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ORIGINAL PAPER

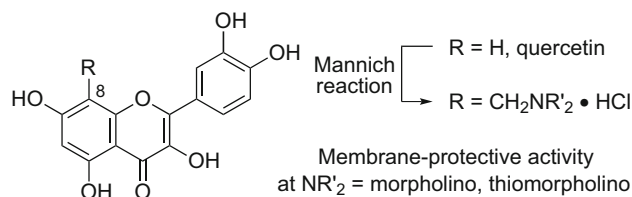# Synthesis and membrane-protective properties of aminomethyl derivatives of quercetin at the C-8 position

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Abstract A set of four C-8-aminomethyl derivatives of quercetin has been synthesized by Mannich reaction. The synthesis was carried out using a simple procedure to give target compounds as hydrochlorides. Study of oxidative hemolysis on mice erythrocytes showed that derivatives with morpholinomethyl or thiomorpholinomethyl groups favorably differ from the original quercetin by the ability to protect cells from acute oxidative stress.

Graphical abstract

Introduction

Natural antioxidants are substances, which are able to inhibit the oxidation processes of other compounds. Their useful effect is reflected in the capacity to protect the human organism against the harmful action of the free radicals (Nasser et al. 2016). Flavonoids are a special group of natural antioxidants which are a class of polyphenols and secondary metabolites of plants (Kao et al. 2008). The high antioxidant activity (AOA) of flavonoids is due to the presence of the OH groups attached to the aromatic rings, along with the electronic delocalization through the entire structure (Donato et al. 2011). Flavonoids exhibit numerous biological and pharmacological effects (Cross et al. 1996; Middleton et al. 2000; Harwood et al. 2007). Well-known flavonoid quercetin (**1**) is a representative of this class of compounds, it is widely encountered in the plants and exhibits anti-carcinogenic, anti-inflammatory, cardioprotective properties (Middleton et al. 2000; Erlund 2004), and high AOA (Zhang et al. 2011; Veverka et al. 2013; Fatokun et al. 2015).

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Mannich reaction is a well-known one-step method for introducing an aminoalkyl moiety by electrophilic substitution (Tramontini and Angiolini 1994). This reaction is often used for drug design and for the synthesis of pharmacologically important molecules (Roman 2015). For example, Mannich bases and their derivatives with the 1-propanone fragment exhibit antimicrobial activity against pathogenic bacteria and fungi (Gul et al. 2005), and showed cytotoxicity against transformed human T-lymphocytes (Kucukoglu et al. 2011). Our previous studies showed that the aminomethylation of 2-isobornyl-4-methylphenol by morpholine led to anti-inflammatory activity of a novel compound (Buravlev et al. 2011). However, the introduction of piperidinomethyl and morpholinomethyl fragments into natural xanthone α-mangostin led to an increase in AOA and membrane-protective activity (MPA) of prepared compounds towards erythrocytes (Buravlev et al. 2015). Quercetin (1) can be used as a suitable scaffold for the production of new compounds with biological activity. It was previously reported that the aminomethylation of compound (1) proceeds with formation of C-6- (Chen et al. 2006; Zhan et al. 2015) or C-8-monosubstituted (Zhang et al. 2008; Joshi et al. 2013; Helgren et al. 2015) quercetin or disubstituted product (Helgren et al. 2015). Some quercetin derivatives with aminomethyl and amine fragments exhibit in vitro cytotoxicity to four lines of human cancer cells, AKT1 (protein kinase B) inhibitory activity (Zhan et al. 2015), and antimalarial activity (Helgren et al. 2015). These compounds can also inhibit photo-oxidation of A2E (component of retinal pigmented epithelial) (Joshi et al. 2013) and exhibit AOA (Ilkei et al. 2017).

