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Synthesis, structure, antioxidant activity, and water solubility of trolox ion conjugates

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ABSTRACT

The interaction of trolox with ammonia, alkylamines of different classes, and amino derivatives of heterocyclic compounds, including nitroxyl radicals and alkaloids, led to the production of ammonium salts called ion conjugates (ICs). Five ICs were characterised by X-ray diffraction. This is the first time a wide range of ICs were made from trolox with amines, and ESI-MS data demonstrated they have the potential to generate pseudomolecular $[(A^{-}B^{+}) + H]^{+}$ ions. For all obtained trolox ICs, a significant increase (1–3 orders of magnitude) in water solubility was achieved while retaining high antioxidant activity. ICs synthesised from two biologically active fragments may be used to create polyfunctional agents with varying solubility and bioavailability.

1. Introduction

Modification of lead molecules with pronounced biological activity is one of the most efficient ways in synthetic organic and medicinal chemistry to develop novel medicinal agents (Christiaans and Timmerman, 1996; Šebestik et al., 2011). Conjugation of two or more pharmacologically active molecules with different essential properties can be used to synthesise new, biologically active compounds (Šebestik et al., 2011, Hadjipavlou-Litina et al., 2010; Anbharasi et al., 2010). As a rule, hybrid compounds generally have better pharmacological activity when compared to their precursors, and in some cases they demonstrate other types of activities. To illustrate this point, it is common knowledge that many conjugates of various classes of biologically active compounds with nitroxyl radicals have considerable antitumor and antioxidant activity as well as a significant decrease in their general toxicity and an increase in their selective cytotoxicity (Grigor'ev et al., 2014).

Molecules with high antioxidant activity, including substances containing a pharmacophoric chromane core such as α-tocopherol, trolox, dihydroquercetin (taxifolin), rutin, etc., are used today in directed chemical transformations for the synthesis of compounds with high pharmacological potential (Grigor'ev et al., 2014; Nepovimova et al., 2015). For example, hybrid compounds containing trimethyl chromane fragments have been shown to be efficient multifunctional agents with antitumor (Nakagawa-Goto et al., 2007; Arya et al., 1998) anti-inflammatory (Goto et al., 2002), cardioprotective (Koufaki et al., 2003) and neuroprotective properties (Koufaki et al., 2009).

Trolox (6-hydroxy-2,5,7,8-tetra methyl chromane-2-carboxylic acid) **1** is one of the most widely known antioxidants (Scott et al., 1974) and is used as an antioxidant platform for the synthesis of polyfunctional hybrids containing different pharmacologically active fragments covalently bonded to base compounds either directly or through a corresponding linker. Based on trolox, a whole series of hybrid compounds with various types of biological activities was synthesised (Stvolinsky et al., 2010; Koufaki et al., 2004). Trolox conjugated to tacrine seems to be a promising polyfunctional drug with cholinergic, antioxidant, neuroprotective and hepatoprotective properties that can cure Alzheimer's disease.

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It is a strong inhibitor of acetylcholine esterase and butyrylcholinesterase, which demonstrates its antioxidative properties and that it can penetrate through hemo-encephalitic barriers. Its hepatotoxicity is significantly lower than tacrine (Xie et al., 2015). Moreover, notable hybrid systems with NO-emitting fragments were obtained from a trolox base (Lopez et al., 2005). Recently, we have shown that trolox conjugates with nitroxyl radicals have antitumor activity in addition to their antioxidative properties (Zakharova et al., 2016).

Most covalently bonded trolox conjugates are hydrophobic compounds, and that limits the scope of their use in medicine as water soluble drugs. Formation of salts of trolox conjugates is a simple and accessible way to increase the aqueous solubility of the active pharmaceutical ingredient (API). Many drugs are pharmaceutical salts, composed of pharmacologically active amines and pharmaceutically acceptable acids (Serajuddin, 2007; Stahl and Wermuth, 2002).

Data on the biological properties of trolox ion hybrids are extremely limited. Cytotoxicity of the 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (EQ) salt of trolox was less than that of EQ and is used as an antioxidant in various food products (Błaszczyk and Skolimowski, 2007). The 1,4-dihydroxy-2,2,6,6-tetramethylpiperidine salt of trolox is a promising antioxidant and radioprotector (Metodiewa et al., 1996).

Using the Twin Drug Approach (Contreras and Sipp, 2008), we synthesised three types of trolox dimers with different types of binding (covalent-covalent, ionic-covalent, ionic-ionic) in which the monomers were connected through an ethylenediamine linker (Yushkova et al., 2015). A similar approach using ionic liquids was used to prepare pharmacologically active conjugates with different types of binding (Egorova et al., 2015).

The aim of this research was to obtain a series of biologically active, antioxidative trolox ion conjugates (ICs) with different amines and study their water solubility and antioxidative properties.

2. Material and methods

2.1. General techniques

Analytical and spectroscopic studies were performed in the Chemical Service Center for collective use at the Siberian Branch of Russian Academy of Sciences (SB RAS). NMR spectra were recorded on Bruker AV-400 and Bruker Drx-500 spectrometers, operating at 400 and 500 MHz for protons and at 100 and 125 MHz for carbons, respectively. Chemical shifts (ppm) were referenced to solvent peaks: δ_H 3.31 and δ_C 49.1 for CD_3OD, δ_H 2.50 for DMSO-d_6. IR spectra were recorded on a Vector-22 infrared spectrometer. UV-spectra were recorded on a Cary-50 Varian spectrometer. EPR spectra were recorded on a Bruker ESP-300 spectrometer (X-band, microwave power 265 mW, modulation frequency 100 kHz, modulation amplitude 0.01 mT) equipped with a dual-resonator. Elemental analyses were performed in a EURO EA 3000 Elemental Analyzer. Melting points were measured on a Metler Toledo FP 90 Central Processor with a heating rate of 5 °C per minute in the temperature range 50–300 °C with an accuracy of ±0.3 °C. All samples were dried using a vacuum pump Becool 3CFM at ~20 °C. High resolution mass spectra were obtained on an Agilent 1200 liquid chromatograph with a diode-array detector and a Bruker micrOTOF-Q hybrid quadrupole-TOF mass spectrometer with a direct mode of sample introduction. Mass detection was performed by electrospray ionisation mass spectrometry (ESI-MS) at atmospheric pressure. Positive and negative ions were scanned in the range *m/z* 80–3000 amu, with a capillary voltage (V_{cap}) of 2500 V, nebuliser pressure of 1.0 bar, temperature of the dry gas at 140 °C, flow rate of the dry gas at 4 L/min. Samples **2a**–**2q** were dissolved in THF or MeOH for analysis at a concentration of 0.5 mg/mL.

