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Dear Editor,

Mass spectral studies of diamide compounds obtained by the Ugi reaction

The Ugi multicomponent reaction[1] involves the combination of an amine, an acid, a carbonyl compound and an isocyanide to produce a diamide. Several diamides obtained by this methodology have shown cytotoxic effects.[2,3] In order to evaluate their cellular metabolism in *in vitro* assays it is necessary to develop techniques that allow the unambiguous identification of these compounds and their metabolites. Most classical methods lack the minimal required sensitivity since the expected concentrations in both culture media and intracellular environments are very low (around 10^{-6} M). The present work reports the synthesis of the two cinnamic acid-derived diamides *N*-(1-(*tert*-butylamino)-2-methyl-1-oxopropan-2-yl)-*N*-phenethylcinnamamide (**1**) and *N*-(1-(*tert*-butylamino)-2-methyl-1-oxopropan-2-yl)-*N*-phenylcinnamamide (**2**) by a "one-step-one-pot" procedure (Fig. 1), as well as the study of their fragmentation pathways by mass spectrometry. The characterization of certain fragment ions could be a tool for the detection of these compounds and their derived metabolites in complex biological matrices.[4–6]

2-Phenylethanamine (2.02 mmol) or aniline and acetone (2.43 mmol) were dissolved in methanol and stirred at roomtemperature. After 3 h, cinnamic acid (1.35 mmol) and *tert*-butyl isocyanide (1.48 mmol) were added and stirred for 24 h. Reaction progress was checked by thin-layer chromatography (TLC) and purification was carried out by flash chromatography using mixtures of *n*-hexane/ethyl acetate as eluent to afford 306.23 mg and 282.94 mg of compounds **1** and **2**, respectively.

Compound ***1****: N-(1-(tert-butylamino)-2-methyl-1-oxopropan-2-yl)-N-phenethyl-cinnamamide*: 306.23 mg (0.78 mmol, yield 58%) of compound **1** was obtained as a colorless oil, which exhibited a molecular ion at *m/z* 392.2462 corresponding to the molecular formula $C_{25}H_{32}N_2O_2$. The ^{1}H-NMR spectrum ($CDCl_3$, 200 MHz) exhibited δ (ppm): 7.68 (d J = 15.3 Hz, 1H, H_3); 7.51–7.23 (*m* 10H, aromatic H); 6.77 (*d* J = 15.3 Hz, 1H, H_2); 3.75 (*dd* J = 7.8, 8.4 Hz, 2H, H_{14}); 3.0 (*dd* J = 7.8, 8.4 Hz, 2H, H_{15}); 1.61 (*s* 6H, $H_{9\ \&\ 10}$); 1.33 (*s* 9H, $H_{11,\ 12\ \&\ 13}$); 5.65 (*s* NH). The ^{13}C-NMR spectrum ($CDCl_3$, 50.25 MHz) exhibited δ (ppm): 167.4 (C_1), 118.8 (*d* C_2); 142.9 (*d* C_3); 62.9 *s* (C_5); 174.3 (*s* C_6); 50.9 (*s* C_8); 46.0 (*t* C_{14}); 37.8 (*t* C_{15}); 25.7 (*q* $C_{9\ \&\ 10}$); 28.5 (*q* $C_{11\text{-}15}$); 135.1 (*s* $C_{1'}$); 138.5 (*s* $C_{1''}$); 129.7, 128.86, 128.82, 128.6, 127.9, 126.8 (aromatic carbons). The gas chromatography/electron ionization low-resolution mass spectrometry (GC/EI-LRMS) spectra showed: *m/z* values (% relative abundance): $[M]^{+\cdot}$392 (0.8), 320 (1.0), 299 (1.1), 293 (6.1), 292 (29.4), 216 (1.4), 209 (1.0), 202 (4.5), 163 (12.7), 162 (100.0), 145 (10.0), 132 (2.6), 131 (21.4), 106 (1.1), 105 (22.7), 104 (1.9), 103 (29.1), 102 (1.3), 79 (2.1), 77 (9.5), 70 (1.0).

