Electronic supplementary materials *Mendeleev Commun.*, 2022, **32**, 680–682

Antioxidant activity and redox properties of *cis*-2,4,5-tris(hydroxyaryl)imidazolines

Daniil R. Bazanov, Victoria Yu. Savitskaya, Natalia A. Maximova, Vladimir Yu Tyurin, Yulia A. Gracheva, Natalia B. Chesnokova, Olga V. Beznos, Sergey E. Sosonyuk and Natalia A. Lozinskaya

Synthesis. All solvents were purified by standard procedures. Thin layer chromatography (TLC) was carried out on Merck TLC silica gel plates (60 F254), using UV light for visualization. Flash column chromatography purification was carried out using silica gel 60 (particle size 40-60 mkm). ^{1}H and ^{13}C NMR spectra were recorded at 25 °C on Agilent-400 MR spectrometer with operating frequency of 400 MHz (^{1}H) and 100 MHz (^{13}C). Calibration was performed using DMSO-d_5 (δ_H = 2.50 ppm) and DMSO-d_6 (δ_C = 39.52 ppm). NMR data were presented as follows: chemical shift (δ ppm), multiplicity (s - singlet, d - doublet, dd - doublet of doublet, t - triplet, q - quartet, m - multiplet, br. - broad), coupling constant (J) in Hertz (Hz), integration.

High-resolution mass spectra (HRMS) were recorded on a Thermo Scientific LTQ Orbitrap instrument using nanoelectrospray ionization (nano-ESI). Aromatic aldehydes were provided by Merk. Compounds **4a-k, 5a-b** were prepared according to a previously described procedures[S1].

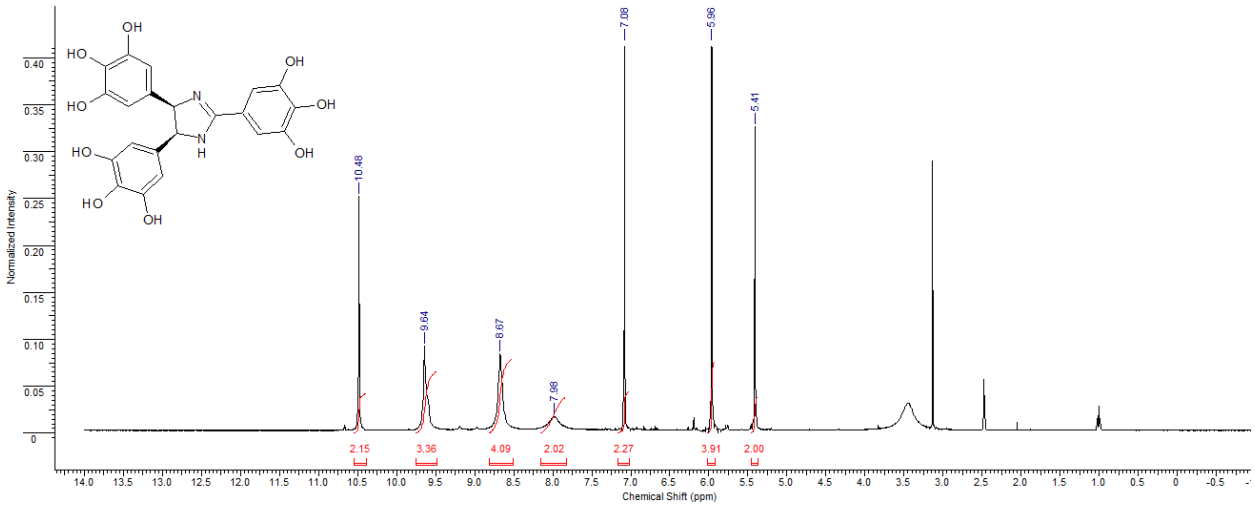
Lead compound, *cis*-2,4,5-tris(3,4,5-trihydroxyphenyl)imidazoline **5c**, was prepared as follows. *cis*-2,4,5-Tris(3,4,5-trihydroxyphenyl)imidazoline **4c** (1.00 g, 1.7 mmol) was dissolved in dry dichloromethane (30 ml), the mixture was cooled with ice bath, and BBr_3 (5.00 ml) was slowly added under vigorous stirring. After 24 h, MeOH (10 ml) was slowly added, and the mixture was evaporated under vacuum to give 0.65 g (1.47 mmol) of dark red solid (87%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.41 (s, 2H, C-H, imidazoline), 5.96 (s, 4H, C-H, Ar), 7.08 (s, 2H, C-H, Ar), 7.98 (br. s, 2H, O-H), 8.67 (br. s, 4H, O-H), 9.64 (br. s, 3H, O-H), 10.48 (br. s, 2H, O-H).