All solvents and chemicals were of analytical grade and were used without further purification. Trolox was from Acros Organics, the amines (**b**–**f**, **j**, **k**, **n**, and **o**) were from ICN Biomedicals and Sigma-Aldrich, the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was from Sigma-Aldrich. The amines (**g**, **h**, and **i**) and the alkaloid (**p**) were obtained from Pilot Plant of Novosibirsk Institute of Organic Chemistry SB RAS, and the alkaloids (**l**, **m**, and **q**) were obtained from the S. Yu. Yunusov Institute of the Chemistry of Plant Substances (ICPS ASRU).

2.2. General procedure for preparation of compounds *2a* and *2b*

For preparation of the compound **2a**, NH_3 (**2b**, CH_3NH_2) in excess was bubbled through a solution of trolox (**1**, 30 mg, 0.12 mmol) in 0.5 mL THF (NH_3/CH_3NH_2 is liberated from a solution of ammonia hydrochloride /methylamine hydrochloride on treatment with sodium hydroxide). After removing the solvent, the raw product was ground in Et_2O and, as a result, a powdery substance was obtained. The precipitate was filtered off, washed with THF, and dried *in vacuo*.

2.3. General procedure for preparation of compounds *2c–2q*

A solution containing the appropriate amine (**c**–**q**, 0.12 mmol) in 0.3 mL THF was added to a solution of trolox (**1**, 30 mg, 0.12 mmol) in 0.5 mL of THF with stirring. After 16–20 h, the reaction mixture was evaporated and the viscous residue was triturated in Et_2O to form a solid powder. The powder was filtered off, washed with EtOAc, and dried *in vacuo*.

2.3.1. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate ammonium (*2a*)

White powder, yield 82%, mp 208.7–210.0 °C. IR (KBr) ν_{max} 1395 and 1557 (COO^-), 1497 (N—H) cm^{-1}; UV (MeOH) λ_{max} (lgε) 292 (3.44); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.44–2.41 (m, 2H, CH_2-4), 2.29–2.24 (m, 1H, CH_2-3), 1.56–1.48 (m, 1H, CH_2-3); 2.03 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H) – methyl groups of the phenolic moiety, 1.36 (s, 3H, CH_3-2). ^{13}C NMR (125 MHz, CD_3OD): δ 179.60 (C9); 146.04, 144.06, (C6, C8a); 122.39, 121.25, 120.01, 116.78 (C5, C7, C8, C4a); 77.61 (C2); 30.45, 20.68 (C3, C4); 23.72 (CH_3-2); 10.97, 10.46, 9.99 – methyl groups of the phenolic moiety; HRESIMS *m/z* (pos): 268.154 $C_{14}H_{22}NO_4$ $[M + H]^+$ (calcd. 268.155).

2.3.2. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate methylammonium (*2b*)

White powder, yield 85%, mp 191.0–191.4 °C. UV (MeOH) λ_{max} (lgε) 293 (3.65); IR (KBr) ν_{max} 1398 and 1634 (COO^-), 1545 (N—H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO d_6): δ 2.44–2.40 (m, 2H, CH_2-4); 2.29–2.24 (m, 1H, CH_2-3); 1.54–1.46 (m, 1H, CH_2-3); 2.03 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H) – methyl groups of the phenolic moiety; 2.25 (s, 3H, $\underline{CH_3}NH_3^+$), 1.35 (s, 3H, CH_3-2); HRESIMS *m/z* (pos): 282.170 $C_{15}H_{24}NO_4$ $[M + H]^+$ (calcd. 282.169).

2.3.3. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate 2-aminoethylanammonium (*2c*)

White powder, yield 99%, mp 155.4–156.5 °C. UV (MeOH) λ_{max} (lgε) 292 (3.60); IR (KBr) ν_{max} 1400 and 1605 (COO^-), 1528 (N—H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO d_6): δ 2.65 (s, 4H, CH_2-groups of ethylenediamine fragment), 2.44–2.40 (m, 2H, CH_2-4), 2.30–2.24 (m, 1H, CH_2-3), 1.54–1.46 (m, 1H, CH_2-3); 2.03 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H) – methyl groups of the phenolic moiety; 1.35 (s, 3H, CH_3-2); HRESIMS *m/z* (pos): 311.196 $C_{16}H_{27}N_2O_4$ $[M + H]^+$ (calcd. 311.197).

*2.3.4. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate 2-methylpropane-2-ammonium (**2d**)*

White powder, yield 95%, mp 199.6–200.0 °C. UV (MeOH) λ_{max} (lgε) 292 (3.57); IR (KBr) ν_{max} 1400 and 1607 (COO^-), 1543 (N—H) cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 2.69–2.55 (m, 2H, CH_2-4), 2.44–2.38 (m, 1H, CH_2-3), 1.78–1.71 (m, 1H, CH_2-3); 2.17 (s, 3H), 2.15 (s, 3H), 2.07 (s, 3H) – methyl groups of the phenolic moiety; 1.52 (s, 3H, CH_3-2), 1.34 (s, 9H, methyl groups of *tert*-butylamine fragment). Anal. Calcd. for $C_{18}H_{29}NO_4$: C, 66.8; H, 9.0; N, 4.3. Found: C, 66.9; H, 9.0; N, 4.3.

*2.3.5. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate 2-hydroxyethylanammonium (**2e**)*

White powder, yield 76%, mp 169.5–171.1 °C. UV (MeOH) λ_{max} (lgε) 292 (3.60); IR (KBr) ν_{max} 1404 and 1570 (COO^-), 1537 (N—H) cm^{-1}; ^1H NMR (400 MHz, DMSO d_6): δ 3.49 (t, 2H, J = 5.3 Hz), 2.73 (t, 2H, J = 5.3 Hz) CH_2-groups of ethanolamine fragment; 2.44–2.41 (m, 2H, CH_2-4), 2.30–2.24 (m, 1H, CH_2-3), 1.56–1.48 (m, 1H, CH_2-3); 2.03 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H) – methyl groups of the phenolic moiety; 1.37 (s, 3H, CH_3-2); HRESIMS m/z (pos): 312.181 $C_{16}H_{26}NO_5$ $[M + H]^+$ (calcd. 312.178).

*2.3.6. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate 1,3-dihydroxy-2-(hydroxymethyl) propan-2-ammonium (**2f**)*

Beige powder, yield 97%, mp 185.2 °C (dec.). UV (MeOHλ_{max} (lgε) 292 (3.46), 270 (3.58); IR (KBr) ν_{max} 1408 and 1604 (COO^-), 1576 (N—H) cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 3.67 (s, 6H, CH_2-groups of Tris fragment), 2.59–2.65 (m, 2H, CH_2-4), 2.44–2.38 (m, 1H, CH_2-3), 1.79–1.72 (m, 1H, CH_2-3); 2.17 (s, 3H), 2.15 (s, 3H), 2.08 (s, 3H) – methyl groups of the phenolic moiety, 1.53 (s, 3H, CH_3-2); HRESIMS m/z (pos): 372.199 $C_{18}H_{30}NO_7$ $[M + H]^+$ (calcd. 372.202).

