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Irradiation of fluvastatin in water Structure elucidation of photoproducts

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Abstract

Fluvastatin is easily transformed in water by solar irradiation. One day-light exposure induces a complete degradation of the drug and formation of a mixture of products. Different chromatographic processes led to the isolation of dihydrobenzocarbazole, benzocarbazole, azonane-2,7-dione and spiro[4.4]azononane derivatives. Structures of photoproducts were elucidated by spectroscopic means. Photocyclization and photooxygenation are the main reactions involved in the formation of the observed products.

Keywords: Fluvastatin; Benzocarbazole derivatives; Lactam; Photocyclization; Photooxygenation

1. Introduction

Risk associated with the introduction of pharmaceuticals and personal care products (PPCPs) into the aquatic environment has become an important issue in environmental research. These chemicals are considered emerging pollutants, because they are continuously introduced into the aquatic environment and have been detected in surface waters in many countries [1,2].

These substances enter into the aquatic environment due to the ineffectiveness of sewage treatment plants, and thus they are potential pollutants for the aquatic ecosystem, with adverse effects on aquatic organisms. Pharmaceuticals, in water, can be subject to abiotic transformations (hydrolytic and photochemical) leading to different products that in some cases are more persistent and more toxic than the parent compound [3–6]. Therefore, the presence and possible effects of these transformation compounds should be investigated, too. While the occurrence of pharmaceuticals in surface waters has been extensively reviewed, data on their fate in water are still limited.

In this context, we are interested in investigating the photochemical behaviour of some of the most commercialized drugs under environmentally relevant conditions, with particular attention to the isolation and identification of photoproducts. Recently our attention has been focused on synthetic statins [7,8]. Results obtained have shown an easy sunlight-induced photodegradation of these drugs giving rise to several photoproducts.

In this work, we have investigated the photochemical transformation processes of fluvastatin (**1**) in water and reported the structure elucidation of the main photoproducts. Fluvastatin sodium is a statin that acts as lipid-lowering agent and is widely used in the prevention of cardiovascular events. In a recent study, the photodegradation kinetics of fluvastatin was determined at λ 365 nm region (high-pressure mercury lamp, interference filter and Wood's filter) in methanol and water evidencing a fast degradation, mainly in the latter solvent [9].

2. Experimental

2.1. Chemicals

Fluvastatin sodium was obtained from KEMPROTEC Limited. Solutions and suspensions of the drug were prepared using

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Milli-Q water. All other solvents were of HPLC grade. Methylene blue and NaN_3 were obtained from Aldrich.

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 (Luna Prep C-18, 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 MeOH on a Perkin-Elmer Lambda 7 spectrophotometer. IR spectra were recorded in $CHCl_3$ on a Nicolet 5700 FT-IR spectrometer. Low resolution electron impact mass spectra were obtained operating at 70 eV on a GC–MS (QP-5050A Shimadzu). A photoreactor (Helios Italquartz) equipped with a 500 W high-pressure mercury lamp (through a Pyrex glass filter, λ > 300 nm) was used for UV irradiation. Analytical TLC was performed on precoated Merck aluminum 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. Irradiation experiments

Experiments in the dark were conducted on solutions of the drug (10^{-4} M) in pure water and at pH 7 using buffered water with NaH_2PO_4/Na_2HPO_4. The preparations were kept in the dark for 1, 4 and 7 days, concentrated and the residues analyzed by TLC and ^{1}H NMR.

A solution of fluvastatin, 10^{-5} M, was exposed to sunlight and analyzed by UV every 15 min (Fig. 1).

Solutions of fluvastatin (40 mg, 10^{-4} M) were exposed to sunlight in pure water on June in Naples in open Pyrex flasks for 1 day-light. Irradiation mixtures were then dried under vacuum and the residue (40 mg) was separated by silica gel preparative TLC-chromatography (1 mm). Eluting with 50 ml of CH_2Cl_2/MeOH (95:5) with two drops of acetic acid (two runs) gave a fraction A (diastereomeric mixture **2**, 15 mg), photoproduct **3** (6 mg), photoproduct **4** (9 mg), and a fraction B (14 mg).

Fraction A was subjected to methylation with excess of diazomethane in ether solution. The resulting mixture was separated by silica gel TLC-chromatography eluting with toluene/ethyl acetate to afford pure diastereomeric methyl esters of **2a** (6 mg) and **2b** (7 mg).

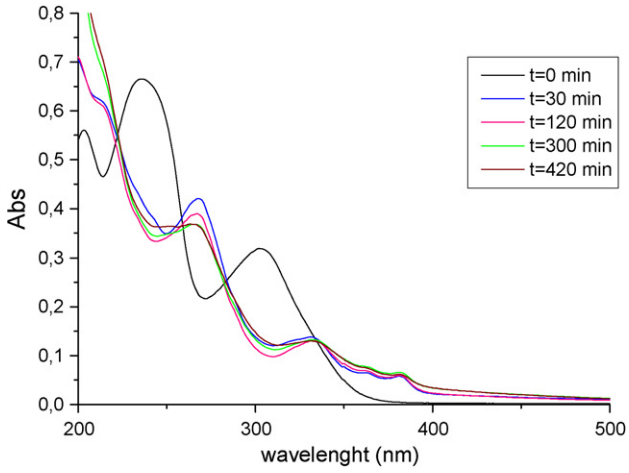


Fig. 1. Changes in UV spectra of water solution of fluvastatin (1.1×10^{-5} M) at different times of solar exposure.