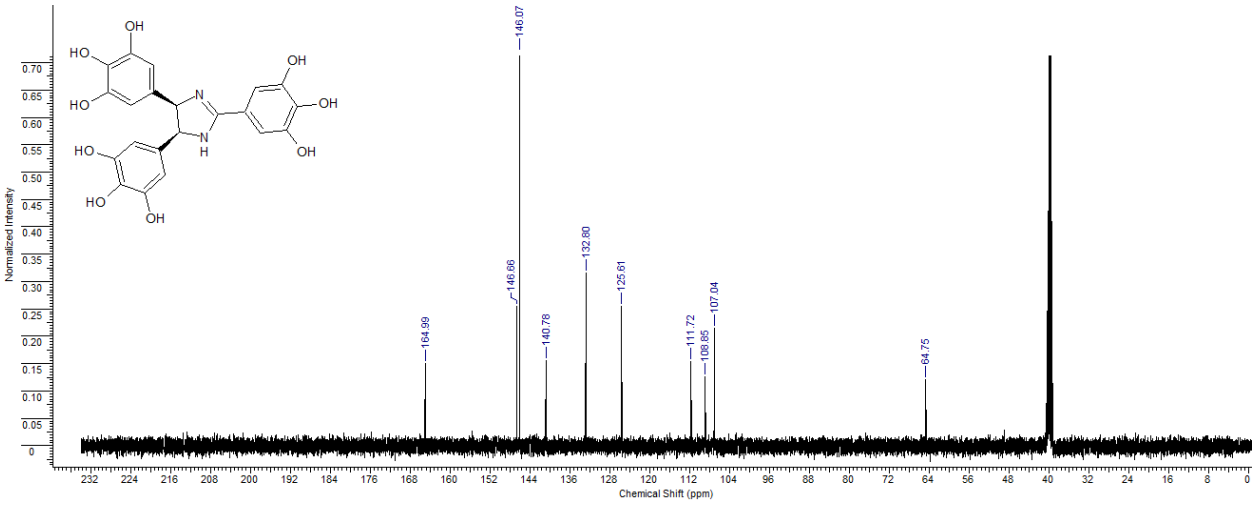
^{13}C NMR (101 MHz, DMSO-d_6) δ 64.75, 107.04, 108.85, 111.72, 125.61, 132.80, 140.78, 146.07, 146.66, 164.99.

HRMS (ESI): m/z calculated for $C_{21}H_{18}N_2O_6$ 442.1012, found 443.1081 $[M+H]^+$.