*2.3.7. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate 1-oxyl-2,2,6,6-tetramethylpiperidine-4-ammonium (**2g**)*

Orange powder, yield 70%, mp 128.1–132.3 °C. UV (MeOH) λ_{max} (lgε) 292 (3.61); IR (KBr) ν_{max} 1398 and 1607 (COO^-), 1543 (N—H) cm^{-1}; EPR/G: a_N = 16.0; HRESIMS m/z (pos): 422.283 $C_{23}H_{38}N_2O_5$ $[M + H]^+$ (calcd. 422.278).

*2.3.8. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate 1-oxyl-2,2,5,5-tetramethylpirrolidin-4-ammonium (**2h**)*

Yellow powder, yield 75%, mp 137.4 °C (dec.). UV (MeOH) λ_{max} (lgε) 292 (3.67); IR (KBr) ν_{max} 1398 and 1618 (COO^-), 1524 (N—H) cm^{-1}; EPR/G: a_N = 14.9; HRESIMS m/z (pos): 408.260 $C_{22}H_{36}N_2O_5$ $[M + H]^+$ (calcd. 408.262).

*2.3.9. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate, 2,2,6,6-tetramethylpiperidine-4-ammonium (**2i**)*

Light yellow powder, yield 84%, mp 119.6–121.2 °C. UV (MeOH) λ_{max} (lgε) 293 (3.64); IR (KBr) ν_{max} 1394 and 1626 (COO^-), 1574 (N—H) cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 3.38–3.30 (m, 1H, H-4′ of piperidine fragment), 2.69–2.55 (m, 2H, CH_2-4); 2.44–2.38 (m, 1H), 1.78–1.71 (m, 1H) CH_2-3; 1.92 (dd, 2H, J_1 = 12.8 Hz, J_2 = 3.7 Hz, CH_2 fragment of piperidine); 2.17 (s, 3H), 2.15 (s, 3H), 2.07 (s, 3H) methyl groups of the phenolic moiety); 1.52 (s, 3H, CH_3-2); 1.34 (s, 6H), 1.31 (s, 6H) – piperidine methyl groups. Anal. Calcd. for $C_{23}H_{38}N_2O_4$: C, 67.9; H, 9.4; N, 6.9. Found C, 67.7; H, 9.3; N, 6.2.

*2.3.10. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate diethylammonium (**2j**)*

White powder, yield 97%, mp 210.5–211.9 °C. UV (MeOH) λ_{max} (lgε) 293 (3.57); IR (KBr) ν_{max} 1393 and 1631 (COO^-), 1558 (N—H) cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 2.92 (q, 4H, J = 7.4 Hz, CH_2-groups of diethylamine fragment), 2.68–2.55 (m, 2H, CH_2-4); 2.45–2.39 (m, 1H), 1.78–1.70 (m, 1H) CH_2-3; 2.17 (s, 3H), 2.15 (s, 3H), 2.07 (s, 3H) methyl groups of the phenolic moiety; 1.53 (s, 3H, CH_3-2); 1.24 (t, 6H, J = 7.4 Hz methyl groups of diethylamine fragment); HRESIMS m/z (pos): 324.214 $C_{18}H_{30}NO_4$ $[M + H]^+$ (calcd. 324.217).

*2.3.11. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate, di(2-hydroxyethyl) ammonium (**2k**)*

White powder, yield 95%, mp 131.8–137.1 °C. UV (MeOH) λ_{max} (lgε) 292 nm (3.51); IR (KBr) ν_{max} 1402 and 1629 (COO^-), 1562 (N—H) cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 3.76–3.73 (m, 4H) and 2.99–2.97 (m, 4H) CH_2-groups of diethanolamine fragment; 2.69–2.56 (m, 2H, CH_2-4); 2.45–2.39 (m, 1H) and 1.78–1.71 (m, 1H) CH_2-3; 2.17 (s, 3H), 2.15 (s, 3H), 2.08 (s, 3H) – methyl groups of the phenolic moiety; 1.53 (s, 3H, CH_3-2); HRESIMS m/z (pos): 356.207 $C_{18}H_{30}NO_6$, $[M + H]^+$ (calcd. 356.207).

*2.3.12. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate, 1,2,3,4,5,6-hexahydro-1,5-methano-8H-pyridinium-[1,2-α][1,5]-diazotsin-8-one (**2l**)*

White powder, yield 78%, mp 150.3 °C (dec.). UV (MeOH) λ_{max} (lgε) 299 (3.98); IR (KBr) ν_{max} 1400 and 1649 (COO^-), 1545 (N—H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.32 (dd, 1H, J_1 = 9.6 Hz, J_2 = 6.9 Hz, H′-10), 6.20 (dd, 1H, J_1 = 9.0 Hz, J_2 = 1.4 Hz, H′-9), 6.05 (dd, 1H, J_1 = 6.9 Hz, J_2 = 1.3 Hz, H′-11), 3.84–3.66 (m, 2H, H′-6), 2.97–2.77 (m, 6H, H′-1, H′-2, H′-4, H′-5), 2.46–2.36 (m, 1H, CH_2-3), 2.29–2.24 (m, 2H, CH_2-4); 2.05 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H) methyl groups of the phenolic moiety, 1.81 (t, 2H, J = 2.9 Hz, H′-7), 1.76–1.66 (m, 1H), 1.47 (s, 3H, CH_3-2); HRESIMS m/z (pos): 463.205 $C_{25}H_{32}N_2O_5Na$ $[M + Na]^+$ (calcd. 463.220).

*2.3.13. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate, 3-(3,4-dimethoxybenzoate)-8-ammoniybicyclo[3.2.1]octane (**2m**)*

White powder, yield 98%, mp 215.2–215.6 °C. UV (MeOH) λ_{max} (lgε) 292 (4.04), 265 (4.16); IR (KBr) ν_{max} 1396 and 1634 (COO^-), 1589 (N—H) cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 7.67 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.0 Hz, H′-6), 7.56 (1H, d, J = 2.0 Hz, H′-2), 7.08 (1H, d, J = 8.5 Hz, H′-5), 3.93 (s, 3H) and 3.91 (s, 3H), 3′-OCH_3, 4′-OCH_3, 2.64–2.60 (m, 2H, CH_2-4); 2.47–2.41 (m, 1H) and 1.78–1.70 (m, 1H) CH_2-3; 2.18 (s, 3H), 2.15 (s, 3H), 2.08 (s, 3H) methyl groups of the phenolic moiety, 1.54 (s, 3H, CH_3-2); HRESIMS m/z (pos): 542.276 $C_{30}H_{40}NO_8$ $[M + H]^+$ (calcd. 542.275).