Experimental

Materials

UV–Vis spectra were recorded on a Shimadzu UV-1700 spectrometer in quartz cuvettes (10 mm) in EtOH–H_2O solutions (2:1 v/v). IR diffuse reflectance spectra were recorded on a Shimadzu IR Prestige 21 IR-FT spectrometer in tablets with KBr. NMR ^1H and ^{13}C spectra were recorded on a Bruker Avance II 300 (300 and 75 MHz) in DMSO-d_6. Chemical shifts were referenced to the residual DMSO signals (δ_H 2.50 ppm, δ_C 39.52 ppm). The signals of carbon atoms were assigned using ^{13}C NMR spectra in J-modulation mode and using the literature data for quercetin (1) (Markham et al. 1978); the other assignments were made using HSQC and HMBC experiments. Mass spectra (HRMS, ESI) were recorded on a Shimadzu LCMS-IT-TOF mass spectrometer. The commercially available quercetin dihydrate (abcr GmbH), pyrrolidine,

piperidine, thiomorpholine (Alfa Aesar), and morpholine (Sigma-Aldrich) were used without additional purification.

Absorption spectra were measured on Thermo Spectronic Genesys 20 instrument. The absorption and fluorescence spectra of hemolysates were analyzed on a Fluorat-02-Panorama spectrofluorimeter. Incubation of brain homogenates and mice erythrocytes were carried out in thermostated Biosan ES-20 shaker. Tested compounds (1–5) were dissolved in an acetone–water mixture (1:1 v/v).

Each experiment was carried out in 4–6 replicates. Statistical analysis was performed by applying software packages Microsoft Office Excel 2007 and Statistica 6.0. Data are presented as mean values ± mean squared error. A statistical significance of differences between quercetin (1) and its derivates was assessed by a non-parametric Mann–Whitney test. Significance was set at $p < 0.05$ and $p < 0.01$.

Synthesis

Synthesis of hydrochlorides 2 and 3

To cooled in a water bath solution of quercetin dihydrate (1 mmol, 13 °C) in 6 mL of 1,4-dioxane, a 37% aqueous solution of formaldehyde (1 mmol) was added followed by amine (1 mmol). The mixture was stirred for 75 min (pyrrolidine) or 100 min (piperidine) with increasing temperature to 25 °C. The intermediate product as crude tertiary amine was filtered off, washed with 3 mL of 1,4-dioxane and dried. 3.5 mL of 2 N HCl in ethanol was added to the solid residue and the mixture was stirred for 5 min at a room temperature. The precipitated hydrochloride was filtered off, and washed with 2 N HCl solution in ethanol. For removing the solvated organic solvents, the final product was treated with a mixture of water–acetone (1:1 v/v), the volatiles were removed under reduced pressure, and the target product was dried in vacuo.

2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-8-(pyrrolidin-1-ylmethyl)-4H-chromen-4-one hydrochloride (2). Yellow powder, mp > 220 °C. Yield 60%. UV–Vis (EtOH–H_2O, 2:1 v/v, λ_{max}, nm): 277, 322, 385. IR (KBr, v, cm^{-1}): 3379, 3194 (O–H); 2974, 1456, 1431 (CH_2); 2731 (CH_2–N); 1653 (C=O); 1612, 1560, 1516 (C=C, C–C_{Ar}, C–C_{Het}); 1271 (=C–OH); 1194 (=C–O–C); 1163, 1134 (C−O); 1005 (C–O–C); 821, 781 (=C–H). NMR ^1H (300 MHz, DMSO-d_6, δ, ppm): 1.70–2.18 m (4H, NCH$_2$C$\underline{H}_2$); 3.02–3.73 m (4H, NC$\underline{H}_2$CH$_2$); 4.49 s (2H, C-8–C$\underline{H}_2$); 6.51 s (1H, H-6); 6.93 d (1H, H-5′, J = 8.2 Hz); 7.66 d (1H, H-6′, J = 8.5 Hz); 7.83 s (1H, H-2′); 9.39–9.81 m, 10.04 br.s, 11.90 br.s (2H, 1H, 1H, C-3–OH, C-7–OH, C-3′–OH, C-4′–OH); 12.76 br.s (1H, C-5–OH). NMR ^{13}C (75 MHz, DMSO-d_6, δ, ppm): 22.46 (NCH$_2$$\underline{C}H_2$); 45.31 (C-8–$\underline{C}H_2$); 53.14 (N$\underline{C}H_2CH_2$); 96.00

(C-8); 97.72 (C-6); 103.00 (C-10); 114.92 (C-2′); 115.79 (C-5′); 120.33 (C-6′); 121.83 (C-1′); 136.00 (C-3); 145.29 (C-3′); 147.12 (C-2); 147.88 (C-4′); 154.79 (C-9); 161.41 (C-5); 163.22 (C-7); 175.89 (C-4). HRMS, ESI (*m/z*): 386.1238 [M+H]$^+$; 385.1240 calcd [M+H]$^+$ for $C_{20}H_{20}NO_7$.

