To the Editor-in-Chief

Dear Sir,

Cleavages of photochromic compounds derived from heterocycles under Electrospray Tandem Mass Spectrometry: Study of the influence of the heteroatom in fragmentation mechanisms.

Benzo- and naphthopyrans constitute an important class of compounds that exhibit photochromic behaviour. In solution or included in polimeric matrices, the UV irradiation of the usualy colorless pyran form (CF) undergoes a clevage of the pyran C-O bond cleavage, producing a set of colored isomeric open forms (TC and TT) which can be thermally and/or photochemically reverted to the original structure.[1] This reversible behaviour opens a wide range of interesting applications where a physical or chemical reversible change can be phototriggered. In recent years NMR studies revealed important details in the photochromic mechanism of this family of compounds, namely on the thermal or photochemical interconversion of the different species and the common involvement of a colourless allenyl-naphthol structure as a key-intermediate.[2,3,4] All these studies were performed in polar solvents, at low temperatures and quite high concentrations (10^{-2} *M*) due to the relatively low instrumental sensitivity. More recently, Maurel *et al.* using surface-enhanced resonance Raman scattering (SERRS) showed that this technique could be used for the study of the photochromic process at ambient temperature and low concentrations (10^{-5} *M*).[5,6] Several chromenes besides the detection of the usual coloured photoisomers (TC and TT) evidenced, again, the involvement of the allenyl-naphthol intermediate.[6]

In a previous work we have shown that positive mode electrospray tandem mass spectrometry (ESI-MS/MS) could be a very useful technique for the structural characterization of the main photoproducts of 2*H*-chromenes derived from carbazoles and that fragmentation could occur either from the CF form or the TC and/or TT forms.[7]

Information about mass spectrometric fragmentation of photochromic compounds, such as benzo- and naphthopyrans, is very scarce and all the studies were based on electron impact mass spectrometry (EI) of simple 2*H*-pyrans and 2*H*-[1]-benzopyrans being the benzopyrilium ion identified as the

major fragment.[8] Therefore we have decided to study the mass spectral characteristics using electrospray tandem mass spectrometry of different chromenes derived from dibenzothiophenes and dibenzofurans with the same annelation of the carbazole derivatives.[7]

In this paper we report the fragmentation pathways of these classes of compounds, under ESI-MS/MS experimental conditions, and its relation with their structural features, specially focused on the heteroatom's effect on the fragmentation mechanisms. Among other results we found evidences on the involvement of the allenyl-naphthol intermediate what indicates that this technique can be a valuable tool for the study of the photochromic mechanisms of 2*H*-chromenes without irradiation, using trace amounts of samples.

Positive ion mode electrospray mass spectrometry (ESI-MS) and tandem mass spectrometry (ESI-MS/MS) spectra were acquired in a Q-TOF 2 instrument (Micromass, Manchester, UK), setting the needle voltage at 3000V with the ion source at 80ºC and sample cone voltage at 35V. Nitrogen was used as nebulizer gas and argon as collision gas. Tandem mass spectra (MS/MS) of molecular ions were performed by Collision Induced Decomposition (CID) using argon as the collision gas and by changing collision energy between 22-27eV. Data acquisition was carried out with a Micromass Masslynx 4.0 data system. Samples for electrospray analysis were prepared by solubilisation in chloroform and diluting 1 μL in 200 μL of MeOH for a final concentration of approximately 10 pmol/μL. Samples were introduced into the mass spectrometer using a flow rate of 10 μL/min. Methanol and chloroform were HPLC grade (Riedel-de Haen). Methanol-d_1 (CH_3OD) (Riedel-de-Haen) used in the solution preparation to obtain the deuterated molecular species $[M+D]^+$. The chromenes were synthesized by a previously described methodology.[9,10]

Electrospray mass spectra (ESI-MS) show the $[M+H]^+$ ions of all compounds (Scheme 1), that can be present in either the open or closed form. The major product ions with their relative abundances (RA) observed in the ESI- MS/MS experiments from the $[M+H]^+$ precursor ions, are listed in Table 1.

