**Supporting information**

# Effects of sponge-derived polybrominated diphenyl ethers on human cancer cell α-N-acetylgalactosaminidase and bacterial α-D-galactosidase and their antioxidant activity

Natalia K. Utkina,* Svetlana P. Ermakova, and Irina Y. Bakunina*

G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, Vladivostok 690022, Russian Federation

* To whom correspondence should be addressed Tel: 7 (423) 231-11-68. Fax: 7 (423) 231-40-50. E-mail: utkinan@mail.ru; E-mail: bakun@list.ru

**Contents**

**Table S1.** Physical properties for compounds **3-5**

**Table S2.** Physical properties for compounds **6** and **7**

**Figure S1.** Dose-response curves of α-PsGal inhibition with compounds **1-3**

**Table S3**. The α-PsGal activity after PBDEs **1** and **2** treatment

**Figure S2.** Effects of compounds **1-7** on cancer cell α-NaGalase

**Table S1.** Physical properties for compounds **3-5** ($^{1}$H NMR, 300 MHz; $^{13}$C NMR, 75 MHz)

| |
|---|
| Compound **3:**<br>2-(3',5'-dibromo-2'-hydroxyphenoxy)-3,4,5,6-tetrabromophenol:<br>colorless needles (acetone); mp 194-196 °C;<br>HREI MS, *m/z:* 675.5204 (calcd for $C_{12}H_4O_3{}^{79}Br_3{}^{81}Br_3$, 675.5198);<br>$^{1}$H NMR ($CDCl_3$), $\delta_H$: 7.45 (1H, *d*, *J* = 2.0 Hz), 7.45 (1H, *d*, *J* = 2.0 Hz), 7.18 (1H, *d*, *J* = 2.0 Hz), 6.77 (1H, d, J = 2.0 Hz), 4.03 (3H, s), 6.65 (1H, brs, OH);<br>$^{13}$C NMR (DMSO-$d_6$), $\delta_C$: 151.5 (C), 150.8 (C), 144.8 (C), 137.7 (C), 127.8 (CH), 125.0 (CH), 119.6 (CH), 118.4 (C), 118.1 (C), 117.5 (C), 116.3 (CH), 115.5 (C), 60.4 (C). |
| Compound **4:**<br>2-(3',5'-dibromo-2'-methoxyphenoxy)-3,5-dibromophenol:<br>colorless needles (hexane); mp 143-145 °C;<br>HREI MS, *m/z:* 531.7178 (calcd for $C_{13}H_8O_3{}^{79}Br_2{}^{81}Br_2$,531.7165);<br>$^{1}$H NMR ($CDCl_3$), $\delta_H$: 7.45 (1H, *d*, *J* = 2.0 Hz), 7.45 (1H, *d*, *J* = 2.0 Hz), 7.18 (1H, *d*, *J* = 2.0 Hz), 6.77 (1H, *d*, *J* = 2.0 Hz), 4.03 (3H, *s*), 6.65 (1H, brs, OH);<br>$^{13}$C NMR (DMSO-$d_6$), $\delta_C$: 151.5 (C), 150.8 (C), 144.8 (C), 137.7 (C), 127.8 (CH), 125.0 (CH), 119.6 (CH), 118.4 (C), 118.1 (C), 117.5 (C), 116.3 (CH), 115.5 (C), 60.4 (C). |
| Compound **5**: 2-(2',4'-dibromophenoxy)-3,5-dibromophenol:<br>colorless needles ($CHCl_3$); mp 169-171°C;<br>HREI MS, *m/z:* 501.7069 (calcd for $C_{12}H_6O_2{}^{79}Br_2{}^{81}Br_2$, 501.7059);<br>$^{1}$H NMR (DMSO-$d_6$), $\delta_H$: 7.29 (1H, *d*, *J* = 2.4 Hz), 6.42 (1H, *d*, *J* = 2.4 Hz), 10.91 (1H, brs, OH);<br>$^{13}$C NMR (DMSO-$d_6$), $\delta_C$: 152.3 (C), 139.4 (C), 148.8 (C), 135.1 (CH), 131.7 (CH), 121.6 (C), 125.5 (C), 120.4 (CH), 117.4 (C), 115.9 (CH), 114.0 (C), 111.8 (C). |

