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Polycyclic compounds by sunlight exposure of the drug rosuvastatin in water

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Abstract

Irradiation of rosuvastatin in water by sunlight or UV lamp (Pyrex) affords cyclic compounds. The main photo-induced reaction is cyclization of the drug to give diastereomeric dihydrobenzoquinazolines. Products derived from side-chain loss were also isolated in the irradiation mixture, and characterized as dihydrobenzoquinazoline and benzoquinazoline derivatives by spectroscopic means. Photoproducts structure elucidation and mechanistic hypothesis are reported.

Keywords: Rosuvastatin; Dihydrobenzoquinazolines; Benzoquinazolines; Photocyclization; Photoxidation

1. Introduction

Rosuvastatin sodium is a statin of new generation that acts as lipid-lowering agent and is widely used in the prevention of cardiovascular events. It works as inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme which catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. This pharmaceutical product is widely prescribed in Europe and in the USA [1,2].

Rosuvastatin presents a *p*-fluorophenyl, a pyrimidinic ring and an unsaturated functionalized side-chain, necessary for the interaction with the active site of the enzyme.

Nowadays, pharmaceuticals and personal care products (PPCPs) are receiving considerable attention as emerging pollutants of the aquatic ecosystem [3]. The continuous introduction of these chemicals in the aquatic environment has been evidenced by the detection of a large number of PPCPs in surface waters in many countries [4,5]. A lot of articles in environmental chemistry reported the occurrence of these compounds, but data on the fate of pharmaceuticals in water are still limited. The large quantities utilized and the bioactive properties of drugs call for a deeper insight on their fate in the environment. Once in water, these chemicals can undergo hydrolytic and photochemical transformations leading to different compounds [6–9]. In such cases the analytical and eco-toxicological investigations should be addressed also to these transformation compounds.

In this context, in a recent work, we have investigated the photochemical behaviour of atorvastatin in water [10]. The data show that the drug is not stable when irradiated by sunlight giving rise to several photoproducts.

In this work the photochemical transformation processes of rosuvastatin in water have been investigated. Here we report the structure elucidation of the transformation products.

2. Experimental

2.1. Chemicals

Rosuvastatin sodium was obtained from KEMPROTEC Limited. This product was used without further treatment. Solutions and suspensions of drugs were prepared using Milli-Q water. All other solvents were of HPLC grade.

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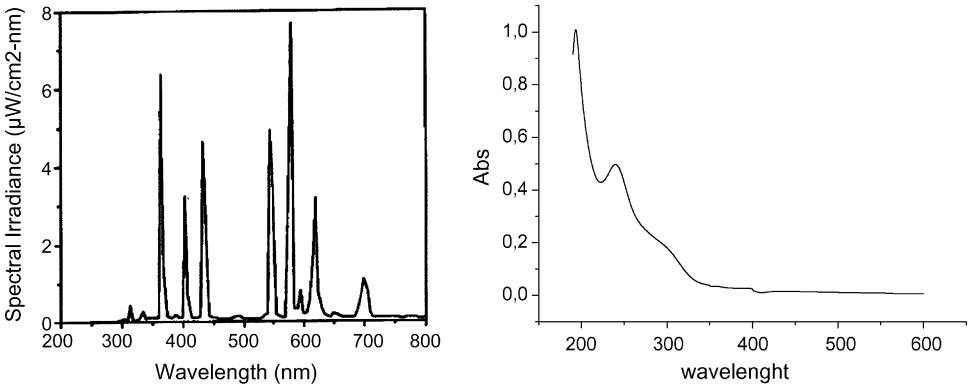


Fig. 1. Emission spectrum Hg lamp and UV spectrum of rosuvastatin in water.

