper(i) are now available, leading to aryl radicals, thanks to photoredox catalysis, for example, using of  $[\text{Ru}(\text{bpy})_3]^{2+}$ .<sup>14</sup> One particular application of the Meerwein reaction involves cinnamic acid: in this case, the reaction leads to the loss of

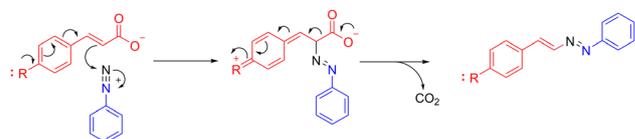


**Scheme 3** Reaction of *o*-coumaric acid with aniline.

(as it happens in case of diazadienes) and the coupling constant remains of 16.1 Hz. The formation of diazo-coupling product is also confirmed by the presence of the carboxylic acid carbon at 167.85 ppm in the  $^{13}\text{C}$ -NMR spectrum. 2D-NMR as well as mass spectrometry confirm the assigned structure to **3d**. From mass spectrometry analysis is possible to find the signal at 269.0  $m/z$ , corresponding to the adduct  $[\text{M} + \text{H}]^+$ , and the signal at 291.0  $m/z$ , corresponding to the adduct  $[\text{M} + \text{Na}]^+$ . In this reaction, diazo-coupling reaction was likely possible due to a less steric hindrance of the aromatic ring in the activated position.

### 2.2 Influence of the aromatic amine on reactivity

The cinnamic acids **2a–c** were further used in the reaction with acetyl-*p*-amino-phenylalanine methyl ester **4** in order to obtain



**Scheme 5** Possible mechanism for the formation of DDs.

a carbon dioxide molecule and to the formation of the corresponding stilbene.<sup>14,15</sup>

In the present work we observed a different reactivity with respect to the Meerwein arylation reaction, although the reaction conditions are not the same, and formation of DDs, which has never been reported in the literature, was the only reaction to take place. We could only find a somehow-correlate reaction reported by Xu and colleagues, in which 4,4-dialkylthio-1,2-diaza-1,3-butadienes are synthesized based on the azo-coupling decarboxylation of  $\alpha$ -carboxy ketene dithioacetals with aryldiazonium salts.<sup>16</sup>

## 2.4 Biological activity

**2.4.1 Antitumor activity.** The biological activity of **5a**, **5b**, **5c**, **3a**, **3b**, **3c** was evaluated by comparing the response of normal hTERT-immortalized human uterine smooth muscle cells and the tumorous counterpart, leiomyosarcoma (LMS) SK-UT-1 cells. LMS are rare and aggressive tumors that arise in various sites of the body and retain some smooth muscle features. Surgical resection is the effective curative treatment, but patients often experience recurrences, and available cytotoxic therapies show modest clinical activity.<sup>17,18</sup>

In a first screening, cells were incubated with the different compounds at a concentration of 10  $\mu$ M for 48 hours. **3c** was the compound that caused the highest percentage of cell death in SK-UT-1 cells compared with normal HUtSMC (Fig. 2A). The number

