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Photolability of potential calcium channel antagonists: Hexahydroquinoline derivatives

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Abstract

Photodegradation of three chlorinated derivatives—Compound I (2′-chloro-), Compound II (3′-chloro-) and Compound III (2′,3′-dichloro-) of methyl 2,6,6-trimethyl-4-phenyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate was studied in the conditions recommended by the International Conference on Harmonization (ICH) in the liquid phase. Photodegradation kinetics of the three compounds in methanolic solutions was studied by means of the spectrophotometric method. Reactions followed the first-order kinetic equation. The measured real quantum yields of photodegradation were 1.2×10^{-4}, 4.0×10^{-5} and 3.0×10^{-5}, respectively. Gas chromatography–mass spectrometry (GC–MS) was used to identify the photodegradation products, with the main products formed by oxidation of the dihydropyridine ring to pyridine derivatives. Mass fragmentation pathways of the photoproducts were also described.

Keywords: Calcium channel blockers; Photostability; Drugs; Photodegradation; Dihydropyridine derivatives

1. Introduction

Calcium channel antagonists from the group of dihydropyridine (DHP) derivatives deserve special attention from a number of points of view [1–3]. Their widespread successful application in therapy has stimulated the search for new derivatives with a possibly even better therapeutic effect, with improved selectivity, stability, and perhaps with different modes of action [4–6]. The chemical structure of dihydropyridine derivatives permits introduction of various modifications, potentially adding new modes of action to the already known useful properties of DHP [7–9]. The specific goals of such modifications may be different; still, they are all motivated by an underlying desire to obtain new compounds with improved therapeutic efficiency [10–12]. One of possible modifications to DHP leads to hexahydroquinoline (HHQ) derivatives, which are successfully explored by the Safak's group [13–18]. The DHP derivatives get photodegraded quite easily; therefore, their photochemical decomposition has to be investigated as it may reduce the therapeutic value of the drugs, and eventually even lead to the appearance of toxic products [19]. These undesirable and unforeseen photochemical products of drugs may induce hypersensitivity to light, possibly leading to phototoxic and photoallergic effects in the patient [20]. Indeed, a long list of photosensitive drugs includes, e.g. various calcium channel antagonists from the group of DHP derivatives [21]. HHQ derivatives, closely related to DHP, are also light sensitive [22]. The main objective of this paper is to evaluate photochemical stability of methyl 2,6,6-trimethyl-4-phenyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate. In this study we report the photochemical properties of the studied compounds, with the emphasis on detection and identification of the respective stable photoproducts. Fig. 1 shows the structure and Table 1 summarises some of the basic information regarding the studied compounds.

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Table 1
Methyl 2,6,6-trimethyl-4-phenyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives (HHQ)

HHQ derivative	Formula	R	R'	Substituent position	M.w. (g mol^{-1})
Compound I (2-Cl HHQ)	$C_{20}H_{22}O_3NCl$	–Cl	–	$R=2'$	359.85
Compound II (3-Cl HHQ)	$C_{20}H_{22}O_3NCl$	–Cl	–	$R=3'$	359.85
Compound III (2-Cl,3-Cl HHQ)	$C_{20}H_{21}O_3NCl_2$	–Cl	–Cl	$R=2'$, $R'=3'$	393.30

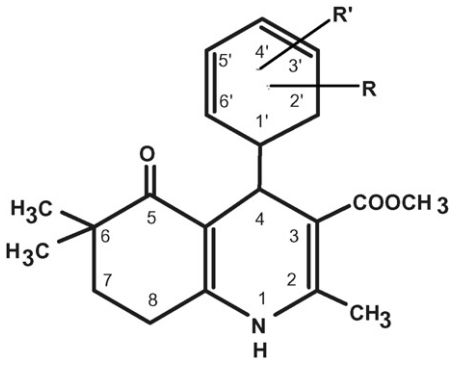

Fig. 1. Structure of methyl 2,6,6-trimethyl-4-phenyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (HHQ).

2. Experimental

2.1. Materials

Methyl 2,6,6-trimethyl-4-phenyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (HHQ), were synthesised using a modified Hantzsch synthesis by the Safak's group [16], and kindly donated for our studies. HPLC grade solvents were used. The pharmacological activity of the hexahydroquinoline derivatives was earlier determined by Aydin and Safak, and their results are presented in the paper [13].