All the compounds in study have the same annelation (5,6) between the heterocyclic and the pyran ring, however small differences in their spectra that

could be associated with the presence of different heteroatom's (N; O and S) were observed.

Generally, all the chromenes used in this work have a common pattern of fragmentation in the ESI-MS/MS experiments, as can see in scheme 2, that occur initially, through a ring opening, similar to that observed upon UV irradiation that can be used for the study of mechanistic aspects of photochromism.

For chromenes derived from carbazole (**1** and **4**) we have observed a fragment ion at m/z 191 that is always the base peak (Figure 1A) and we have already shown that the formation of this ion indicates that the charge is not predominantly located on the heteroatom of the precursor ion, which was demonstrated by the MS/MS spectra of deuterated molecules, $[M+D]^+$. Therefore the fragment ion must be generated from fragmentation of the open forms (structures E and F, Scheme 2).[7]

After the ring opening we can consider the possibility of two kinds of species of the open form, a zwitterionic form that can be formed in solution and allows, upon the acquisition of a proton by the negatively charged oxygen, the generation of a very stable tertiary carbocation (structure A). This carbocation produces the ion at *m/z* 191 after cleavage of the bond adjacent to the charged carbon. The presence of fragments at *m/z* 268 (**1**), 269 (**2**) and 285 (**3**) due to the loss of 106 Da ($CO + C_6H_6$) from the protonated molecule indicates that the open structures can also be quinoidal species because the elimination of CO is a typical fragmentation of cyclic ketones.[11]

The formation of fragment ions with *m/z* 182 (**1**), 183 (**2**) and 199 (**3**) results from the loss of 192 Da ($C_5H_{12}^{\bullet}$) and *m/z* 196 (**1**), 197 (**2**) and 213 (**3**) results from the loss of 179 Da (C_4H_{11}). To compounds **2** (derived from dibenzofuran) and **3** (derived from dibenzothiophene) the peak at *m/z* 191 was always observed together with the fragment at m/z 192 (Figure 1B-C).

Thus, besides the common fragmentations reported for all the classes, the dibenzofuran and dibenzothiophene derivatives showed also the ion at *m/z* 192 as base peak, which was minor ion for carbazoles.[7] In order to understand what was happening we have made the MS/MS spectra of deuterated molecules $[M+D]^+$ (Figure 2). In the previous ESI-MS/MS study of carbazole derivatives, it

was found that the fragment at *m/z* 191 is observed as the base peak for both $[M+H]^+$ and $[M+D]^+$ MS/MS spectra (Fig.1A and 2A).[7]

In figure 2B and 2C, for compounds **2** and **3**, a major ion at *m/z* 193 is observed which indicates the presence of an ionizing D^+, instead of H^+, as observed for fragmentation of $[M+H]^+$ ions. The presence of this labile H-atom led us to consider a different preferential cleavage for dibenzofuran and dibenzothiophene derivatives that is not relevant for the fragmentation of carbazoles. So we proposed a fragmentation pathway that involves the initial formation of allenyl-naphtol with subsequent 1,5-sigmatropic shift leading to an intermediate that can cleave on the α-bond relative to the charge by homolytic cleavage (Scheme 3).

In conclusion, we have found differences in the fragmentation mechanism of carbazole, dibenzofuran and dibenzothiophene derivatives with the same annelation and relative position of oxygen atom of the pyran ring. This results suggests the involvement of the allenyl-naphthol intermediate on the photochromic mechanism what makes electrospray tandem mass spectrometry a valuable tool for the study of the photochromic mechanisms of 2*H*-chromenes.

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Yours,

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Captions

Figure 1. ES-MS/MS spectra of $[M+H]^+$ ions of chromenes **1**-**3**.

Figure 1. ES-MS/MS spectra of $[M+D]^+$ ions of chromenes **1**-**3**.