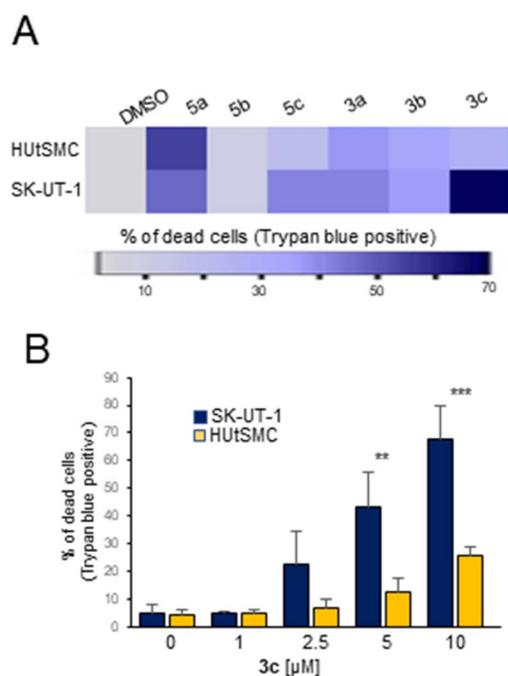


Fig. 2 The cell death activity of the different compounds (A) the heatmap shows the percentage of Trypan blue positive cells after the treatment with the indicated compounds at the concentration of 10  $\mu$ M for 48 h in HUtSMC or SK-UT-1 cells. DMSO was used as control. (B) The histogram illustrates the percentage of cell death measured by Trypan blue staining after treatment with **3c** for 48 h at the indicated concentration in HUtSMC or SK-UT-1 cells. DMSO was used as control. Data are the average of four independent experiments, SD is indicated in error bars.

Table 3 Minimum inhibitory concentrations (MIC) of compounds **3a**, **3b**, **3c**, **5a**, **5b**, **5c**, in Mueller–Hinton broth

Compound	MIC ( $\mu$ M)	
	<i>S. aureus</i> (ATCC 25923)	<i>E. coli</i> (ATCC 25922)
<b>3a</b>	8	>64
<b>3b</b>	8	>64
<b>3c</b>	>64	>64
<b>5a</b>	64	>64
<b>5b</b>	32	>64
<b>5c</b>	>64	>64

of Trypan blue-positive cells was approximately 70% in cancer cells and 25% in normal cells. The other compounds were either ineffective (**5b**) or showed no differential effect between normal and cancer cells (**3a**, **3b**, **5a**). **5c**, similar to **3c**, elicited a differential cell death response in cancer and normal cells, but was less effective than **3c**. Therefore, we investigated the death-promoting effect of **3c** in more detail. Dose-dependent studies confirmed the specific cell-killing effect of **3c** against LMS cells (Fig. 2B).

**2.4.2 Antibacterial activity.** The antibacterial activity of the compounds **5a**, **5b**, **5c**, **3a**, **3b**, **3c**, was also investigated. Minimum concentrations inhibiting the growth (MIC) were measured on a standard strain of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), as representative of a Gram-negative and -positive pathogen, respectively, of serious concern for the human health due to the development of antibiotic resistance (Table 3).

The compounds **3a**, **3b**, **5a** and **5b** inhibited the growth of *S. aureus* cells when given at 8  $\mu$ M (**3a**, **3b**), 32  $\mu$ M (**5b**) or at 64  $\mu$ M (**5a**), whereas *E. coli* cells were not susceptible to any of the compounds, at least up to 64  $\mu$ M. It is worth noting that **3c**, which caused the highest percentage of cell death in leiomyosarcoma SK-UT-1 cells, did not affect the viability of either bacterial strain, indicating selective cytotoxicity. In contrast, **3b**, which showed anti-*Staphylococcus* activity, did not affect heavily human cells (regardless their tumoral origin or not) and thus may have selective antibacterial effect. On the other hand, the high insensitivity of *E. coli* to these compounds is not surprising since Gram-negative bacteria, that possess a second membrane barrier, are generally more resistant to small hydrophobic molecules<sup>19</sup> such as DDs.

## 3 Materials and methods

### 3.1 Chemicals and materials

Acetyl-*p*-amino-phenylalanine methyl ester was purchased from Bachem, aniline was purchased from BDH.

All the other reagents were purchased from Sigma-Aldrich (Milano, Italy).

### 3.2 Instrumentation

NMR spectra were recorded on Varian 400 or 500 MHz spectrometers (Palo Alto, CA, USA) using DMSO- $d_6$  as the solvent if not otherwise stated. Coupling constants are given in Hertz.

Accurate masses were determined on a microTOF-Q (Bruker, Billerica, MA, USA). Measurements were registered in positive mode.

Infrared spectra were registered on an ATR-IR IRAffinity-1S Fourier transform infrared spectrophotometer (Shimadzu).

### 3.3 General procedure for the synthesis of compounds 3a–c and 5a–c

To a solution of aniline (1 mmol, 1 eq.) in 32 mL of 0.1 M hydrochloric acid, cooled in an ice bath, a solution of sodium nitrite (1.2 mmol, 1.2 eq.) in 6.4 mL of distilled water was added dropwise. The reaction mixture was stirred in an ice bath for 15 minutes to enable the formation of the diazonium salt. Subsequently, a cooled solution of cinnamic acid (1 or more eq.) in 32 mL of 0.1 M sodium carbonate was added dropwise to the diazonium salt solution. The reaction mixture suddenly turned colored, and it was left to react 30 minutes at 0 °C and for 30 more minutes at room temperature.

