Supporting Information

Binding and Activity of Polybrominated Diphenyl Ethers Sulfates to Thyroid Hormone Transport Proteins and Nuclear Receptors

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1. OH-PBDEs and PBDE sulfates synthesis and characterization

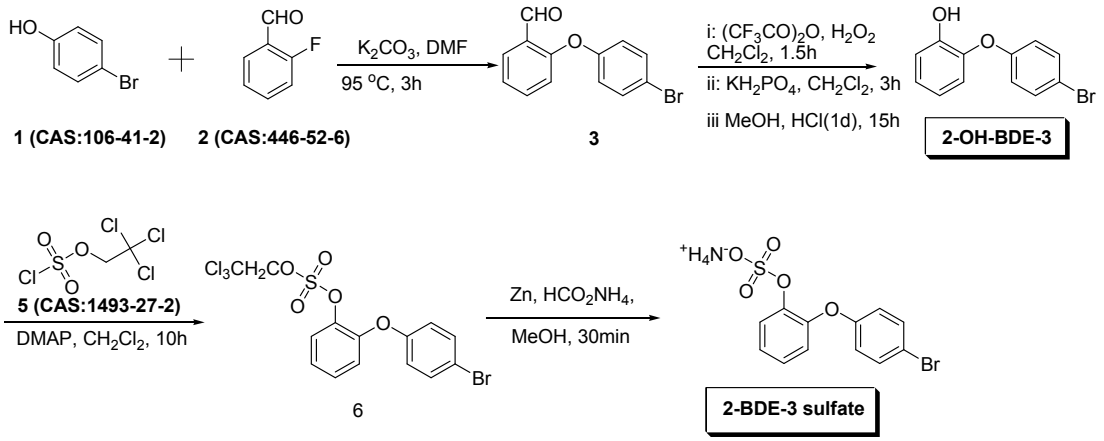
Four OH-PBDEs including 2'-hydroxy-4-monobromodiphenyl ether (2'-OH-BDE 3), 2'-hydroxy-2,4-dibromodiphenyl ether (2'-OH-BDE 7), 3'-hydroxy-2,4-dibromodiphenyl ether (3'-OH-BDE 7), and 2'-hydroxy-2,4,4'-tribromodiphenyl ether (2'-OH-BDE 28) were synthesized by Kabin Biotechnology Co. Ltd. (Beijing, China) based on the method reported previously (Marsh et al., 2003). The ammonium salts of four PBDE sulfates including 2'-sulfooxy-4-monobromodiphenyl ether (2'-BDE 3 sulfate), 2'-sulfooxy-2,4-dibromodiphenyl ether (2'-BDE 7 sulfate), 3'-sulfooxy-2,4-dibromodiphenyl ether (3'-BDE 7 sulfate), and 2'-sulfooxy-2,4,4'-tribromodiphenyl ether (2'-BDE 28 sulfate) were also synthesized by Kabin Biotechnology Co. Ltd. The structures of the tested chemicals are listed in Figure S1. The details of the synthesis process of the chemicals are showed in the paragraphs 2-5. The characterization results of OH-PBDEs and PBDE sulfates are shown in Figure S1-S25. The OH-PBDEs and PBDE sulfates were stored at -80 ℃ until to be used. The OH-PBDEs and PBDE sulfates were dissolved in dimethyl sulfoxide (DMSO) to give 50 mM stock solutions.

We analyzed the purity of produces by high-performance liquid chromatography (HPLC, Agilent Technologies, Palo Alto, California, USA) equipped with a XBridge C18 S-3.5 μm column (Waters, Milford, MA, USA). The mobile phase condition used was as follows: H_2O (0.05% TFA)/CH_3CN (0.05% TFA) in the linear gradient from 90/10 to 0/100 at a flow rate of 1 mL/min. The elution composition was detected at 254 nm. The products of OH-PBDEs were characterized by using proton nuclear magnetic resonance (^{1}H-NMR) (Bruker, Karlsruhe, Germany) and mass spectrometry (MS) (Agilent Technologies). The products of PBDE sulfates were characterized just by ^{1}H-NMR. For ^{1}H-NMR analysis, 3 mg product was dissolved in deuterated DMSO and then the solution was subjected to detection by Bruker Ultra Shield-300 MHz NMR spectrometer. Chemical shifts (f1) were given in parts per million (ppm). Tetramethylsilane was used as an internal standard. MS analysis was performed by a HP-1100 liquid chromatography-mass spectrometer (LC-MS) instrument equipped

with a Capcell Pak UG 120 ODS column (Shiseido Co., Ltd., Tokyo, Japan). The mobile phase condition used was as follows: H_2O (0.05% TFA)/CH_3CN (0.05% TFA) in the linear gradient from 90/10 to 5/95 at a flow rate of 0.8 mL/min. The elution composition was detected by MS. Positive ion mode was used in all MS experiments.

