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Polycarboxylic fullerene derivatives as protein tyrosine phosphatases inhibitors

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Enzyme inhibition assay

The enzyme preparations of CD45, PTP1B, PTPβ, TC-PTP, SHP2, and compounds for the assays were obtained from Sigma-Aldrich. Before using, the preparations of protein tyrosine phosphatases were diluted in buffer solution that consisted of 50 mM Bis-Tris (Bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane; pH 7.2), 3 mM ethylenediamine tetraacetic acid (EDTA), 2 mM dithiothreitol (DTT), 75 mM sodium chloride, 30% glycerol and 0.05% Tween-20. These solutions were stored at -70°C. The test system for the analysis of the inhibitory activities of compounds **1** - **8** was composed of *p*-nitrophenyl phosphate as enzyme substrate, 50 mM Bis-Tris (pH 7.2), 1 mM DTT, 2 mM EDTA, 100 mM NaCl, and 1% DMSO. The assay mixture was incubated during 5-min at 30 °C in case with CD45, SHP2, TC-PTP, and PTPβ, and 37 °C in case with PTP1B. After that, the reaction was initiated by adding the enzyme. Enzyme activity was assessed spectrophotometrically, measuring the absorbance of *p*-nitrophenol at 410 nm.

Spectral characteristics of the prepared fullerene derivatives

Compound 1 (in the form of the pentacarboxylic acid). ^{1}H NMR (600 MHz, CS_2-acetone-D_6, δ, ppm): 3.45-3.70 (m, 10H); 5.45 (s, 1H); 7.06 (d, 2H); 7.16 (d, 4H); 7.30 (d, 4H); 7.33 (d, 2H), 7.58 (d, 4H); 7.80 (d, 4H). ^{13}C NMR (150 MHz, CS_2-acetone-D_6, δ, ppm): 40.25 (*C*H_2); 40.34 (*C*H_2); 40.39 (*C*H_2); 58.64 (sp^3 cage); 58.82 (sp^3 cage); 60.83 (sp^3 cage); 63.02 (sp^3 cage); 127.74 (Ar); 128.15 (Ar); 128.28 (Ar); 129.85 (Ar); 129.98 (Ar); 130.14 (Ar); 130.24 (Ar); 130.27 (Ar); 134.40; 137.77; 143.27; 144.07; 144.18; 144.25; 144.34; 144.48; 144.79; 145.65; 145.91; 146.11; 146.97; 147.18; 147.28; 147.80; 148.13; 148.32; 148.45; 148.73; 148.79; 151.95; 152.64; 156.61; 170.81 (*COOH*); 171.54 (*COOH*); 171.82 (*COOH*). ESI MS: m/z = 1396.2 ($[M-H]^-$).

Compound 2 (in the form of the pentacarboxylic acid). ^{1}H NMR (600 MHz, CS_2-acetone-D_6, δ, ppm): 2.56 (t, 2H); 2.62 (t, 4H); 2.68 (t, 4H); 2.85 (t, 2H); 2.90 (t, 4H); 2.98 (t, 4H); 5.43 (s, 1H); 7.03 (d, 2H); 7.12 (d, 4H); 7.25 (d, 2H); 7.27 (d, 4H); 7.59 (d, 4H); 7.79 (d, 4H). ^{13}C NMR (150 MHz, CS_2-acetone-D_6, δ, ppm): 30.36 (*C*H_2); 30.43 (*C*H_2); 30.53 (*C*H_2); 35.03 (*C*H_2); 35.11 (*C*H_2); 35.17 (*C*H_2); 58.60 (sp^3 cage); 58.79 (sp^3 cage); 60.81 (sp^3 cage); 63.17 (sp^3 cage); 125.40 (Ar); 127.82 (Ar); 128.18 (Ar); 128.29 (Ar); 128.84 (Ar); 129.00 (Ar); 129.16 (Ar); 137.00; 137.04; 139.96; 140.27; 140.58; 143.24; 143.27; 144.07; 144.13; 144.19; 144.21; 144.25; 144.30; 144.44; 144.84; 145.70; 145.99; 146.13; 146.77; 146.95; 147.15; 147.25; 147.76; 148.11; 148.28; 148.41; 148.69; 148.74; 151.91; 152.80; 156.58; 174.24 (*COOH*); 174.39 (*COOH*); 174.54 (*COOH*). ESI MS: m/z = 1466.3 ($[M-H]^-$).