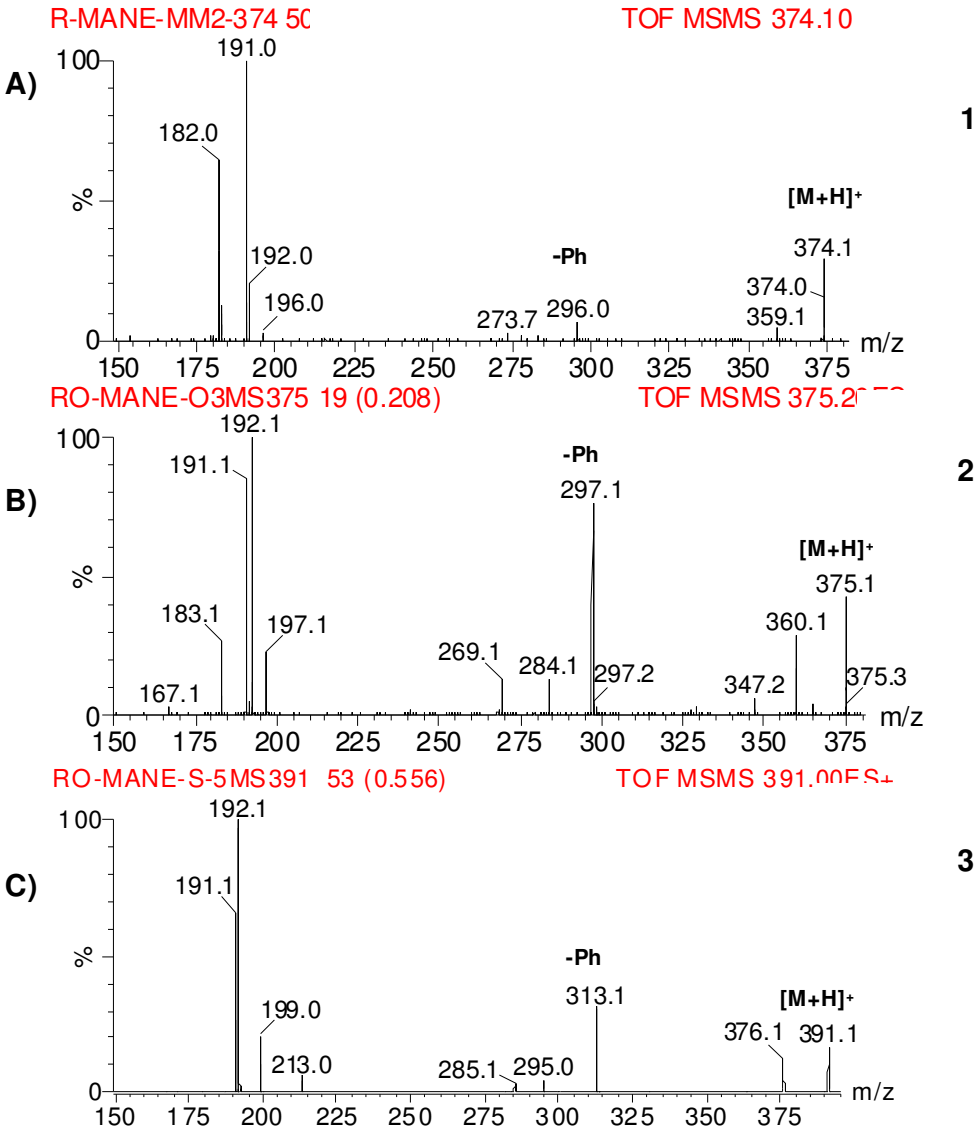
Scheme 1. Compounds **1**-**4** and **Ref 1**

Scheme 2**.** Common fragmentation pathways of compounds **1**, **2**, **3** and **4**

Scheme 3. Proposed MS/MS fragmentation mechanism of dibenzofuran and dibenzothiophene derivatives.