2. Synthesis of 2-OH-BDE-3 and 2-BDE-3 sulfate

The scheme of synthesis of 2-OH-BDE-3 and 2-BDE-3 sulfate was shown as follows:



2.1 Synthesis of 2-(4-bromophenoxy)benzaldehyde (3)

K_2CO_3 (24 g, 174.3 mmol), 4-bromophenol (10 g, 58.1 mmol) and 2-fluorobenzaldehyde (7.2 g, 58.1 mmol) were added to dimethyl formamide (DMF, 150 mL). The mixture was stirred at 100 °C for 3 h. Then, the solution was cooled to room temperature, diluted with ice-water (200 mL), and extracted with ethyl acetate (EA, 200 mL). The organic phase was washed by H_2O, saturated brine and dried over by anhydrous Na_2SO_4. Then, the solvent was concentrated and the residue was further purified by silica gel chromatography [with petroleum ether (PE):EA=30:1 to PE:EA=20:1]. The solvent was removed under reduced pressure at temperature below 30 °C to get the title compound as a light yellow solid (11.7 g, 73%).

2.2 Synthesis of 2-(4-bromophenoxy)phenol (2-OH-BDE-3)

KH_2PO_4 (113 g, 834 mmol) was added to a solution of 2-(4-bromophenoxy)benzaldehyde (11.5 g, 41.7 mmol) in CH_2Cl_2 (200 mL). The reaction mixture was cooled to 0 °C. A TFPA solution was prepared by dropwise addition of trifluoroacetic anhydride (52 g, 250.1 mmol) to aqueous hydrogen peroxide (7.1 g,

62.2 mmol, 30%) in CH_2Cl_2 (200 mL) at 0 °C, and further stirred at 0 °C for 1 h. Then, 200 mL TFPA solution was added to the reaction mixture by dropwise over 0.5 h. The mixture was stirred at 0 °C for 3 h. Then, the reaction mixture was diluted with saturated brine (200 mL) and aqueous $NaHSO_3$ (20%, 200 mL) at 0 °C. The organic and aqueous phases were separated. The aqueous phase was extracted once with an equal volume of CH_2Cl_2. The combined organic phases were washed once with an equal volume of saturated $NaHCO_3$ and H_2O. The crude formate ester was dissolved in MeOH (300 mL) containing one drop of concentrated hydrochloric acid. The solution was stirred for 15 h and then concentrated in vacuo. The crude product was further purified on a silica gel column chromatography (with PE: EA=20:1 to PE: EA=10:1). The solvent was removed under reduced pressure at temperature below 30 ^oC to get the title compound as a white solid (3.4 g, 31%). MS: m/e =263 $(M-H)^+$. HPLC (254 nm ＞ 98%). ^{1}HNMR($CDCl_3$, 400MHz) : δ (ppm) = 7.48-7.45 (m, 2H) , 7.09-7.07 (m, 2H), 6.95-6.86 (m, 1H), 5.56 (s, 1H). Abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublets of doublet, br = broad.

2.3 Synthesis of 2-(4-bromophenoxy)phenyl 2,2,2-trichloroethyl sulfate (6)

A solution of 2,2,2-trichloroethyl sulfochloridate (2.2 g, 9.1 mmol) in CH_2Cl_2 (10 mL) was prepared. Then, this solution was dripwise added to a solution of 2-(4-bromophenoxy)phenol (2-OH-BDE-3) (2.3 g, 8.7 mmol) and 4-dimethylaminopyridine (DMAP) (1.6 g, 13.1 mmol) in CH_2Cl_2 (30 mL) at 0 ^oC. The mixture was stirred at 0 ^oC for 30 min and kept at room temperature for overnight. Then, the solvent was concentrated by vacuo and the residue was diluted with EA (60 mL). Then, the organic phase solution was washed by 1 mol/L HCl (40 mL×3) and H_2O (50 mL), dried over by anhydrous Na_2SO_4 and evaporated in vacuo. The crude product was further purified by silica gel column chromatography with PE: EA=10:1. The solvent was removed under reduced pressure at temperature below 30 ^oC to get title compound as a white solid (2.7 g, 65%).

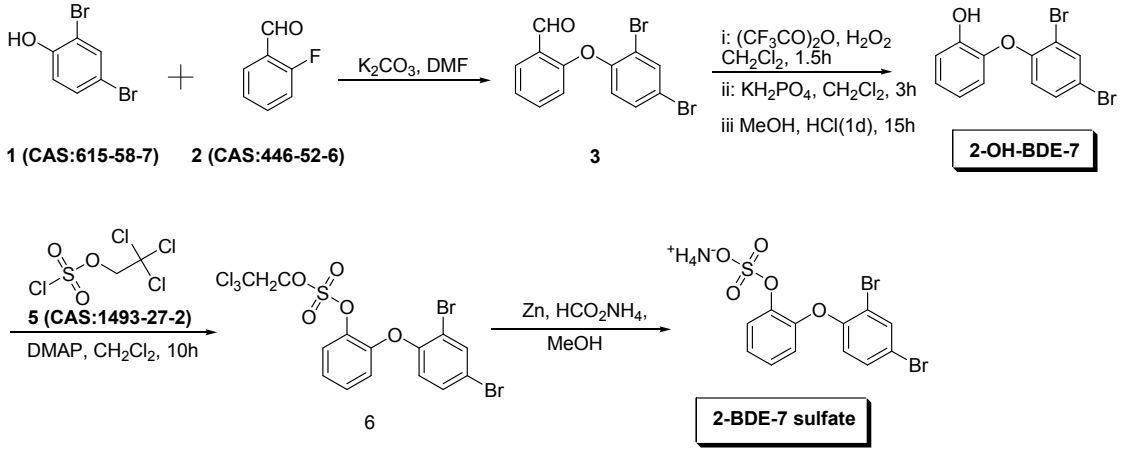
2.4 Synthesis of ammonium 2-(4-bromophenoxy)phenyl sulfate (2-BDE-3 sulfate)