Fraction B was a mixture of polar products that were separated by reverse-phase HPLC with a RP-18 column. The flow was set to 1.7 ml/min. The column was equilibrated with a mixture of A (H_2O containing 0.1% acetic acid)–B (methanol) 9:1 (v/v) and using the following program: an increase of B up to 50% in 11 min and a further increase to 100% in 11 min. The detector wavelength was set at 260 nm. Pure compounds **5a** (2 mg), **5b** (1 mg), **6a** (6 mg) and **6b** (5 mg) were obtained.

In order to evaluate the stability of the irradiation mixtures for longer times, solutions of fluvastatin (10^{-4} M) were exposed to sunlight in water for 10 days on June in Naples in open Pyrex flasks.

Irradiation experiments with the 500 W high-pressure mercury lamp in open Pyrex tube (distance of 15 cm from the lamp at r.t.) were conducted for different times (1, 2, and 4 h).

A solution of the drug (2 mg/20 ml, ca. 10^{-4} M) saturated with argon for 15 min was irradiated in a closed pyrex flask with the UV-lamp for 2 h.

A solution of drug (2 mg/20 ml, ca. 10^{-4} M) in the presence of NaN_3 (0.3 equiv.) was irradiated with UV-lamp in an open pyrex tube for 1, 2, and 4 h.

Compounds **2**, **3** and **4** (each 2 mg/20 ml) were irradiated in water in open pyrex tubes with the UV-lamp for different times.

A drug solution (2 mg/20 ml, ca. 10^{-4} M), after adding methylene blue (10^{-5} M) and bubbling oxygen for 15 min, was irradiated with 650 W halogen lamp for 10 min in a closed pyrex flask. Similar treatment was used starting from diastereomeric mixture **2**.

2.3.2. Compound *1*

White powder; UV spectrum shown in Fig. 1. IR $\nu_{max}(CHCl_3)$ 3676, 3588, 3300 br band, 2972, 1697, 1595, 1502, 1406, 1343 cm^{-1}. ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2.

2.3.3. Compound *2* (mixture of diastereomers 1:1)

One isomer as methyl ester, i.e. **2a**: white powder; UV λ_{max} (CH_3OH) nm; 313 (log ε 4.1). IR $\nu_{max}(CHCl_3)$ 3693, 3604, 3484, 2923, 1726, 1601, 1503, 1459, 1370 cm^{-1}; EI–MS *m/z*

Table 1
^{1}H NMR Data of **1–3** (CD_3OD)

Position	**1**	**2a**[a]	**2b**[a]	**3**
2	2.35 dd (4.8, 15.6); 2.27 dd (7.8, 15.6)	2.24 dd (3.9, 14.6); 2.13 dd (7.8, 14.6)	2.30 dd (4.8, 15.6); 2.13 dd (7.8, 15.6)	2.52 m; 2.47 m
3	3.97 m	4.01 m	3.97 m	4.24 m
4	1.70 m, 1.52 m	1.46 m, 1.36 m	1.58 m, 1.51 m	2.15 m
5	4.39 m	3.83 m	3.61 m	5.71 m
6	5.73 dd (6.8, 16.6)	3.16 m	2.99 m	
7	6.70 d (16.6)	3.40 obscured, 3.06 dd (7.8, 16.6)	3.61 m, 2.93 m	8.17 s
11	7.57 d (8.9)	7.12 d (8.8)	7.91 d (8.0)	8.54 d (8.0)
12	7.13	7.11 m	7.11 m	7.31 t (8.2)
13	7.00 t (7.8)	7.11 m	7.11 m	7.42 t (8.2)
14	7.41	7.55 d (8.8)	7.53 d (8.8)	7.78 d (8.2)
16	4.95sept (7.0)	4.88 sept (7.5)	4.88 sept (7.5)	5.29 sept (6.8)
17	1.65d (7.0)	1.66* d (7.5)	1.65* d (7.5)	1.77 d (6.8)
18	1.65d (7.0)	1.65* d (7.5)	1.64* d (7.5)	1.77 d (6.8)
2′	7.41	7.81 dd (8.8, 7.8)	7.78 dd (8.8, 7.8)	8.88 dd (5.4, 3.6)
3′	7.15	7.01 m	7.03 m	7.47 m
5′	7.15	7.01 m	7.03 m	8.03 dd (5.7, 1.5)
6′	7.41			
OMe		3.43 s	3.48 s	

J values (in Hz) in parentheses. Values with same superscript were exchangeable.
[a] Data derived from methyl ester derivative.

(%): 425 (15), 393 (18), 278 (67), 236 (100). ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2. The other isomer as methyl ester, i.e. **2b**: white powder; UV λ_{max} (CH_3OH) nm: 312 (log ε 4.1). IR ν_{max}($CHCl_3$) 3693, 3604, 3484, 2923, 1726, 1601, 1503, 1459, 1370 cm^{-1}; EI–MS *m/z* (%): 425 (15), 393 (18), 278 (67), 236 (100). ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2.