2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-8-(piperidin-1-ylmethyl)-4*H*-chromen-4-one hydrochloride (**3**). Yellow powder, mp > 220 °C. Yield 33%. UV–Vis (EtOH–H$_2$O, 2:1 v/v, λ_{max}, nm): 276, 322, 385. IR (KBr, v, cm^{-1}): 3211, 3142 (O–H); 2953, 1454, 1431 (CH$_2$); 2734 (CH$_2$–N); 1653 (C=O); 1605, 1566, 1516 (C=C, C–C$_{Ar}$, C–C$_{Het}$); 1273 (=C–OH); 1194 (=C–O–C); 1166, 1130 (C−O); 1001 (C–O–C); 819 (=C–H). NMR ^{1}H (300 MHz, DMSO-d_6, δ, ppm): 1.28–1.63 m (2H, N(CH$_2$CH$_2$)$_2$CH$_2$), 1.63–1.94 m (4H, N(CH$_2$CH$_2$)$_2$CH$_2$); 2.84–3.84 m (4H, N(CH$_2$CH$_2$)$_2$CH$_2$); 4.40 s (2H, C-8–CH$_2$); 6.51 s (1H, H-6); 6.93 d (1H, H-5′, J = 8.8 Hz); 7.65 dd (1H, H-6′, J = 8.3 Hz, J = 2.1 Hz); 7.82 d (1H, H-2′, J = 2.2 Hz); 9.20–9.92 m, 11.86 br.s (3H, 1H, C-3–OH, C-7–OH, C-3′–OH, C-4′–OH); 12.80 br.s (1H, C-5–OH). NMR ^{13}C (75 MHz, DMSO-d_6, δ, ppm): 20.95 (N(CH$_2$CH$_2$)$_2$CH$_2$); 22.34 (N(CH$_2$CH$_2$)$_2$CH$_2$); 48.39 (C-8–CH$_2$); 52.33 (N(CH$_2$CH$_2$)$_2$CH$_2$); 95.07 (C-8); 97.74 (C-6); 103.04 (C-10); 114.91 (C-2′); 115.78 (C-5′); 120.31 (C-6′); 121.82 (C-1′); 135.99 (C-3); 145.27 (C-3′); 147.14 (C-2); 147.84 (C-4′); 155.05 (C-9); 161.51 (C-5); 163.53 (C-7); 175.86 (C-4). HRMS, ESI (*m/z*): 400.1401 [M+H]$^+$; 400.1396 calcd [M+H]$^+$ for $C_{21}H_{22}NO_7$.

Synthesis of hydrochlorides 4 and 5

A 37% aqueous solution of formaldehyde (1 mmol) was added to a suspension of quercetin dihydrate (1 mmol) in EtOH (6 mL), and then amine (1 mmol) was added. The mixture was heated at 60 °C for 2 h, then cooled to room temperature. The intermediate product as crude tertiary amine was filtered off, washed with 3 mL of EtOH and dried. 2 N HCl solution in EtOH (30 mL) was added to the solid residue, the stirred mixture was heated to reflux in a water bath, then cooled to room temperature. The precipitated hydrochloride was filtered off, washed with 3 mL of a 2 N HCl in EtOH. Solvated organic solvents were removed as described above, and the target product was dried in vacuo.