Ammonium formate (2.2 g, 34.2 mmol) was added to a solution of 2-(4-

bromophenoxy)phenyl 2,2,2-trichloroethyl sulfate (2.7 g, 5.7 mmol) in CH_3OH (20 mL). Zinc power (0.74 g, 11.4 mmol) was added, and stirred until the 2-(4-bromophenoxy)phenyl 2,2,2-trichloroethyl sulfate was completely consumed as determined by thin layer chromatography (TLC). The solution was filtered and concentrated under reduced pressure at temperature below 30 °C. The residue was further purified by silica gel column chromatography (using DCM: CH_3OH: ammonium hydroxide=10:1:0.1, V:V:V as a dilute). The solvent was removed under reduced pressure at temperature below 30 °C to yield the final product as a white solid (0.91 g, 44%). HPLC (254 nm＞99%). ^{1}HNMR(CD_3OD, 400MHz) : δ (ppm) = 7.44-7.40 (m, 2H), 7.07-7.05 (m, 1H), 6.96-6.93 (m, 2H), 6.85-6.81 (m, 3H).

3. Synthesis of 2-OH-BDE-7 and 2-BDE-7 sulfate

The scheme of synthesis of 2-OH-BDE-7 and 2-BDE-7 sulfate was shown as follows**:**



3.1 Synthesis of 2-(2,4-dibromophenoxy)benzaldehyde (3)

K_2CO_3 (16.6 g, 120 mmol), 2,4-dibromophenol (10 g, 40 mmol) and 2-fluorobenzaldehyde (5.0 g, 40 mmol) were added to DMF (150 mL). The mixture was stirred at 100 °C for 3 h. Then, the mixture was cooled to room temperature, diluted with ice-water (200 mL), and extracted with EA (200 mL). The organic phase was washed by H_2O, saturated brine and dried over by anhydrous Na_2SO_4. Then, the solvent was concentrated and the residue was further purified by silica gel chromatography [with petroleum ether (PE):EA=30:1 to PE:EA=20:1] to get the title compound as a light yellow solid (8.8 g, 62%).

3.2 Synthesis of 2-(2,4-dibromophenoxy)phenol (2-OH-BDE-7)

KH_2PO_4 (67 g, 497 mmol) was added to a solution of 2-(2,4-dibromophenoxy)benzaldehyde (8.8 g, 24.8 mmol) in CH_2Cl_2 (150 mL). The reaction mixture was cooled to 0 °C. 100 mL TFPA solution was added to the reaction mixture by dropwise over 0.5 h. The mixture was stirred at 0 °C for 3 h. Then, the reaction mixture was diluted with saturated brine (200 mL) and aqueous $NaHSO_3$ (20%, 200 mL) at 0 °C. The organic and aqueous phases were separated. The aqueous phase was extracted once with an equal volume of CH_2Cl_2. The combined organic phases were washed once with an equal volume of saturated $NaHCO_3$ and H_2O. The crude formate ester was dissolved in MeOH (300 mL) containing one drop of concentrated hydrochloric acid. The solution was stirred for 15 h and then concentrated in vacuo. The crude product was further purified on a silica gel column chromatography (with PE: EA=20:1 to PE: EA=10:1) to get the title compound as a white solid (3.1 g, 37%). MS: m/e =341 $(M-H)^+$. HPLC (214 nm＞96%, 254 nm＞ 99%). 1HNMR($CDCl_3$, 400MHz) : δ (ppm) = 7.80 (s, 2H) , 7.39-7.38 (m, 1H), 7.09-7.07 (m, 2H), 6.88-6.85 (m, 2H), 6.81-6.79 (m, 1H), 5.57 (s, 1H).