2.2. General procedures

HPLC experiments were carried out on an Agilent 1100 HPLC system equipped with an UV detector, the column used was a RP-18 column (Prodigy Prep ODS, 10 μm, 250 mm × 10 mm). Nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz for [^{1}H] and 125 MHz for [^{13}C] on a Fourier Transform NMR Varian 500 Unity Inova spectrometer and at 400 MHz for [^{1}H] and 100 MHz for [^{13}C] on a Bruker AC 400 spectrometer. The carbon multiplicity was evidenced by DEPT experiments. The proton couplings were evidenced by ^{1}H–^{1}H COSY experiments. The heteronuclear chemical shift correlations were determined by HMQC and HMBC pulse sequences. ^{1}H–^{1}H proximities through space within a molecule were determined by NOESY. UV/vis spectra were recorded in CH_3OH on a Perkin-Elmer Lambda 7 spectrophotometer. Low resolution electron impact mass spectra were obtained operating at 70 eV on a GC–MS (QP-5050A Shimadzu). IR spectra were recorded in CH_2Cl_2 on a Nicolet 5700 FT-IR spectrometer. Analytical TLC was performed on precoated Merck aluminium sheet (DC-Alufolien Kielselgel 60 F_{254}, 0.2 mm) or RP-18 F_{254} plates with 0.2 mm film thickness. The spots were visualized by UV light or by spraying with H_2SO_4–AcOH–H_2O (1:20:4). The plates were then heated for 5 min at 110 °C. Prep. TLC was performed on a Merck Kiesegel 60 F_{254} plates, with 0.5 or 1 mm film thickness.

2.3. Experimental procedure

2.3.1. Rosuvastatin irradiation experiments

Rosuvastatin calcium is slightly soluble in water. Experiments in the dark were conducted on solution (10^{-5} M) or dispersion of the drug (80 ppm) in pure water and in buffered water pH 7 with NaH_2PO_4/Na_2HPO_4.

The preparations were kept in the dark for 1, 4 and 7 days, then the water was concentrated, dissolved with CH_2Cl_2 and filtered on Millex; the residue was analyzed by TLC and ^{1}H NMR.

Solutions of rosuvastatin (10^{-5} M) were exposed to sunlight in water for 4 days on January in Naples in open Pyrex flasks.

Other irradiation experiments were conducted with a photoreactor equipped with a 500 W mercury vapor lamp (Helios Italquarz, emission spectrum shown in Fig. 1), through a Pyrex filter, for 8 h in open Pyrex tube at room temperature under stirring at a distance of 15 cm from the lamp.

A dispersion of rosuvastatin calcium (40 mg in 500 ml of water) was exposed to sunlight for 4 days, the irradiation mixture was dried under vacuum and separated by silica gel TLC-chromatography eluting with CH_2Cl_2/MeOH (95:5) with drops of AcOH (two runs) to obtain three main fractions.

Fraction 1 (5%) was a mixture of polar products in which aromatic functions were absent, fraction 2 (45%) was a diastereomeric mixture and fraction 3 (50%) contained two apolar products.

Separation of the diastereomeric mixture **2** was performed on RP-18 HPLC column eluting with MeOH/H_2O (0.2% HCOOH) (7:3) to obtain pure isomers **2a** (6 mg, 15%) and **2b** (7 mg, 17%).

Two compounds, **3** (12 mg, 30%) and **4** (4 mg, 10%), from fraction 3 were separated by silica gel TLC-chromatography eluting with hexane/CH_2Cl_2 (8:2) (five runs).

2.3.2. Compound *1*

White powder; UV spectrum shown in Fig. 1. ν_{max}($CHCl_3$) 3688, 3612, 3300, 2971, 1596, 1547, 1378 cm^{-1}. ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2.

2.3.3. Compound *2a*

White powder; UV λ_{max} (CH_3OH) nm: 314 (log ε 3.8). ν_{max}($CHCl_3$) 3686, 3602, 3056, 2928, 1724, 1612, 1558, 1383 cm^{-1}; EI-MS (as methyl ester) *m/z* (%): 463 (10), 445 (5), 384 (65), 366 (37), 348 (100). Anal. calcd for $C_{22}H_{28}FN_3O_6S$: C, 54.88, H, 5.82, F, 3.95, N, 8.73. Found: C, 55.00, H, 5.75, F, 3.99, N, 8.77. ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2.

2.3.4. Compound *2b*

White powder; UV λ_{max} (CH_3OH) nm: 312 (log ε 3.8). ν_{max}($CHCl_3$) 3686, 3599, 3056, 2928, 1724, 1612, 1558, 1420, 1383 cm^{-1}; EI-MS (as methyl ester) *m/z* (%): 463 (10), 445 (5), 384 (65), 366 (37), 348 (100). Anal. calcd for $C_{22}H_{28}FN_3O_6S$: C, 54.88, H, 5.82, F, 3.95, N, 8.73. Found: C, 54.92, H, 5.70, F, 3.92, N, 8.73. ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2.