(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-8-(morpholinomethyl)-4*H*-chromen-4-one hydrochloride (**4**). Yellow powder, mp > 220 °C. Yield 69%. UV–Vis (EtOH–H$_2$O, 2:1 v/v, λ_{max}, nm): 259, 270 (shoulder), 307 (shoulder), 380. IR (KBr, v, cm^{-1}): 3431, 3157 (O–H); 2941, 1429 (CH$_2$); 2719 (CH$_2$–N); 1651 (C=O); 1603, 1553, 1514 (C=C, C–C$_{Ar}$, C–C$_{Het}$); 1288, 1269 (=C–OH); 1167, 1134 (C−O); 1005, 866 (C–O–C); 793 (=C–H). NMR ^{1}H (300 MHz, DMSO-d_6, δ, ppm): 3.00–3.60 m (4H,

N(CH$_2$CH$_2$)$_2$O); 3.60–4.11 m (4H, N(CH$_2$CH$_2$)$_2$O); 4.47 s (2H, C-8–CH$_2$); 6.52 s (1H, H-6); 6.92 d (1H, H-5′, J = 8.4 Hz); 7.65 d (1H, H-6′, J = 8.0 Hz); 7.84 d (1H, H-2′, J = 2.0 Hz); 9.25–9.88 m, 10.21 br.s, 11.93 br.s (2H, 1H, 1H, C-3–OH, C-7–OH, C-3′–OH, C-4′–OH); 12.83 br.s (1H, C-5–OH). NMR ^{13}C (75 MHz, DMSO-d_6, δ, ppm): 48.61 (C-8–CH$_2$); 51.32 (N(CH$_2$CH$_2$)$_2$O); 63.10 (N(CH$_2$CH$_2$)$_2$O); 94.57 (C-8); 97.75 (C-6); 103.08 (C-10); 114.95 (C-2′); 115.85 (C-5′); 120.41 (C-6′); 121.82 (C-1′); 136.03 (C-3); 145.26 (C-3′); 147.19 (C-2); 147.87 (C-4′); 155.12 (C-9); 161.68 (C-5); 163.61 (C-7); 175.89 (C-4). HRMS, ESI (*m/z*): 402.1192 [M+H]$^+$; 402.1189 calcd [M+H]$^+$ for $C_{20}H_{20}NO_8$.

2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-8-(thiomorpholinomethyl)-4*H*-chromen-4-one hydrochloride (**5**). Yellow powder, mp > 220 °C. Yield 68%. UV–Vis (EtOH–H$_2$O, 2:1 v/v, λ_{max}, nm): 259, 272 (shoulder), 308 (shoulder), 381. IR (KBr, v, cm^{-1}): 3207 (O–H); 2816, 1435 (CH$_2$); 2816 (CH$_2$–N); 2700 (CH$_2$–S); 1651 (C=O); 1651, 1564, 1514 (C=C, C–C$_{Ar}$, C–C$_{Het}$); 1263 (=C–OH); 1194 (=C–O–C); 1165, 1130 (C−O); 1003 (C–O–C); 819 (=C–H). NMR ^{1}H (300 MHz, DMSO-d_6, δ, ppm): 2.63–3.98 m (8H, N(CH$_2$CH$_2$)$_2$S); 4.46 s (2H, C-8–CH$_2$); 6.51 s (1H, H-6); 6.92 d (1H, H-5′, J = 8.5 Hz); 7.66 d (1H, H-6′, J = 8.3 Hz); 7.82 s (1H, H-2′); 9.25–9.83 m, 10.05 br.s, 11.93 br.s (2H, 1H, 1H, C-3–OH, C-7–OH, C-3′–OH, C-4′–OH); 12.82 br.s (1H, C-5–OH). NMR ^{13}C (75 MHz, DMSO-d_6, δ, ppm): 23.85 (N(CH$_2$CH$_2$)$_2$S); 49.25 (C-8–CH$_2$); 53.25 (N(CH$_2$CH$_2$)$_2$S); 94.68 (C-8); 97.72 (C-6); 103.10 (C-10); 115.02 (C-2′); 115.81 (C-5′); 120.34 (C-6′); 121.84 (C-1′); 136.02 (C-3); 145.24 (C-3′); 147.22 (C-2); 147.84 (C-4′); 155.12 (C-9); 161.69 (C-5); 163.55 (C-7); 175.89 (C-4). HRMS, ESI (*m/z*): 418.0955 [M+H]$^+$; 418.0960 calcd [M+H]$^+$ for $C_{20}H_{20}NO_7S$.