Compound ***2****: N-(1-(tert-butylamino)-2-methyl-1-oxopropan-2-yl)-N-phenyl-cinnamamide*: 287.94 mg (0.79 mmol, yield 59%) of compound **2** was obtained as a clear and colorless oil, which exhibited a molecular ion at *m/z* 364.2166 corresponding to the molecular formula $C_{23}H_{28}N_2O_2$. The ^{1}H-NMR spectrum ($CDCl_3$, 200 MHz) exhibited δ (ppm): 7.59 (*d* J = 15.3 Hz, 1H, H_3); 7.51–7.23 (*m* 10H, aromatic H); 6.06 (*d* J = 15.3 Hz, 1H, H_2); 1.41 (*s* 6H, $H_{9\ \&\ 10}$); 1.42 (*s* 9H, $H_{11,\ 12\ \&\ 13}$); 5.82 (*s* NH). The ^{13}C-NMR spectrum ($CDCl_3$, 50.25 MHz) exhibited δ (ppm): 165.8 (*s* C_1); 119.8 (*d* C_2); 141.5 (*d* C_3); 63.1 (*s* C_5); 173.8 (*s* C_6); 50.9 (*s* C_8); 25.5 (*q* $C_{9,\ 10}$); 28.6 (*q* $C_{11–13}$); 135.1 (*s* $C_{1'}$); 139.5 (*s* $C_{1''}$); 130.6, 129.4, 129.2, 128.5, 127.7 (aromatic carbons). The mass spectral data by GC/EI-LRMS: *m/z* values (% relative abundance): $[M]^{+\cdot}$364 (0.4), 292 (1.49), 291 (1.96), 266 (1.5), 265 (11.8), 264 (29.9), 188 (1.3), 146 (1.9), 135 (9.1), 134 (100.0), 132 (5.0), 131 (49.9), 130 (2.3), 118 (7.6), 115 (1.6), 106 (2.3), 104 (6.1), 103 (31.1), 102 (2.7), 94 (1.3), 93 (2.0).

The EI-HRMS measurements were performed on a Micromass VG AutoSpec (Manchester, UK), at the Instituto Universitario de Bioorgánica (Universidad de La Laguna), at a resolution of 5000 (5% valley definition), by 70 eV electron ionization, at an accelerating voltage of 8 kV. EI-LRMS was performed at 70 eV using an ion trap (GCQ Plus) with MS^n (Finnigan, Thermo-Quest, Austin, TX, USA), operated at a fundamental rf-drive of 1.03 MHz. Helium was used as the damping gas at an uncorrected gauge reading of 6×10^{-5} Torr. For the analysis of tandem mass spectrometric (MS/MS) product ions, the precursor ion was selected using a MS/MS standard function, with a peak width of 0.5–1.0 *m/z* units, and dynamically programmed scans. The supplementary voltage was in the range 0.5–1.0 V, as described previously.[7]

Although peptides usually yield protonated molecules $[M+H]^+$ when studied by LC/MS using positive ion electrospray ionization (ESI) or atmospheric pressure ionization (API) methods, the diamines considered here are not really peptidic and their high thermal stability facilitate their study by GC/MS and GC/MS^n. Thus, the compounds have been studied by electron ionization low-resolution mass spectrometry (LR-EIMS), EI-MS^n (n = 1–5) using an ion trap mass spectrometer, and electron ionization high-resolution mass spectrometry (HR-EIMS). The combination of these techniques allows us to establish the genesis[7] and the possible structures of the fragment ions. Figures 2(a) and 2(b) show the nomenclature[8–10] used in the present work to describe the different bond types and the possible fragment ions that could arise from fragmentation of the molecular ions of compounds **1** and **2**.

The EI-LRMS spectrum of compound **1** and the study of its fragment ions by MS^n, combined with the EI-HRMS spectra, led to the fragmentation pathway shown in Scheme 1 (Tables 1 and 2).

Routes $\mathbf{R_1}$ and $\mathbf{R_2}$ involve the elimination of 71 and 72 Da as neutral species from the molelcular ion at *m/z* 392. These pathways can be described as removal of *t*-butene and a methyl group, and the *N*-*t*-butylamino radical, respectively, to form the fragment ions at *m/z* 321 ($[C_{20}H_{21}N_2O_2]^+$**)** and 320 ($[C_{21}H_{22}NO_2]^+$**)**.