Ratio of cinnamic acid/aniline and workup for each product obtained will be described in the specific paragraph.

**3.3.1 4-((E)-2-((E)-Phenyldiazenyl)vinyl)phenol 3a.** According to the general procedure, a solution of 91  $\mu\text{L}$  (1.00 mmol, 1 eq.) of aniline in 32 mL of 0.1 M HCl and 0.0835 g of sodium nitrite (1.21 mmol, 1.2 eq.) in 6.4 mL of water reacted with 0.3287 g of *p*-coumaric acid (2.00 mmol, 2 eq.) in 32 mL of 0.1 M sodium carbonate. The red precipitate formed was filtered under vacuum on a Gooch crucible and the solid was washed with few mL of water and dried. Compound **3a** was obtained in 92% yield as a red-purple solid.

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.02 (1H, d,  $J = 13.7$  Hz, H-7), 7.73 (3H, m, H-1, H-5, H-8), 7.64 (2H, d,  $J = 8.6$  Hz, H-10, H-14), 7.53 (2H, m, H-2, H-4), 7.47 (1H, m, H-3), 6.85 (2H, d,  $J = 8.6$  Hz, H-11, H-13).  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  159.70 (C-12), 152.46 (C-6), 143.94 (C-7), 143.49 (C-8), 130.49 (C-3), 130.31 (C-10, C-14), 129.39 (C-2, C-4), 125.63 (C-9), 122.02 (C-1, C-5), 116.05 (C-11, C-13). MS (ESI+) 225.1023 [M + H] $^+$  (calculated 225.1022); 247.0843 [M + Na] $^+$  (calculated 247.0842). IR ( $\nu_{\text{max}}$ /cm $^{-1}$ ) 1602.85 (stretching N=N).

**3.3.2 2-Methoxy-4-((E)-2-((E)-phenyldiazenyl)vinyl)phenol 3b.** According to the general procedure, a solution of 91  $\mu\text{L}$  (1.00 mmol, 1 eq.) of aniline in 32 mL of 0.1 M HCl and 0.0856 g of sodium nitrite (1.24 mmol, 1.2 eq.) in 6.4 mL of water reacted with 0.3881 g of ferulic acid (2.00 mmol, 2 eq.) in 32 mL of 0.1 M sodium carbonate. The reaction mixture turned red, and extraction was performed with ethyl acetate four times. The organic phase was dried over anhydrous sodium sulfate and then the solvent was removed using a rotary evaporator. Compound **3b** was obtained in 98% yield as a dark red solid.

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.11 (1H, d,  $J = 13.6$  Hz, H-7), 7.74 (2H, m, H-1, H-5), 7.70 (1H, d,  $J = 13.6$  Hz, H-8), 7.53 (2H, m, H-2, H-4), 7.48 (1H, m, H-3), 7.40 (1H, d,  $J = 2.0$  Hz, H-10), 7.22 (1H, dd,  $J = 8.1, 2.0$  Hz, H-14), 6.85 (1H, d,  $J = 8.1$  Hz, H-13), 3.85 (3H, s, OCH $_3$ ).  $^{13}\text{C}$  NMR (126 MHz, DMSO- $d_6$ )  $\delta$  152.48 (C-6), 149.18 (C-12), 148.05 (C-11), 144.29 (C-7), 143.71 (C-8), 130.43 (C-3), 129.35 (C-2, C-4), 126.19 (C-9), 123.21 (C-14), 121.99 (C-1, C-5), 115.79 (C-13), 111.09 (C-10), 55.72 (OCH $_3$ ). MS

(ESI+): 255.1120 [M + H] $^+$  (calculated 255.1128), 277.0949 [M + Na] $^+$  (calculated 277.0948). IR ( $\nu_{\text{max}}$ /cm $^{-1}$ ) 1587.42 (stretching N=N).