Radical scavenging activity, brains lipids test, and erythrocytes test for antioxidant and membrane-protective activity

Radical scavenging activity (DPPH-test)

The radical scavenging activity (RSA) of compounds was assessed by their ability to interact with 2,2-diphenyl-1-picrylhydrazyl (DPPH). Tested compounds (10 μM) were added into DPPH solution in methanol. The mixture was then shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The decrease in absorption was measured at λ 517 nm. The RSA was calculated as percentage of DPPH discoloration using the equation: RSA, % = $100 \times (A_c - A_s)/A_c$, where A_c is the absorbance of the control reaction (containing all reagents except the test compound), and A_s is the absorbance of the test compound (Sevgi et al. 2015).

Antioxidant activity (brain lipids test)

The antioxidant activity of the compounds was evaluated in vitro as an ability to inhibit the accumulation of secondary lipid peroxidation (LPO) products in the brain lipids of laboratory mice (Lim et al. 2002; Acker et al. 2009; Wu et al. 2011; Kim 2013; Stefanello et al. 2013). The brain was homogenised (10%) in physiological saline (pH 7.4) and centrifuged for 10 min. The low-speed supernatant (S1) containing water, proteins, DNA, RNA, and lipids (cholesterol, galactolipids, individual phospholipids and gangliosides) was separated (Bellé et al. 2004; Acker et al. 2009). The test compounds were added to the supernatant (final concentration 1, 10, and 100 μM). After 30 min, LPO was initiated by the addition of a freshly prepared FeCl$_2$ solution and ascorbic acid (Chawla et al. 2005; Stefanello et al. 2013). Then the samples were incubated while slow stirring for 1 h at 37 °C. The reaction was stopped by adding trichloroacetic acid and 2-thiobarbituric acid. The solution was heated in a boiling water bath for 15 min. After cooling, the precipitate was removed by centrifugation. The concentration of secondary LPO products reacting with 2-thiobarbituric acid (TBA reactive substances, TBA-RS) was determined at λ 532 nm, the extinction coefficient was 1.56×10^5 M^{-1} cm^{-1} (Asakawa et al. 1980; Lim et al. 2002; Kim et al. 2013).

Toxicity, antioxidant activity, membrane-protective activity (erythrocytes test)

The toxicity, antioxidant activity, and membrane-protective activity of compounds were evaluated on 0.5% (v/v) suspension of mice erythrocytes in phosphate buffered saline (PBS, pH 7.4, Sigma-Aldrich). Toxicity was assessed by erythrocytes hemolysis after 1–5 h of incubation with test compounds. Membrane-protective and antioxidant activity were determined by inhibition of H$_2$O$_2$-induced hemolysis, inhibition of lipid peroxidation secondary products accumulation and oxidation of oxyhemoglobin in erythrocytes, and also by the ability of compounds to prevent oxidative heme degradation. After addition of the test compound solutions (at 1- and 10-μM concentration), the suspension of erythrocytes was incubated for 30 min, hemolysis was initiated by addition of hydrogen peroxide solution (1.8 mM). Then the reaction mixture was shaken gently for 5 h at 37 °C while slow stirring. The aliquot was taken from incubation medium each hour and centrifuged for 5 min (1600g). Hemolysis was determined as hemoglobin content in the supernatant at λ 524 nm (Takebayashi et al. 2010). The percentage of hemolysis was calculated relative to complete hemolysis of the sample. The content of TBA-RS was determined by spectrophotometric measurements as indicated above. To assess the accumulation

of hemoglobin oxidation products, the absorption spectrum of hemolysate was analysed in the interval of λ 540–640 nm. The content of oxyhemoglobin (oxyHb) and methemoglobin (metHb) was calculated with corresponding extinction coefficients (van den Berg et al. 1992). To evaluate the concentration of heme degradation products formed during the oxidation of hemoglobin with active forms of oxygen, the fluorescence intensity of the hemolysate was measured at maximum λ 470 nm (excitation at λ 321 nm, emission at λ 400–600 nm) (Nagababu and Rifkind 1998, 2008, 2010).