Table 2
^{13}C NMR Data of **1–3** (CD_3OD)

Position	**1**	**2a**[a]	**2b**[a]	**3**
1	180.2	174.0	174.1	179.0
2	45.3*	43.1	43.1	45.5
3	68.8	69.2	69.0	69.8
4	45.6*	39.8	43.1	47.5
5	72.2	74.8	71.2	71.5
6	141.4	47.6	48.5	140.4
7	123.1	22.8	24.8	112.5
8	130.2	137.4	137.4	140.4
9	113.3	110.4	110.6	128.8
10	130.2	126.6	126.4	125.1
11	120.6	120.6	120.5	123.3
12	120.6	121.4	121.3	121.1
13	121.0	122.1	122.1	125.5
14	113.3	112.8	112.6	112.5
15	138.0	138.0	138.1	140.4
16	48.9	48.7	48.5	48.5
17	22.4	22.5	22.3	21.9
18	22.4	22.5	22.3	21.9
1′	134.6	132.3	132.2	127.6
2′	133.7	124.9	124.9	127.3
3′	116.7	117.8	118.3	116.9
4′	163.5	161.7	161.4	160.5
5′	116.7	115.1	115.1	110.2
6′	133.7	137.9	138.1	129.4
OMe		52.3	52.4	

Values with same superscript were exchangeable.
[a] Data derived from methyl ester derivative.

2.3.4. Compound **3**

White powder; UV λ_{max} (CH_3OH) nm: 332 (log ε 4.2), 363 (log ε 3.9), 378 (log ε 3.9). IR ν_{max}($CHCl_3$) 3688, 3608, 2932, 2402, 1726, 1605, 1468, 1370 cm^{-1}; EI–MS *m/z* (%): 409 (5), 391 (58), 373 (38), 236 (100). ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2.

2.3.5. Compound **4**

White powder; UV λ_{max} (CH_3OH) nm: 332 (log ε 4.3), 359 (log ε 3.9), 379 (log ε 3.9); IR ν_{max}($CHCl_3$) 3693, 2932, 2861, 1722, 1624, 1615, 1526, 1463, 1365 cm^{-1}; EI–MS *m/z* (%): 277 (97), 262 (100), 235 (90); δ_H (500 MHz, $CDCl_3$) 8.80 (1 H, dd, *J* 9.5, 5.5 Hz, H-5), 8.57 (1 H, d, *J* 7.5 Hz, H-4), 7.82 and 7.81 (2 H, d, *J* 8.0 Hz, H-9 and H-10), 7.70 (1 H, d, *J* 8.5 Hz, H-1), 7.63 (1 H, dd, *J* 8.5, 2.5 Hz, H-8), 7.49 (2 H, m, H-2 and H-6), 7.37 (1 H, t, *J* 7.0 Hz, H-3), 5.21 (1 H, m, H-11), 1.78 (6 H, d, *J* 7.5 Hz, H-12 and H-13), δ_C (125 MHz, $CDCl_3$) 158.9 (C-7), 138.6 (C-1a), 137.2 (C-10a), 129.5 (C-8a), 127.1 (C-5a), 126.0 (C-9), 125.3 (C-5), 124.2 (C-4), 123.7 (C-4a), 122.3 (C-2), 119.7 (C-3 and C-4b), 116.5 (C-6), 113.3 (C-1), 112.7 (C-8), 112.5 (C-8), 111.0 (C-10), 47.1 (C-11), 21.5 (C-12 and C-13).

2.3.6. Compound **5** (mixture of diastereomers 1:1)

One isomer, i.e. **5a**: white powder; UV λ_{max} (CH_3OH) nm: 257 (log ε 3.3). IR ν_{max}($CHCl_3$) 3300 (broad), 2940, 2397, 2352, 1636 cm^{-1}; EI–MS *m/z* (%): 443 (10), 355 (5), 277 (31), 262 (28). ^{1}H and ^{13}C NMR data are listed in Table 3. The other isomer, i.e. **5b**: White powder; UV λ_{max} (CH_3OH) nm: 257 (log ε 3.3). IR ν_{max}($CHCl_3$) 3300 (broad), 2940, 2397, 2352, 1636 cm^{-1}; EI–MS *m/z* (%): 443 (10), 355 (5), 277 (31), 262 (28). δ_H (500 MHz, CD_3OD) 7.91 (1 H, dd, *J* 8.8, 6.8 Hz, H-2′), 7.89 (1 H, d, *J* 8.0 Hz, H-11), 7.74 and 7.70 (2 H, m, H-12 and H-13), 7.31 (1 H, d, *J* 7.0 Hz, H-14), 7.26 (1 H, dd, *J* 10.6, 2.5 Hz, H-5′), 7.14 (1 H, ddd, *J* 10.6, 8.8, 2.5 Hz, H-3′), 4.24 (1 H, sept, *J* 6.8 Hz, H-16), 4.03 (1 H, m, H-5), 3.87 (1 H, m, H-3), 2.96 (1