2.2. Sample preparation and photodegradation conditions

The methods applied for assessment of the photochemical decomposition of HHQ derivatives were UV spectrophotometry and GC–MS.

Metanolic solutions of the HHQ derivatives were tested for photodegradation according to the requirements of the first version of the document issued by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), currently in force in photochemical studies of drugs and therapeutic substances [23]. The solutions were placed in a quartz cell of 2.8 ml in capacity, and irradiated with a high-pressure HBO-50 mercury lamp from a distance of 3.5 cm, using a Wood's filter (λ_{max} = 365 nm).

For GC–MS analysis after specific time intervals, 1.4 ml aliquots (at a concentration of ca. 10^{-4} mol/l) were placed into

Table 2
Spectral and analytical properties of methyl 2,6,6-trimethyl-4-phenyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (HHQ) in methanol used in photodegradation experiments

HHQ derivative	λ_{max} (nm)	c ($\times 10^5$ mol l^{-1})	ε (l mol^{-1} cm^{-1})
Compound I (2-Cl HHQ)	367	8.96	7730
Compound II (3-Cl HHQ)	362	5.61	7830
Compound III (2-Cl,3-Cl HHQ)	367	3.58	8550

λ_{max}: analytical wavelength; c: concentration used in the experiments; ε: molar absorption coefficient

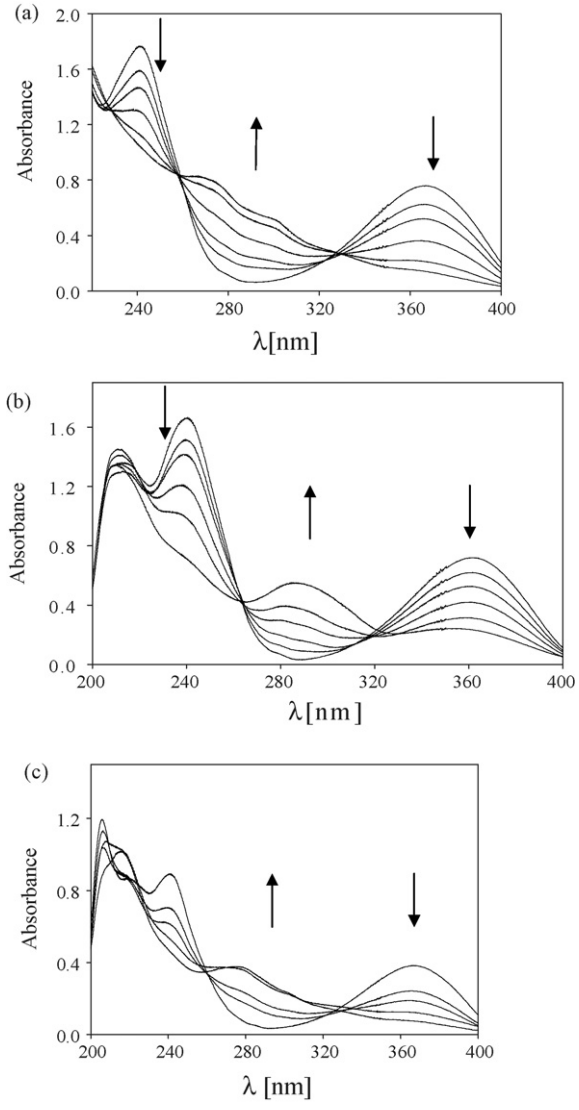

Fig. 2. (a) Spectral changes of Compound I (2-Cl HHQ) in methanol after different times of irradiation (0, 30, 60, 120, 200, and 255 min) at 365 nm, (b) spectral changes of Compound II (3-Cl HHQ) in methanol after different times of irradiation (0, 120, 230, 270, 330, and 480 min) at 365 nm, and (c) spectral changes of Compound III (2-Cl 3-Cl HHQ) in methanol after different times of irradiation (0, 60, 110, 190, and 200 min) at 365 nm.