Results and discussion

Synthesis

C-8 monoaminomethyl derivatives as hydrochlorides (**2–5**) were obtained from quercetin (**1**) and heterocyclic secondary amines. The intermediate products as crude tertiary amines were formed in reaction of flavonoid (**1**) with aqueous formaldehyde and amines in 1,4-dioxane or ethanol. Then the amines were treated with HCl and solvated organic solvents were removed (Fig. 1). The preparative yields of salts (**2–5**) were 33–69%.

Formation of target products was confirmed by spectral data. The peaks of protonated molecular ions of aminomethyl derivatives were observed in the ESI mass spectra. The signals of C-8–CH$_2$ (δ_H 4.4–4.5 ppm, δ_C 45.3–49.3 ppm) fragment and CH$_2$-groups bounded to a nitrogen atom were found in the NMR spectra of the salts (**2–5**), these signals confirmed the presence of aminomethyl substituents. The protons of the quercetin ring A gave only one signal in the ^{1}H NMR spectra of salts (**2–5**) in DMSO-d_6, therefore this ring seems to be monosubstituted; however, the signal of proton H-6 in the spectra of salts (**2–5**) appears to be in a weaker field (δ_H 6.5 ppm) compared with the spectrum of the original quercetin (**1**) (δ_H 6.0–6.5 ppm). In the HSQC spectrum, the signal of proton H-6 in ^{1}H NMR spectrum correlates with the signal of unsubstituted carbon atom C-6 (δ_C 98 ppm) in the ^{13}C

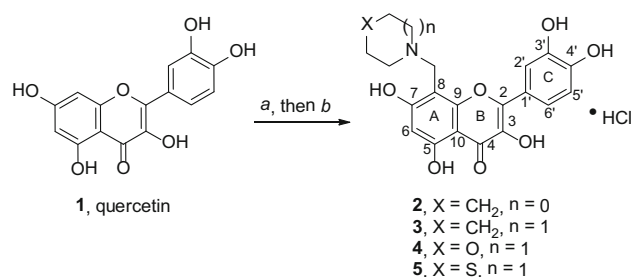**Fig. 1** Synthesis of compounds **2–5**. Conditions: *a* HCHO (aq.), amine, 1,4-dioxane or EtOH; *b* HCl, EtOH

Table 1 Comparative evaluation of RSA and AOA of compounds (1–5) on non-cellular models

Sample[a]	DPPH-test	Brains lipids		
	10 μM RSA/(%)	100 μM	10 μM	1 μM
		TBA-RS concentration/(nmol/mL)[b]		
Control	–	82.0 ± 0.9	88.3 ± 1.6	54.9 ± 0.3
1	91.1 ± 0.4	3.4 ± 0.4	3.5 ± 0.2	12.2 ± 0.4
2	93.7 ± 0.1	3.0 ± 0.2	3.6 ± 0.2	48.2 ± 0.2[c]
3	89.9 ± 0.5	3.0 ± 0.5	3.3 ± 0.2	37.8 ± 0.4[c]
4	74.3 ± 0.8[c]	2.5 ± 0.2[c]	4.9 ± 0.2[c]	12.6 ± 0.2
5	78.9 ± 1.9[c]	2.6 ± 0.1[c]	3.9 ± 0.1	29.2 ± 0.5[c]
Intact	–	25.7 ± 0.2	22.4 ± 0.2	16.7 ± 0.8[c]

[a] Control, sample without investigated compounds; Intact, sample without oxidation initiator

[b] Concentration of secondary LPO products in brain homogenates (1 h incubation, ascorbate/Fe^{2+} as oxidation initiator)

[c] $p < 0.05$

NMR spectrum. The data of the HMBC spectra confirm the substitution at C-8 position as well: interactions $^1H \rightarrow ^{13}C$ were observed between the protons of the C-8–CH_2 fragment and the C-7, C-9 atoms, between the proton H-6 and the atoms C-5, C-7, C-10, between the proton of the C-5–OH group and C-5 and C-6 atoms. All other signals of quercetin remain unchanged in the spectra of all its derivatives. The selected NMR spectra are presented in Fig. S1–S12 (see Electronic supplementary material).

Evaluation of radical scavenging activity, antioxidant and membrane-protective activity of compounds 1–5

The study of RSA of derivatives (2–5) showed that all compounds exhibited high activity at a concentration of 10 μM, but their effect did not exceed the effect of original quercetin (1) (Table 1).