Table 3
NMR spectral data of compound **5a** in CD_3OD

Position	δ_H[a]	*J* (Hz)	NOESY	δ_C[b]	HMBC[c]
1				179.0 (q)	
2	2.30 m, 2.35 m			44.0 (s)	1, 3, 4
3	3.98 m			68.5 (t)	1, 2
4	1.20 m, 1.45 m			42.3 (s)	2, 3, 5
5	3.98 m		5′	72.8 (t)	3, 7, 6′
6	2.97 q brd	8.8		45.3 (t)	4, 5, 1′, 5′, 6′
7	2.30 dd, 2.65 dd	13.7, 7.8, 15.8		44.0 (s)	5, 6, 8, 6′
8				172.8 (q)	
9				197.0 (q)	
10				143.7 (q)	
11	7.88 d	7.8		132.4 (t)	9, 10, 12, 15
12	7.72 m			131.9 (t)	10, 11
13	7.72 m			134.7 (t)	12, 14, 15
14	7.31 d	6.5	17/18	133.2 (t)	10, 12
15				135.2 (q)	
16	4.24 sept	6.8		48.5 (t)	8, 15, 17, 18
17	1.01* d	6.8	14	21.8 (p)	16, 18
18	0.63* d	6.8	14	20.2 (p)	16, 17
1′				135.2 (q)	
2′	7.91 dd	8.8, 6.8		134.7 (t)	9, 3′, 6′
3′	7.13 ddd	10.7, 8.8, 2.9		115.9 (t)	1′, 4′, 5′
4′				165.0 (q)	
5′	7.42 dd	10.7, 2.9	5	116.3 (t)	6, 1′, 3′, 4′
6′				145.4 (q)	

Values with same superscript were exchangeable.
[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (*J* in Hz).
[b] Letters, p, s, t and q, in parentheses indicate, respectively, the primary, secondary, tertiary and quaternary carbons, assigned by DEPT.
[c] HMBC correlations from H to C.

Table 4
NMR spectral data of compound **6a** in CD_3OD

Position	δ_H[a]	*J* (Hz)	NOESY	δ_C[b]	HMBC[c]
1				179.4 (q)	
2	2.55 dd, 2.44 dd	12.7, 7.8, 2.0		44.0 (s)	
3	4.34 m			69.3 (t)	
4	2.01 m		6, 7, 5′	43.5 (s)	2, 3, 5
5	4.13 m		7	73.3 (t)	3, 4, 6, 7, 6′
6	3.81 dd	9.3, 5.8	4	55.0 (t)	5, 7, 1′, 6′
7	4.58 d	9.3	4, 5, 11	85.5 (t)	5, 6, 8, 9, 10
8				178.2 (q)	
9				65.8 (q)	
10				134.0 (q)	
11	7.15 d	7.5	7	125.4 (t)	9, 10, 12, 14, 15
12	7.11 t	7.5		124.3 (t)	10, 13, 14
13	7.34 t	7.5		130.2 (t)	11
14	7.20 d	7.5	17/18	111.5 (t)	10, 12
15				145.7 (q)	
16	4.57 m			46.0 (t)	8, 15, 17, 18
17	1.49* d	6.8		20.1 (p)	16, 18
18	1.48* d	6.8		20.1 (p)	16, 17
1′				139.0 (q)	
2′	6.53 dd	8.0, 5.5		124.6 (t)	9, 3′, 6′
3′	6.86 ddd	10.7, 8.0, 1.9		115.9 (t)	2′, 4′
4′				164.7 (q)	
5′	7.47 dd	8.8, 1.9	4	114.6 (t)	1′, 3′, 4′
6′				148.0 (q)	

Values with same superscript were exchangeable.
[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (*J* in Hz).
[b] Letters, p, s, t and q, in parentheses indicate, respectively, the primary, secondary, tertiary and quaternary carbons, assigned by DEPT. Values with same superscript were exchangeable.
[c] HMBC correlations from H to C.

H, q, *J* 8.8 Hz, H-6), 2.85 and 2.43 (2 H, dd, *J* 13.7, 7.8, 15.8 Hz, H-7), 2.29 and 2.19 (2 H, m, H-2), 1.30 and 1.21 (2 H, m, H-4), 1.01 and 0.62 (6 H, m, H-17 and H-18); δ_C (125 MHz, CD_3OD) 198.0 (C-9), 179.1 (C-1), 172.4 (C-8), 164.0 (C-4′), 143.4 (C-10 and C-6′), 135.4 (C-15, C-1′ and C-2′), 134.8 (C-13), 133.3 (C-14), 132.4 (C-11), 132.0 (C-12), 116.2 (C-3′), 115.8 (C-5′), 73.9 (C-5), 68.1 (C-3), 49.9 (C-16), 45.3 (C-6), 44.4 (C-2 and C-7), 43.6 (C-4), 21.8 and 20.2 (C-17 and C-18).