Table 1
^{1}H NMR data of **1–2** (CD_3OD)[a]

Position	1	2a	2b
2	2.37 dd (3.9, 15.6); 2.27 dd (8.8, 15.6)	2.37 dd (2.0, 14.6); 2.26 dd (6.8, 14.6)	2.33 m, 2.27 m
3	4.01 m	4.11 m	4.03 m
4	1.65 m, 1.50 m	1.64 m, 1.52 m	1.62 m, 1.57 m
5	4.35 m	3.74 m	3.59 obscured
6	5.55 dd (5.9, 15.6)	3.08 m	2.99 m
7	6.62 d (15.6)	3.29 obscured, 2.98 dd (6.8, 16.6)	3.59 obscured, 2.85 dd (6.8, 17.6)
14	3.48 m	3.41 m	3.41 m
15	1.27 d (6.5)	1.35$^{\#}$ d (6.5)	1.36$^{\#}$ d (6.5)
16	1.27 d (6.5)	1.25$^{\#}$ d (6.5)	1.26$^{\#}$ d (6.5)
17	3.53* s	3.57* s	3.57* s
18	3.51* s	3.54* s	3.55* s
2′	7.70 dd (4.9, 8.9)	8.34 dd (5.8, 7.8)	8.31 dd (5.8, 7.8)
3′	7.16 t (8.9)	7.12 m	7.14 m
5′	7.16 t (8.9)	7.12 m	7.14 m
6′	7.70 dd (4.9, 8.9)		

Values with same superscript were exchangeable.
[a] *J* values (in Hz) in parentheses.

2.3.5. Compound **3**

White powder; UV λ_{max} (CH_3OH) nm: 312 ($\log \varepsilon$ 4.1). ν_{max}($CHCl_3$) 3683, 3056, 2922, 1616, 1555, 1383 cm^{-1}; EI-MS *m/z* (%): 349 (54), 270 (100), 254 (54). Anal. calcd for $C_{17}H_{20}FN_3O_2S$: C, 58.45, H, 5.73, F, 5.44, N, 12.03. Found: C, 58.50, H, 5.75, F, 5.48, N, 12.07. ^{1}H and ^{13}C NMR data are listed in Table 3.

2.3.6. Compound **4**

White powder; UV λ_{max} (CH_3OH) nm: 357 ($\log \varepsilon$ 4.0). ν_{max}($CHCl_3$) 3686, 3053, 2981, 2922, 1621, 1561, 1511, 1420, 1370, 1261 cm^{-1}. EI-MS *m/z* (%): 347 (68), 332 (50), 268 (100), 253 (55). Anal. calcd for $C_{17}H_{18}FN_3O_2S$: C, 58.79, H, 5.19, F, 5.48, N, 12.10. Found: C, 58.85, H, 5.15, F, 5.55, N, 12.15. ^{1}H and ^{13}C NMR data are listed in Table 3.

Table 2
^{13}C NMR data of **1–2** (CD_3OD)

Position[a]	1	2a	2b
1	182.4	175.0	175.7
2	45.4	42.5	40.1
3	69.1	68.9	69.2
4	44.7	40.9	42.5
5	72.1	73.9	72.3
6	142.3	45.5	45.7
7	124.4	25.0	24.6
8	123.6	119.4	119.5
9	163.6	160.4	160.3
11	159.3	159.5	160.3
13	176.9	175.0	175.7
14	33.7	32.8	32.9
15	22.6	22.1	22.1
16	22.6	22.1	22.1
17	42.8	41.6	42.5
18	34.3	34.3	34.4
1′	136.6	130.9	131.0
2′	134.0	130.0	130.2
3′	116.6	115.9	116.1
4′	165.2	167.3	165.1
5′	116.6	118.1	117.7
6′	134.0	144.5	144.7

[a] Assigned by DEPT, HSQC and HMBC experiments.