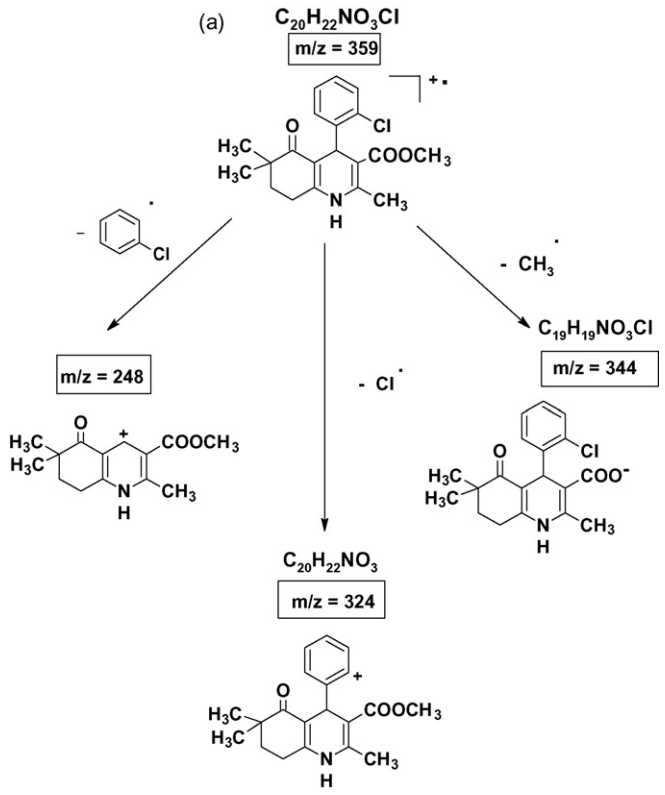

Fig. 3. Mass fragmentation scheme of (a) methyl 2,6,6-trimethyl-4-(2′-chlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound I), (b) methyl 2,6,6-trimethyl-4-(3′-chlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound II), and (c) methyl 2,6,6-trimethyl-4-(2′,3′-dichlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Compound III).

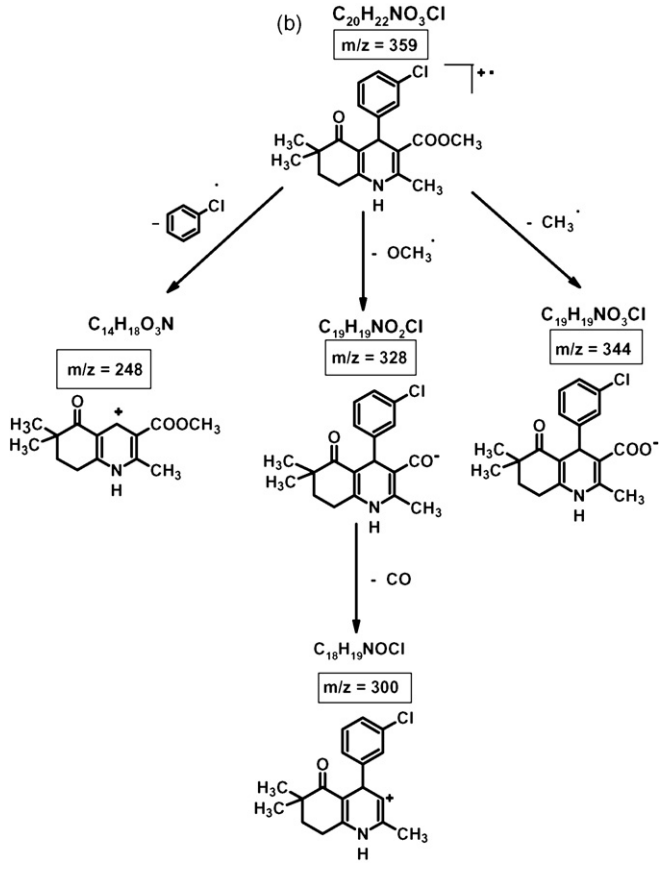

Fig. 3. (*Continued*).

conical vials, concentrated to dryness in nitrogen atmosphere, and dissolved in 10 μl of methanol.

To analyse the process of photodegradation by UV spectroscopy, the UV spectra of the compounds (see Table 2 for the concentrations) were taken at fixed time intervals and their absorbance was measured. After a proper exposure time, the UV spectra were recorded in the range 200–400 nm on an UV-160 A Shimadzu spectrophotometer at room temperature.

The irradiation doses were monitored by a Reinecke salt chemical actinometer, with *trans*-tetrathiocyandiammine-chromate(III) potassium obtained from the ammonium salt [24,25]. Actinometer solutions were irradiated using the same irradiation geometry and Wood filter. The measured number of quanta absorbed by the actinometer in 85 s was 1.42×10^{17}, corresponding to the light intensity of 2.77×10^{-9} Es s^{-1}.