2.3.7. *Compound* ***6*** *(mixture of diastereomers 1:1)*

One isomer, i.e. **6a**. White powder; UV λ_{max} (CH_3OH) nm: 260 (log ε 3.8). IR ν_{max}($CHCl_3$) 3300 (broad), 2936, 2357, 1693, 1599, 1484 cm^{-1}; EI–MS *m*/*z* (%): 443 (10), 381 (5), 363 (5), 311 (50), 283 (59), 268 (26), 251 (100). ^1H and ^{13}C NMR data are listed in Table 4. The other isomer, i.e. **6b**. White powder; UV λ_{max} (CH_3OH) nm: 260 (log ε 3.8). IR ν_{max}($CHCl_3$) 3300 (broad), 2936, 2357, 1693, 1599, 1484 cm^{-1}; EI–MS *m*/*z* (%): 443 (10), 381 (5), 363 (5), 311 (50), 283 (59), 268 (26), 251 (100). δ_H (500 MHz, CD_3OD) 7.34 (1 H, t, *J* 7.5 Hz, H-13), 7.20 (2 H, overlapped, H-14 and H-5′), 7.17 (1 H, d, *J* 7.0 Hz, H-11), 7.10 (1 H, t, *J* 7.5 Hz, H-12), 6.85 (1 H, dt, *J* 7.5 and 1.5 Hz, H-3′), 6.53 (1 H, dd, *J* 7.5 and 5.5 Hz, H-2′), 4.88 (1 H, d, *J* 9.3 Hz, H-7), 4.58 (3 H, H-3, H-5 and H-16), 3.85 (1 H, d br, H-6), 2.50 and 2.42 (2 H, m, H-2), 1.94 and 1.88 (2 H, m H-4), 1.49 and 1.48 (6 H, d, *J* 6.8 Hz, H-17 and H-18); δ_C (125 MHz, CD_3OD) 179.4 (C-1), 178.4 (C-8), 164.0 (C-4′), 147.8 (C-6′), 145.7 (C-15), 139.4 (C-1′), 134.4 (C-10), 130.1 (C-13), 126.6 (C-2′), 125.4 (C-11), 124.3 (C-12), 115.8 (C-3′), 113.6 (C-5′), 83.6 (C-7), 70.2 (C-3 and C-5), 65.6 (C-9), 54.6 (C-6), 41.9 (C-2 and C-4), 20.1 (C-17 and C-18).

3. Results

In a first stage fluvastatin (**1**) was kept in the dark in order to evaluate its hydrolytic behaviour. The drug dissolved in pure water or at buffered pH 7.0 was recovered unchanged even after 7 days. The photochemical behaviour of the drug in pure water was examined under solar irradiation. UV analysis showed fast alteration of the drug (Fig. 1). This behaviour is in agreement with the absorption spectrum of fluvastatin that exhibits a tail in the UVA region, and fits with previously reported data [9]. NMR analysis, after 1 h of solar exposition, showed the presence of products **2**–**4**, and **6**, while after 1 day-light exposure also compound **5** was detected. Chromatographic separation of the

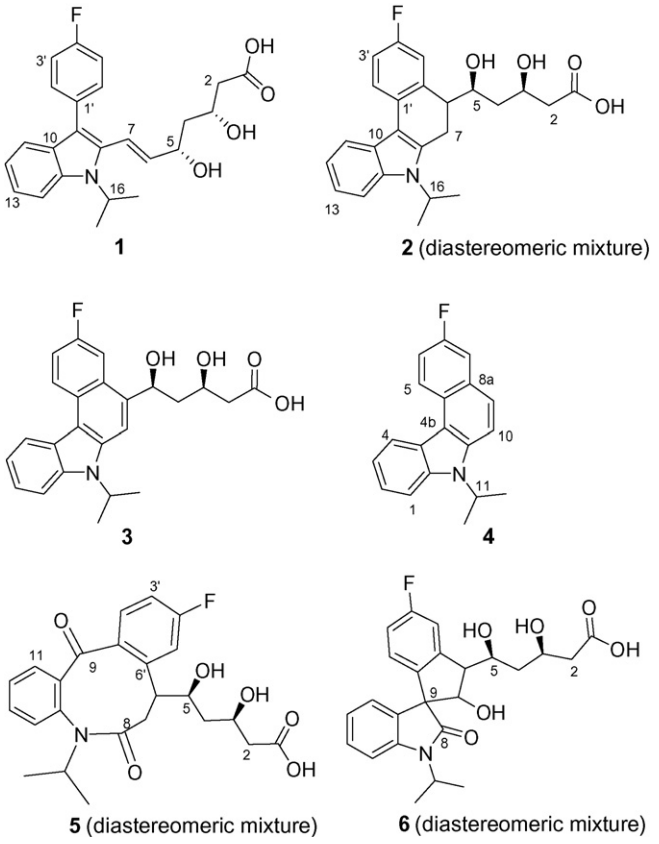


Fig. 2. Fluvastatin and photoproducts **2**–**6**.

products could be obtained with different stationary and mobile phases. These separations led to the isolation of substituted dihydrobenzocarbazole **2** (32%) as diastereomeric mixture, 3,5-dihydroxypentanoic chain substituted benzocarbazole derivative **3** (12%), benzocarbazole derivative **4** (22%), lactam **5** (6%) and spiro-compound **6** (24%), the latter two as diastereomeric mixtures (Fig. 2). Separation of diastereomeric mixture **2** was achieved by methylation and RP-HPLC giving methyl esters **2a** and **2b**. The structures of compounds were elucidated by NMR spectra (^{1}H, ^{13}C, DEPT, COSY, HSQC, HMBC, NOESY) and EI–MS (see Section 3.1).

The ^{1}H NMR analysis of a prolonged solar irradiation mixture (10 days) of the drug showed the presence of compounds **4** (about 90%) and **5** (traces). In order to get a further insight into the photochemical behaviour of the pharmaceutical and its photoproducts irradiation experiments were run under different conditions, and to shorten the reaction times UV-lamp (Pyrex filter) was also used as light source. With this lamp the parent drug was completely converted within 1 h to the same mixture as that obtained after 1 day of solar exposure. Under argon atmosphere the drug gave only compounds **2**, thus indicating that products **3**–**6** require the presence of oxygen. The photostability of photoproducts **2**–**4** was evaluated irradiating them for 4 h with UV light. The NMR analysis showed that diastereomers **2** were transformed in compounds **3**, **4** and **5**, while compounds **3** and **4** were stable in these conditions. Prolonged irradiation (further 4 h) of compounds **3** and **4** indicated that the first decomposed slowly, while **4** remained unaltered.