3.3 Synthesis of 2-(2,4-dibromophenoxy)phenyl 2,2,2-trichloroethyl sulfate (6)

A solution of 2,2,2-trichloroethyl sulfochloridate (1.5 g, 6.1 mmol) in DCM (30 mL) was prepared. Then, this solution was dripwise added to a solution of 2-(2,4-dibromophenoxy)phenol (2-OH-BDE-7) (2.0 g, 5.8 mmol) and DMAP (1.1 g, 8.8 mmol) in DCM (30 mL) at 0 ^oC. The mixture was stirred at 0 ^oC for 30 min and warmed to room temperature for overnight. The solvent was concentrated and the residue diluted with EA (60 mL). Then, the EA solution was washed by 1 mol/L HCl (40 mL×3) and H_2O (50 mL), dried over by anhydrous Na_2SO_4 and evaporated in vacuo. The crude product was further purified by silica gel column chromatography with PE: EA=10:1 to get title compound as a white solid (1.9 g, 59%).

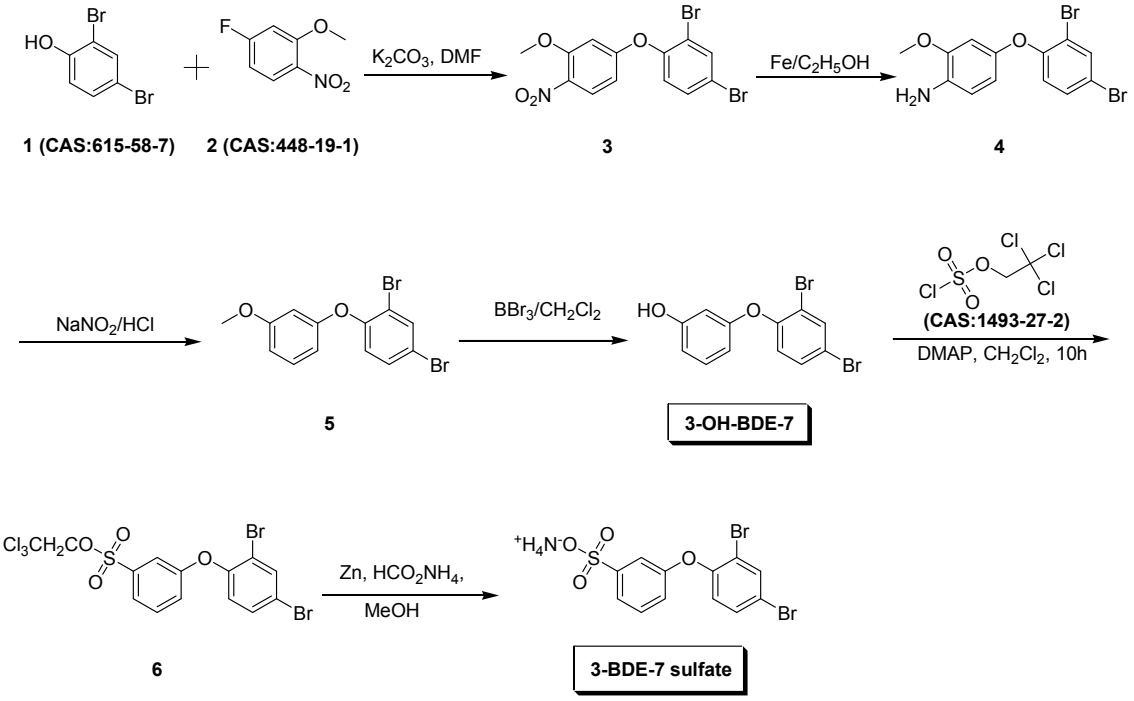
3.4 Synthesis of ammonium 2-(2,4-dibromophenoxy)phenyl sulfate (2-BDE-7 sulfate)

Ammonium formate (1.3 g, 20.6 mmol) was added to a solution of the 2-(2,4-dibromophenoxy)phenyl 2,2,2-trichloroethyl sulfate (1.9 g, 3.4 mmol) in methanol

(15 mL). Zinc power (0.44 g, 6.8 mmol) was added, and stirred until the 2-(2,4-dibromophenoxy)phenyl 2,2,2-trichloroethyl sulfate was consumed completely as determined by TLC. The solution was filtered and concentrated under reduced pressure at room temperature below 30 °C. The residue was further purified by silica gel column chromatography (using DCM: CH_3OH: ammonium hydroxide=10:1:0.1, V:V:V as a dilute). The solvent was removed under reduced pressure at temperature below 30 °C to yield the final product as a white solid (0.82 g, 55%). HPLC (254 nm＞99%). ^{1}HNMR(CD_3OD, 400MHz) : δ (ppm) = 7.80 (s, 1H), 7.79-7.68 (m, 1H) , 7.39-7.38 (m, 1H), 7.15-7.13 (m, 2H), 6.92-6.90 (m, 1H), 6.83-6.81 (m, 3H).

4. Synthesis of 3-OH-BDE-7 and 3-BDE-7 sulfate

The scheme of synthesis of 3-OH-BDE-7 and 3-BDE-7 sulfate was shown as follows.