2.3. Chromatographic analysis of the photodegradation products

A model 5890II gas chromatograph, equipped with a 5971 A selective mass detector (Hewlett-Packard) was used in this study. The separation was conducted on a DB-5 silica capillary column (J&W, USA), of 0.25 mm internal diameter, 30 m length and 0.25 μm film thickness. The following temperature program was used: the injection chamber temperature 250 °C, the initial temperature in the oven 140 °C kept for 2 min, 5 °C/min temperature ramp up to 200 °C, and then 10 °C/min up to 300 °C, with the final temperature maintained for 13 min. The carrier gas was helium, 99.99% purity, 1.0 ml/min flow-rate, 5 bar pressure, and was divided between the column and the injection chamber at the 1:2 ratio.

Gas chromatograph was connected to the mass spectrometer by an AMD402 interface kept at 300 °C.

Table 3
Real quantum yields of photodegradation (φ) of hexahydroquinoline derivatives in methanolic solutions after irradiation at 365 nm, and kinetic parameters of photodegradation of HHQ derivatives obtained by the spectroscopic method: the rate constant of photodegradation (k), the half-life time ($t_{0.5}$) and the time of degradation of 10% of the compound ($t_{0.1}$)

HHQ derivative	$k \pm \Delta k$ (s^{-1})	$t_{0.1}$ (min)	$t_{0.5}$ (min)	$\varphi \pm \Delta\varphi$
Compound I (2-Cl HHQ)	$(1.20 \pm 0.01) \times 10^{-4}$	14.17	96.17	$(1.58 \pm 0.02) \times 10^{-4}$
Compound II (3-Cl HHQ)	$(3.95 \pm 0.13) \times 10^{-5}$	44.44	292.18	$(4.37 \pm 0.21) \times 10^{-5}$
Compound III (2-Cl,3-Cl HHQ)	$(2.95 \pm 0.19) \times 10^{-5}$	59.55	391.53	$(3.95 \pm 0.11) \times 10^{-5}$

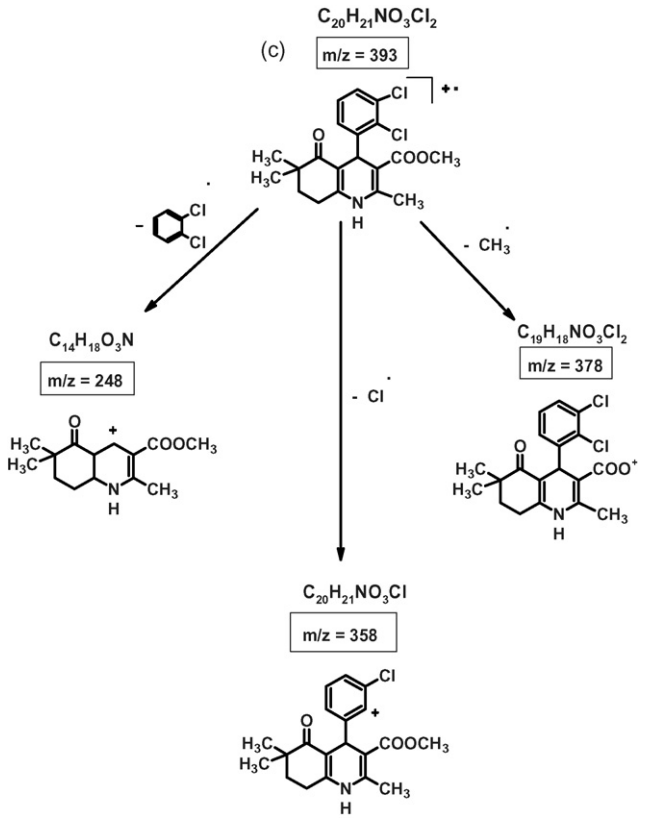

Fig. 3. (*Continued*).

2.4. Low-resolution mass spectra of the photoproducts

The low-resolution mass spectra of the photodegradation products of HHQ derivatives were taken on an AMD 402 two-sector B/E type mass spectrometer, in the Nier–Johnson geometry. The unit resolution was $R = 1000$. The ionisation was performed by an electron beam at 70 eV, and 8 kV accelerating voltage was used. The temperature of the electron source was 200 °C; while the evaporation temperature varied from 100 to 250 °C. The low-resolution mass spectra in the normalised form are shown in the range from 50 to 400 *m*/*z*.