3.1. Identification of photoproducts

Compound **2** was a ca. 1:1 mixture of diastereomers, which were separated and characterized as methyl esters. One isomer of **2**, i.e. **2a**, showed a molecular peak at *m*/*z* 425 $[M]^+$ in the EI–MS spectrum suggesting, along with the elemental analysis, a molecular formula $C_{25}H_{28}FNO_4$. The UV spectrum revealed a band at 313 nm. The ^{1}H NMR spectrum (Table 1) showed seven aromatic protons, four methines at δ 4.88, 4.01, 3.83, and 3.16, six methylene protons at δ 3.40/3.06, 2.24/2.13, and 1.46, and three methyls at δ 3.43, 1.66 and 1.65. The ^{13}C NMR spectrum showed 24 carbon signals. The DEPT spectrum showed 2 methyls, 3 methylenes, and 11 methines that were correlated to the corresponding protons by the HSQC experiment. The absence of the olefin bond and one aromatic proton by comparison with the spectral data of fluvastatin **1** (Table 1) suggested the presence of an additional ring. This hypothesis was confirmed by other NMR experiments. ^{1}H–^{1}H COSY spectrum showed the correlations of the 3,5-dihydroxypentanoic chain, indicating that the side-chain was still present and linked to the additional ring. In the same experiment the H-5 methine was correlated to the H-6 methine, which was in turn correlated to the H-7 methylene. The planar structure was completely determined on the basis of an HMBC experiment. In assigning the structure very important were the long-range correlations observed from the H-6 proton to C-7, C-4, C-5, C-5′, C-1′ and C-8, and from the H-7 protons to the C-6, C-5, C-9, C-6′ and C-8 carbons. The correlations from H-16 proton (δ 4.88) to the C-8 and C-15, the H-2′ and H-11 with C-9 were also present. These correlations were consistent with structure **2** as depicted.

The general features of the NMR spectra of isomer **2b** closely resembled those of its isomer (Tables 1 and 2), except for the ^{1}H and ^{13}C chemical shifts at the C-4, C-5, C-6 and C-7 positions.

Compound **3** showed a molecular peak at *m*/*z* 409 $[M]^+$ in the mass spectrum suggesting, along with the elemental analysis, a molecular formula $C_{24}H_{24}FNO_4$. The UV spectrum revealed three bands at 332, 363 and 378 nm. The ^{13}C NMR spectrum (Table 2), showed 20 carbon signals. The DEPT spectrum showed 2 methyls, 2 methylenes, and 11 methines. A close inspection of the ^{1}H (Table 1) and ^{13}C NMR spectra of **3** by an HSQC experiment allowed the protons to be attributed to the corresponding carbons. The planar structure was determined on the basis of ^{1}H–^{1}H COSY and HMBC correlations. Long-range correlations from the H-7 proton at δ 8.17 to the C-5 carbon, C-9, C-6′, and C-6/C-8, and from the H-2′ proton at δ 8.88 to C-3′, C-6′, and C-4′ were observed in the HMBC spectrum and were consistent with a benzocarbazole structure linked to a dihydroxypentanoic chain.

Compound **4** had the molecular formula $C_{19}H_{16}FN$ according to the molecular ion at *m*/*z* 277 in its EI–MS spectrum and the elemental analysis. The UV spectrum revealed three bands at 332, 359 and 379 nm. At a first glance the ^{13}C and ^{1}H NMR spectra showed the absence of signals relative to a side-chain by comparison with the spectra of fluvastatin and products **2**–**3**. The

^{13}C NMR spectrum showed 18 carbon signals that were assigned by a DEPT experiment to 2 methyls, and 10 methines. The ^{1}H NMR spectrum showed nine aromatic protons, one methine and two methyls. The protons were correlated to the corresponding carbons by an HSQC experiment. In the HMBC experiment the H-4 proton (δ 8.57) was correlated to the C-4a (δ 123.7), C-4b (δ 119.7), and C-2 (δ 122.3) carbons. The H-5 proton (δ 8.79) gave cross peaks with the C-7 (δ 158.9), C-8a (δ 129.5), and C-4b carbons. The H-11 proton (δ 5.21) was correlated with the C-1a (δ 138.6) and C-10a (δ 137.2) carbons. These correlations were consistent with the benzocarbazole structure indicated for compound **4**.

Compound **5** was a ca. 1:1 mixture of diastereomers, which were separated by reverse-phase HPLC. One isomer of **5**, i.e. **5a**, showed a molecular peak at *m/z* 443 $[M]^+$ in the EI–MS spectrum suggesting, along with the elemental analysis, a molecular formula $C_{24}H_{26}FNO_6$. The UV spectrum revealed a band at 257 nm. The ^{1}H NMR spectrum (Table 3) showed seven aromatic protons, four methines at δ 4.24, 3.98 (×2), and 2.97, six methylene protons at δ 2.65/2.30, 2.35/2.30, and 1.45/1.20, and two methyls at δ 1.01 and 0.63 in the aliphatic region. The ^{13}C NMR spectrum showed 21 carbon signals. The DEPT spectrum showed 2 methyls, 2 methylenes, and 10 methines that were correlated to the corresponding protons by the HSQC experiment. In the ^{1}H–^{1}H COSY spectrum the correlations of the 3,5-dihydroxypentanoic chain indicated that the side-chain was still present. In the same experiment the H-5 methine was correlated to the H-6 methine, which was in turn correlated to the H-7 methylene. The presence of two additional oxygen atoms was evidenced in the mass spectrum. The signals at δ 197.0 and 172.8, in the ^{13}C NMR spectrum, were consistent with the presence of two carbonyl functions. In the HMBC spectrum (Table 3) the first carbonyl carbon was correlated to the H-11 and H-2′ protons, while the second one was correlated to H-7 and H-16 protons. These correlations indicated an oxidation of the heteroaromatic ring giving an azonane-2,7-dione. All the other correlations in the HMBC experiment confirm the planar structure of isomer **5a**.