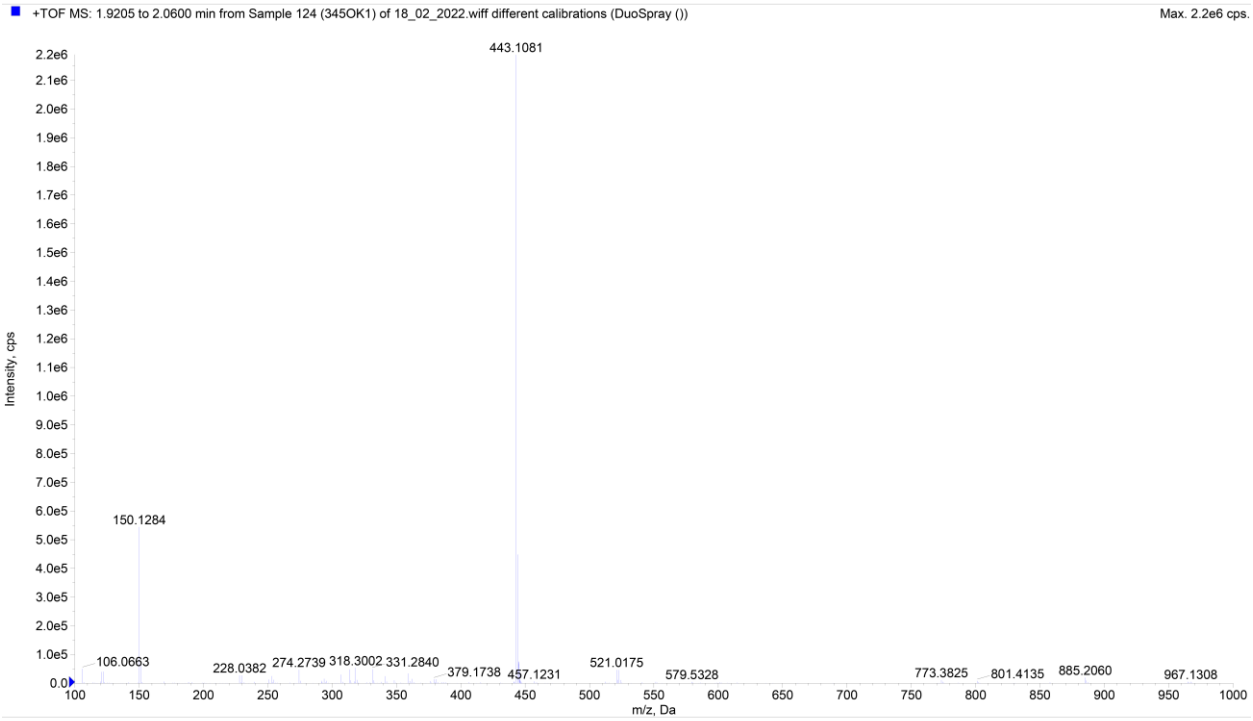
^{1}H NMR *cis*-2,4,5-tris(3,4,5-trihydroxyphenyl)imidazoline (**5c**)



^{13}C NMR *cis*-2,4,5-tris(3,4,5-trihydroxyphenyl)imidazoline (**5c**)



HRMS *cis*-2,4,5-tris(3,4,5-trihydroxyphenyl)imidazoline (**5c**)



Chemiluminescence method (hemoglobin-luminol-H_2O_2 model system). Total antioxidant activity of melatonin analogs was determined via registration of chemiluminescence kinetics in hemoglobin-H_2O_2-luminol model system as described in literature[S2,S3], where luminol worked as oxygen radical detection reagent. Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used for calibration. Briefly, incubation mixture contained luminol (10 μM), hemoglobin (0.5 mM) (all reagents from Sigma-Aldrich, USA) in PBS (0.05 M, pH=7.4), and a sample. The reaction was started by the addition of H_2O_2 (final concentration 58 μM). The chemiluminescence kinetic curve was recorded using chemiluminometer Lum-1200 (DISoft, Russia). The presence of an antioxidant leads to the inhibition of the free radical-caused oxidation of luminol. Hydroxyl radical elimination caused the appearance of a lag period between the H_2O_2 addition and the beginning of luminescence emission. Superoxide radical anion suppression results in the decrease in the maximal luminescence intensity. Thus the antioxidant potential was assayed by means of the lag phase and maximal luminescence intensity at different concentrations. Results for the anti-hydroxyl activity were expressed in equivalent Trolox concentrations calculated on the basis of a Trolox standard curve (μM Trolox), and for the antisuperoxide anion – in percent of free luminol oxidation inhibition.

Spectrophotometric measurements (DPPH test). Tablet spectrophotometer "Zenyth 200rt" (Austria). The reaction proceeded in the wells of the tablet. Measurements were taken at 25°C every 20 seconds for 30 minutes at a wavelength of 517 nm. The initial concentration of DPPH C_0 in all cases was 100 μM. The absorbance of the control solution (DPPH in ethanol) was determined separately for each substance. The values of the optical density of the solution according to the Bouguer-Lambert-Beer law were recalculated in concentration to plot curves in the coordinates C (DPPH)-t (extinction coefficient ε_{517} = 1.16×10^4 dm^3 mol^{-1} cm^{-1}). The values of effective concentrations are determined by the approximation method.