2.5. High-resolution mass spectra of the photoproducts

In order to identify the fragmentation pathway of the photodegradation products the method of peak superposition was applied and the elemental compositions of the fragment ions were determined using perfluoroxene as a standard. The measurements were made on JMS D100a mass spectrometer, with a resolution of $R = 10{,}000$. The error in determination of the elemental composition of the ions did not exceed 0.05 Da, relative to the results of theoretical calculations.

3. Results and discussion

3.1. Spectral and photochemical studies

Spectral properties of dihydropyridine derivatives including HHQ derivatives have been the subject of intense studies [22]. The absorption spectra of the presently studied HHQ derivatives in methanol reveal a characteristic band at longer wavelengths, with the maximum at approximately 367 nm (for Compounds I and II) and at about 362 nm (for Compound III) and are shown in Fig. 2. Table 2 presents parameters characterising analytical properties of the compounds used in photodegradation experiments. As shown, the spectral properties of all three derivatives are rather similar; analogously, the spectral changes observed during photolysis of HHQ solutions in methanol are also similar.

The visually noticeable relatively rapid photodegradation is also supported by the experimentally determined quantum yields for particular irradiation times, as described in paper [25]. To facilitate comparison, the experimentally determined quantum yields of photodegradation were extrapolated to the starting concentrations (0% conversion), thus producing real quantum yields. The results for the real quantum yields are given in Table 3 together with the kinetic parameters of photodegradation of HHQ derivatives as obtained from the UV–vis spectra: the rate constant of photodegradation (k), the half-life time ($t_{0.5}$) and the time of degradation of 10% of the compound ($t_{0.1}$). All the kinetics parameters were extracted assuming changes in the concentration of HHQ derivatives during irradiation can be described by the equation of the first-order reaction [24]:

$$\ln(A_t - A_\infty) = \ln(A_0 - A_\infty) - k_{\text{obs}}t \quad (1)$$

where A_0, A_t and A_∞ stand for the absorbency measured at $t = 0$, t and t_∞; k is the rate constant of photodegradation and t is the time of irradiation (min). The Lambert–Beer law was used to relate concentrations and absorption values. We get from Eq. (1) that $t_{0.1} = \ln 1.11/k = 0.1054/k$, and $t_{0.5} = \ln 2/k = 0.693/k$.

The values obtained for the real quantum yields of photodegradation are in the 10^{-4} to 10^{-5} range, indicating the

Table 4
Chromatographic parameters (t_R) and *m*/*z* positions of the molecular and fragmentation ions of the photoproducts of the HHQ derivatives

HHQ derivative	Retention time t_R (min)	Molecular ion (*m*/*z*)	Fragmentation ions (*m*/*z*)
Compound I	22.18	322	307, 266
	22.41	353	339, 338, 323, 322
Compound II	22.40	357	342, 328, 314, 301, 269
Compound III	23.43	391	356
	24.05	387	372, 356, 340, 331, 316, 301, 207
	27.59	341	313, 298, 285, 256