**Table S2.** Physical properties for compounds **6** and **7** ($^{1}$H NMR, 300 MHz; $^{13}$C NMR, 75 MHz)

| |
|---|
| Compound **6:**<br>2-(2',4'-dibromophenoxy)-3,4,5-tribromophenol:<br>colorless needles (hexane); mp 198-199 °C;<br>HREI MS, *m/z:* 579.6171, 581.6155 (calcd for $C_{12}H_5O_2{}^{79}Br_3{}^{81}Br_2$, 579.6164; for $C_{12}H_5O_2{}^{79}Br_2{}^{81}Br_3$, 581.6144);<br>$^{1}$H NMR (DMSO-$d_6$), $\delta_H$: 7.90 (1H, *d*, *J* = 2.4 Hz), 7.45 (1H, *s*), 7.40 (1H, *dd*, *J* = 8.8 Hz, *J* = 2.4), 6.51 (1H, *d*, *J* = 8.8 Hz), 6.46 (1H, *d*, *J* = 9.0 Hz), 10.96 (1H, brs, OH);<br>$^{13}$C NMR (DMSO-$d_6$), $\delta_C$: 152.3 (C), 150.8 (C), 139.4 (C), 135.1 (CH), 131.7 (CH), 121.6 (C), 121.6 (C), 120.5 (CH), 116.0 (C), 115.9 (CH), 114.0 (C), 111.8 (C). |
| Compound **7:**<br>2-(2',4'-dibromophenoxy)-3,4,5,6-tetrabromophenol:<br>colorless needles (hexane); mp 151-153 °C;<br>HREI MS, *m/z:* 659.5263 (calcd for $C_{12}H_4O_2{}^{79}Br_3{}^{81}Br_3$,659.5249);<br>$^{1}$H NMR (DMSO-$d_6$), $\delta_H$: 7.79 (1H, *d*, *J* = 2.4 Hz), 7.29 (1H, *dd*, *J* = 8.8 Hz, *J* = 2.4), 6.42 (1H, *d*, *J* = 8.8 Hz), 10.94 (1H, brs, OH);<br>$^{13}$C NMR (DMSO-$d_6$), $\delta_C$: 152.0 (C), 148.8 (C), 139.8 (C), 135.0 (CH), 131.6 (CH), 125.2 (C), 119.8 (C), 117.0 2 (C), 116.1 (CH), 115.8 (CH), 114.3 (C), 112.1 (C). |

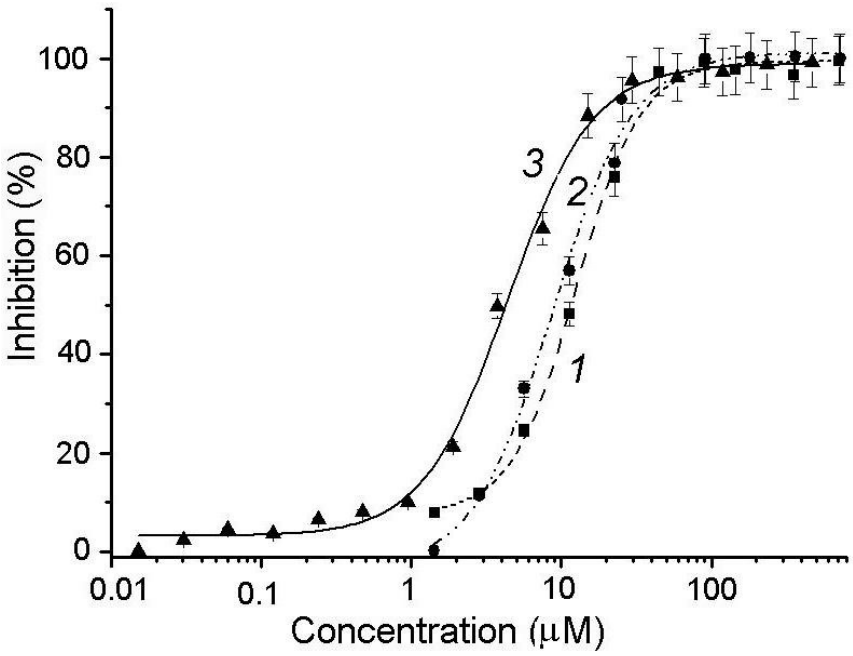


**Figure S1.** Dose-response curves of α-PsGal inhibition after preincubation with compound **1** (1), compound **2** (2), and compound **3** (3) for 30 min followed by addition of substrate and 10 min reaction with the enzyme and substrate *p*NP-α-Gal (1 mg $mL^{-1}$ 15 μL (3.3 mM) 0.05 M sodium phosphate buffer (pH 7.3)) at 20°C. Inhibition (%) are plotted against concentration of compounds on a logarithmic scale. The $IC_{50}$ value of compound **3** is 4.48±0.24 μM.