**3.3.3 *N,N*-Dimethyl-4-((E)-2-((E)-phenyldiazenyl)vinyl)aniline 3c.** According to the general procedure, a solution of 91  $\mu\text{L}$  (1.00 mmol, 1 eq.) of aniline in 32 mL of 0.1 M HCl and 0.0851 g of sodium nitrite (1.23 mmol, 1.2 eq.) in 6.4 mL of water, reacted with 0.1961 g of 4-(dimethylamino)cinnamic acid (1.03 mmol, 1 eq.) in 32 mL of 0.1 M sodium carbonate. The dark orange precipitate formed was filtered under vacuum on a Gooch crucible and the solid was washed with few mL of water and dried. Compound **3c** was obtained in 81% yield as a dark orange solid.

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.02 (1H, d,  $J = 13.6$  Hz, H-7), 7.70 (2H, m, H-1, H-5), 7.67 (1H, d,  $J = 13.6$  Hz, H-8) 7.61 (2H, d,  $J = 8.9$  Hz, H-10, H-14), 7.51 (2H, m, H-2, H-4), 7.43 (1H, m, H-3), 6.76 (2H, d,  $J = 8.9$  Hz, H-11, H-13), 3.00 (6H, s, N(CH $_3$ ) $_2$ ).  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  152.64 (C-6), 151.49 (C-12), 144.01 (C-8), 142.59 (C-7), 129.97 (C-10, C-14), 129.90 (C-3), 129.28 (C-2, C-4), 122.00 (C-9), 121.82 (C-1, C-5), 111.98 (C-11, C-13), 39.5 (N(CH $_3$ ) $_2$ ). MS (ESI+): 252.1492 [M + H] $^+$  (calculated 252.1495), 274.1310 [M + Na] $^+$  (calculated 274.1315). IR ( $\nu_{\text{max}}$ /cm $^{-1}$ ) 1600.92 (stretching N=N).

**3.3.4 Methyl 2-acetamido-3-(4-((E)-((E)-4-hydroxystyryl)diazenyl)phenyl)propanoate 5a.** According to the general procedure, a solution of 0.0150 g (0.063 mmol, 1 eq.) of acetyl-*p*-amino-phenylalanine methyl ester in 2 mL of 0.1 M HCl and 0.0053 g of sodium nitrite (0.077 mmol, 1.2 eq.) in 0.4 mL of water, reacted with 0.0312 g of *p*-coumaric acid (0.19 mmol, 3 eq.) in 3 mL of 0.1 M sodium carbonate. The dark precipitate formed was filtered under vacuum on a Gooch crucible and the solid was washed with few mL of water and dried. Compound **5a** was obtained in 96% yield as a brick red solid.

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.38 (1H, d,  $J = 7.8$  Hz, NH), 8.00 (1H, d,  $J = 13.7$  Hz, H-7), 7.64 (5H, m, H-1, H-5, H-8, H-10, H-14), 7.36 (2H, d,  $J = 8.3$  Hz, H-2, H-4), 6.84 (2H, d,  $J = 8.7$  Hz, H-11, H-13), 4.51 (1H, ddd,  $J = 9.3, 7.8, 5.6$  Hz, H-16), 3.61 (3H, s, H-18), 3.08 (1H, dd,  $J = 13.8, 5.6$  Hz, H-15), 2.95 (1H, dd,  $J = 13.8, 9.3$  Hz, H-15), 1.80 (3H, s, H-20).  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  172.05 (C-17), 169.37 (C-19), 159.63 (C-12), 151.23 (C-6), 143.94 (C-7), 143.06 (C-8), 140.05 (C-3), 130.22 (C-10, C-14), 130.04 (C-2, C-4), 125.64 (C-9), 121.91 (C-1, C-5), 116.01 (C-11, C-13), 53.37 (C-16), 51.89 (C-18), 36.53 (C-15), 22.24 (C-20). MS (ESI+): 368.1595 [M + H] $^+$  (calculated 368.1605), 390.1417 [M + Na] $^+$  (calculated 390.1424). IR ( $\nu_{\text{max}}$ /cm $^{-1}$ ) 1602.85 (stretching N=N).