Table 3
NMR data of **3–4** ($CDCl_3$)[a]

Position	3		4	
	δ_H	δ_C[b]	δ_H	δ_C[b]
1		173.1		175.8
1a		118.5		126.9
3		157.6		156.0
4a		158.6		151.0
5a		129.8		129.7
5	8.26 dd (6.0, 8.7)	128.4	9.15 dd (6.0, 8.7)	128.1
6	7.04 dt (9.0, 2.7)	114.5	7.44 dt (9.0, 2.4)	116.3
7		165.6		162.6
8	6.95 dd (9.0, 2.4)	114.5	7.52 dd (8.7, 2.4)	112.1
8a		141.8		137.0
9	2.93 s	27.8	7.67 d (9.0)	125.4
10	2.93 s	21.9	7.96 d (9.0)	121.8
11	3.24 m	31.3	3.92 m	31.3
12	1.24 d (6.5)	21.2	1.45 d (6.5)	21.8
13	1.24 d (6.5)	21.2	1.45 d (6.5)	21.8
14	3.61* s	42.2	3.77* s	42.3
15	3.58* s	33.2	3.67* s	33.4

Values with same superscript were exchangeable.
[a] *J* values (in Hz) in parentheses.
[b] Assigned by DEPT, HSQC and HMBC experiments.

3. Results and discussion

In a first stage the drug was kept in the dark in order to evaluate its transformation in the absence of light. The drug dissolved in pure water or at buffered pH 7.0 was recovered unchanged by keeping it in the dark even after 7 days.

The photochemical behaviour of rosuvastatin in pure water was then studied under solar irradiation.

In a first experiment a solution of rosuvastatin (10^{-5} M) was exposed to sunlight. The TLC and the ^{1}H NMR analyses of the irradiation mixture indicated that the drug was completely

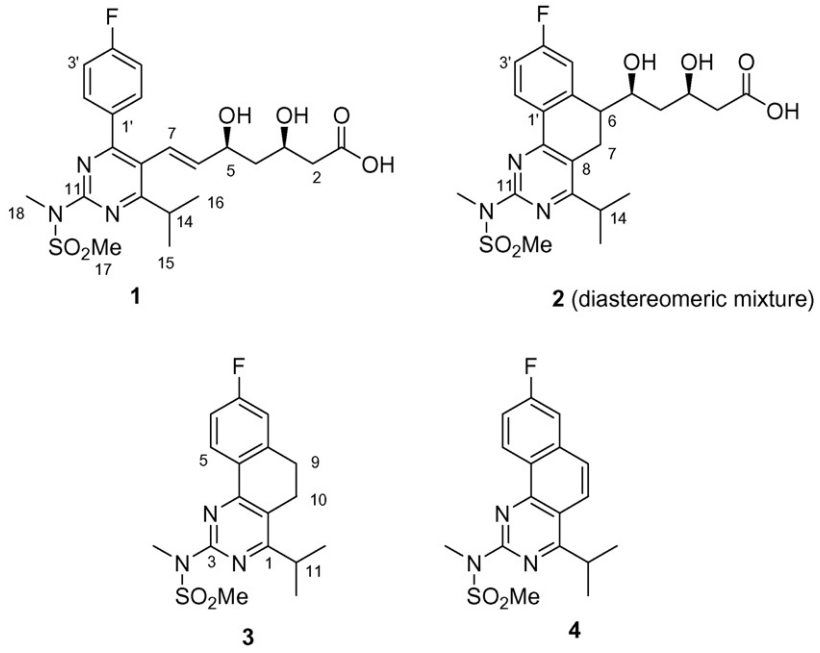


Fig. 2. Rosuvastatin and phototoproducts **2**–**4**.

transformed after 4 days into photoproducts **2**–**4** (Fig. 2). In order to get higher amounts of photoproducts the irradiation was carried out on a water dispersion of **1** (80 ppm) for 4 days. The ^{1}H NMR analysis showed that the same photoproducts were obtained in these conditions.

Irradiation experiments were also run using UV-light (Pyrex filter) to shorten the reaction times. When rosuvastatin was irradiated in an open tube, with a UV lamp, it was completely transformed after 8 h of irradiation. The ^{1}H NMR and TLC analyses showed the same irradiation mixture as after 4 days of solar exposure. The irradiation was carried out also under argon atmosphere and the TLC and the NMR analyses showed that only compound **2** (as diastereomeric mixture) and **3** were formed.

