Saudi Pharmaceutical Journal xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

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

Yuliya V. Yushkova [a], Elena I. Chernyak [a,*], Yuriy V. Gatilov [a,b], Vladimir G. Vasil'ev [a], Sergey V. Morozov [a,b], Igor A. Grigor'ev [a,c]

[a] N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry Siberian Branch of Russian Academy of Sciences (NIOCH SB RAS), 9, acad. Lavrent, ev Ave., Novosibirsk 630090, Russian Federation
[b] Novosibirsk State University (NSU), 2, Pirogova Str., Novosibirsk 630090, Russian Federation
[c] Academician E. N. Meshalkin State Research Institute of Circulation Pathology (SRICP), 15, Rechkunovskaya Str., Novosibirsk 630055, Russian Federation

ARTICLE INFO

Article history:
Received 31 March 2017
Accepted 8 October 2017
Available online xxxx

Keywords:
Trolox
Amines
Ion conjugates
Antioxidants
Mass-spectrometry

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.

* Corresponding author.
E-mail addresses: yushkova@nioch.nsc.ru (Y.V. Yushkova), chernyak@nioch.nsc.ru (E.I. Chernyak), gatilov@nioch.nsc.ru (Y.V. Gatilov), vgvasil@nioch.nsc.ru (V.G. Vasil'ev), moroz@nioch.nsc.ru (S.V. Morozov), grig@nioch.nsc.ru (I.A. Grigor'ev).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2017.10.008
1319-0164/

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).

Please cite this article in press as: Yushkova, Y.V., et al. Synthesis, structure, antioxidant activity, and water solubility of trolox ion conjugates. Saudi Pharmaceutical Journal (2017), https://doi.org/10.1016/j.jsps.2017.10.008