**3.3.5 Methyl 2-acetamido-3-(4-((E)-((E)-4-hydroxy-3-methoxystyryl)diazenyl)phenyl)propanoate 5b.** According to the general procedure, a solution of 0.0939 g (0.397 mmol, 1 eq.) of acetyl-*p*-amino-phenylalanine methyl ester in 12.8 mL of 0.1 M HCl and 0.0341 g of sodium nitrite (0.494 mmol, 1.24 eq.) in 2.6 mL of water, reacted with 0.0781 g of ferulic acid (0.402 mmol, 1 eq.) in 12.8 mL of 0.1 M sodium carbonate. The dark red precipitate formed was filtered under vacuum on a Gooch crucible and the solid was washed with few mL of water and dried. Compound **5b** was obtained in 77% yield as a dark red solid.

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.38 (1H, d,  $J$  = 7.8 Hz, NH), 8.09 (1H, d,  $J$  = 13.7 Hz, H-7), 7.66 (3H, m, H-1, H-5, H-8), 7.37 (2H, d,  $J$  = 8.4 Hz, H-2, H-4), 7.39 (1H, d,  $J$  = 2 Hz, H-10), 7.20 (1H, dd,  $J$  = 8.2, 2.0 Hz, H-14), 6.84 (1H, d,  $J$  = 8.2 Hz, H-13), 4.51 (1H, ddd,  $J$  = 9.3, 7.8, 5.6 Hz, H-16), 3.84 (3H, s, OCH $_3$ ), 3.61 (3H, s, H-18), 3.09 (1H, dd,  $J$  = 13.7, 5.6 Hz, H-15), 2.95 (1H, dd,  $J$  = 13.7, 9.3 Hz, H-15), 1.80 (3H, s, H-20).  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  172.07 (C-17), 169.40 (C-19), 151.26 (C-6), 149.17 (C-12), 148.07 (C-11), 144.30 (C-7), 143.33 (C-8), 140.05 (C-3), 130.07 (C-2, C-4), 126.21 (C-9), 123.19 (C-14), 121.92 (C-1, C-5), 115.80 (C-13), 111.04 (C-10), 55.73 (OCH $_3$ ), 53.39 (C-16), 51.91 (C-18), 36.55 (C-15), 22.26 (C-20). MS (ESI $^+$ ): 398.1704 [M + H] $^+$  (calculated 398.1710), 420.1528 [M + Na] $^+$  (calculated 420.1530). IR ( $\nu_{\text{max}}/\text{cm}^{-1}$ ) 1598.99 (stretching N=N).

**3.3.6 Methyl 2-acetamido-3-(4-((E)-((E)-4-(dimethylamino)styryl)diazanyl)phenyl)propanoate 5c.** According to the general procedure, a solution of 0.0983 g (0.416 mmol, 1 eq.) of acetyl-*p*-amino-phenylalanine methyl ester in 12.8 mL of 0.1 M HCl and 0.0406 g of sodium nitrite (0.588 mmol, 1.4 eq.) in 2.6 mL of water, reacted with 0.0768 g of 4-(dimethylamino)cinnamic acid (0.402 mmol, 1 eq.) in 12.8 mL of 0.1 M sodium carbonate. The dark orange precipitate formed was filtered under vacuum on a Gooch crucible and the solid was washed with few mL of water and dried. Compound **5c** was obtained in 71% yield as a dark orange solid.