Table 1**.** Main fragment ions observed in the ES-MS/MS spectra of the $[M+H]^+$ ions of the compounds studied. **1** - 2,2-diphenyl-2*H*-pyrano[5,6-*a*]carbazole; **2** – 2,2-diphenyl-2*H*-pyran[5,6-*a*]dibenzofurane; **3** - 2,2-diphenyl-2*H*-pyran[5,6-*a*]dibenzothiophene; **4** - 2,2-diphenyl-5-methyl-2*H*-pyrano[5,6-*a*]carbazole; **Ref 1** - 3,3-diphenyl-3*H*-naphtho[2,1-*b*]pyran;

Figure 1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TOF MSMS 374.10
A)
1
191.0
182.0
192.0
196.0
-Ph
273.7
296.0
[M+H]+
374.1
374.0
359.1
m/z
RO-MANE-O3MS375 19 (0.208)
TOF MSMS 375.2
B)
2
192.1
191.1
-Ph
297.1
[M+H]+
375.1
183.1
197.1
360.1
269.1
284.1
297.2
347.2
375.3
167.1
m/z
RO-MANE-S-5MS391 53 (0.556)
TOF MSMS 391.00ES+
C)
3
192.1
191.1
-Ph
313.1
199.0
[M+H]+
376.1
391.1
213.0
285.1
295.0
m/z

Figure 2

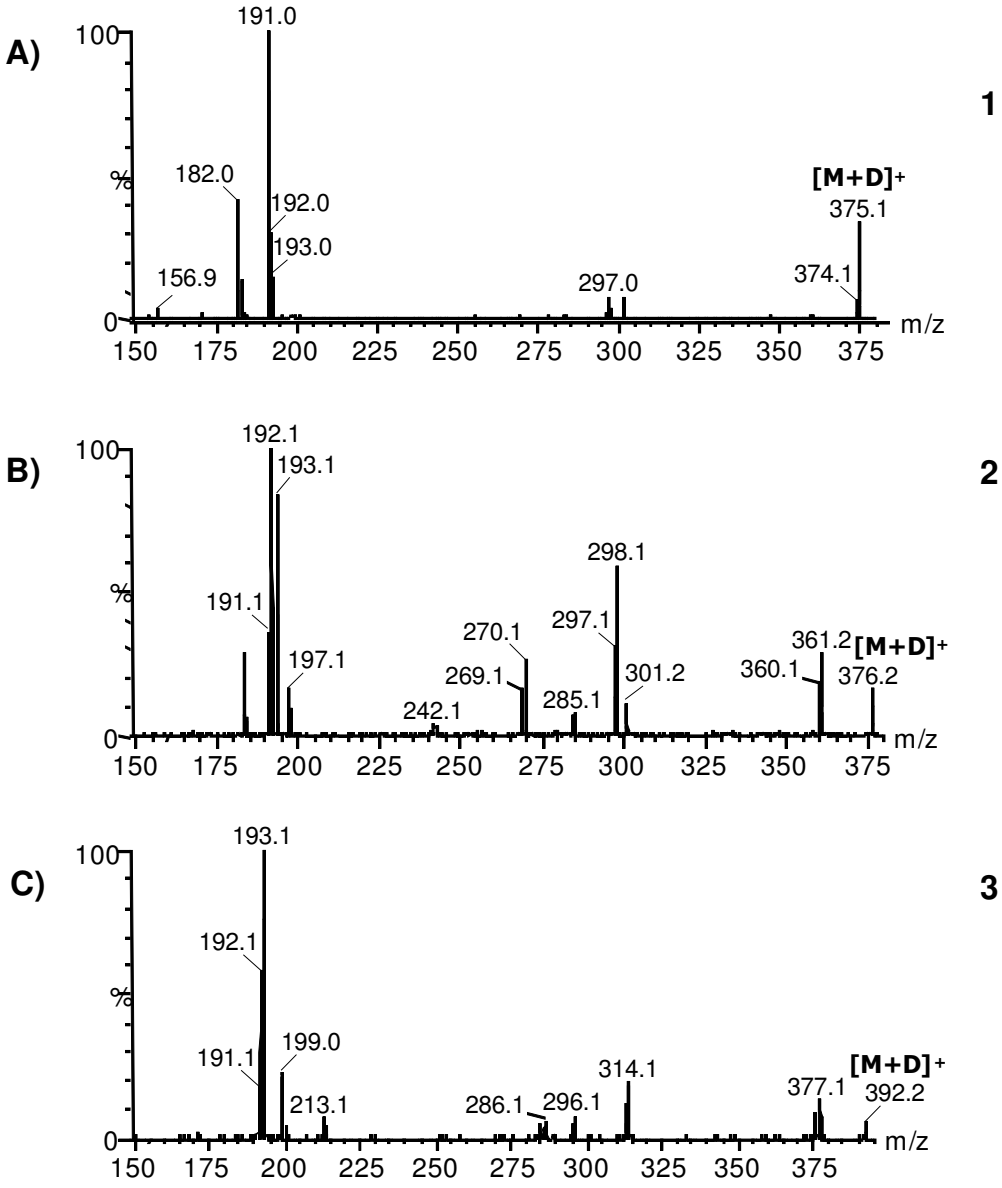
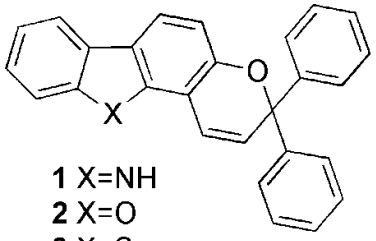