4.1 Synthesis of 2,4-dibromo-1-(3-methoxy-4-nitrophenoxy)benzene (3)

K_2CO_3 (50 g, 360 mmol), 2,4-dibromophenol (30 g, 120 mmol) and 4-fluoro-2-methoxy-1-nitrobenzene (20.5 g, 120 mmol) were added to DMF (500 mL). The mixture was stirred at 100 °C for 3 h. Then, the mixture was cooled to room temperature, diluted with ice-water (500 mL), and extracted with EA (500 mL). The organic phase was washed by H_2O, saturated brine and dried over by anhydrous

Na_2SO_4. Then, the solvent was concentrated and the residue was further purified by silica gel chromatography [with petroleum ether (PE):EA=20:1 to PE:EA=10:1]. The solvent was removed under reduced pressure at temperature below 30 ^oC to get the title compound as a light yellow solid (34 g, 71%).

4.2 Synthesis of 4-(2,4-dibromophenoxy)-2-methoxybenzenamine (4)

A mixture of 2,4-dibromo-1-(3-methoxy-4-nitrophenoxy)benzene (3) (34 g, 85 mmol), NH_4Cl (22.5 g, 425 mmol) and ferrous powder (47.6 g, 850 mmol) in C_2H_5OH/H_2O (600 mL，V:V=5:1) was prepared and heated at 60 ^oC for 5 h. Then, the mixture was cooled to room temperature and filtered by Buchner funnel. The filtrate was concentrated by vacuo. Then, the reside was diluted with EA (300 mL), washed by H_2O (200 mL), saturated brine, dried over by anhydrous Na_2SO_4. Then, the solvent was concentrated by vacuo and the residue was further purified by silica gel chromatography (with PE: EA=10:1 to PE: EA=1:2). The solvent was removed under reduced pressure at temperature below 30 ^oC to get the title compound as a brown oil (20.5 g, 65%).

4.3 Synthesis of 2,4-dibromo-1-(3-methoxyphenoxy)benzene (5)

Concentrated hydrochloric acid (20 mL) was added to a solution of 4-(2,4-dibromophenoxy)-2-methoxybenzenamine (4) (10 g, 27 mmol) in C_2H_5OH (120 mL) and cooled to 0 ^oC. Then, $NaNO_2$ (1.9 g, 27 mmol) in 5 mL H_2O was dropwise added. The resulting solution was refluxed for 3 h, cooled to room temperature. The solvent was concentrated and the reside was diluted with EA (100 mL). The organic phase was washed by H_2O (100 mL), aqueous $NaHCO_3$ (100 mL), saturated brine, dried over by anhydrous Na_2SO_4. The organic phase was concentrated and further purified by silica gel chromatography (with PE:EA=50:1 to PE:EA=20:1) to get the title compound as a yellow solid (4.6 g, 48%).

4.4 Synthesis of 3-(2,4-dibromophenoxy)phenol (3-OH-BDE-7)

BBr_3 (38.7 mL, 38.7 mmol, 1 mol/L in CH_2Cl_2) was dropwise added to a solution of 2,4-dibromo-1-(3-methoxyphenoxy)benzene (5) (4.6 g, 12.9 mmol) in CH_2Cl_2 (80 mL) at 0 ^oC. The mixture was stirred for 5 h and quenched by ice-water (50 mL). The organic phase was washed by aqueous saturated $NaHCO_3$ (100 mL), saturated brine

and dried over by anhydrous Na_2SO_4. The organic phase was concentrated by vacuo and further purified by silica gel chromatography (with PE:EA=20:1 to PE:EA=10:1). The solvent was removed under reduced pressure at temperature below 30 ^oC to get the title compound as a white solid (3.0 g, 69%). HPLC (254 nm＞ 99%). MS: m/e =341 $(M-H)^+$. ^{1}HNMR (DMSO, 300MHz) : δ (ppm) = 9.67 (s, 1H), 7.98-7.97 (m, 1H), 7.60-7.57 (m, 1H) , 7.56-7.00 (m, 2H) , 6.56-6.53 (m, 1H) , 6.39-6.36 (m, 1H) , 6.31-6.29 (m, 1H).

4.5 Synthesis of 2,2,2-trichloroethyl 3-(2,4-dibromophenoxy)benzenesulfonate (6)

2,2,2-trichloroethyl sulfochloridate (1.5 g, 5.9 mmol) in DCM (10 mL) was dripwise added to a solution of 3-(2,4-dibromophenoxy)phenol (3-OH-BDE-7) (1.9 g, 5.6 mmol) and DMAP (1.0 g, 8.3 mmol) in DCM (30 mL) at 0 ^oC. Then the mixture was stirred at 0 ^oC for 30 min and kept in room temperature for overnight. The solvent was concentrated by vacuo and the residue was diluted with EA (70 mL). Then, the EA solution was washed by 1 mol/L HCl (50 mL×3) and H_2O (70 mL), dried over by anhydrous Na_2SO_4. The solution was evaporated in vacuo and the residue was further purified by silica gel column chromatography with PE: EA=10:1. The solvent was removed under reduced pressure at temperature below 30 ^oC to get the title compound as a white solid (1.8 g, 62%).