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.37 (1H, d,  $J$  = 7.8 Hz, NH), 8.01 (1H, d,  $J$  = 13.6 Hz, H-7), 7.63 (5H, m, H-1, H-5, H-8, H-10, H-14), 7.34 (2H, d,  $J$  = 8.4 Hz, H-2, H-4), 6.76 (2H, d,  $J$  = 9.0 Hz, H-11, H-13), 4.50 (1H, ddd,  $J$  = 9.3, 7.8, 5.6 Hz, H-16), 3.61 (3H, s, H-18), 3.07 (1H, dd,  $J$  = 13.8, 5.6 Hz, H-15), 3.00 (6H, s, N(CH $_3$ ) $_2$ ), 2.94 (1H, dd,  $J$  = 13.8, 9.3 Hz, H-15), 1.80 (3H, s, H-20).  $^{13}\text{C}$  NMR (126 MHz, DMSO- $d_6$ )  $\delta$  172.14 (C-17), 169.55 (C-19), 151.54 (C-6 or C-12), 151.48 (C-6 or C-12), 143.71 (C-8), 142.66 (C-7), 139.50 (C-3), 130.05 (C-2, C-4), 130.00 (C-10, C-14), 122.09 (C-9), 121.80 (C-1, C-5), 112.07 (C-11, C-13), 53.49 (C-16), 51.96 (C-18), 39.52 (N(CH $_3$ ) $_2$ ), 36.58 (C-15), 22.30 (C-20). MS (ESI $^+$ ): 395.2076 [M + H] $^+$  (calculated 395.2078), 417.1905 [M + Na] $^+$  (calculated 417.1897). IR ( $\nu_{\text{max}}/\text{cm}^{-1}$ ) 1598.99 (stretching N=N).

**3.3.7 (E)-3-(2-Hydroxy-5-((E)-phenyldiazanyl)phenyl)acrylic acid 3d.** According to the general procedure, a solution of 23  $\mu\text{L}$  (0.25 mmol, 1 eq.) of aniline in 8 mL of 0.1 M HCl and 0.0212 g of sodium nitrite (0.31 mmol, 1.2 eq.) in 1.6 mL of water reacted with 0.0824 g of *o*-coumaric acid (0.5 mmol, 2 eq.) in 8 mL of 0.1 M sodium carbonate. The reaction mixture turned orange and extraction was performed with ethyl acetate four times. The organic phase was dried over anhydrous sodium sulfate and then the solvent was removed using a rotary evaporator. Compound **3d** was obtained in 40% yield as an orange solid.

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.16 (1H, d,  $J$  = 2.4 Hz, H-12), 7.85 (4H, m, H-1, H-5, H-8, H-13), 7.58 (2H, m, H-2, H-4), 7.52 (1H, m, H-3), 7.10 (1H, d,  $J$  = 8.8 Hz, H-9), 6.66 (1H, d,  $J$  = 16.1 Hz, H-14).  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  167.85 (C-15), 159.74 (C-10), 152.00 (C-6), 145.15 (C-7), 138.68 (C-13), 130.80 (C-3), 129.38 (C-2, C-4), 124.84 (C-12), 124.70 (C-8), 122.25 (C-1, C-5), 121.53 (C-11), 119.87 (C-14), 116.94 (C-9). MS (ESI $^+$ ): 269.0 [M + H] $^+$ , 291.0 [M + Na] $^+$ .

### 3.4 Cell culture, drug treatment and cell death evaluation

Human uterine smooth muscle cells (HUtSMC) immortalized with hTERT and SK-UT-1 cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS), L-glutamine (2 mM), penicillin (100 U mL $^{-1}$ ), and streptomycin (100  $\mu\text{g}$  mL $^{-1}$ ) (Lonza). Cells were tested regularly for mycoplasma contamination through microscope after Hoechst 33342 (Sigma) staining. For cell death evaluation, cells were treated with the indicated compounds for 48 h. Cell death was evaluated through Countess II FL automated cell counter (Invitrogen) after 0.1% Trypan blue (Sigma) staining.

### 3.5 Bacterial strains, antimicrobial activity

The bacterial strains used in this work were *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Bacterial strains were grown overnight at 37  $^\circ\text{C}$  in Mueller–Hinton broth (MHB, Difco) with shaking (140 rpm). The day after, the bacterial cultures were diluted 1 : 40 in fresh MHB and incubated at 37  $^\circ\text{C}$  with shaking (140 rpm) to mid-log phase, until an optical density (OD) at 600 nm  $\approx$  0.3 was reached. Mid-log bacterial cultures were diluted to  $5 \times 10^5$  colony-forming units (cfu) per mL in Mueller–Hinton Broth (MHB). To test the antimicrobial activity, the DDs, diluted in MHB to the concentration of 128  $\mu\text{M}$  in the presence of 1.5–2% DMSO, were added to the first wells of a round-bottom 96-well microtiter plate and serially twofold diluted in a final volume of 50  $\mu\text{L}$  of MHB into successive wells. Subsequently, 50  $\mu\text{L}$  of the bacterial suspension  $5 \times 10^5$  cfu mL $^{-1}$  (see above) was added to each well. This reduced the final bacterial load to  $2.5 \times 10^4$  cfu per well and halved the DD concentration in the wells. The same amount of untreated bacteria in MHB + 2% DMSO, without any other added compound, was used as bacterial growth control. MHB only (100  $\mu\text{L}$ ) was used to control the medium sterility. The plate was sealed with Parafilm to minimize evaporation and incubated overnight at 37  $^\circ\text{C}$  (for approx. 18 h). Data are the median of 3 independent experiments performed as internal duplicates ( $n = 6$ ).