The flavonoids tested at 100 and 10 μM demonstrated a high AOA on the substrate obtained from brain tissue and containing natural lipids. Compounds (4) and (5) are more active than the original quercetin at a concentration of 100 μM. When the concentration was reduced to 1 μM, AOA as under quercetin exposure was retained only for the morpholinomethyl derivative (4) (Table 1).

None of aminomethyl derivatives of quercetin at a concentration of 10 μM demonstrated significant cytotoxicity against erythrocytes: the hemolysis level in the experiment without the initiator of oxidation did not exceed 3% during all incubation period (data not shown). It allowed us to carry out a study of MPA and AOA of these compounds using the model of H_2O_2-induced hemolysis (Table 2).

All flavonoids tested at concentration 10 μM had high MPA, and the activity of morpholinomethyl or thiomorpholinomethyl derivatives (4 and 5) was higher than activity of original quercetin (1) with statistically significant difference. The least active in this system was the compound with piperidinomethyl fragment (3). The high antioxidant activity of all studied compounds in the cellular model system was confirmed by their ability to actively inhibit the oxidation of native hemoglobin and its transformation to the methemoglobin as well as the ability to protect heme from H_2O_2-induced disintegration (see results of fluorescence analysis of heme degradation products).

H_2O_2-induced hemolysis of tested compounds (1–5) at decreased concentration (1 μM) was measured to clarify the differences of their activity. The result was the same as in the previous experiment: compounds (4) and (5) with morpholinomethyl or thiomorpholine methyl group showed statistically higher erythrocyte protective activity against H_2O_2-induced acute oxidative stress comparing with quercetin (1) (Table 3).

The molecular mechanisms of the antioxidant action of flavonoids have yet to be fully elucidated and are still a matter of considerable debate (Suwalsky et al. 2008). As consequence of their polyphenolic structure, these compounds may act as hydrogen donors and are able to suppress free radical processes at three stages: the formation of superoxide ion, the generation of hydroxyl radicals in the Fenton reaction, and the formation of lipid radicals. The protective effects of flavonoids in biological systems are ascribed to their capacity to transfer electrons free radicals, chelate metal catalysts, activate antioxidant enzymes, suppress lipid peroxidation by recycling other antioxidants, such as α-tocopherol and inhibit oxidases (Arora et al. 2000; Heim et al. 2002; López-Revuelta et al. 2006). Another factor that contributes decisively to the effectiveness of certain phenolic compounds as antioxidants is their degree of incorporation, uniformity of distribution, and orientation in the membrane lipid bilayer, and their ability to stabilize membranes by decreasing membrane fluidity (Arora et al. 2000; López-Revuelta et al. 2006; Chen and Deuster 2009). The flavonoids, similar to cholesterol and α-tocopherol, part into the hydrophobic core of the membrane, cause a dramatic decrease in lipid fluidity in this region of the membrane, and could reduce the mobility of free radicals in the lipid bilayer (Arora et al. 2000). Thus, direct interactions of flavonoids with erythrocytes membranes may alter their antioxidative and membrane-protective properties (Chen and Deuster 2009; Hapner et al. 2010). This incorporation is affected by electrostatic interactions, the formation of hydrogen bonds with the polar groups of phospholipids, hydrophobic interactions with fatty acyl chains, and by the molecular geometry of phospholipids. The lipophilicity of flavonoids

Table 2 Comparative evaluation of AOA and MPA of compounds (**1–5**) at concentration 10 μM on erythrocytes under H_2O_2-induced oxidative stress

	Hemolysis/(%)[a]	TBA-RS concentration/(nmol/mL)[b]	metHb/oxyHb/(ratio)[c]	I_{fl}/(rel. units)[d]
Control	51.3 ± 0.1	2.21 ± 0.05	4.46 ± 0.25	9.4 ± 0.2
1	10.5 ± 0.1	1.37 ± 0.09	1.27 ± 0.11	6.1 ± 0.2
2	13.9 ± 0.1	1.56 ± 0.10	1.20 ± 0.12	6.4 ± 0.1
3	19.6 ± 0.1[f]	2.00 ± 0.11[f]	1.30 ± 0.07	7.2 ± 0.3[e]
4	6.6 ± 0.1[e]	1.52 ± 0.14	1.01 ± 0.05	6.4 ± 0.1
5	5.0 ± 0.1[e]	1.26 ± 0.05	1.00 ± 0.06[e]	6.5 ± 0.2
Intact	2.3 ± 0	–	0.11 ± 0	1.9 ± 0.6