**Table S3**. The α-PsGal activity after PBDEs **1** and **2** treatment.

| **Inhibitor** (Stock concentration - 0.645 mM) | Volume of inhibitor solution (μL) or EtOH added to 75 μL of the enzyme | Standard activity (μM/min/mL) | |
|---|---|---|---|
| | | Before dialysis | After dialysis |
| **Compound 1** | 15 | 0 | 0 |
| **Compound 2** | 15 | 0 | 0 |
| Ethanol | 15 | 0.032± 0.003 | 0.033 ± 0.001 |

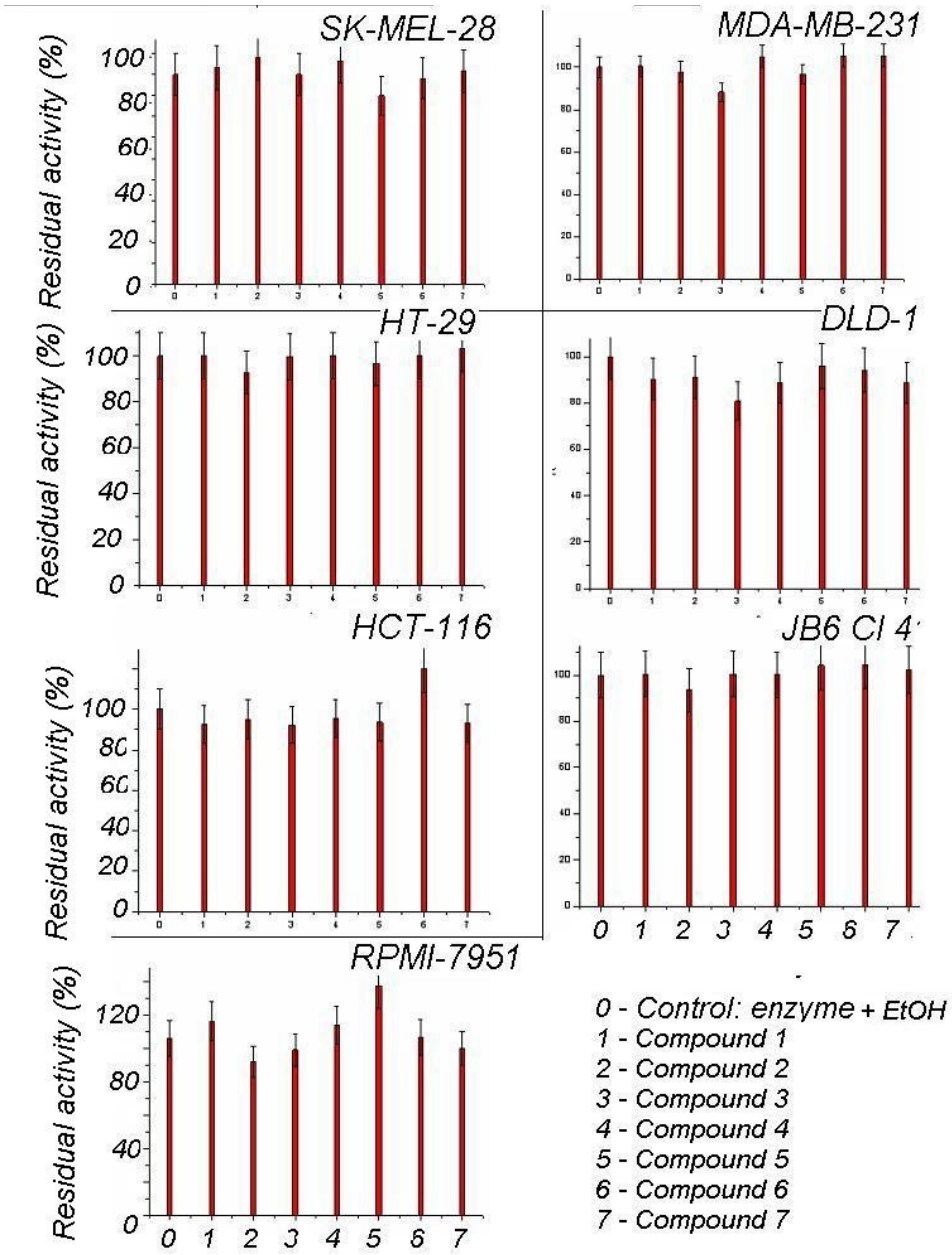


**Figure S2**. Effects of compounds **1**-**7** on cancer cell α-NaGalase of the lines RPMI-7951 (ATCC#no. HTB-66™), MDA-MB-231 (ATCC#no.HTB-26™), DLD-1 (ATCC#no.CCL-221), HT-29 (ATCC#no.HTB-38™), HCT-116 (ATCC #no.CCL-247), SK-MEL-28 (ATCC #no. HTB-72™) and mouse healthy epidermal cells JB6 Cl 41 (ATCC #no. CRL-2010™). Standard activities of enzyme preparations used in the experiment were 546, 778, 808, 837, 616, 132, 477 units, respectively. One unit of the standard activity was determined as the amount of the α-NaGalase that releases 1 nmol of pNP per 1 hour at 37 °C.