## 4 Conclusions

In this work, the synthesis of six 1,2-diaza-1,3-dienes from substituted cinnamic acids and aromatic amines has been described. The obtained diazadienes have never been reported in literature and their structures have been fully characterized, even if the real novelty is the synthetic procedure adopted to obtain them. In fact, for the first time a new reactivity of diazonium salts from anilines with cinnamic acids has been reported: in particular, the reaction has been carried out with three substituted cinnamic acids, *i.e.* *p*-coumaric acid, ferulic acid and 4(dimethylamino)cinnamic acid, and two model anilines, namely aniline and acetyl-*p*-amino-phenylalanine methyl ester. All the synthesized compounds have also been used for preliminary biological activity evaluation, to establish potential antitumor and antimicrobial activity. Compound **3c** is the most effective compound in terms of antitumor activity, since it is the only compound that causes a higher percentage of cell death in LMS cells compared to normal HUtSMC cells, and

this was confirmed also through promising dose-dependent studies. Interestingly, compound **3c** is completely inactive as an antibacterial against *S. aureus*, but compound **3b**, which do not affect much human cells, shows an anti-*Staphylococcus* activity with a MIC of 8  $\mu\text{M}$ , so it might have a selective action as antibacterial.

## Author contributions

Veronica Vida: conceptualization, investigation, methodology, formal analysis, data curation, validation, visualization, writing original draft and writing review and editing. Martina Minisini: investigation, data curation, validation, visualization, writing original draft. Mario Mardirossian: investigation, data curation, validation, writing original draft. Claudio Brancolini: data curation, validation, visualization, writing original draft, writing review and editing, resources and supervision. Marco Scocchi: data curation, validation, visualization, writing original draft, writing review and editing, resources and supervision. Cristina Forzato: conceptualization, validation, visualization, writing original draft, writing review and editing, supervision. Federico Berti: conceptualization, validation, visualization, writing review and editing, supervision, resources, project administration.

## Conflicts of interest

There are no conflicts to declare.