Route $\mathbf{R_3}$ corresponds to the loss of a neutral radical of 91 Da ($[C_7H_7]^\cdot$) involving the B ring, to form the fragment ion at *m/z* 301 ($[C_{18}H_{25}N_2O_2]^+$). Route $\mathbf{R_4}$ involves cleavage of

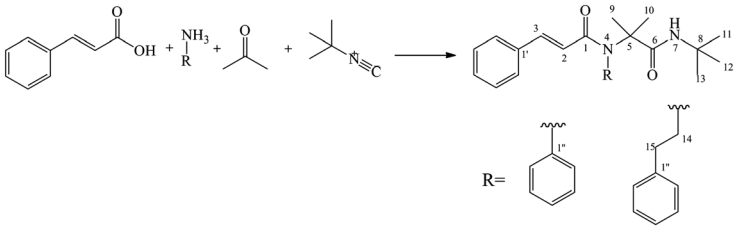


Figure 1. One-step-one-pot preparation of compounds **1** and **2** by the Ugi multicomponent reaction.

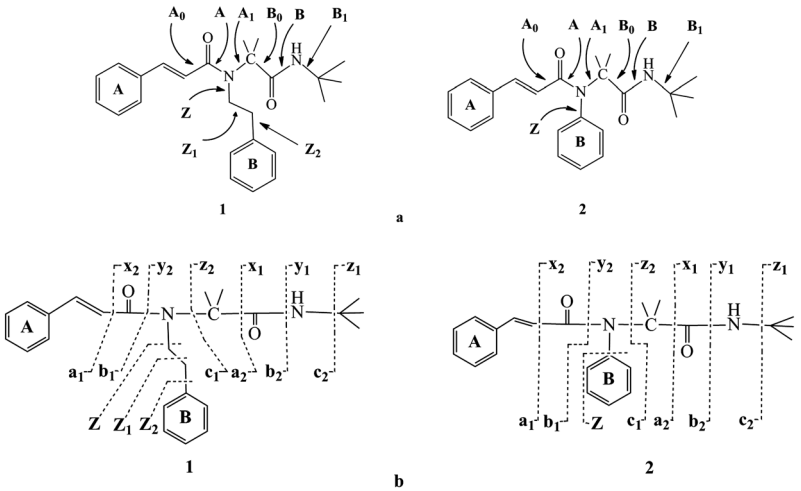


Figure 2. (a) Compounds **1** and **2** and their different bond types and (b) possible fragment ions from these compounds.

the B_0 bond bwetween the *gem*-dimethyl group and the carbonyl of the *N*-*t*-butylamide group with loss of the latter group (99 Da) to form the fragment ion at *m/z* 292 ($[C_{20}H_{22}NO]^+$).

The simultaneous loss of both *N*-t-butylamide (99 Da) and the benzyl group formed from ring B (91 Da) depicted in route $\mathbf{R_5}$ yields the fragment ion at *m/z* 202 ($[C_{13}H_{16}NO]^+$). The fragment ion at *m/z* 162, $[C_{11}H_{16}N]^+$, can be explained as involving the simultaneous removal of *N*-*t*-butylamide (99 Da), phenylacetylene (102 Da) and HCO (Da) as shown in route $\mathbf{R_6}$.

Route $\mathbf{R_7}$ involves the formation of the cinnamoyl fragment ion at *m/z* 131, $[C_9H_7O]^+$, that, when subjected to MS^3, yields a product ion at *m/z* 103 ($[C_8H_7]^+$). The latter ion can also be formed directly from the molecular ion, as depicted in route $\mathbf{R_8}$. The ion at *m/z* 103 can then eliminate acetylene, producing a phenyl ion at *m/z* 77 ($[C_6H_5]^+$), coming from ring A.