The general features of the NMR spectra of isomer **5b** closely resembled those of its isomer **5a**, except for small differences of the ^{1}H chemical shifts at the H-3, H-4, H-5, H-7, H-2′ and H-5′ positions, and of the ^{13}C chemical shifts at the C-4, C-16, C-2′and C-6′ positions.

Compound **6** was a ca. 1:1 mixture of diastereomers, which were separated by reverse-phase HPLC. One isomer of **6**, i.e. **6a**, showed a molecular peak at *m/z* 443 $[M]^+$ in the EI–MS spectrum suggesting, along with the elemental analysis, a molecular formula $C_{24}H_{26}FNO_6$. The UV spectrum revealed a band at 260 nm. The ^{1}H NMR spectrum (Table 4) showed seven aromatic protons, and five methines at δ 4.58, 4.57, 4.34, 4.13 and 3.81, four methylene protons at δ 2.55/2.44 and 2.01, and two methyls at δ 1.49 and 1.48 were in the aliphatic region. The ^{13}C NMR spectrum showed 23 carbon signals. The DEPT spectrum showed one methyl, two methylenes, and twelve methines that were correlated to the corresponding protons by the HSQC experiment. ^{1}H–^{1}H COSY spectrum showed the correlations of the 3,5-dihydroxypentanoic chain, indicating that the side-chain was still present. In the same experiment the H-5 methine was correlated to the H-6 methine, which was in turn correlated to the H-7 methine. The presence of two additional oxygen atoms was evidenced in the mass spectrum. The signals at δ 178.2 and 85.5, in the ^{13}C NMR spectrum, indicated the presence of a carbonyl and a carbinol functions. The carbinol carbon at δ 85.5 was correlated to the proton at δ 4.58 in the HSQC spectrum, and to H-5 and H-6 protons in the HMBC spectrum (Table 4). In the same experiment the proton at δ 4.58 was correlated to C-5–C-10 carbons. The quaternary carbon at δ 65.8 was assigned to C-9 on the basis of correlations with H-7 and H-11 protons. Furthermore, the carbonyl carbon at δ 178.2 was correlated to the H-7 and H-16 protons. All NMR data are consistent with the planar structure of isomer **6a**. The value of ^{3}J coupling constant of 9.3 Hz between H-6 and H-7 indicated a *cis* relative configuration. According to the structure the analysis of NOESY spectrum evidenced NOEs of H-4 with H-6 and H-7, H-5 with H-7, H-7 with H-11, and H-14 with the methyls 17/18.

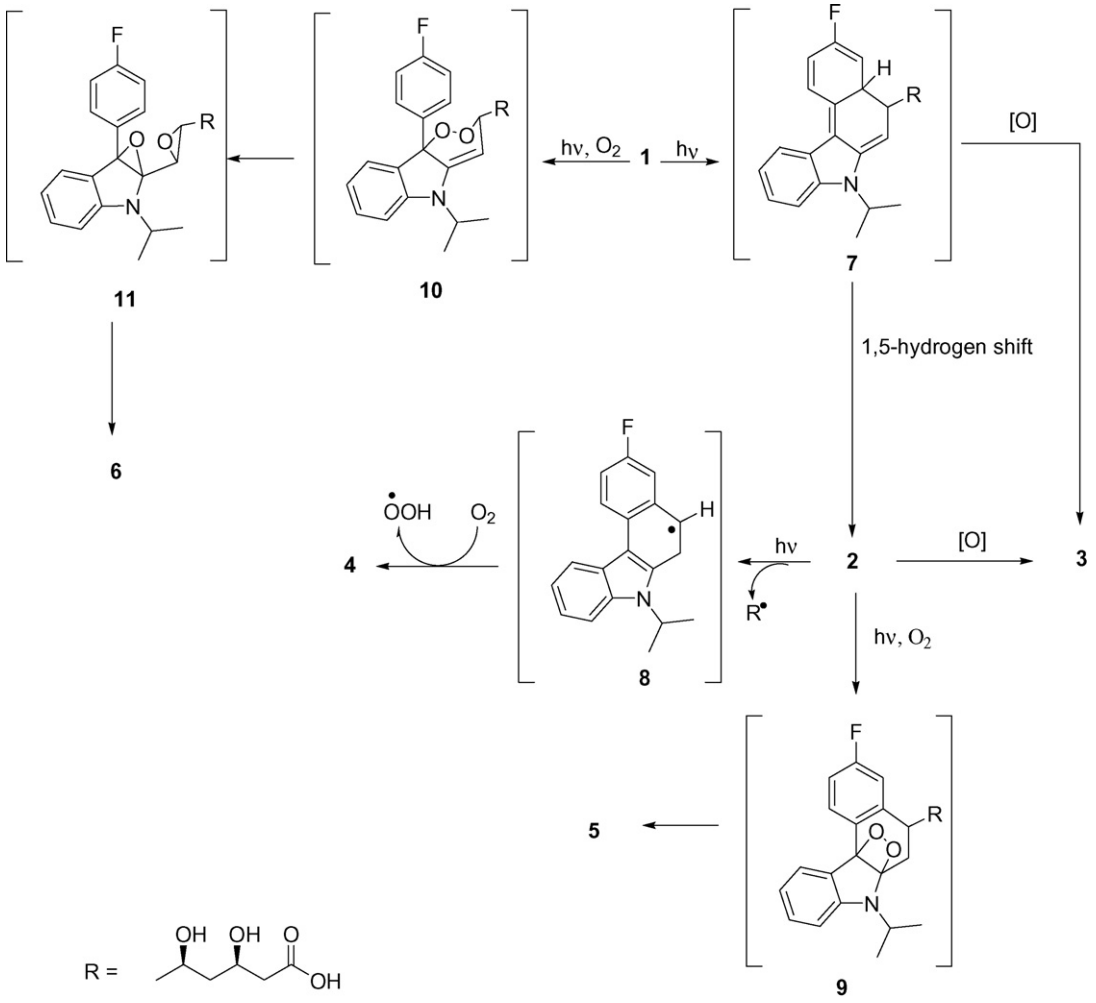
The other isomer of **6**, i.e. **6b**, showed NMR spectra closely resembling those of its isomer, except for small differences of the ^{1}H chemical shifts at the H-3, H-4, H-5, H-7 and H-5′ positions, and of the ^{13}C chemical shifts at the C-5, C-7, C-5′positions. The analysis of NOESY spectrum evidenced NOEs of H-14 with the methyls 17/18.