Compound 3 (in the form of the pentacarboxylic acid). ^{1}H NMR (600 MHz, CS_2-acetone-D_6, δ, ppm): 1.82 (m, 2H); 1.88 (m, 4H); 1.95 (m, 4H); 2.26 (m, 6H); 2.30 (t, 4H); 2.59 (t, 2H); 2.65 (t, 4H); 2.74 (t, 4H); 5.41 (s, 1H); 7.01 (d, 2H); 7.07 (d, 4H); 7.24 (d, 4H); 7.27 (d, 2H); 7.56 (d, 4H); 7.78 (d, 4H). ^{13}C NMR (150 MHz, CS_2-acetone-D_6, δ, ppm): 26.61 (*C*H_2); 26.69 (*C*H_2); 32.59 (*C*H_2); 32.60 (*C*H_2); 32.78 (*C*H_2); 34.56 (*C*H_2); 34.63 (*C*H_2); 58.69 (sp^3 cage); 58.83 (sp^3 cage); 60.85 (sp^3 cage); 63.20 (sp^3 cage); 127.82 (Ar); 128.22 (Ar); 128.35 (Ar); 128.92 (Ar); 129.05 (Ar); 129.27 (Ar); 136.91; 136.95; 141.02; 141.24; 141.47; 143.05; 143.24; 144.08; 144.15; 144.22; 144.26; 144.31; 144.45; 144.86; 145.70; 145.98; 146.18; 146.78; 146.97; 147.17; 147.27; 147.78; 148.11; 148.28; 148.29; 148.43; 148.69; 148.72; 148.75; 151.98; 152.63; 152.76; 156.63; 173.58 (*COOH*); 173.68 (*COOH*); 173.79 (*COOH*). ESI MS: m/z = 1536.5 ($[M-H]^-$).

Compound 4 (mixture of isomers, in the form of the pentacarboxylic acid). ^{1}H NMR (600 MHz, DMSO-D_6, δ, ppm): 2.80-4.20 (m, 10H); 6.70-8.90 (m, 30H). ^{13}C NMR (150 MHz, DMSO-D_6, δ, ppm): 38.4-38.9 (*C*H_2); 58.9 (sp^3 cage); 61.9 (sp^3 cage); 123.0-124.4; 142.7-148.9; 172.2-173.2 (*COOH*). ESI MS: m/z = 1680.5 ($[M-H]^-$).

Compound 5 (in the form of the pentacarboxylic acid). ^{1}H NMR (600 MHz, DMSO-D_6, δ, ppm): 3.55 (s, 2H); 3.61 (s, 8H); 7.27 (d, 2H); 7.35 (m, 8H); 7.44 (d, 2H); 7.55 (d, 4H); 7.59 (d, 4H); 7.62 (d, 4H); 7.70 (d, 4H); 7,84 (d, 4H); 8.12 (d, 4H). ^{13}C NMR (150 MHz, DMSO-D_6, δ, ppm): 40.37 (*C*H_2); 40.44 (*C*H_2); 58.05 (sp^3 cage); 60.73 (sp^3 cage); 63.38 (sp^3 cage); 76.49 (*C*-Cl); 126.62; 126.97; 127.08; 127.54; 127.59; 129.11; 129.24; 129.95; 129.99; 130.06; 130.75; 134.24; 134.33; 134.38; 135.95; 137.35; 138.47; 138.56; 138.63; 139.93; 140.62; 140.70; 142.42; 143.15; 143.62; 143.73; 143.94; 144.20; 144.42; 144.53; 144.61; 144.79; 145.51;

145.59; 146.94; 147.41; 147.54; 148.02; 148.28; 148.43; 148.63; 148.79; 148.83; 148.94; 150.61; 151.35; 153.96; 156.92; 171.42 (*COOH*); 171.49 (*COOH*); 171.53 (*COOH*). ESI MS: m/z = 1776.5 ($[M-H]^-$).