A)
191.0
100
%
182.0
192.0
193.0
156.9
297.0
374.1
[M+D]+
375.1
0
m/z
150 175 200 225 250 275 300 325 350 375
1
B)
192.1
193.1
100
%
191.1
197.1
242.1
269.1
270.1
285.1
297.1
298.1
301.2
360.1
361.2
[M+D]+
376.2
0
m/z
150 175 200 225 250 275 300 325 350 375
2
C)
193.1
100
%
192.1
191.1
199.0
213.1
286.1
296.1
314.1
377.1
[M+D]+
392.2
0
m/z
150 175 200 225 250 275 300 325 350 375
3

Table 1

Chromenes	***m/z* [M+H]$^+$**	***m/z* (relative abundance)**
1	374	359 (2); 296 (7); 283 (4); 268 (5); 196(18); 192 (20); **191** (100); 182 (18)
2	375	360 (35); 347 (8); 297 (87); 284 (12); 269 (14); 197 (16); **192** (100); **191** (90); 183 (23)
3	391	376 (8); 313 (27); 295 (5); 285 (6); 213 (7); 199 (22); **192** (100); **191** (66)
4[7]	388	373 (10); 360 (3); 345 (2); 310 (10); 297 (6); 296 (9); 284 (8); 282 (6); 220 (7); 210 (5); 197 (15); 196 (30); **191** (100)
Ref 1[7]	335	335 (21); 320 (10); 317 (11); 307 (2); 257 (88), 244 (12); 239 (4); 229 (7); **192** (100); **191** (60); 167 (20); 157 (4)

