**Supporting Information**

**for**

**Characterization of two new degradation products of atorvastatin-calcium formed upon treatment with strong acids**

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**1. Materials and methods**

Atorvastatin-calcium trihydrate (Ph. Eur.) was a gift of Prof. Dr. W. Frieß, LMU Munich. All solvents used were of HPLC grade or p.a. grade and/or purified according to standard procedures. Chemical reagents were purchased from Sigma Aldrich (Schnelldorf, Germany) and Acros (Geel, Belgium). IR-spectra: Jasco FT/IR 4600 series (KBr pellet method); MS: Hewlett Packard MS-Engine, electron ionisation (EI) 70 eV, chemical ionisation (CI) with CH4 (300 eV); MS spectra: Thermo Q Exactive GC Orbitrap or Finnigan MAT 95 spectrometer, HR-ESI-MS spectra: Thermo Finnigan LTQ FT. NMR: Avance III HD 400 MHz Bruker BioSpin (1H: 400 MHz, 13C: 100 MHz); 500 MHz Avance III HD 500 MHz Bruker BioSpin (1H: 500 MHz, 13C: 125 MHz); melting points: Büchi Melting Point B-540 (not corrected); flash column chromatography (FCC): silica gel 60 (230 – 400 mesh, E. Merck, Darmstadt); HPLC: Shimadzu LC 10 pump, , Shimadzu column oven CTO-10AS, Shimadzu autosampler SIL 10A, UV-detector Shimadzu LC 10 AS, column: Eurospere 100 – C18, 4 mm ID, (Knauer). Polarimeter: Perkin Elmer 241.

**2. Stress tests and analytical data of the products obtained thereby**

**5-(4-Fluorophenyl)-1-(2-((2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)-2-isopropyl-N,4-diphenyl-1*H*-pyrrole-3-carboxamide** (**2**)

1.025 g (0.848 mmol) of atorvastatin-calcium trihydrate (**1**) was dissolved in 50 mL 2 M aqueous hydrochloric acid and stirred for 2 h. Then the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na2SO4 and the solvent was evaporated. The residue was purified by flash column chromatography (ethyl acetate) to give 503 mg (55 %) of **2** as a white solid. The same product could be isolated when **1** was treated with 2 M aqueous hydrochloric acid under reflux for 4 h to give 595 mg (65 %) of **2**. M.p. 165 °C (ref. [1] 160 – 162 °C). [α]D25 = + 19.6 (c 0.745, DMF); 1H NMR (400 MHz, CDCl3) δ 7.16 – 7.04 (m, 9 H, 9 arom. CH), 7.02 – 6.89 (m, 5 H, 5 arom. CH), 6.80 (s, 1 H, NH), 4.45 (ddt, *J* = 12.2, 9.2, 3.2 Hz, 1 H, CH), 4.24 (dq, *J* = 7.4, 3.5 Hz, 1 H, CH), 4.15 (ddd, *J* = 15.0, 10.3, 5.0 Hz, 1 H, CH2), 3.96 (ddd, *J* = 14.8, 9.9, 5.7 Hz, 1 H, CH2), 3.55 – 3.43 (m, 1 H, CH), 2.59 (dd, *J* = 17.7, 4.8 Hz, 1 H, CH2), 2.49 (ddd, *J* = 17.8, 3.5, 1.6 Hz, 1 H, CH2), 1.81 (qt, *J* = 9.0, 4.9 Hz, 1 H, CH2), 1.74 – 1.60 (m, 2 H, CH2), 1.56 – 1.49 (m, 1 H, CH2), 1.49 – 1.43 (m, 6 H, 2 CH3). 13C NMR (125 MHz, CDCl3) δ 169.34 (CO), 164.85 (CO), 162.38 (d, *J* = 249.6 Hz, quat. C), 141.35 (quat. C), 138.23 (quat. C), 134.40 (quat. C), 133.09 (d, *J* = 8.99 Hz, 2 arom. CH), 130.41 (2 arom. CH), 128.74 (quat. C), 128.70 (2 arom. CH), 128.39 (2 arom. CH), 128.01 (d, *J* = 3.16 Hz, quat. C), 126.66 (arom. CH), 123.68 (arom. CH), 122.10 (quat. C), 119.69 (2 arom. CH), 115.70 (quat. C), 115.63 (d, *J* = 20.76 Hz, 2 arom. CH), 73.00 (CH), 62.53 (CH), 40.76 (CH2), 38.51 (CH2), 37.16 (CH2), 35.66 (CH2), 26.19 (CH), 22.01 (CH3), 21.71 (CH3). IR (KBr): ν (cm-1) = 3405, 2963, 2930, 1724, 1653, 1507, 1437, 1314, 1225, 1156, 1110, 1074, 1031, 1011, 915, 885, 808, 754, 694, 617. HR-ESI-MS calcd. for C33H34FN2O4 [M++1]: 541.2503, found: 541.2498.