*2.3.14. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate, triethylammonium (**2n**)*

White powder, yield 96%, mp 58.6 °C (dec.). UV (MeOH) λ_{max} (lgε) 292 (3.57); IR (KBr) ν_{max} 1395 and 1593 (COO^-), 1580 (N—H) cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 3.10 (q, 6H, J = 7.3 Hz, CH_2-groups of triethylamine fragment), 2.63–2.60 (m, 2H, CH_2-4), 2.46–2.40 (m, 1H) and 1.80–1.73 (m, 1H) CH_2-3, 2.16 (s, 3H), 2.15 (s, 3H), 2.07 (s, 3H) – methyl groups of the phenolic moiety, 1.55 (s, 3H, CH_3-2), 1.26 (t, 9H, J = 7.3 Hz, methyl groups of triethylamine frafment); HRESIMS m/z (pos): 352.248 $C_{20}H_{34}NO_4$ $[M + H]^+$ (calcd. 352.248).

*2.3.15. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate, tri(2-hydroxyethyl) ammonium (**2o**)*

White powder, yield 94%, mp 109.6 °C (dec.). UV (MeOH) λ_{max} (lgε) 292 (3.51); IR (KBr) ν_{max} 1402 and 1580 (COO^-), 1560 (N—H) cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 3.80–3.77 (m, 6H) and 3.13–3.11 (m, 6H) CH_2-groups of triethanolamine fragment, 2.64–2.60 (m, 2H) CH_2-4, 2.45–2.39 (m, 1H) and 1.80–1.73 (m, 1H) CH_2-3; 2.17 (s, 3H), 2.15 (s, 3H), 2.08 (s, 3H) methyl groups of the phenolic moiety; 1.54 (3H, s, CH_3-2); HRESIMS m/z (neg): 398.219 $C_{20}H_{32}NO_7$ $[M-H]^-$ (calcd. 398.218).

*2.3.16. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate, 4-(N-acetylantranoiloxy)-8,9-dihydroxy-1α,14α,16β-trimethoxy-N-ethylammonium-18-norakonan (**2p**)*

White powder, yield 95%, mp 175.2 °C (dec.). UV (MeOH) λ_{max} (lgε) 296 (3.96), 254 (4.13); IR (KBr) ν_{max} 1379 and 1589 (COO^-), 1528 (N—H) cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 8.67 (d, 1H, *J* = 8.4 Hz), 7.94 (dd, 1H, J_1 = 8.1 Hz, J_2 = 1.5 Hz), 7.53–7.493 (m, 1H), 7.06–7.03 (m, 1H) hydrogen atoms from benzene ring; 3.41 (s, 3H), 3.33 (s, 3H), 3.30 (s, 3H) OCH_3 groups; 2.24 (s, 3H) – CH_3 group of N-acetylate, 2.17 (s, 3H), 2.16 (s, 3H), 2.08 (s, 3H) methyl groups of the phenolic moiety; 1.62 (s, 3H, CH_3-2); HRESIMS *m*/*z* (neg): 833.430$C_{46}H_{61}N_2O_{12}$ $[M-H]^-$ (calcd. 833.422).

*2.3.17. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylate, 1-(1H-indol-3-yl)-N,N-dimetilmetanammonium (**2q**)*

White powder, yield 67%, mp 155. °C (dec.). UV (MeOH) λ_{max} (lgε) 288 (3.92); IR (KBr) ν_{max} 1400 and 1587 (COO^-), 1547 (N—H) cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 7.68 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1.0 Hz, H′-4), 7.46 (s, 1H, H′-6), 7.44 (dd,1H, J_1 = 8.0 Hz, J_2 0.9 = Hz, H′-1), 7.22–7.12 (m, 2H, H-2, H-3), 4.33 (s, 2H, 9′-CH_2), 2.69 (s, 6H, $N(CH_3)_2$), 2.64–2.60 (m, 2H, CH_2-4), 1.78–1.70 (m, 1H), 2.47–2.41 (1H, m) CH_2-3; 2.18 (s, 3H), 2.15 (s, 3H), 2.08 (s, 3H) methyl groups of the phenolic moiety; 1.54 (s, 3H, CH_3-2); HRESIMS *m*/*z* (pos): 425.242 $C_{25}H_{33}N_2O_4$ $[M+H]^+$ (calcd. 425.243).

*2.4. Structural analysis of **2a**, **2b**, **2c**, **2e**, and **2l** by X-ray diffraction*

X-ray crystal structure analysis was performed on a Bruker Kappa Apex II CCD diffractometer (graphite monochromator, λ (Mo-Kα) = 0.71073 Å, ω,φ-scanning). The absorption was taken into account empirically by the program SADABS (Bruker/Siemens area detector absorption and other Corrections) (Sheldrick and Madison, 2002). The structure was solved by direct methods. The positions of the atoms and the temperature parameters were specified by the anisotropic approximation. The hydrogen atoms of the hydroxyl and ammonium groups in **2a**, **2b**, and **2c** were refined isotropically, while in **2e** and **2 l** they were treated like all other hydrogen atoms and refined in a riding model. All calculations were performed using the programs SHELX-97.

Crystallographic data for structural analysis of the trolox salts has been deposited with the Cambridge Crystallographic Data Centre: CCDC 1055443 (**2a**); CCDC 1055444 (**2b**); CCDC 1055445 (**2c**); CCDC 1055446 (**2e**); CCDC 1055447 (**2l**). These data may be obtained free of charge from the Cambridge Crystallographic Data Center through www.ccdc.cam.ac.uk/data_request/cif.

*2.5. Solubility of the compounds **1**, **2a–q**, **3g**, and **3h***

Solubility of the compounds **1**, **2a–q**, **3 g**, and **3 h** was determined according to the (State pharmacopeia of the Russian Federation, 2008). Synthesis of substances **1**, **2a–q** is described in the Sections 2.2 and 2.3; compounds **3g** and **3h** were synthesized previously (Yushkova et al., 2013). Compounds **1**, **2a–q**, **3g**, and **3h** (2–5 mg) were pounded into powder, then 0.1 mL of distilled water was added, and samples were shaken for 10 min. Water was added in this manner until the substances dissolved completely. The errors were ±15% for the range 0.1–10.0 g/L; ±10% for the range 10.0–100.0 g/L.