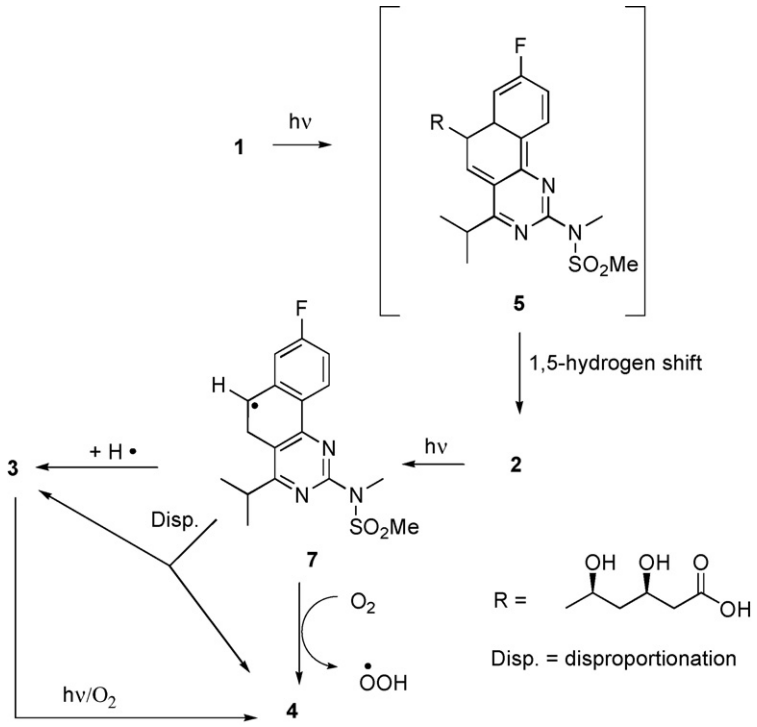
The photostability of compound **2** was evaluated irradiating (10^{-5} M) for 3 days with solar light. The NMR analysis showed that most of the starting products were transformed in compounds **3** and **4**.

Photoproducts **2**–**4** were separated by repeated chromatographies employing different stationary and mobile phases. All structures were elucidated by IR, UV, NMR techniques (COSY, TOCSY, HSQC, HMBC, NOESY) and EI-MS experiments.

Compound **2** was a ca. 1:1 mixture of diastereomers. One isomer of **2** (as methyl ester), i.e. **2a**, showed a peak at *m*/*z* 463 $[M - CH_3OH]^+$ in the EI-MS spectrum suggesting, along with the elemental analysis, a molecular formula $C_{22}H_{28}FN_3O_6S$. The UV spectrum revealed a band at 314 nm. The ^{1}H NMR spectrum (Table 1) showed three aromatic protons, and four methines at δ 4.11, 3.74, 3.41, and 3.08, six methylene protons at δ 3.29/2.98, 2.37/2.26, and 1.64/1.52, and four methyls at δ 3.57, 3.54, 1.35 and 1.25 were in the aliphatic region. The ^{13}C NMR spectrum showed twenty carbon signals (Table 2). The DEPT spectrum showed three methyls, three methylenes, and six methines that were correlated to the corresponding protons by the HSQC experiment. Comparison with the spectral data of rosuvastatin **1** (Tables 1 and 2) revealed the presence of an additional ring and the absence of the olefinic bond and one aromatic proton. ^{1}H–^{1}H COSY spectrum showed the correlations of the 3,5-dihydroxypentanoic chain. The H-5 methine was correlated, in the same experiment, to the H-6 methine, which was in turn correlated to the H-7 methylene. The planar structure was determined on the basis of an HMBC experiment. Long-range correlations from the H-6 proton to C-7 (δ 25.0), C-4 (δ 40.9), C-5 (δ 73.9), C-5′ (δ 118.1), C-1′ (δ 130.9) and C-8 (δ 119.4), the H-7 protons to the C-6 (δ 45.5), C-5, C-9 (δ 160.4), C-6′ (δ 144.5) and C-8 carbons were observed. The correlations from H-14 proton (δ 3.41) to the C-13 (δ 175.0), C-8, and C-15/C-16 (δ 22.1), the H-2′ with C-9 and C-6′ were present. These correlations were consistent with structure **2** (Fig. 2).