Scheme 1



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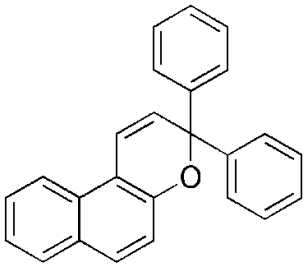
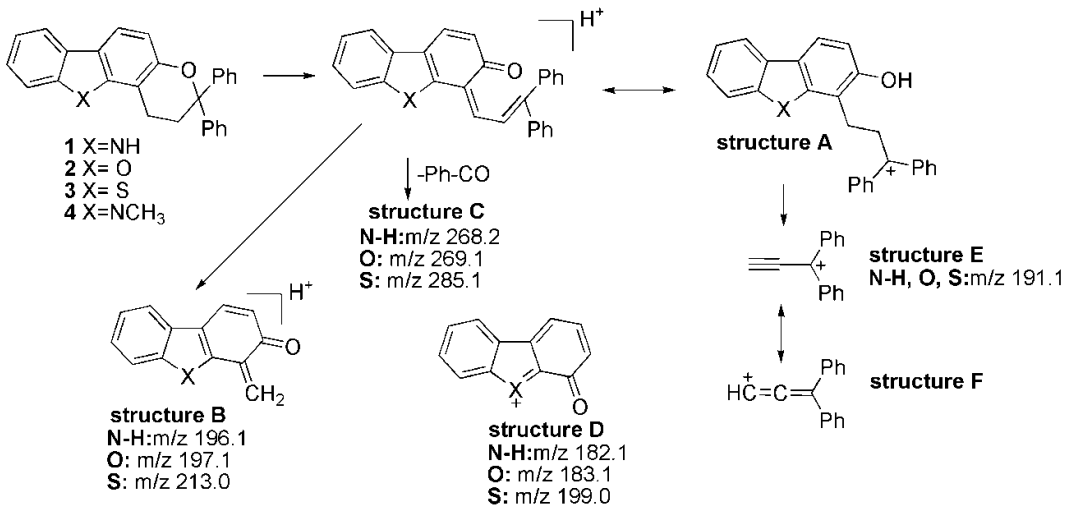
2 X=O
3 X=S
4 X=NCH_3

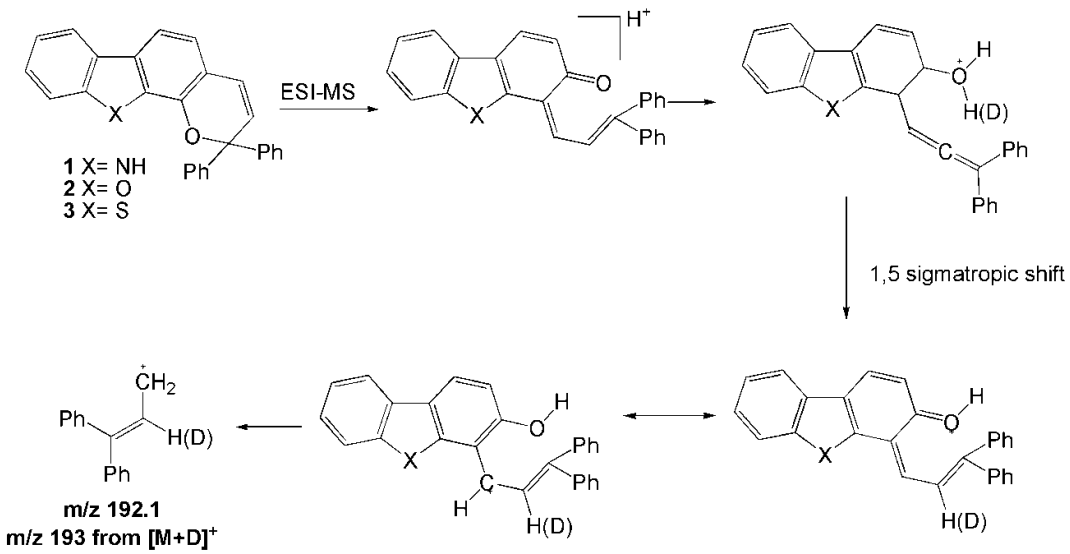


Ref 1

Scheme 2


1 X=NH
2 X= O
3 X= S
4 X=NCH3
-Ph-CO
structure C
N-H:m/z 268.2
O: m/z 269.1
S: m/z 285.1
structure A
structure B
N-H:m/z 196.1
O: m/z 197.1
S: m/z 213.0
structure D
N-H:m/z 182.1
O: m/z 183.1
S: m/z 199.0
structure E
N-H, O, S:m/z 191.1
structure F

Scheme 3


ESI-MS
H+
1 X= NH
2 X= O
3 X= S
1,5 sigmatropic shift
m/z 192.1
m/z 193 from [M+D]+