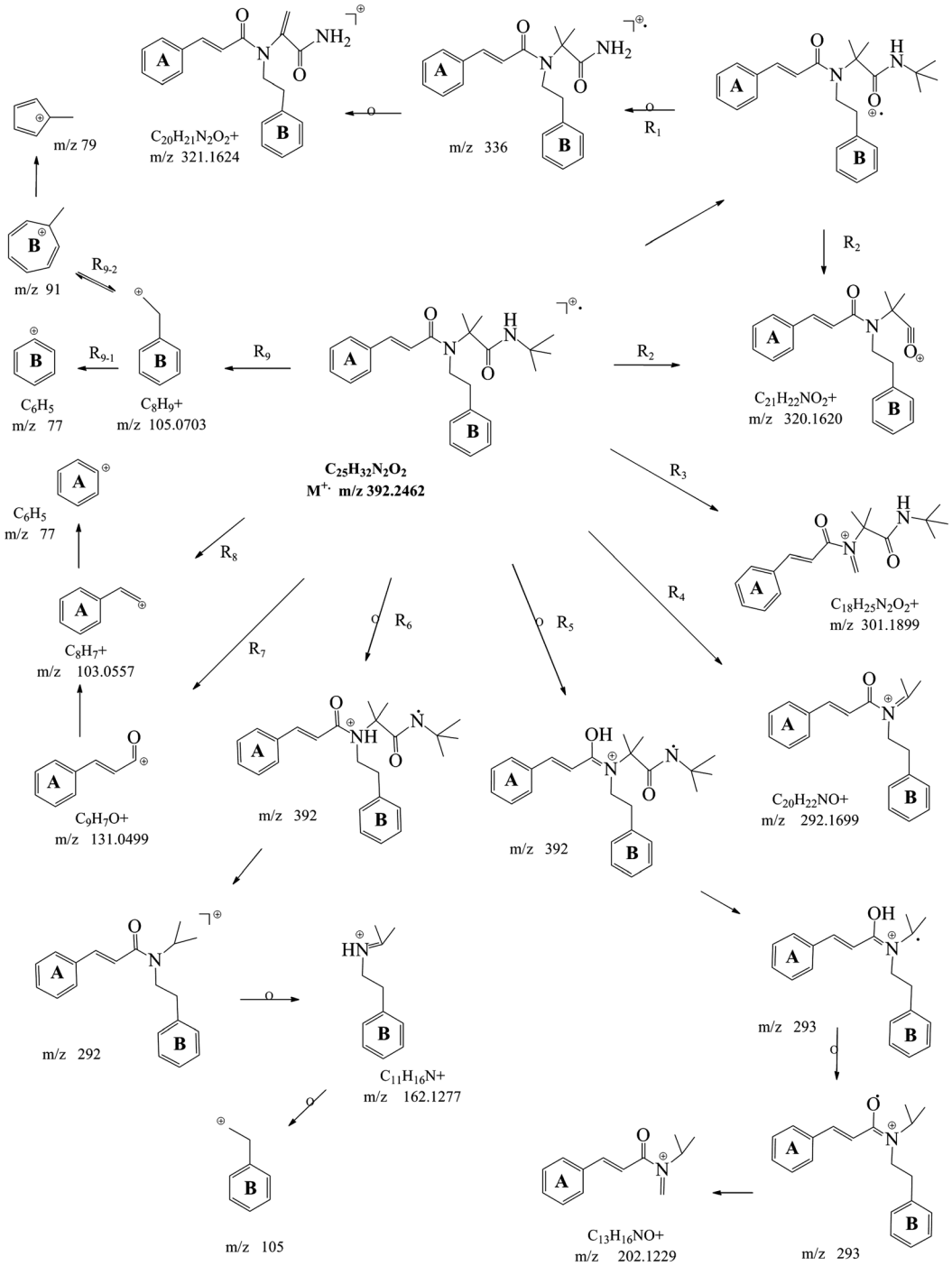
In addition, route $\mathbf{R_9}$ shows the direct formation of an ethylphenyl cation at *m/z* 105 ($[C_8H_9]^+$) from the molecular ion. This ion can then either lose ethylene (route $\mathbf{R_{9\text{-}1}}$), affording the phenyl ion at *m/z* 77 ($[C_6H_5]^+$), or tautomerize into a methyl-tropylium-type cation at *m/z* 105 (route $\mathbf{R_{9\text{-}2}}$), which can eliminate acetylene to form the methylcyclopentadienyl ion at *m/z* 79 ($[C_6H_7]^+$).

Scheme 2 (Tables 3 and 4) shows the EI fragmentation pathway of compound **2**. The fragment ions that are shared with compound **1** are not depicted nor discussed here.