2.6. DPPH radical scavenging activity

The free radical scavenging activity of compounds **1**, **2a–q**, **3g**, and **3h** was measured by reduction of the stable free radical DPPH following the method of (Kamkar et al., 2010). Solutions of the samples (0.1 mL, in methanol) in the concentration range 0.05–0.65 mg/ml were added to 4 mL of DPPH solution in methanol (70 μM), and maintained for 10 min at 20–25 °C. Absorbance at 517 nm was measured for the prepared solutions and the DPPH solution (A and A_0, respectively). The antioxidative activity of compounds **1**, **2a–2q**, **3g**, and **3h** was expressed as the concentration required to quench the stable radical DPPH by 50% (IC_{50}, μM). Error was ±10%. (n = 3).

3. Results and discussion

3.1. Synthesis and characterisation of trolox ammonium salts

In order to synthesise the trolox ammonium salts **2a–2l** (Scheme 1), we chose amines from different groups of compounds. Ammonia (**a**) and alkylamines (**b–f**, **j**, **k**, **n**, and **o**) are often used in the production of pharmaceutical salts (Berge et al., 1977), and heterocyclic compounds containing amino groups (**g–i**) are very promising in the design of new types of pharmacologically active compounds. Of special interest are nitroxyl radicals, which are widely used to synthesise spin-labelled, biologically active natural compounds (Grigor'ev et al., 2014). Finally, we used compounds of particular interest that belonged to a group of pharmacologically active plant alkaloids. Compounds in this group such as cytisine (**l**), convolvine (**m**), lappaconitine (**p**), and donaxine (**q**) have psychoactive, bronchiolitis, local anesthetic, antiarrhythmic, vasodilating and other types of pharmacological activities. Today, some of these compounds are used in the production of medicinal agents (Fattorusso and Taglialatela-Scafati, 2008).

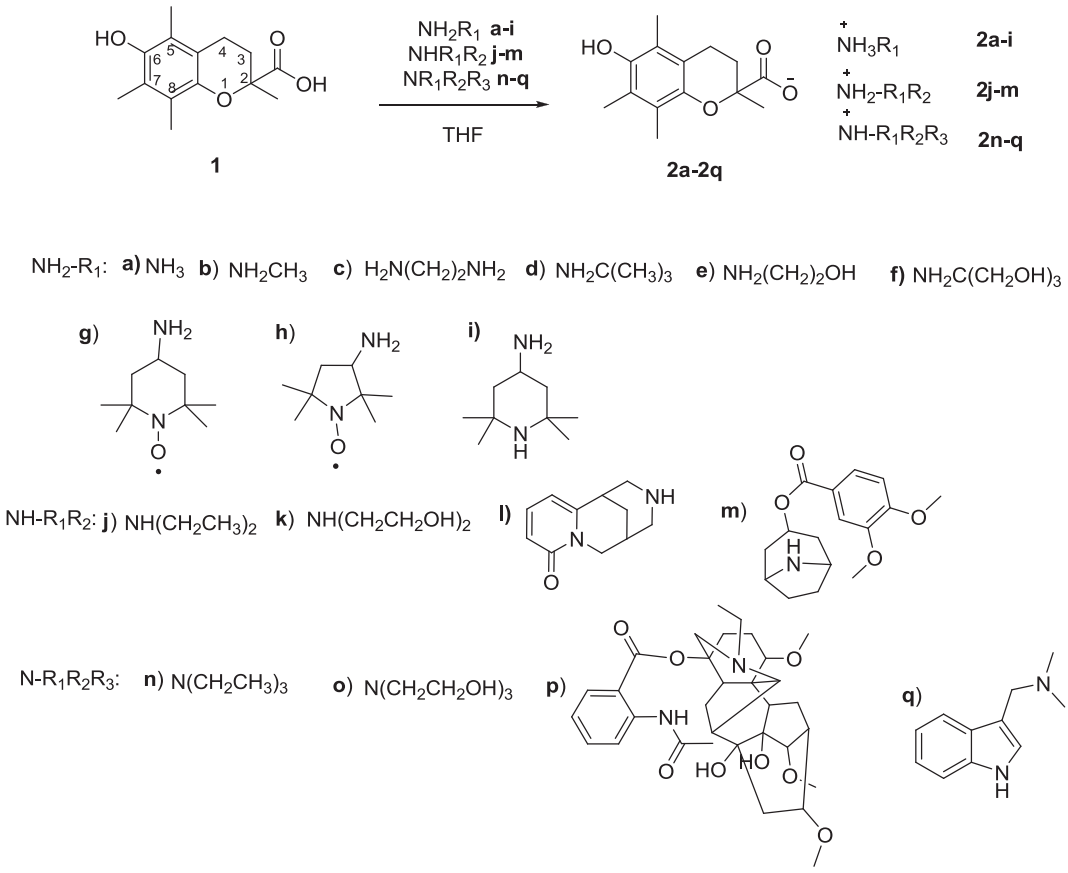
Trolox ICs were produced either by passing gaseous amines (**a**, **b**) through a trolox solution in THF or adding the amine solutions in THF (**c–q**) to trolox solutions in THF at 20–25 °C (Scheme 1).

In IR spectra of the obtained trolox ICs **2a–2q**, the band for stretching vibrations of the trolox carboxyl group at 1711 cm^{-1} was lacking; instead, there were bands for the asymmetric and symmetric stretching vibrations of carboxylate anions at 1557–1649 cm^{-1} and 1379–1408 cm^{-1}, respectively, as well as bending vibrations for the N—H bonds of ammonium groups at 1497–1589 cm^{-1} (Socrales, 1994). These signals confirmed that compounds **2a–2q** have ionic structures. Evidence of the ionic structure was also found in the ^{13}C NMR spectrum of compound **2a**, which had the characteristic downfield shifts of a carboxylate and nodal C-2 carbon atoms at 3.5 ppm and 1.2 ppm, respectively. These peaks are typical for carbon atoms of carboxylic acids when salts are generated (Kalinowski et al., 1998). Thus, the data obtained by IR- and ^{13}C NMR-spectroscopy explicitly demonstrated the formation of an ionic bond between the trolox carboxyl group and the corresponding amine.

In UV spectra of the obtained compounds **2a–2q**, maximums of absorption were observed in the range of 288–299 nm, which is typical for trolox derivatives (λ_{max} = 292 nm) (Gorecki et al., 2014) and verified the existence of a chromane core. In UV spectra of ICs with convolvine **2m** and lappaconitine **2p**, additional aromatic ring absorption bands were observed at 265 and 254 nm, respectively.

The data on molecular masses and molecular formulas of the trolox ICs were obtained by atmospheric pressure ESI-MS and direct infusion.