**(*S*)-5-(4-Fluorophenyl)-2-isopropyl-1-(2-(6-oxo-3,6-dihydro-2H-pyran-2-yl)ethyl)-N,4-diphenyl-1*H*-pyrrole-3-carboxamide (3)**

1.00 g (0.841 mmol) of atorvastatin-calcium trihydrate (**1**) was dissolved in 200 mL toluene, 200 mg (1.07 mmol) *p*-toluenesulfonic acid were added and the suspension was refluxed for 5 h at a water separator. The solvent was evaporated, the residue dissolved in 50 mL aqueous 2 M hydrochloric acid and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na2SO4, the solvent was evaporated and the residue was purified by flash column chromatography (isohexane-ethyl acetate 1:1) to give 795 mg (95%) of **3** as an amorphous solid with an extremely broad melting interval (168 – 195 °C). [α]D25 = -49.6 (c 0.74, DMF). The same product could be isolated in >98% yield after refluxing atorvastatin-calcium trihydrate (**1**) for two hours with 20% aqueous sulfuric acid. 1H NMR (500 MHz, CDCl3) δ 7.21 – 7.11 (m, 9 H, 9 arom. CH), 7.06 (d, *J* = 7.7 Hz, 2 H, 2 arom. CH), 7.04 – 6.94 (m, 3 H, 3 arom. CH), 6.87 (s, 1 H, NH), 6.78 (ddd, *J* = 8.9, 5.7, 2.6 Hz, 1 H, arom. CH), 5.98 (ddd, *J* = 9.8, 2.5, 1.2 Hz, 1 H, CH=), 4.22 (tt, *J* = 10.1, 4.5 Hz, 2 H, CH2), 4.04 (ddd, *J* = 15.2, 10.1, 5.6 Hz, 1 H, CH), 3.56 (hept, *J* = 6.9 Hz, 1 H, CH), 2.13 (tdd, *J* = 18.5, 12.4, 7.0 Hz, 2 H, CH2), 1.98 (tq, *J* = 10.7, 6.0, 5.4 Hz, 1 H, CH2), 1.84 – 1.73 (m, 1 H, CH2), 1.54 (t, *J* = 6.8 Hz, 6 H, 2 CH3). 13C NMR (125 MHz, CDCl3) δ 164.68 (CO), 163.47 (CO), 162.32 (d, *J* = 247.8 Hz, quat. C), 144.68 (CH=), 141.34 (quat. C), 138.32 (quat. C), 136.35 (quat. C), 133.09 (d, *J* = 8.1 Hz, 2 arom. CH), 130.42 (2 arom. CH), 128.69 (2 arom. CH), 128.40 (2 arom. CH), 128.05 (d, *J* = 4.2 Hz, quat. C), 127.49 (quat. C), 126.69 (arom. CH), 123.61 (arom. CH), 122.13 (quat. C), 121.33 (CH=), 119.60 (2 arom. CH), 115.66 (d, *J* = 21.4 Hz, 2 arom CH), 75.17 (CH), 40.58 (CH2), 36.39 (CH2), 29.08 (CH2), 26.20 (CH), 21.98 (CH3), 21.74 (CH3). IR (KBr): ν (cm-1) = 3402, 2959, 2928, 1722, 1665, 1595, 1526, 1242, 1155, 1043, 816, 753, 693. MS (EI): m/z = 522 (10), 430 (100). HR-MS calcd. for C33H31O3N2F [M+]: 522.2319, found: 522.2313.