The fragmentation routes described as $\mathbf{R_{1\text{-}1}}$, $\mathbf{R_{1\text{-}2}}$ and $\mathbf{R_{1\text{-}3}}$ show the single losses of methyl, hydroxyl, and *t*-butene from the molecular ion at *m/z* 364 yielding the ions at *m/z* 349, 347 and 308, respectively. Elimination of the *N*-*t*-butylamide group following cleavage of the $\mathbf{B_0}$ bond (routes $\mathbf{R_{2\text{-}2}}$ and $\mathbf{R_{2\text{-}3}}$) leads to the generation of the ions at *m/z* 265 ($[C_{18}H_{19}NO]^{+\cdot}$) and *m/z* 264 ($[C_{18}H_{18}NO]^+$), respectively. Route $\mathbf{R_3}$ involves the simultaneous removal of the *N*-*t*-butylamide and one of the methyl groups, leading to the fragment ion at *m/z* 250 ($[C_{17}H_{16}NO]^+$), while route $\mathbf{R_4}$ involves the elimination of a neutral group of 141 Da ($C_8H_{16}NO$) to yield the fragment ion at *m/z* 223 ($[C_{15}H_{13}NO]^{+\cdot}$). Route $\mathbf{R_5}$ involves the successive losses of the *N*-*t*-butylamide and cinnamoyl groups to form the fragment ion at *m/z* 134 ($[C_9H_{12}N]^+$) that then loses a methyl group to generate the ion at *m/z* 119 ($[C_8H_9N]^{+\cdot}$). As in compound **1** cleavage of the **A** bond yields the cinnamoyl ion at *m/z* 131 (route $\mathbf{R_6}$), while route $\mathbf{R_7}$ is analogous to route $\mathbf{R_8}$ proposed for compound **1**.

As was pointed out above, compounds **1** and **2** both possess two peptide-type bonds, named **A** and **B**. Their cleavages in EI and MS/MS processes show similarities to, as well as, major differences from the breaking of bonds in peptides, peptoids and retropeptides,[15] as well as remarkable differences and similarities between themselves. For example, cleavage of the $\mathbf{A_0}$ bond leads to formation of the styryl fragment ion, $\mathbf{a_1}$, at *m/z* 103, while the $\mathbf{x_2}$ fragments are neutral for both compounds. The cinnamoyl ion ($\mathbf{b_1}$) at *m/z* 131 that is formed by **A** bond fragmentation have relative intensities of 20.5% and 49.0% for compounds **1** and **2**, respectively.

On the other hand, the very low abundance of the $\mathbf{c_1}$ and/or $\mathbf{z_2}$ fragment ions indicates that $\mathbf{A_1}$ bonds in compounds **1** and **2** are not easily cleaved, or that the products are extremely unstable, although this would be a common bond cleavage in classical peptide chemistry.[9]



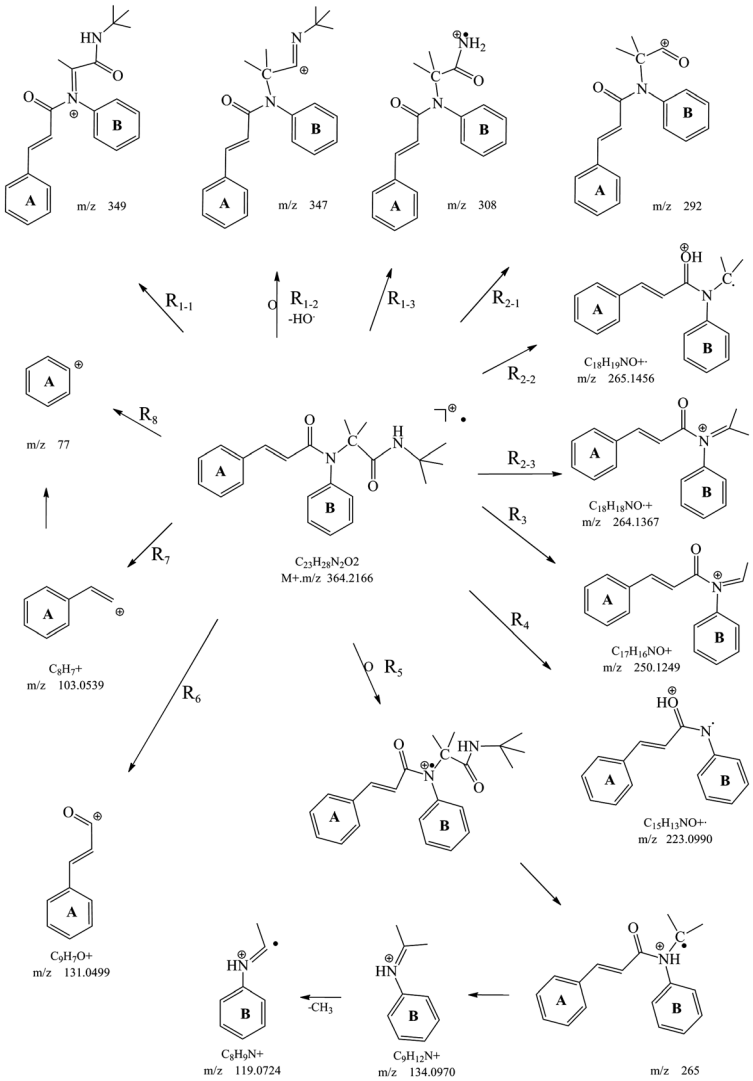
Scheme 1. Molecular ion fragmentation pattern, compound **1**, by GC/EI-MS^n.