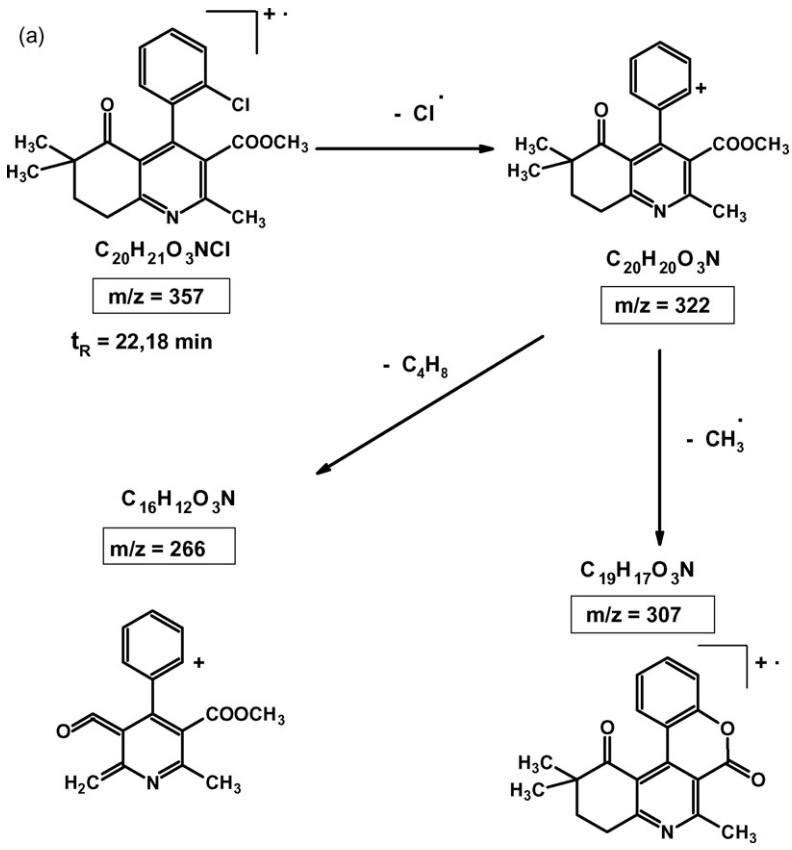

Fig. 4. Scheme of mass fragmentation of the photoproducts (a) $t_R = 22.18$ min formed during photodegradation Compound I (2-Cl HHQ) after 120 min of irradiation at 365 nm; this photoproduct was identified as methyl 2,6,6-trimethyl-4-(2′-chlorophenyl)-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate and (b) $t_R = 22.41$ min, this photoproduct was identified as methyl 2,6,6-trimethyl-4-(2′-methoxyphenyl)-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate.

presence of some differences depending on the position of the chloro substituent. Results of our earlier studies and literature data have revealed that similar trends have been observed for calcium antagonists from the group of 1,4-dihydropyridine [26]. Summing up the results of studies on the photochemical stability of the DHP derivatives, we conclude that the rate of their degradation depends on the type and position of substituents in the phenyl ring. Previously obtained results suggest that DHP derivatives containing a substituent group in the *ortho*-position of the phenyl ring are particularly photolabile. Under the same irradiation conditions, the *meta*-isomer appears to be more stable. This is in agreement with our present results, where the real quantum yields of photodegradation are the lowest for Compound II, the highest for Compound I, whereas Compound III produced an intermediate value.

3.2. Identification of the photoproducts

Identification of photoproducts was done using GC–MS method [27,28]. In the first step, the method of gas chromatography was employed, which after an appropriate optimisation permitted to separate the products of photochemical degradation.

The results of the GC–MS analysis are displayed in Table 4 showing retention times of Compounds I, II, and III and their photoproducts subjected to total photodegradation.

Analysis of the values obtained indicates that exposition of different HHQ derivatives to light leads to formation of a different number of photoproducts. As shown in Table 4, the chromatogram of Compound I indicates the presence of two photoproducts characterised by the retention times: $t_R = 22.18$ min ($m/z = 357$) and 22.41 min ($m/z = 353$). Photodegradation of Compound II leads to formation of only one photoproduct characterised by the retention time, $t_R = 22.40$ min ($m/z = 357$). The photoproducts appearing as a result of photodegradation of Compound III are characterised by the retention time, $t_R = 23.43$ min ($m/z = 356$), $t_R = 24.05$ min ($m/z = 387$), $t_R = 27.59$ min ($m/z = 341$).

The separated photoproducts were identified with the use of a MS detector, on ionisation by electron impact (EI). The low- and high-resolution mass spectra permitted determination of the m/z values and of the elemental composition of molecular and fragmentation ions. A comparison of the data presented in Figs. 3 and 4 and Table 4 has shown that the photodegradation of all HHQ derivatives analysed leads to the formation of pyridine derivatives as the main products. In particular, for Compound I the two photoproducts were identified as ($t_R = 22.41$ min) methyl 2,6,6-trimethyl-4-(2′-chlorophenyl)-5-oxo-5,6,7,8-tetrahydroquinoline-3carboxylate and ($t_R = 22.41$ min) methyl 2,6,6-trimethyl-4-(2′-methoxyphenyl)-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate. In the case of photodegradation of Compound II, only one photoproduct was found ($t_R = 22.40$ min)