**(1*R*,5*R*)-9-(4-Fluorophenyl)-11-isopropyl-10-phenyl-1,2,6,7-tetrahydro-3*H*,5*H*-1,5-methanopyrrolo[1,2-e][1,5]oxazonin-3-one (6)**

2.41 g (2.00 mmol) of atorvastatin-calcium trihydrate (**1**) was dissolved in 50 mL concentrated (37%) aqueous hydrochloric acid and refluxed for 5 h. After addition of 40 mL water the mixture was extracted with ethyl acetate (3 × 40 mL). The combined organic layers were dried over Na2SO4 and the solvent was evaporated. The residue was purified by flash column chromatography (isohexane-ethyl acetate 1:1) to give 775 mg (96%) of **6** as a white solid. M.p.: 208 °C. [α]D25 = -68.7 (c 2.07, DMF); 1H NMR (500 MHz, CDCl3) δ 7.16 – 7.03 (m, 3 H, 3 arom. CH), 7.02 – 6.91 (m, 4 H, 4 arom. CH), 6.90 – 6.79 (m, 2 H, 2 arom. CH), 5.07 (t, *J* = 4.9 Hz, 1 H, CH), 3.95 (ddd, *J* = 15.7, 4.8, 2.8 Hz, 1 H, CH), 3.87 (dd, *J* = 9.3, 5.8 Hz, 1 H, CH), 3.63 (dd, *J* = 15.5, 12.2 Hz, 1 H, CH), 3.06 (dd, *J* = 19.5, 10.2 Hz, 1 H, CH), 2.92 – 2.78 (m, 2 H, CH2), 2.51 – 2.41 (m, 1 H, CH2), 2.35 – 2.20 (m, 1 H, CH2), 2.06 – 1.96 (m, 1 H, CH2), 1.90 (ddt, *J* = 14.8, 12.5, 2.5 Hz, 1 H, CH2), 1.10 (dd, *J* = 7.2, 2.0 Hz, 6 H, 2 CH3). 13C NMR (101 MHz, CDCl3) δ 170.30 (CO), 161.76 (d, *J* = 247 Hz, quat. C), 136.54 (quat. C), 132.67 (d, *J* = 7.9 Hz, 2 arom. CH), 131.65 (quat. C), 131.13 (2 arom. CH), 129.93 (quat. C), 128.34 (d, *J* = 3.4 Hz, quat. C), 127.62 (2 arom. CH), 125.71 (arom. CH), 125.44 (quat. C), 122.32 (quat. C) 115.07 (d, *J* = 21.24 Hz, 2 arom. CH), 77.26 (CH), 39.58 (CH2), 34.67 (CH2), 34.27 (CH2), 30.57 (CH2), 26.54 (CH), 25.20 (CH), 24.19 (CH3), 23.99 (CH3). IR (KBr): ν (cm-1) = 2961, 2939, 1742, 1719, 1602, 1526, 1508, 1469, 1347, 1241, 1222, 1087, 1057, 846, 704. HR-ESI-MS calcd. for C26H27FNO2 [M++H]: 404.2026, found: 404.2020.

**(*S*)-6-(2-(2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-1H-pyrrol-1-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (7)**