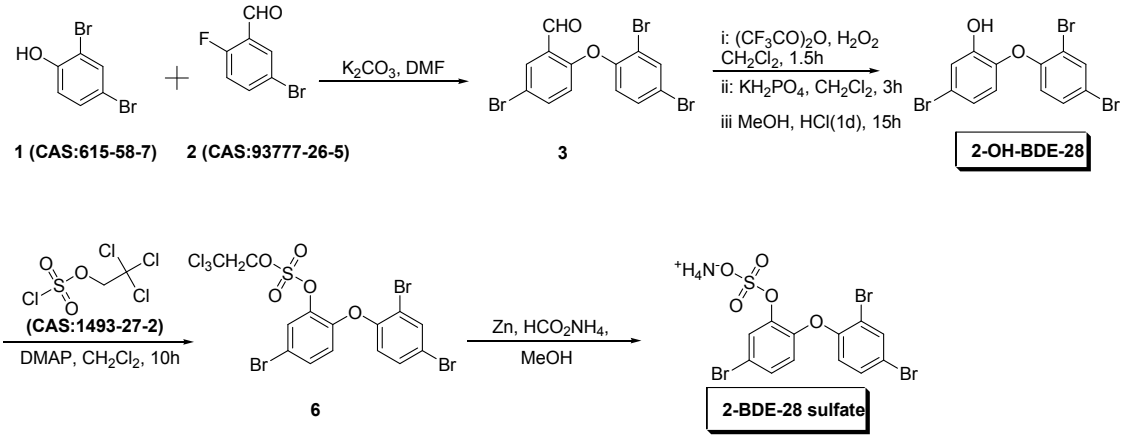
4.6 Synthesis of ammonium 3-(2,4-dibromophenoxy)benzenesulfonate (3-BDE-7 sulfate)

Ammonium formate (1.3 g, 20.1 mmol) was added to a solution of 2,2,2-trichloroethyl 3-(2,4-dibromophenoxy)benzenesulfonate (1.8 g, 3.4 mmol) in methanol (20 mL). Zinc powder (0.44 g, 6.8 mmol) was added, and stirred until the 2,2,2-trichloroethyl 3-(2,4-dibromophenoxy)benzenesulfonate was consumed completely as determined by TLC. The solution was filtered and concentrated under reduced pressure at room temperature below 30 ^oC. Then, the residue was further purified by silica gel column chromatography (using DCM: CH_3OH: ammonium hydroxide=10:1:0.1, V:V:V as a dilute). The solvent was removed under reduced pressure at temperature below 30 ^oC to yield the final product as a white solid (0.95 g,

69%). HPLC (254 nm＞ 95%). ^{1}HNMR(CD_3OD, 400MHz) : δ (ppm) = 7.86-7.84 (m, 1H), 7.49-7.48 (m, 1H), 7.32-7.30 (m, 1H), 7.11-7.09 (m, 1H) , 6.99-6.93 (m, 2H) , 6.78-6.75 (m, 1H).

5. Synthesis of 2-OH-BDE-28 and 2-BDE-28 sulfate

The scheme of synthesis of 2-OH-BDE-28 and 2-BDE-28 sulfate was shown as follows:



5.1 Synthesis of 5-bromo-2-(2,4-dibromophenoxy)benzaldehyde (3)

K_2CO_3 (16.6 g, 120 mmol), 2,4-dibromophenol (10 g, 40 mmol) and 5-bromo-2-fluorobenzaldehyde (8.1 g, 40 mmol) were added to DMF (150 mL). The mixture was stirred at 100 ^oC for 3 h. Then, the mixture was cooled to room temperature, diluted with ice-water (200 mL), and extracted with EA (200 mL). The organic phase was washed by H_2O, saturated brine and dried over by anhydrous Na_2SO_4. Then, the solvent was concentrated and the residue was further purified by silica gel chromatography [with petroleum ether (PE):EA=30:1 to PE:EA=10:1] to get the title compound as a light yellow solid (12.1 g, 70%).

5.2 Synthesis of 5-bromo-2-(2,4-dibromophenoxy)phenol (2-OH-BDE-28)

KH_2PO_4 (75 g, 555 mmol) was added to a solution of 2-(2,4-dibromophenoxy)benzaldehyde (12 g, 27.8 mmol) in CH_2Cl_2 (200 mL). The reaction mixture was cooled to 0 °C. Then, 150 mL TFPA solution was added to the reaction mixture by dropwise over 0.5 h. The mixture was stirred at 0 °C for 3 h. Then, the reaction mixture was diluted with saturated brine (200 mL) and aqueous $NaHSO_3$

(20%, 200 mL) at 0 °C. The organic and aqueous phases were separated. The aqueous phase was extracted once with an equal volume of CH_2Cl_2. The combined organic phases were washed once with an equal volume of saturated $NaHCO_3$ and H_2O. The crude formate ester was dissolved in MeOH (300 mL) containing one drop of concentrated hydrochloric acid. The solution was stirred for 15 h and then concentrated in vacuo. The crude product was further purified on a silica gel column chromatography (with PE: EA=20:1 to PE: EA=10:1) to get the title compound as a white solid (3.9 g, 34%). MS: m/e =419 $(M-H)^+$. HPLC (254 nm＞ 99%). ^{1}HNMR($CDCl_3$, 400MHz) : δ (ppm) = 7.81 (s, 2H) , 7.44-7.41 (m, 1H), 7.25-7.2 (m, 1H), 6.99-6.97 (m, 1H), 6.89-6.87 (m, 1H), 6.65-6.62 (m, 1H), 5.66 (s, 1H).