Several other features are noticeable when comparing the spectra of compounds **1** and **2**. The $\mathbf{a_2}$ fragment ions at *m/z* 292 and 264 appeared in the EI spectra of both compounds. However, the MS/MS spectrum of the molecular ion of the diamide **1** does not show a product ion at *m/z* 292. On the other hand, the ion at *m/z* 264 is the base peak in the MS/MS spectrum of the molecular ion of compound **2**. For compound **2**, this might be attributed to the stabilization of the positive charge generated on the α carbon by cleavage of the $\mathbf{B_0}$ bond (fragment ion $\mathbf{a_2}$), caused by delocalization of the π electrons of phenyl ring B.

Fragment ions $\mathbf{b_2}$ and $\mathbf{y_1}$ are generated by cleavage of the **B** bonds. In the EI spectrum the $\mathbf{b_2}$ fragment ions (*m/z* 320 and 292) have similar and very low relative intensities of 1.0% and 1.5% for compounds **1** and **2**, respectively, and $\mathbf{y_1}$ is neutral in both cases.

Cleavage of the $\mathbf{B_1}$ bonds produces the low intensity fragment ions $\mathbf{c_2}$ (*m/z* 336 and 308), while the $\mathbf{z_1}$ fragment ion, of low abundance for both compounds, is undetectable in both MS/MS spectra.

Cleavage of the **Z** bonds is unlikely by MS/MS, since no peaks for the expected ions appeared were detected in the



Scheme 2. Molecular ion fragmentation pattern, compound **2**, by GC/EI-MS^n.

spectrum. However, the ion at *m/z* 301 corresponding to cleavage of the benzylic bond ($\mathbf{Z_1}$) is the base peak in the MS/MS spectrum of the molecular ion of compound **1**. On the contrary, in the EI spectrum this ion is of very low intensity, and the tropylium ion (*m/z* 91) is present at only very low relative abundance (0.9%). Likeness, the phenyl ion (*m/z* 77) is detected for diamides **1** and **2** (10.2% and 23.0%, respectively) only in the EI spectra.

In the MS/MS spectrum of $[M]^{+\cdot}$ of compound **1**, the base peak is the ion at *m/z* 301, formed by loss of 91 Da, whereas for the MS/MS of $[M]^{+\cdot}$ ion of compound **2**, the base peak appeared at *m/z* 264, corresponding to cleavage of the $\mathbf{B_0}$ bond.

Interestingly, the ions of *m/z* 162 and 134 are the base peaks of both the EI spectra and the MS/MS spectra of the $\mathbf{a_2}$ fragment ions (*m/z* 292 and 264) of compounds **1** and **2**, respectively.