4. Discussion

Fluvastatin (**1**) is easily transformed in water by solar irradiation leading to products **2**–**6**. Under argon conditions only diastereomeric mixture **2** is obtained. In the presence of oxygen fluvastatin also gives compounds **3**–**6**. Control experiments showed that under the same oxidative conditions **2** led easily to compounds **3**–**5**, while **3** and **4** were recovered unchanged.

These results and literature data suggest hypotheses about the pathways of formation of photoproducts **2**–**6** that are depicted in Scheme 1.

As expected on the basis of the behaviour of *o*-vinylbiphenyl compounds [10], the photochemical electrocyclization is the main reaction leading to dihydrobenzocarbazoles **2**. These compounds are formed by 1,5-hydrogen shift of undetected dihydro intermediate **7**. Compound **3** should be formed by the easy oxidation of **1**, via **7**, and/or **2**. The latter gives compound **4** through a photoinduced C6–C7 bond cleavage, loss of the side-chain and oxidation of the well-stabilized benzylic radical **8** [11]. Photooxygenation of the indole moiety of **2** gives compounds **5**. These should be formed via O–O and C–C bond cleavage of an undetected dioxetane intermediate **9** resulting from oxygen addition to the heterocyclic ring. Oxo-amide macrocycles have previously been observed in photooxygenation reactions of indole alkaloids [12]. Oxygen-addition products **6** should derive from **1**. Indeed, irradiation experiments starting from **2** and **3** did not show formation of **6**. It is likely that intermediate **10**, formed by oxygen addition to the diene moiety, undergoes a structural reorganization, likely via diepoxide **11**. It is well-known that in vinyl derivatives of five membered heterocycles singlet oxygen (see below) gives 1,4-addition involving the exocyclic double bond [13,14]. Furthermore, examples of oxida-



Scheme 1. Suggested pathways for compounds **2**–**6**.

tive ring contraction and hydroxylation have been described [12,15].

Control experiments showed that the phototransformation of the drug slowed down significantly in the presence of NaN_3, a well-known singlet oxygen quencher [13]. Furthermore, the irradiation of **1** in the presence of methylene blue, an efficient singlet oxygen sensitizer [12], was accelerated giving mainly **6**, while **2** under the same conditions afforded **5**. These experiments suggest that singlet oxygen may be involved in the photooxygenation pathways, probably formed through a self-sensitized mechanism. The capacity of polycyclic nitrogen heterocycles to generate singlet oxygen is reported [16]. On the other hand, due to the complexity of the model and the use of water as solvent, it cannot be excluded that different oxygenated species (e.g. $O_2^{-}\bullet$) can be involved in the formation of peroxides **9** and **10**. This should fit on with the reported superoxide scavenging properties of fluvastatin and its metabolites [17].

It is interesting to compare the photochemical behaviour of fluvastatin with those of the previously studied statin drugs (atorvastatin and rosuvastatin).

Rosuvastatin presents a pyrimidinic ring substituted with a *p*-fluorophenyl and an unsaturated side-chain (*o*-vinylbiphenyl-like structure), and no oxygen addition was observed in its irradiation. In this case photocyclyzation is the main reaction [8]. On contrast, oxygenated products were found in the irradiation of atorvastatin which presents a pyrrole moiety [7]. The presence of both functionalities in fluvastatin accounts for the photochemical behaviour of this drug where both photocyclization and photooxygenation are observed.

The easy light-induced production, from these statins, of polycyclic compounds that may present biological activity [18] calls for eco-toxicological and toxicological investigations addressed towards their environmental metabolites.

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