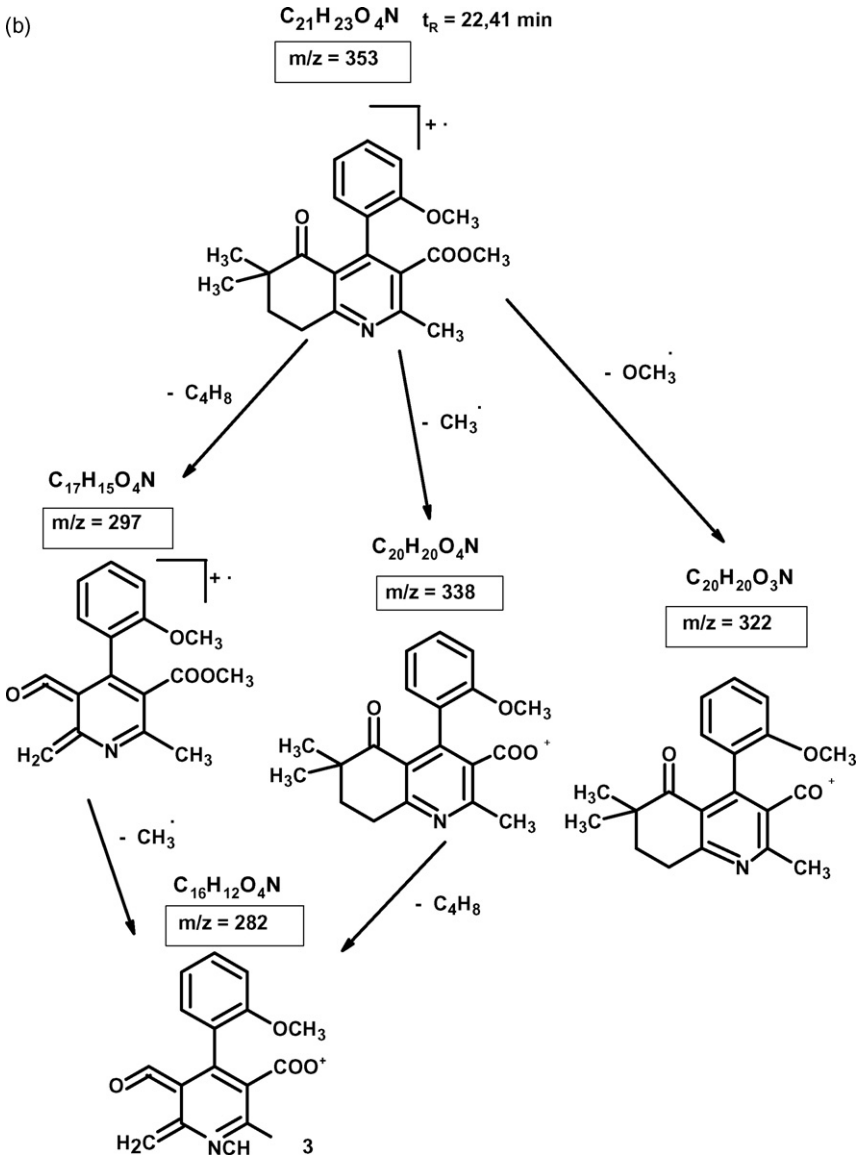

Fig. 4. (*Continued*).

and identified as methyl 2,6,6-trimethyl-(3′-chlorophenyl)-5-oxo-5,6,7,8-tetrahydroquinoline-3-carboxylate.

Mass fragmentation patterns of the photoproducts of all the HHQ derivatives studied show that their fragmentation involves elimination of the chlorine. This is a novel fragmentation path, which has not been previously observed for molecules containing a chlorine atom at the *para*-position of the phenyl ring [26]. On the basis of the fragmentation pathways analysis, mass fragmentation schemes were proposed as shown in Fig. 3a–c. Fragmentation of HHQ derivatives leads to formation of a different number of products; however, all of them have shown aromatic properties. The mass fragmentation patterns of all photoproducts formed did not show a peak at $m/z = 248$.

It should be emphasized that as follows from Fig. 4, the favoured direction of mass fragmentation of all HHQ derivatives (I–III) involves breaking up of the C–C bond between the phenyl ring and the hexahydroquinoline ring, leading to ions at $m/z = 248$. According to the m/z positions of the molecular and fragmentation ions and the mode of mass fragmentation these compounds are formed as a result of dehydrogenation of the dihydropyridine ring yielding an aromatic pyridine ring.

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References

[1] M.J. Eisenberg, A. Brox, A.N. Bestawros, Am. J. Med. 116 (2004) 35–43.
[2] T. Kobayashi, Y. Mori, Eur. J. Pharmacol. 363 (1998) 1–15.
[3] D. Inzitari, A. Pogessi, Aging Clin. Exp. Res. 17 (2005) 16–30.
[4] T.J. Cleophas, R. van Marum, Am. J. Cardiol. 87 (2001) 487–490.
[5] D. Ilavsky, V. Milata, Collect. Czech. Chem. Commun. 61 (1996) 1233–1243.
[6] R. Miri, H. Niknahad, G. Vesal, A. Shafiee, Farmaco 57 (2002) 123–128.