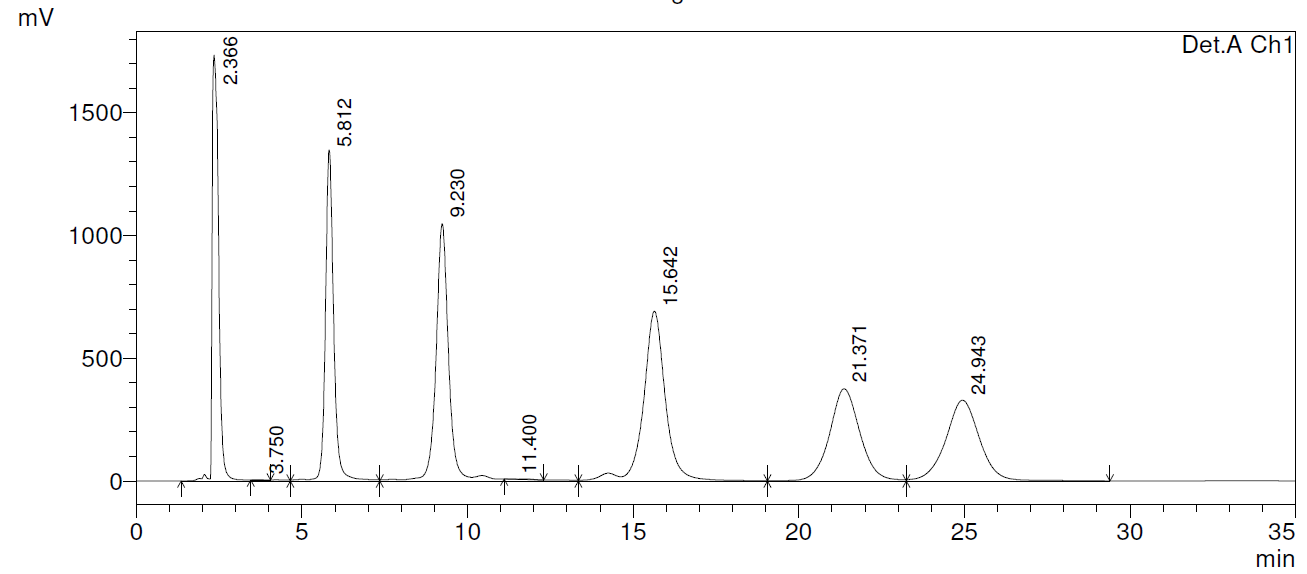
992 mg (0.821 mmol) of atorvastatin-calcium trihydrate (**1**) was dissolved in 20 mL concentrated sulfuric acid and the mixture was stirred for 2 h at 60 °C. The mixture was carefully diluted with 40 mL ice-water and extracted with ethyl acetate (3 × 40 mL). The combined organic layers were dried over Na2SO4, the solvent was evaporated and the residue purified by flash column chromatography (isohexane-ethyl acetate 1:1) to give 117 mg (18%) of **7** as a grey solid. [α]D25 = -46.7 (c 0.51, DMF); 1H NMR (400 MHz, CDCl3) δ 7.30 – 7.24 (m, 2 H, 2 arom. CH), 7.19 – 6.99 (m, 7 H, 7 arom. CH), 6.77 (ddd, *J* = 9.7, 5.9, 2.6 Hz, 1 H, CH=), 6.20 (s, 1 H, arom. CH), 5.96 (ddd, *J* = 9.7, 2.6, 1.0 Hz, 1 H, CH=), 4.21 – 4.06 (m, 2 H, CH2), 3.98 (ddd, *J* = 14.9, 9.1, 6.5 Hz, 1 H, CH), 3.02 (p, *J* = 6.8 Hz, 1 H, CH), 2.17 – 1.93 (m, 2 H, CH2), 1.86 (dtd, *J* = 13.9, 8.9, 5.0 Hz, 1 H, CH2), 1.74 – 1.57 (m, 1 H, CH2), 1.39 – 1.29 (m, 6 H, 2 CH3). 13C NMR (100 MHz, CDCl3) δ 163.70 (CO), 162.29 (d, *J* = 248.4 Hz, Hz, quat. C), 144.72 (CH=), 140.64 (quat. C), 136.26 (quat. C), 132.85 (d, *J* = 8.2 Hz, 2 arom. CH), 129.57 (d, *J* = 3.3 Hz, quat. C), 128.02 (2 arom. CH), 127.65 (quat. C), 127.54 (2 arom. CH), 124.98 (arom. CH), 122.59 (quat. C), 121.33 (CH=), 115.88 (d, *J* = 16.0 Hz, 2 arom. CH), 103.90 (arom. CH), 75.13 (CH), 39.51 (CH2), 36.23 (CH2), 29.06 (CH), 25.67 (CH2), 23.79 (CH3), 23.48 (CH3). MS (EI) m/z = 403 (M+, 100), 388 (59), 360 (19), 276 (56). IR (KBr): ν (cm-1) = 2962, 2925, 1720, 1599, 1523, 1507, 1348, 1240, 1222, 1087, 851, 695. HR-MS calcd. for C26H26FNO2 [M+]: 403.1948, found: 403.1951.

[1] J. Stach, J. Havlíček, L. Plaček, S. Rádl, Synthesis of some impurities and/or degradation products of atorvastatin, Coll. Czech. Chem. Commun. 73 (2008) 229-246. <https://doi.org/10.1135/cccc20080229>

**3. HPLC method for the detection of the novel impurities**

We worked out an isocratic HPLC protocol, which prettily separates the four artefacts **2**, **3**, **6** and **7** from atorvastatin (**1**). This method uses an RP18 stationary phase (Eurospere 100 – C18), isocratic elution with 54% buffer (0.01 M ammonium acetate buffer (pH 4)-acetonitrile 54:46 (v/v) at a flow rate of 1 mL/min at 40 °C, with UV detection at 246 nm (Figure S1).

**Figure S1.** Separation of atorvastatin (**1**; retention time: 5.8 min) from the four decomposition products **2** (retention time: 9.2 min), **3** (retention time: 15.6 min), **6** (retention time: 21.4 min) and **7** (retention time: 24.9 min). Chromatogram obtained with a solution containing 10 mg each in DMF (retention time 2.3 min), diluted 1:5 with the eluent buffer before injection.