[a] The degree of erythrocyte hemolysis is indicated (5 h incubation)

[b] Concentration of secondary LPO products in erythrocytes (4 h incubation)

[c] metHb/oxyHb—the ratio of methemoglobin to oxyhemoglobin in erythrocytes (5 h incubation)

[d] I_{fl}—the fluorescence intensity of heme degradation products in erythrocytes (5 h incubation)

[e] $p < 0.05$

[f] $p < 0.01$

Table 3 Comparative evaluation of AOA and MPA of compounds (**1–5**) at a concentration of 1 μM on erythrocytes under H_2O_2-induced oxidative stress

	Hemolysis/(%)	TBA-RS concentration/(nmol/mL)	metHb/oxyHb/(ratio)
Control	45.3 ± 1.0	2.48 ± 0.14	1.44 ± 0.09
1	43.0 ± 1.5	2.18 ± 0.11	0.81 ± 0.03
2	46.8 ± 1.8	2.14 ± 0.04	1.06 ± 0.10[a]
3	40.2 ± 0.9	2.44 ± 0.09	0.75 ± 0.03
4	37.8 ± 0.7[a]	2.26 ± 0.13	0.72 ± 0.02[a]
5	34.2 ± 0.9[b]	2.12 ± 0.05	0.66 ± 0.03[a]

[a] $p < 0.05$

[b] $p < 0.01$

is an important feature for the biological activity of these compounds, as they become more hydrophilic, their location in the membrane is shifted further towards the aqueous environment (López-Revuelta et al. 2006).

Morpholine derivatives are very essential in the drug discovery process and stimulate research in the broad spectrum of biological activity study, including antioxidative (Naim et al. 2015; Al-Ghorbani et al. 2015). The number of morpholine derivatives (e.g., 2-biphenylyl morpholines) is found to inhibit the ascorbate/Fe^{2+}-induced lipid peroxidation of microsomal membrane lipids (Chrysselis et al. 2000). It was now high popularity of the morpholine moiety caused by several factors. First, the oxygen atom in the morpholine core can participate in the donor–acceptor type interactions with the corresponding receptor, increasing binding affinity. Second, the electronegative effect of the oxygen atom reduces the basicity of the nitrogen atom (Al-Ghorbani et al. 2015). Perhaps the high AOA and MPA of the morpholinomethyl derivative (**4**) and its structural analog with thiomorpholine substituent (**5**) in the cellular system depend on the polarity of these compounds.

Because the multidimensional effects of flavonoids confound the correlation of chemical structure with a particular mechanism, it is not unexpected that some in vitro experiments generate data that are inconsistent with outcomes from simpler assays of aqueous radicals (Heim et al. 2002).

Conclusions

Thus, C-8-aminomethyl derivatives of quercetin with heterocyclic amines were obtained as hydrochlorides using the simple synthetic procedure and for the first time described in detail by the NMR spectroscopy. It was shown that all compounds (**1–5**) at concentrations of 10–100 μM had a high AOA on the model of ascorbate/Fe^{2+}-induced lipid peroxidation in brain homogenate. Quercetin (**1**) and its morpholinomethyl derivative (**4**) showed the highest activity at low concentration. Despite the fact that none of the obtained aminomethyl derivatives exceeded antiradical activity of quercetin in the DPPH-test, the derivatives (**4**) and (**5**) surpassed quercetin in the ability to protect erythrocytes from acute oxidative stress induced by H_2O_2. The difference in activity was statistically significant. We believe that the high MPA of the compounds obtained in the biological model system can be due to the peculiarities

of their interaction with the erythrocyte membrane. The results define the perspectives of further investigation of the pharmacological properties of C-8-aminomethyl derivatives.

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