Compound 6 (in the form of the pentacarboxylic acid). ^{1}H NMR (600 MHz, acetone-D_6, δ, ppm): 4.55 (s, 2H); 4.67 (s, 4H); 4.68 (s, 4H); 6.67 (d, 2H); 6.90 (d, 4H); 6.94 (d, 4H); 7.21 (d, 2H); 7.61 (d, 4H); 7.86 (d, 4H). ^{13}C NMR (150 MHz, acetone-D_6, δ, ppm): 57.47 (sp^3 cage); 60.15 (sp^3 cage); 62.86 (sp^3 cage); 64.40 (*C*H_2); 64.51 (*C*H_2); 64.55 (*C*H_2); 76.97 (*C*-Cl); 114.00 (Ar); 114.83 (Ar); 115.06 (Ar); 115.12 (Ar); 128.51; 129.63; 129.81; 129.93; 130.11; 130.67; 131.27; 131.37; 136.87; 143.03; 143.37; 143.42; 143.65; 143.82; 143.88; 144.30; 144.41; 144.54; 144.67; 145.10; 145.36; 146.77; 147.18; 147.31; 147.40; 147.77; 148.36; 148.49; 148.69; 148.83; 148.91; 150.52; 151.30; 153.79; 156.79; 157.17; 157.76; 169.30 (*COOH*); 169.42 (*COOH*); 169.51 (*COOH*). ESI MS: m/z = 1476.4 ($[M-H]^-$).

Compound 7 (in the form of the pentacarboxylic acid). ^{1}H NMR (600 MHz, CS_2-acetone-D_6, δ, ppm): 2.69 (t, 2H), 2.78 (m, 8H), 4.15 (t, 2H), 4.27 (m, 8H), 6.68 (d, 2H), 6.90 (d, 4H), 6.95 (d, 4H), 7.18 (d, 2H), 7.58 (d, 4H), 7.86 (d, 4H). ^{13}C NMR (150 MHz, CS_2-acetone-D_6, δ, ppm): 33.89 (*C*H_2); 33.92 (*C*H_2); 33.97 (*C*H_2); 57.56 (sp^3 cage); 60.26 (sp^3 cage); 62.96 (sp^3 cage); 63.65 (O*C*H_2); 63.77 (O*C*H_2); 63.86 (O*C*H_2); 76.97 (*C*-Cl); 113.85 (Ar); 114.91 (Ar); 114.97 (Ar); 128.94; 129.72; 129.85; 130.48; 131.36; 136.12; 142.95; 143.65; 143.83; 143.94; 144.24; 144.29; 144.34; 144.46; 144.60; 145.47; 145.61; 147.10; 147.31; 147.34; 147.47; 147.90; 148.17; 148.32; 148.50; 148.65; 148.72; 148.81; 150.83; 151.49; 153.72; 157.06; 158.02; 158.59; 171.09 (*COOH*); 171.21 (*COOH*); 171.31 (*COOH*). ESI MS: m/z = 1546.3 ($[M-H]^-$).

Compound 8 (in the form of the pentacarboxylic acid). ^{1}H NMR (600 MHz, CS_2-acetone-D_6, δ, ppm): 2.00 (m, 2H), 2.09 (m, 8H), 2.45 (t, 2H), 2.53 (m, 8H), 3.96 (t, 2H), 4.08 (m, 8H), 6.70 (d, 2H), 6.92 (d, 4H), 6.96 (d, 4H), 7.20 (d, 2H), 7.61 (d, 4H), 7.88 (d, 4H). ^{13}C NMR (150 MHz, CS_2-acetone-D_6, δ, ppm): 24.61 (*C*H_2); 24.64 (*C*H_2); 24.69 (*C*H_2); 29.85 (*C*H_2); 29.88 (*C*H_2); 57.55 (sp^3 cage); 60.27 (sp^3 cage); 62.96 (sp^3 cage); 66.76 (*C*H_2); 66.85 (*C*H_2); 66.93 (*C*H_2); 77.04 (*C*-Cl); 113.79 (Ar); 114.68 (Ar); 114.87 (Ar); 114.92 (Ar); 115.01 (Ar); 128.82; 129.20; 129.30; 129.66; 129.81; 130.35; 131.33; 136.03; 142.88; 143.59; 143.77; 143.83; 143.89; 144.17; 144.21; 144.28; 144.42; 144.52; 145.43; 145.57; 147.10; 147.23; 147.26; 147.39; 147.81; 148.09; 148.26; 148.42; 148.56; 148.64; 148.66; 148.73; 150.81; 151.53; 153.81; 157.03; 158.25; 158.79; 158.81; 173.55 (*COOH*); 173.68 (*COOH*); 173.85 (*COOH*). ESI MS: m/z = 1616.1 ($[M-H]^-$).