5.3 Synthesis of 5-bromo-2-(2,4-dibromophenoxy)phenyl 2,2,2-trichloroethyl sulfate (6)

A solution of 2,2,2-trichloroethyl sulfochloridate (1.7 g, 6.9 mmol) in DCM (10 mL) was prepared. Then, this solution was dripwise added to a solution of 5-bromo-2-(2,4-dibromophenoxy)phenol (2-OH-BDE-28) (2.8 g, 6.6 mmol) and DMAP (1.2 g, 10 mmol) in DCM (30 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and warmed to room temperature for overnight. The solvent was concentrated and the residue diluted with EA (60 mL). Then, the EA solution was washed by 1 mol/L HCl (40 mL×3) and H_2O (50 mL), dried over by anhydrous Na_2SO_4 and evaporated in vacuo. The crude product was further purified by silica gel column chromatography with PE: EA=10:1 to get title compound as a white solid (1.6 g, 40%).

5.4 Synthesis of ammonium 5-bromo-2-(2,4-dibromophenoxy)phenyl sulfate (2-BDE-28 sulfate)

Ammonium formate (2.2 g, 34.2 mmol) was added to a solution of 5-bromo-2-(2,4-dibromophenoxy)phenyl 2,2,2-trichloroethyl sulfate (1.6 g, 2.5 mmol) in methanol (15 mL). Zinc power (0.33 g, 5.0 mmol) was added, and stirred until the 5-bromo-2-(2,4-dibromophenoxy)phenyl 2,2,2-trichloroethyl sulfate was consumed completely as determined by TLC. The solution was filtered and concentrated under reduced pressure at room temperature below 30 °C. The residue was further purified by silica gel column chromatography (using DCM: CH_3OH: ammonium

hydroxide=10:1:0.1, V:V;V as a dilute). The solvent was removed under reduced pressure at temperature below 30 ^{o}C to yield the final product as a white solid (0.84 g, 65%). HPLC (254 nm＞99%). 1HNMR(CD3OD, 400MHz) : δ (ppm) = 7.87 (s, 1H), 7.80 (s, 1H), 7.43-7.41 (m, 1H), 7.20-7.28 (m, 1H) , 6.86-6.82 (m, 2H).

6. Fluorescence competitive binding assays

Fluorescence polarization based competitive binding assays were used to determine the binding potency of PBDE sulfates and OH-PBDEs with TH transport proteins (TBG and TTR) and nuclear receptors (TRα-LBD and TRβ-LBD). Fluorescence probes, fluorescein-thyroxine (F-T4) and fluorescein-triiodothyronine (F-T3) were prepared in our laboratory previously (Ren et al., 2012; 2013). For TTR competitive binding assay, 200 nM TTR and 50 nM F-T4 were used. For TBG competitive binding assay, 100 nM TBG and 50 nM F-T4 were used. For TR-LBDs competitive binding assay, 200 nM TRα-LBD or 200 nM TRβ-LBD and 50 nM F-T3 were used. All these competitive binding assays were performed in the potassium phosphate buffer (50 mM potassium phosphate, 150 mM KCl, 0.5 mM EDTA, pH 8.0). Protein, probe and different concentrations of competitor were mixed in a total volume of 100 μL and incubated for 5 min at room temperature (controlled at 25 ℃) before the fluorescence polarization were measured. The content of DMSO in the final solution was kept below 1% to avoid the solvent effect. Fluorescence polarization detection was carried out on a Horiba Fluoromax-4 spectrofluorimeter (Edison, New Jersey, USA). The excitation and emission wavelengths were 490 nm and 520 nm respectively. The fluorescence polarization value was plotted as a function of competitor concentration. The competition curves were fitted with a sigmoidal model (OriginLab, Northampton, MA) to derive the value of IC_{50} value (the concentration of the ligand required to displace half of the probe from the protein). Relative binding potency (RP) was calculated by dividing the IC_{50} of T4 (for TTR and TBG) or T3 (for TRα-LBD and TRβ-LBD) by that of the competitor.