**4. Details of characterization of 6 and 7 by X-ray data**

The data of **6** have been collected at room temperature on a Bruker D8 Quest IµS diffractometer while those of **7** have been collected at 112 K on a Bruker D8 Venture TXS diffractometer. Mo Kα radiation monochromated by multilayer mirror optics was applied in both experiments. The frames were integrated with the Bruker SAINT software package [1] using a narrow-frame algorithm. Data were corrected for absorption effects using the Multi-Scan method of SADABS [2]. The structures were solved and refined using the Bruker SHELXTL Software Package [3]. The hydrogen atoms were calculated in ideal geometry riding on their parent atoms. The data quality of **6** did not allow the determination of the correct absolute structure (the randomly chosen structure solution used to draw Figure 2 in the manuscript shows (*R*)-configuration at both stereocenters, named C3 and C5 in the graphic, as well as the stereocenters of the two independent molecules not displayed herein: C29, C31, C55 and C57). Hence, this structure has been refined as perfect inversion twin. However, the data of **7** led to a Flack parameter of 0.1(2) indicating the correct absolute structure as that with an (*S*)-configured stereocenter (data have been collected up to a resolution of 0.60 Å). The figures were drawn at the 50% ellipsoid probability level (ORTEP [4]).

Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1907390 (**6**), 1907391 (**7**)). These supplementary crystallographic data can be obtained free of charge from The Cambridge Crystallographic Data Centre via <https://www.ccdc.cam.ac.uk/structures/>.

[1] Bruker (2012). *SAINT*. Bruker AXS Inc., Madison, Wisconsin, USA.

[2] G.M. Sheldrick (1996). *SADABS.* University of Göttingen, Germany.

[3] G.M. Sheldrick, SHELXT- Integrated space-group and crystal-structure determination, Acta Crystallogr. A, Found. Adv. 71 (2015) 3-8. <http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=100256836&site=ehost-live>.

[4] L. Farrugia, WinGX and ORTEP for Windows: an update, J. Appl. Crystallogr. 45 (2012) 849-854. <https://doi.org/10.1107/S0021889812029111>.

<https://doi.org/10.1016/j.bmcl.2015.12.069>

**5. Crystallographic data for 6 and 7**

**Table S1**

|  |  |  |
| --- | --- | --- |
|  | **6** | **7** |
| CCDC | 1907390 | 1907391 |
| net formula | C26H26FNO2 | C26H26FNO2 |
| *M*r/g mol−1 | 403.48 | 403.48 |
| crystal size/mm | 0.100 × 0.030 × 0.020 | 0.100 × 0.070 × 0.060 |
| crystal system | orthorhombic | orthorhombic |
| space group | 'P 21 21 21' | 'P 21 21 21' |
| *a*/Å | 9.6001(8) | 9.7398(4) |
| *b*/Å | 17.4122(16) | 10.4787(5) |
| *c*/Å | 38.547(4) | 20.4221(9) |
| α/° | 90 | 90 |
| β/° | 90 | 90 |
| γ/° | 90 | 90 |
| *V*/Å3 | 6443.5(10) | 2084.29(16) |
| *Z* | 12 | 4 |
| calc. density/g cm−3 | 1.248 | 1.286 |
| μ/mm−1 | 0.084 | 0.087 |
| transmission factor range | 0.96–1.00 | 0.96–0.99 |
| refls. measured | 77036 | 48775 |
| *R*int | 0.1383 | 0.0545 |
| mean σ(*I*)/*I* | 0.0889 | 0.0418 |
| θ range | 2.339–25.350 | 3.484–36.313 |
| observed refls. | 7567 | 8541 |
| *x, y* (weighting scheme) | 0.0342, 2.1173 | 0.0756, 0.0638 |
| Flack parameter | 0.5 | 0.1(2) |
| refls in refinement | 11793 | 10078 |
| parameters | 818 | 273 |
| restraints | 0 | 0 |
| *R*(*F*obs) | 0.0667 | 0.0524 |
| *R*w(*F*2) | 0.1283 | 0.1307 |
| *S* | 1.038 | 1.082 |
| shift/errormax | 0.001 | 0.001 |
| max electron density/e Å−3 | 0.172 | 0.490 |
| min electron density/e Å−3 | −0.190 | −0.287 |