The other isomer of **2**, i.e. **2b**, had the molecular formula $C_{22}H_{24}FN_3O_6S$ as deduced from the peak at *m*/*z* 463 $[M - CH_3OH]^+$ in the EI-MS spectrum. The UV spectrum revealed a band at 312 nm. The general features of the NMR spectra closely resembled those of its isomer (Table 1), except for the ^{1}H chemical shifts at the H-5, H-6 and H-7 positions.

Compound **3** showed a molecular peak at *m*/*z* 349 $[M]^+$ in the EI-MS spectrum suggesting, along with the elemental analysis, a molecular formula $C_{17}H_{20}FN_3O_2S$. The UV spectrum revealed a band at 312 nm. The ^{13}C NMR spectrum (Table 3), showed sixteen carbon signals identified by the DEPT spectrum as three methyls, two methylenes, and four methines. A close inspection of the ^{1}H and ^{13}C NMR spectra of **3** by HSQC experiment allowed to attribute the protons to the corresponding carbons (Table 3). The planar structure was determined on the basis of HMBC correlations. Long-range correlations from the H-8 proton at δ 6.95 to the C-9 carbon (δ 27.8), C-5a (δ 129.8), C-8a (δ 141.8), the H-9/H-10 protons at δ 2.93 to C-1 (δ 173.1), C-1a (δ



Scheme 1. Suggested pathways for compounds **2**–**4**.

118.5), and C-8 (δ 114.5) in the HMBC spectrum were consistent with the dihydrobenzoquinazoline structure **3** (Fig. 2).

Compound **4** had the molecular formula $C_{17}H_{18}FN_3O_2S$ according to the molecular ion at *m*/*z* 347 in its EI-MS spectrum and the elemental analysis. The UV spectrum revealed a band at 357 nm. The ^{13}C NMR spectrum showed sixteen carbon signals that were assigned by DEPT experiment to three methyls, and six methines (Table 3). The ^{1}H NMR spectrum showed five aromatic protons, one methine and three methyls (Table 3). The protons were correlated to the corresponding carbons by an HSQC experiment. In the HMBC experiment the H-5 proton (δ 9.15) was correlated to the C-7 (δ 162.6), C-4a (δ 151.0), and C-8a (δ 137.0) carbons. The H-8 proton (δ 7.52) gave cross peaks with the C-9 (δ 125.4), C-5a (δ 129.7), and C-7 carbons. The H-11 proton (δ 3.92) was correlated with the C-1a (δ 126.9) and C-1 (δ 175.8) carbons. These correlations were consistent with structure **4** (Fig. 2).

The main photo-induced reaction is cyclization of the drug to give diastereomeric mixture **2**. As known, the photochemical electrocyclization works well for *o*-vinylbiphenyl molecules leading to stable dihydrophenantrenes [11], and rosuvastatin could be considered to belong to this class of molecules. By irradiation, the drug gives the undetected dihydro intermediate **5** which undergoes self-trapping by 1,5-hydrogen shift leading to **2** (Scheme 1). Compounds **3** and **4** could be considered deriving from **2**, as proven by control experiments starting from compounds **2**. They could be formed by photo-induced C6–C7 bond cleavage with loss of the side-chain and formation of the well-stabilized benzylic radical **7**. This latter could give dihydroderivative **3** by abstraction of a hydrogen from the side-chain radical fragment or other surrounding molecules. Alternatively, even if at a lesser extent, it could disproportionate giving both **3** and **4** [12,13]. Under aerobic conditions, dissolved oxygen serves as the hydrogen acceptor favouring mainly the benzoquinazoline **4**, which can also be formed by photooxidation of **3**.

To summarize, rosuvastatin by sunlight exposure in simulated environmental conditions is easily transformed into polycyclic compounds, dihydrobenzoquinazolines **2**–**3** and benzoquinazoline **4**. It would be ineffective trying to detect this drug in surface water. However, for a complete environmental risk assessment of rosuvastatin the analytical, eco-toxicological and toxicological investigations should be addressed towards its environmental metabolites. In this context recently benzoquinazoline derivatives have been found to exhibit biological activities, in particular they have been classified as peroxidizing herbicides affecting a crucial enzyme in the chlorophyll biosynthesis pathway and producing ethane by light-induced radicals in the cells [14].

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