Electrochemical measurements. The electrochemical potentials of compounds **5a-c** (Table 1) were measured in a MICRO-CELL K0264 three-electrode cell using an IPC Pro%M digital potentiostat-galvanostat (Volta, Russia). Working electrode: stationary glassy carbon electrode with a diameter of 2 mm. Oxygen was removed from the cell by purging with dry argon. The potentials were measured relative to a saturated silver chloride electrode. A Pt electrode was used as an auxiliary electrode. Background electrolyte 0.5M Bu_4NBF_4. The concentration of solutions of the studied compounds in acetonitrile was $1*10^{-3}$ mol/L.

Figure S1 Cyclic voltammogram of **5′a** in MeCN (GC electrode, 200 mV s^{-1}, C= 10^{-3} M)

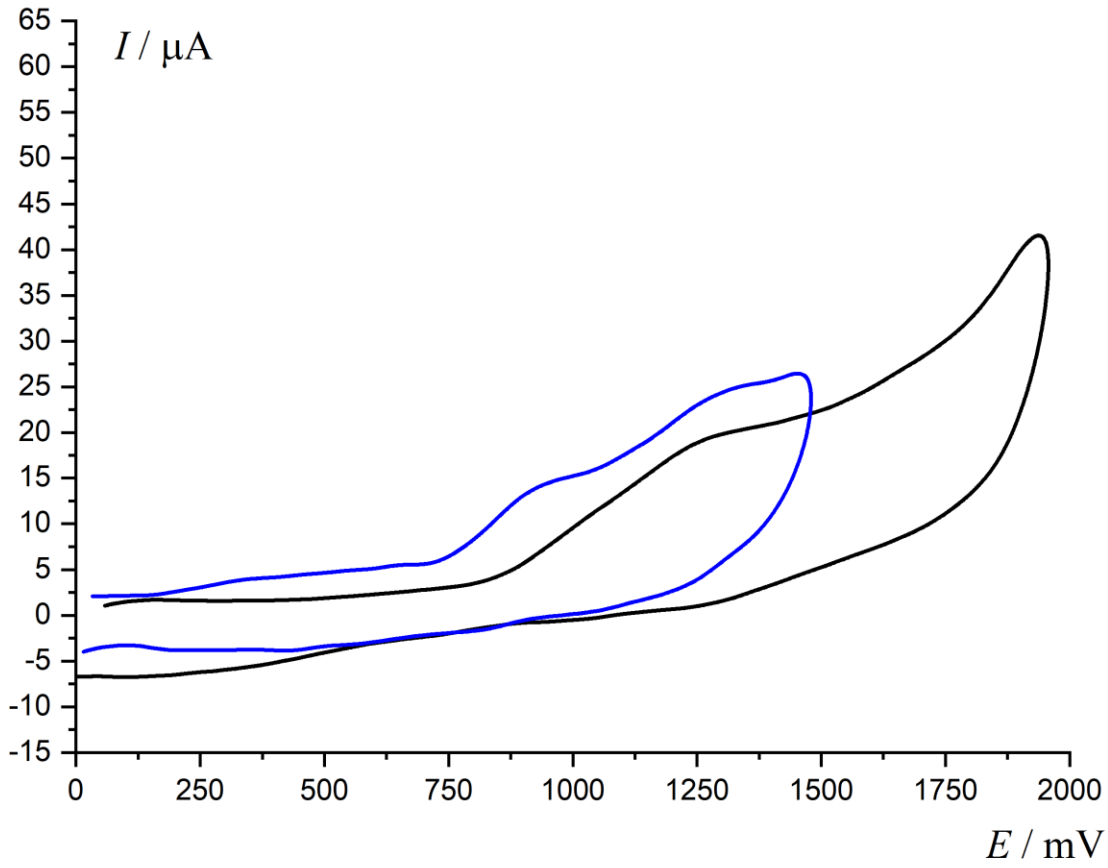
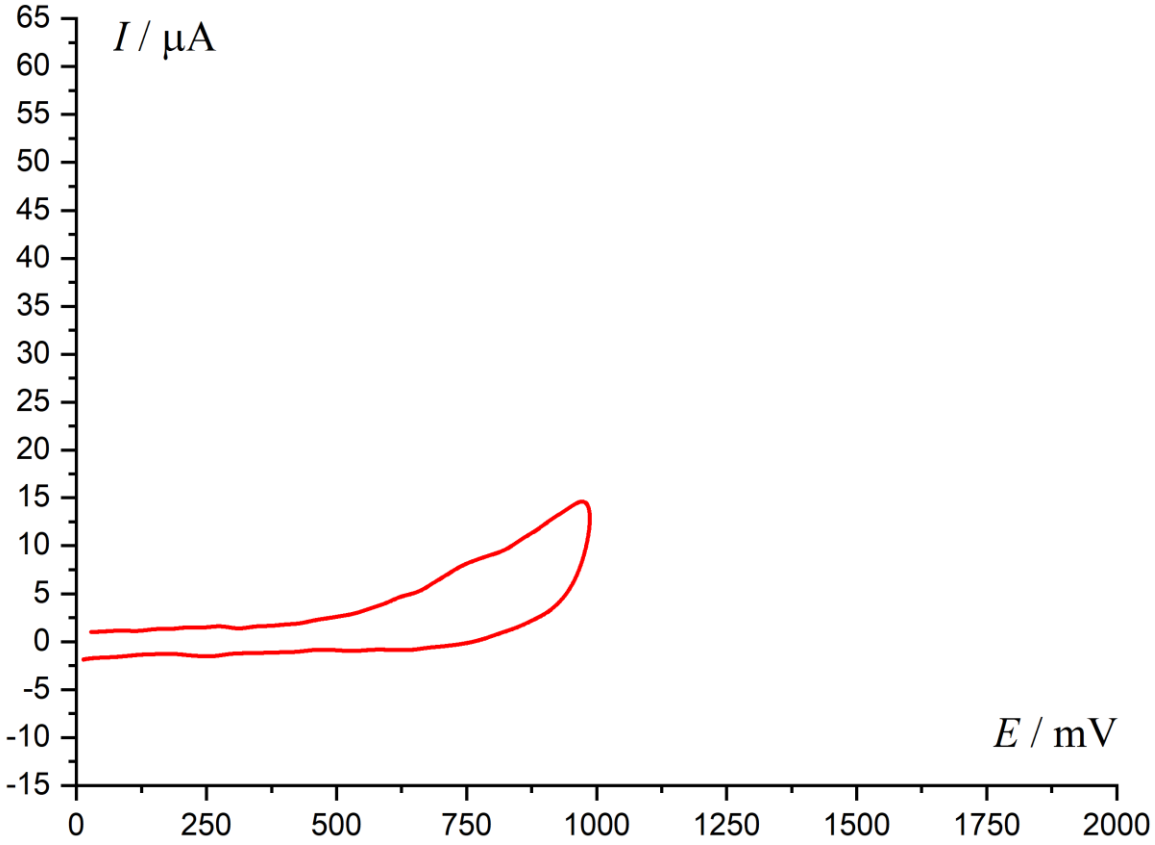


Figure S2 Cyclic voltammogram of **5c** in MeCN (GC electrode, 200 mV s^{-1}, $C = 10^{-3}$ M)



References

S1 D. R. Bazanov, N. V. Pervushin, V. Yu. Savitskaya, L. V. Anikina, M. V. Proskurnina, N. A. Lozinskaya and G. S. Kopeina, *Bioorg. Med. Chem. Lett.*, 2019, **29**, 2364–2368.

S2 O. V. Gulidova, O. B. Lyubitskii, G. I. Klebanov and N. B. Chesnokova, *Bull. Exp. Biol. Med.*, 1999, **128**, 1155–1158.

S3 K. Schlesier, M. Harwat, V. Böhm and R. Bitsch, *Free Radical Res.*, 2002, **36**, 177–187.