Solutions of the ICs **2a–2c**, **2e–2h**, **2j**, **2m**, **2n**, and **2q** in THF were analysed in positive ionisation mode and exhibited intense peaks for the pseudomolecular ion $[(A^-B^+)+H]^+$ and less intense peaks for the ICs **2k** and **2l** (where A^- is a deprotonated trolox molecule and B^+ is a protonated amine). As an example, mass spectra of the ICs **2m** and **2f** with intense pseudomolecular ion peaks are shown in Fig. 1a and b. For solutions of the synthesised ICs **2a**, **2b**, and **2f–2h** in methanol, pseudomolecular ions $[(A^-B^+)+H]^+$ were observable.



Scheme 1. Synthesis of trolox ICs **2a**–**2q**.

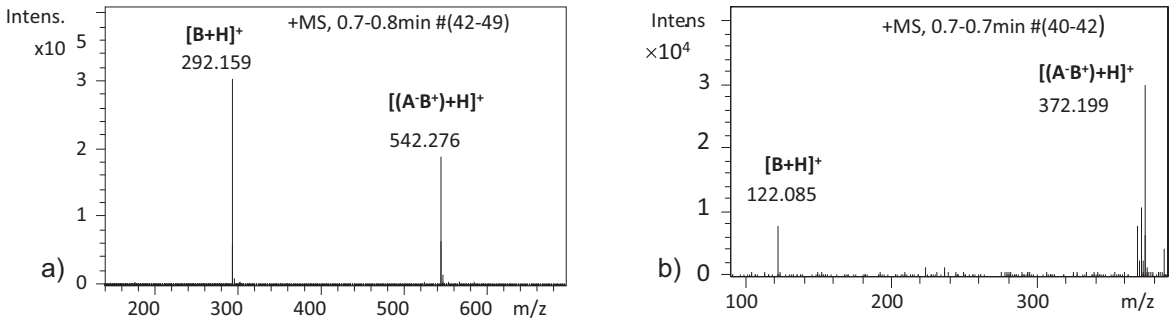


Fig. 1. Positive ion ESI mass spectra of the ICs **2m** (a) and **2f** (b) in THF.

For the ICs **2o** and **2p** in THF, we obtained mass spectra with intense pseudomolecular ion peaks $[(A^-B^+)-H]^-$ in negative ionisation.

It is noteworthy that protic or aprotic solvents (e.g. methanol, water and acetonitrile) were used for ESI-MS (Cech and Enke, 2001). This method was used to study organic ammonium salts, but pseudomolecular ions of the cation type $[(A^-B^+)+H]^+$ have not been observed before now (Kalariya et al., 2014; Wang and Cole, 1996). There are only few examples where pseudomolecular ions of the anion type $[(A^-B^+)-H]^-$ have been observed by ESI-MS analysis, which include the ICs of retinoid amine derivatives with dicarboxylic acids (Um et al., 2004).

The intensity of the pseudomolecular ion peaks for the ICs **2f**, **2g**, **2h**, and **2p** in methanol was considerably lower than in THF. For example, the peak intensity of the protonated conjugate **2f** was higher in THF than in methanol (100 and 56%, respectively). This phenomenon is likely influenced by the nature of the solvent and the degree to which salts are dissociated.

For solutions of the ICs **2d** and **2i** in THF and MeOH, the corresponding pseudomolecular ions $[(A^-B^+)+H]^+$ or $[(A^-B^+)-H]^-$ were not detected. Data for the molecular formulas of the ICs **2d** and **2i** were obtained by elemental analysis.

In ^{1}H NMR spectra of the ICs **2a**–**2f** and **2i**–**2q**, all typical signals for hydrogen atoms of trolox and amine fragments were obtained. Methyl groups in the phenol fragment of trolox were observed in the range of 1.95–2.18 ppm, methyl group in the second position of the pyran ring were observed in the range of 1.35–1.62 ppm, 3-CH_2 groups were found at 1.46–1.80 ppm and 2.24–2.47 ppm, and the 4-CH_2 group was found at 2.24–2.69 ppm. ^{1}H NMR spectra of compounds **2g** and **2h** had extremely broad signals due to the presence of nitroxyl radicals in these molecules. Very often in order to confirm the structure of spin-labelled compounds by high

resolution NMR they are reduced to their corresponding hydroxylamines or amines (Yushkova et al., 2013; Kosheleva et al., 2014). The diamagnetic analogue of the nitroxyl radical **2g** is the secondary amine **2i** whose NMR spectrum corresponded to its structure and, as a consequence, confirmed the structure of the corresponding nitroxyl radical **2g**.

Values of the hyperfine interaction (HFI) constants, a_N, in EPR spectra of the spin-labelled ICs **2g** and **2h** were equal to 16.0 and 14.9 G, respectively, and corresponded to HFI constants for nitroxyl radicals of the piperidine **2g** and pyrrolidine **2h** types (Yushkova et al., 2013).

3.2. X-Ray structure determination

Compounds **2a**, **2b**, **2c**, **2e**, and **2 l** were crystallised and their structures were characterised by X-ray diffraction analysis. Crystal data and refinement results are summarised in Table 1.

The Cambridge Structural Database does not contain information on the structure of the trolox anion with a COO^- group and the structure of a protonated cytisine with an NH_2^+. In the ICs **2a**, **2b**, **2c**, **2e**, and **2 l**, the difference in the C—O bond lengths of the carboxylate anion did not exceed 0.03 Å, which is typical for a deprotonated carboxyl group, whereas, the difference between average C—O and C═O bond lengths in the carboxyl group was approximately 0.13 Å (Allen et al., 1987). Structures of the ICs **2b** and **2l** are illustrated in Fig. 2.

The data obtained confirmed that the dihydropyran cycle in the examined compounds was close to the half-chair form with an axial carboxylate group. In the ICs **2a** and **2b**, the C1 atom deviated from the plane more than atom C2 (0.49, 0.48 and 0.24, 0.20 Å, respectively). By contrast, in the ICs **2c**, **2d**, and **2 l**, deviation of these atoms did not go beyond the range of 0.18–0.35 and 0.40–0.56 Å (0.31–0.32 and 0.40–0.42 Å in trolox polymorphs (Burton et al., 1985)). Another distinction was related to the orientation of the carboxylate group. In the ICs **2c**, **2e**, and **2l** and trolox **1**, polymorphs of the OCCO torsion angle did not exceed 6.1°, while in the ICs **2a** and **2b** it was equal to 22.8 and 26.8°, respectively. Hydrogen bonds are highly likely to be the cause of these differences. In compounds **2a** and **2b**, each oxygen atom of the carboxylate group plays a role as an acceptor of two hydrogen bonds, whereas in **2c**, **2e**, and **2l**, one out of the two carboxylate oxygen atoms forms only one hydrogen bond. The conformations of heterocycles (envelope with a deviation in atom C35 by 0.76 Å and chair) in the cytisine cation of compound **2l** were similar to those in a neutral cytosine (Freer et al., 1987). We note that the C—NH_2^+ bonds to 1.474(4) and 1.488(3) in the cytisine cation (ordered) of **2l** compound were longer than the 1.445–1.461 Å found in the initial cytisine (Barlow and Johnson, 1989).