[7] A. Andreani, A. Leoni, M. Rambaldi, A. Locatelli, R. Bossa, I. Galatulas, M. Chiericozzi, M. Bissoli, Eur. J. Med. Chem. 32 (1997) 151–157.
[8] I. Kruk, A. Kladna, K. Lichszteld, T. Michalska, H.Y. Boul-Enein, M. Tuncbilek, R. Ertan, Biopolymers 62 (2001) 163–167.
[9] M. Nakajima, I. Adachi, M. Ueda, Arzneim.-Forsch./Drug Res. 43 (1993) 1276–1282.
[10] M. Ueda, S. Matsumura, M. Masui, E. Matsuura, M. Kawakami, H. Fujitomo, T. Umeda, H. Kagawa, S. Hirohata, K. Shima, I. Adachi, Arzneim.-Forsch./Drug Res. 43 (1993) 1282–1290.
[11] R.J.M. de Vries, D.J. van Veldhuisen, P.H.J.M. Dunselman, Am. Heart J. 139 (2000) 185–194.
[12] T. Yamamoto, S. Niwa, S. Ohno, T. Onishi, H. Matsueda, H. Koganei, H. Uneyama, S.I. Fujita, T. Takeda, M. Kito, Y. Ono, Y. Saitou, A. Takahara, S. Iwata, M. Shoji, Bioorg. Med. Chem. Lett. 16 (2006) 798–802.
[13] F. Aydin, C. Safak, R. Simsek, K. Erol, M. Ulgen, A. Linden, Pharmazie 61 (2006) 655–659.
[14] E. Kismetli, C. Safak, K. Erol, B. Sirmagül, A. Linden, Arzneim.-Forsch./Drug Res. 54 (2004) 371–375.
[15] R. Simsek, C. Safak, K. Erol, B. Sirmagül, Arzneim.-Forsch./Drug Res. 51 (2001) 959–963.
[16] R. Simsek, C. Safak, K. Erol, B. Sirmagül, Pharmazie 56 (2001) 665–666.
[17] R. Simsek, U.B. Ismailoglu, C. Safak, I. Sahin-Erdemli, Farmaco 55 (2000) 665–668.
[18] R. Simsek, C. Safak, K. Erol, S. Ataman, M. Ulgen, A. Linden, Arzneim.-Forsch./Drug Res. 53 (2003) 159–166.
[19] G.M.J. Beijersbergen van Henegouwen, Medicinal Photochemistry: Phototoxic and Phototherapeutic Aspects of Drugs, Advances in Drug Research, Academic Press, New York, 1997.
[20] W. Aman, K. Thoma, Pharmazie 58 (2003) 877–880.
[21] J. Mielcarek, W. Augustyniak, P. Grobelny, G. Nowacka, Int. J. Pharm. 304 (2005) 145–151.
[22] J. Mielcarek, C. Safak, R. Simsek, A. Matloka, Arch. Pharm. 335 (2002) 77–82.
[23] H.D. Drew, Photostability of Drug Substances and Drug Products: A Validated Reference Method for Implementing the ICH Photostability Study Guidelines, in: A. Albini, E. Fasani (Eds.), Drugs Photochemistry and Photostability, The Royal Society of Chemistry, Cambridge, 1998, pp. 227–242.
[24] G. Favaro, Actinometry: Concepts and Experiments, in: A. Albini, E. Fasani (Eds.), Drugs Photochemistry and Photostability, The Royal Society of Chemistry, Cambridge, 1998, pp. 296–304.
[25] W. Augustyniak, J. Mielcarek, M. Milewski, O. Szamburska, Drug Dev. Ind. Pharm. 27 (2001) 1031–1038.
[26] J. Mielcarek, J. Pharm. Biomed. Anal. 15 (1997) 681–686.
[27] A.B. Baranda, C.A. Mueller, R.M. Alonso, R.M. Jimenez, W. Weinmann, Ther. Drug Monit. 27 (2005) 44–52.
[28] F. Barbato, L. Grumetto, P. Morrica, Farmaco 49 (1994) 461–466.