In summary, the EI mass spectra of the two compounds are very similar. On the contrary notable differences are observed mainly between the MS/MS spectra of the fragment ions at *m/z* 292 (Table 2) and 264 (Table 4). As was mentioned above, this is attributable to the presence of a dimethylenic bridge in the $\mathbf{a_2}$ fragment ion of compound **1** that interrupts the conjugation of the aromatic B ring and thus reduces the stability of the fragment ion at *m/z* 292 relative to the one at *m/z* 264. In both cases, however, fragmentation of these ions

Table 1. Accurate mass measurements and elemental formulae of the main abundant ionic species of compound **1** obtained by EI-HRMS

Mass	Calc. mass	ppm	Formula
392.2462	392.2464	−0.5	$C_{25}H_{32}N_2O_2$
321.1624	321.1603	6.5	$C_{20}H_{21}N_2O_2$
320.1620	320.1651	−9.7	$C_{21}H_{22}NO_2$
301.1899	301.1916	−5.6	$C_{18}H_{25}N_2O_2$
292.1699	292.1701	−0.7	$C_{20}H_{22}NO$
291.1601	291.1623	−7.6	$C_{20}H_{21}NO$
202.1229	202.1232	−1.5	$C_{13}H_{16}NO$
162.1277	162.1283	−3.7	$C_{11}H_{16}N$
160.1138	160.1126	7.5	$C_{11}H_{14}N$
131.0499	131.0497	1.5	C_9H_7O
105.0703	105.0704	−1	C_8H_9
103.0557	103.0548	8.7	C_8H_7

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Table 2. Product ion spectra of the main abundant ionic species of compound **1** obtained by GC/EI-CID-MS/MS

Precursor ions	Product ions			
m/z (%)	*m/z* (%)	*m/z* (%)	*m/z* (%)	*m/z* (%)
392 (27.0)	358 (28.2)	335 (26.1)	301 (100.0)	269 (23.1)
320 (26.9)	292 (40.0)	277 (14.3)	269 (11.6)	162 (100.0)
292 (1.0)	162 (100.0)	131 (1.7)	105 (3.1)	103 (0.3)
202 (100.0)	173 (4.7)	145 (12.2)		
162 (1.0)	105 (100.0)	104 (1.2)	103 (1.2)	
131 (70.4)	103 (100.0)			
105 (100.0)	79 (13.5)	77 (3.5)		
103 (100.0)	77 (2.5)			

Table 3. Accurate mass measurements and elemental formulae of the main abundant ionic species of compound **2** obtained by EI-HRMS

Mass	Calc. mass	ppm	Formula
364.2166	364.2151	4.1	$C_{23}H_{28}N_2O_2$
265.1456	265.1467	−4.1	$C_{18}H_{19}NO$
264.1367	264.1388	−8	$C_{18}H_{18}NO$
250.1249	250.1232	6.8	$C_{17}H_{16}NO$
223.0990	223.0997	−3.1	$C_{15}H_{13}NO$
134.0970	134.0970	0	$C_9H_{12}N$
131.0499	131.0497	1.5	C_9H_7O
118.0667	118.0657	8.5	C_8H_8N
103.0539	103.0548	−8.7	C_8H_7

leads to the corresponding imine ions at *m/z* 162 and 134 that are the base peaks of both spectra.

For compound **2**, the MS/MS spectrum of the fragment ion at *m/z* 264 showed that this ion was the precursor of the product ions at *m/z* 249, 236, 222, 134, and 131. The first three ions could only come from the elimination of internal fragments from the precursor ion, such as a methyl group, C = O or C_2H_4 and ketene (Table 4). It is noticeable that the loss of 28 Da (to form *m/z* 236, 96% in the *cis* isomer) is attributable to C = O and/or C_2H_4, since the ion at *m/z* 264 does not contain the dimethylenic bridge.

This suggests the occurrence of rearrangements that involve the formation of a third ring (Scheme 3). These new structures could easily expel neutral fragments, particularly C = O or C_2H_4, to form the ion at *m/z* 236. In this sense, we and other authors have already reported similar rearrangements in the EI spectra of chalconoid and stilbene compounds.[7,11–15] The high intensity of the peak at *m/z* 236 in the *cis* isomer discussed above could be attributable to the easier formation of the third ring in this isomer than in the *trans* one.

Unlike the data reported from peptides, peptoids and retropeptoids,[15] the base peaks in the EI spectra of compounds **1** and **2** correspond to imine fragment ions ($[C_{11}H_{16}N]^+$) at *m/z* 162 and 134, respectively (Schemes 1 and 2).