Numerous O—H···O and N—H···O hydrogen bonds were observed by crystallography, and the crystal **2c** also had N—H···N hydrogen bonds. These hydrogen bonds formed the 3D-architecture of crystals **2a**, **2c**, and **2e** and the 2D-architecture of crystals **2b** and **2l**.

In summary, data from IR-, UV-, NMR- and EPR-spectroscopy, mass-spectrometry, and X-ray and elemental analysis on the obtained compounds all strongly support the ionic structure of the trolox conjugates **2a**–**2q**.

3.3. Water solubility and antioxidant activity of trolox ammonium salts 2a-2q

In publications on trolox, authors often use the term "water-soluble analogue of α-tocopherol" (Wu et al., 1991), which indicates better water solubility than α-tocopherol. According to this classification of drug solubility (Jouyban, 2010), trolox belongs to a group of very slightly soluble compounds (1.0–0.1 g/L), while α-tocopherol is a practically insoluble compound (<0.1 g/L)

Table 1
Crystal data and structure refinement details.

Compound	2a	2b	2c	2e	2 l
Chemical formula	$C_{14}H_{21}NO_4$	$C_{15}H_{23}NO_4$	$C_{16}H_{26}N_2O_4$	$C_{16}H_{25}NO_5$	$C_{25}H_{32}N_2O_{5.25}$
Formula weight [g mol^{-1}]	267.32	281.34	310.39	311.37	444.53
Temperature [K]	296(2)	296(2)	296(2)	200(2)	200(2)
Crystal system	Monoclinic	Triclinic	Monoclinic	Monoclinic	Orthorhombic
Space group	$P2_1/n$	P-1	$P2_1/c$	$P2_1/c$	$P2_12_12_1$
Crystal colour	Colourless	Colourless	Colourless	Colourless	Colourless
a [Å]	6.1753(2)	6.3672(3)	14.187(2)	14.016(3)	13.9403(4)
b [Å]	10.2741(5)	9.8169(5)	25.474(4)	25.527(5)	15.7776(4)
c [Å]	21.6500(10)	12.6802(6)	8.8259(12)	8.7038(15)	20.9671(5)
α [°]	90.00	105.088(2)	90.00	90.00	90.00
β [°]	93.484(2)	99.153(2)	92.745(6)	95.460(7)	90.00
γ [°]	90.00	103.935(2)	90.00	90.00	90.00
Volume [Å^3]	1371.06(10)	721.65(6)	3186.0(8)	3100.0(10)	4611.6(2)
Z	4	2	8	8	8
Dcalc [g cm^{-3}]	1.295	1.295	1.294	1.334	1.281
Absorption coefficient [mm^{-1}]	0.094	0.093	0.093	0.099	0.090
F(0 0 0)	576	304	1344	1344	1904
θ range [°]	1.88–27.23	1.71–27.55	1.44–25.16	1.46–25.40	1.62–26.08
Index ranges	$-7 \le h \le 7$, $-13 \le k \le 13$, $-27 \le l \le 27$	$-8 \le h \le 8$, $-12 \le k \le 12$, $-16 \le l \le 16$	$-16 \le h \le 13$, $-30 \le k \le 30$, $-10 \le l \le 10$	$-16 \le h \le 16$, $-30 \le k \le 30$, $-9 \le l \le 10$	$-17 \le h \le 15$, $-19 \le k \le 19$, $-24 \le l \le 25$
Reflections collected	26837	17850	36693	40384	36621
Independent reflections	3048 [R(int) = 0.0496]	3326 [R(int) = 0.0363]	5645 [R(int) = 0.1337]	5641 [R(int) = 0.0864]	8781 [R(int) = 0.0459]
Goodness-of-fit on F^2	0.952	1.010	0.994	1.111	1.033
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0427, wR_2 = 0.1223	R_1 = 0.0427, wR_2 = 0.1239	R_1 = 0.0965, wR_2 = 0.2362	R_1 = 0.1381, wR_2 = 0.4131	R_1 = 0.0521, wR_2 = 0.1643
R indices (all data)	R_1 = 0.0594, wR_2 = 0.1532	R_1 = 0.0519, wR_2 = 0.1391	R_1 = 0.1765, wR_2 = 0.2829	R_1 = 0.1814, wR_2 = 0.4400	R_1 = 0.0688, wR_2 = 0.1844
Largest diff. peak and hole [eÅ^{-3}]	0.401, −0.347	0.326, −0.209	0.451, −0.349	0.928, −0.908	0.484, −0.237

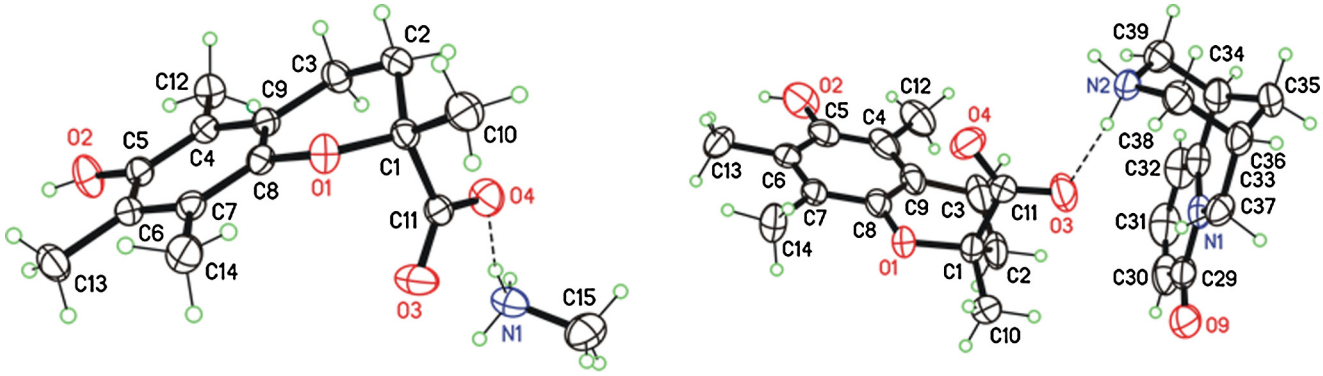


Fig. 2. Structures of the ICs **2b** and **2l** (the disordered cytisine cation is not shown).