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## Notes and references

- 1 O. A. Attanasi, L. De Crescentini, G. Favi, P. Filippone, F. Mantellini, F. R. Perrulli and S. Santeusano, Cultivating the passion to build heterocycles from 1,2-diaza-1,3-dienes: the force of imagination, *Eur. J. Org. Chem.*, 2009, **2009**(19), 3109–3127.
- 2 O. A. Attanasi and L. Caglioti, Conjugated Azoalkenes: Attractive Products and Versatile Intermediates, *Org. Prep. Proced. Int.*, 1986, **18**(5), 299–327.
- 3 O. A. Attanasi, L. D. Crescentini, P. Filippone, F. Mantellini and S. Santeusano, 1,2-Diaza-1,3-butadienes; just a nice class of compounds, or powerful tools in organic chemistry? reviewing an experience, *Arkivoc*, 2003, **2002**(11), 274–292.
- 4 J. Guzman, Natural Cinnamic Acids, Synthetic Derivatives and Hybrids with Antimicrobial Activity, *Molecules*, 2014, **19**(12), 19292–19349.
- 5 V. Sinisi, A. Stevaert, F. Berti, C. Forzato, F. Benedetti, L. Navarini, A. Camps, L. Persoons and K. Vermeire, Chlorogenic Compounds from Coffee Beans Exert Activity against Respiratory Viruses, *Planta Med.*, 2016, **83**(07), 615–623.
- 6 V. Sinisi, K. Boronová, S. Colombaro, L. Navarini, F. Berti and C. Forzato, Synthesis of Mono-, Di-, and Tri-3,4-dimethoxycinnamoyl-1,5- $\gamma$ -quinides, *Eur. J. Org. Chem.*, 2014, **2014**(6), 1321–1326.
- 7 A. L. Gutiérrez Ortiz, F. Berti, L. Navarini, P. Crisafulli, S. Colombaro and C. Forzato, Aqueous extracts of walnut (*Juglans regia* L.) leaves: quantitative analyses of hydroxycinnamic and chlorogenic acids, *J. Chromatogr. Sci.*, 2018, **56**(8), 753–760.
- 8 A. L. Gutiérrez Ortiz, F. Berti, W. Solano Sánchez, L. Navarini, S. Colombaro, P. Crisafulli and C. Forzato, Distribution of *p*-coumaroylquinic acids in commercial *Coffea* spp. of different geographical origin and in other wild coffee species, *Food Chem.*, 2019, **286**, 459–466.
- 9 A. L. Gutiérrez Ortiz, F. Berti, L. Navarini, A. Monteiro, M. Resmini and C. Forzato, Synthesis of *p*-coumaroylquinic acids and analysis of their interconversion, *Tetrahedron: Asymmetry*, 2017, **28**(3), 419–427.
- 10 F. Berti, L. Navarini, S. Colombaro and C. Forzato, Hydroxycinnamoyl Amino Acids Conjugates: A Chiral Pool to Distinguish Commercially Exploited *Coffea* spp, *Molecules*, 2020, **25**(7), 1704.
- 11 A. J. Borah and G. Yan, Decarboxylative functionalization of cinnamic acids, *Org. Biomol. Chem.*, 2015, **13**(30), 8094–8115.
- 12 M. Gupta and B. P. Wakhloo, Tetrabutylammoniumbromide mediated Knoevenagel condensation in water: synthesis of cinnamic acids, *Arkivoc*, 2007, **2007**(1), 94–98.
- 13 L. Chen, L. Zhang, G. Yan and D. Huang, Recent Advances of Cinnamic Acids in Organic Synthesis, *Asian J. Org. Chem.*, 2020, **9**(6), 842–862.
- 14 P. Schroll, D. P. Hari and B. König, Photocatalytic Arylation of Alkenes, Alkynes and Enones with Diazonium Salts, *ChemistryOpen*, 2012, **1**(3), 130–133.
- 15 C. S. Rondstvedt Jr, Arylation of Unsaturated Compounds by Diazonium salts (The Meerwein Arylation Reaction), *Org. React.*, 2004, **11**, 189–260.
- 16 X.-X. Xu, M. Wang, Q. Liu, L. Pan and Y.-L. Zhao, Azocoupling Decarboxylation Reaction of  $\alpha$ -Carboxy Ketene Dithioacetals in Water—a New Route to 1,2-Diaza-1,3-butadienes, *Chin. J. Chem.*, 2006, **24**(10), 1431–1434.
- 17 S. George, C. Serrano, M. L. Hensley and I. Ray-Coquard, Soft tissue and uterine leiomyosarcoma, *J. Clin. Oncol.*, 2018, **36**(2), 144–150.
- 18 L. Iuliano, S. Drioli, Y. Pignochino, C. M. Cafiero, M. Minisini, F. D'Este, R. Picco, E. Dalla, G. Giordano, G. Grignani, E. Di Giorgio, F. Benedetti, F. Felluga and C. Brancolini, Enhancing Proteotoxic Stress in Leiomyosarcoma Cells Triggers Mitochondrial Dysfunctions, Cell Death, and Antitumor Activity *in vivo*, *Mol. Cancer Ther.*, 2021, **20**(6), 1039–1051.
- 19 D. G. Brown, T. L. May-Dracka, M. M. Gagnon and R. Tommasi, Trends and Exceptions of Physical Properties on Antibacterial Activity for Gram-Positive And Gram-Negative Pathogens, *J. Med. Chem.*, 2014, **57**(23), 10144–10161.