In the above-mentioned report[15] the MS/MS spectra of the protonated molecules present a wide range of product ions. Immonium, imine and ylide moieties are eliminated as neutral groups in the spectra of peptides, retropeptides and peptoids.[9] On the contrary, for our compounds **1** and **2** these are ionic fragments, so detectable, which gives them diagnostic importance for recognizing metabolites in complex biological matrices in order to recognize the presence of the diamides and their possible metabolites, even at very low concentrations.

The losses of 100 Da ($[C_5H_{10}NO]^{\cdot}$) affords two characteristic fragment ions at *m/z* 292 and 264 for compounds **1** and **2**, respectively. The further loss of the cinnamoyl radicals ($[C_9H_7O]^{\cdot}$) yields fragment ions at *m/z* 162 and 134, respectively, that also constitute two characteristic peaks to be investigated in biological samples. Exclusively for compound **2** another peak marker could be the ion at *m/z* 236 that comes from the rearrangement proposed for the *cis* isomer of *m/z* 264 ($[C_{18}H_{18}NO]^+$).

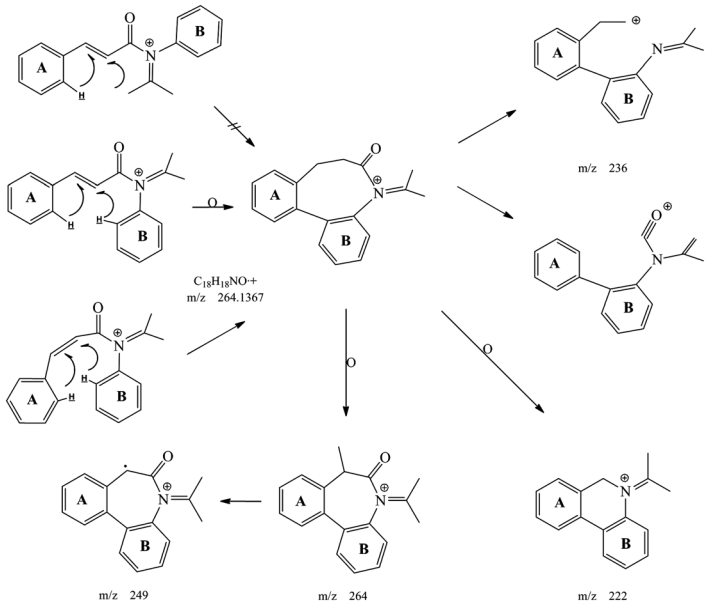
SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Table 4. Product ion spectra of the main abundant ionic species of compound **2** obtained by GC/EI-CID-MS/MS

Precursor ions	Product ions					
m/z (%)	*m/z* (%)	*m/z* (%)	*m/z* (%)	*m/z* (%)	*m/z* (%)	*m/z* (%)
364 (6.9)	349 (32.6)	346 (6.1)	308 (52.0)	307 (16.0)	291 (26.4)	276 (7.0)
	264 (100.0)	238 (10.3)	231 (16.0)	145 (11.7)	138 (10.9)	134 (49.3)
292 (12.8)	265 (10.7)	264 (91.0)	134 (100.0)	131 (27.3)	103 (1.7)	77 (2.2)
265 (32.4)	264 (35.2)	250 (27.0)	174 (10.4)	172 (10.1)	147 (21.9)	146 (17.4)
	135 (72.6)	134 (100.0)	132 (21.6)	131 (27.5)		
264 (7.2)	249 (0.4/0.4)*	236 (0.2/95.6)*	222 (0.8/–)*	134 (100.0)*	131(22/20)*	
223 (42.6)	222 (100.0)	207 (3.1)				
222 (100.0)	221 (11.1)	181 (1.4)				
135 (11.1)	134 (100.0)	120 (19.7)	119 (1.6)	118 (7.9)	106 (9.0)	
134 (100.0)	117 (37.0)	115 (25.8)	106 (16.7)			
132 (7.5)	131 (1.9)	104 (100.0)	103 (8.1)			
131 (5.1)	103 (100.0)					

*% *trans*/% *cis* isomers



Scheme 3. Rearrangements in the fragment ion at *m/z* 264 from compound **2**, to eliminate methyl, C = O or C_2H_4, and ketene.

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