(Takagi et al., 2006). Thus, both of these compounds are poorly soluble in water.

The water solubility of the synthesised ICs **2a**–**2q** (Table 2) varied over a wide concentration range (1–330 g/L), which was 1–3 orders of magnitude higher than trolox.

The most soluble ICs were the ethanolamine derivatives **2e**, **2k**, and **2o** and the most water soluble compound was the triethanolamine derivative **2o** (3300-fold increase in solubility). The trolox ICs with nitroxyl radicals, **2g** and **2h**, had a 10 and 20–fold increase in solubility, respectively. Trolox conjugates with alkaloids, **2l**, **2m**, **2p**, and **2q**, exhibited a 20–590-fold increase in solubility relative to trolox. Alkaloids *per se* are highly active pharmacological compounds that are widely used in medicine; however, they are poorly soluble in water and are mainly used in their salt forms (e.g. hydrochlorides or hydrobromides) (Fattorusso and Taglialatela-Scafati, 2008). Increasing the solubility of compounds usually increases their bioavailability (Rath et al., 2013; Newa et al., 2008; Skyner et al., 2015). Solubility is one of the key physicochemical parameters of a new molecule that needs to be assessed and understood very early on in drug discovery and drug candidate selection process. Membrane permeability is another key property in the drug design pipeline, which also needs to be estimated. (Bennion et al., 2017; Kawabata et al., 2011). This will be the subject of further research for the compounds we are studying. Thus the synthesis of water-soluble trolox ion hybrids is a useful approach for the production of novel medicinal agents with synergistic effects relative to their fragment components.

And earlier study (Yushkova et al., 2013) found that the interaction of trolox with piperidine and pyrrolidine nitroxyl radicals resulted in the synthesis of spin-labelled amides of trolox **3g** and **3h** (Fig. 3), which are *per se* covalent hybrid systems. As expected, the covalent conjugates were significantly less water soluble than their ion analogues, **2g** and **2h** (see Table 2). Thus synthesis of the water soluble, spin-labelled ICs **2g** and **2h** may be a new way to create pharmacologically active compounds and imaging agents for magnetic resonance methods.

To estimate the antioxidative activity of the ICs **2a**–**q** and the covalent trolox conjugates **3g** and **3h,** spectrophotometric methods with the stable DPPH radical were applied (Kamkar et al., 2010). Quantitative analysis of hydrogen transfer from the antioxidants to DPPH was an easy, simple, and efficient way to estimate their antioxidative properties.

This technique is based on detecting a decrease in the absorption intensity of the stable DPPH radical at 517 nm in the absence or presence of an antioxidant. The inhibition rate of DPPH (P,%) was calculated according to the formula:

$$P = \frac{A_0 - A}{A_0} \times 100\%$$

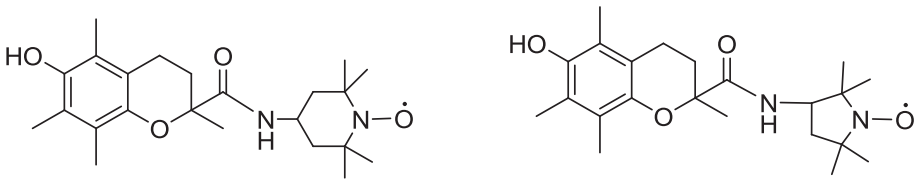
where A_0 and A are the absorbance of DPPH at 517 nm before and after adding the sample of interest.

Antioxidative properties of the synthesised compounds are presented in the form of IC_{50} values (Table 2), which are comparable to similar data published previously (Koufaki et al., 2001). The whole series of ICs **2a**–**2q** and the covalent conjugates **3g** and **3h** preserved their high antioxidant activity, which is typical of trolox compounds modified at position 2 of the chromane core when the phenol group is still present. It should be noted that antioxidant activity is an important characteristic of the biological activity of these compounds. The obtained trolox ICs **2a**–**2q** contain various biologically active fragments that are highly likely to be useful in studies of other types of biological activity.

Table 2
Water solubility and antioxidant activity of compounds **1**, **2a**–**2q**, **3g**, and **3h**.

Entry	Water solubility[a], g/L	DPPH scavenging ([b]IC_{50}, μM)	Entry	Water solubility[a], g/L	DPPH scavenging ([b]IC_{50}, μM)
1	0.1[c]	58	**2j**	46	55
2a	2	62	**2k**	310	68
2b	120	60	**2l**	59	65
2c	35	65	**2m**	2	63
2d	67	63	**2n**	3	63
2e	150	54	**2o**	330	61
2f	78	68	**2p**	7	65
2g	1	76	**2q**	4	57
2h	2	58	**3g**	0.2	63
2i	170	66	**3h**	0.3	69

[a] Water solubility was determined by the gravimetric method (The State Pharmacopoeia of the Russian Federation, 2008).
[b] Concentration at which there was a 50% loss in the initial level of DPPH free radical scavenging.
[c] According to (Arellano et al., 2011), the solubility of trolox in water is <0.2 g/L.



3g 3h

Fig. 3. Structure of covalent trolox conjugates **3g** and **3h**.

4. Conclusions

Interaction between the antioxidant trolox and ammonia, a series of alkylamines, amino derivatives of heterocyclic compounds (including nitroxyl radicals and alkaloids) led to the production of ICs in high yield. The structures of all synthesised compounds were confirmed by MS-spectrometry, IR, UV, EPR, ^{1}H and ^{13}C NMR spectroscopy. The five trolox-amine ICs were characterised by X-ray diffraction. The conditions needed to generate the pseudomolecular ions $[(A^{-}B^{+}) + H]^{+}$ and $[(A^{-}B^{+}) - H]^{-}$ was optimised by atmospheric pressure ESI-MS. A significant increase in water solubility (1–3 orders of magnitude) was observed for all synthesised trolox-amine ICs, and their antioxidative activities remained high. We anticipate that the ICs containing two APIs will have new biological properties. These results support ICs as a promising general approach to create polyfunctional pharmacological salts with a broad range of solubility and bioavailability that could be used for complex therapies and the creation of target drug delivery systems.

The aim of this research was to promote further investigation into the biological activity of this novel group of water soluble hybrid antioxidants. From our point of view, expansion of this approach to other molecules with pronounced biological activity, such as lead molecules and current drugs, will simultaneously carry out two important tasks: to increase water solubility and to synthesise new polyfunctional pharmacological agents. Apart from this, the synthesis of water soluble spin-labelled compounds may also be used to create new types of imaging agents for applications in medicinal magnetic resonance tomography. Given the high potential for this methodology, it deserves significant attention and further development.

Acknowledgements

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Conflict of interest

Authors declare no financial/commercial conflicts of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jsps.2017.10.008.

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