7. Cell culture and GH3 cell proliferation assay

Rat GH3 pituitary cancer cells were purchased from Cell Resource Center, IBMS, CAMS/PUMC (Beijing, China). Dulbecco's modified Eagle

medium: nutrient mixture F-12 (DMEM/F12, Life Technologies, Carlsbad, CA, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Life Technologies) and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Life Technologies) was used to culture GH3 cells. Cells were cultured in a humidified atmosphere composed of 95% air and 5% CO_2 at 37 ℃. The activity of PBDE sulfates and OH-PBDEs towards TR signaling pathway were studied by using GH3 cell proliferation assay according to a previous paper (Ren et al., 2015). Briefly, the culture medium was replaced with test medium: DMEM/F12 medium without FBS but containing bovine insulin (10 mg/ml), ethanolamine (10 mM), sodium selenite (10 ng/ml), bovine serum albumin (500 mg/ml) and human apotransferrin (10 mg/ml) for 24 h. Then, the GH3 cells were seeded in 96-well plates (Corning, NY, USA) at a density of 5×10^3 cells per well in the test medium. After 24 h, the cells were exposed to different concentrations of chemicals (PBDE sulfates, OH-PBDEs and T3) with or without 2.0 μM amiodarone (a specific antagonist of TR) for 96 h. The proliferation effects were assessed using CCK-8 assay.

References

1. Marsh G, Stenutz R, Bergman Å. 2003. Synthesis of hydroxylated and methoxylated polybrominated diphenyl ethers − natural products and potential polybrominated diphenyl ether metabolites. Eur J Org Chem. 2003(14): 2566-2576.
2. Ren XM, Guo LH. 2012. Assessment of the binding of hydroxylated polybrominated diphenyl ethers to thyroid hormone transport proteins using a site-specific fluorescence probe. Environ Sci Technol. 46(8): 4633-4640.
3. Ren XM, Guo LH, Gao Y, Zhang BT, Wan B. 2013. Hydroxylated polybrominated diphenyl ethers exhibit different activities on thyroid hormone receptors depending on their degree of bromination. Toxicol Appl Pharmacol. 268(3): 256-263.
4. Ren XM, Zhang YF, Guo LH, Qin ZF, Lv QY, Zhang LY. 2015. Structure–activity relations in binding of perfluoroalkyl compounds to human thyroid hormone T3 receptor. Arch Toxicol. 89(2): 233-242.

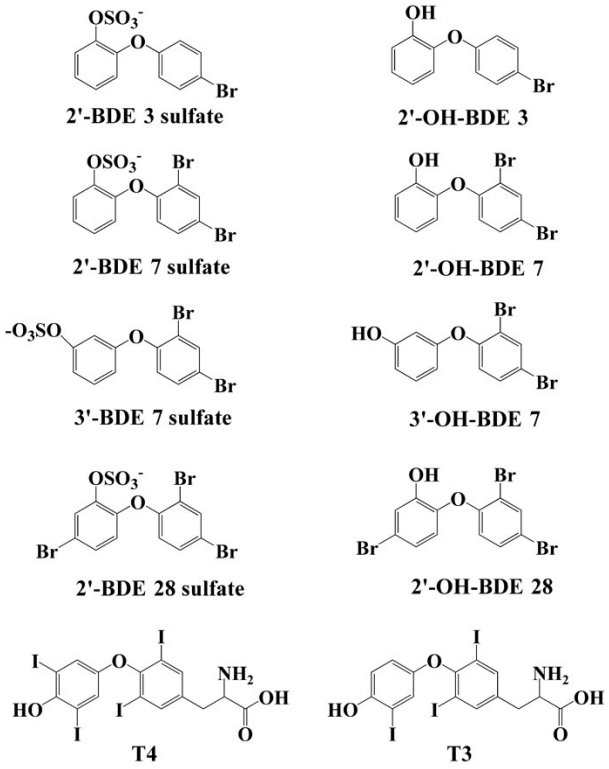


Figure S1. Chemical structures of PBDE sulfates, OH-PBDEs and thyroid hormones used in this study.

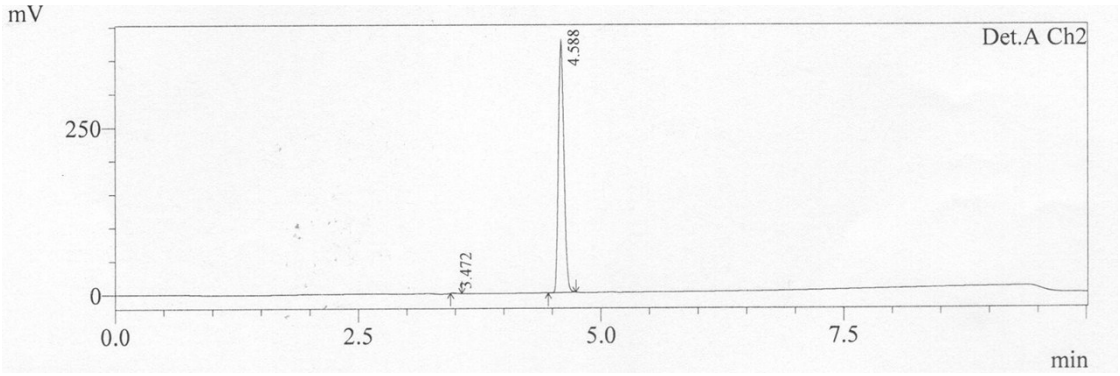


Figure S2. HPLC analysis of 2'-BDE 3 sulfate

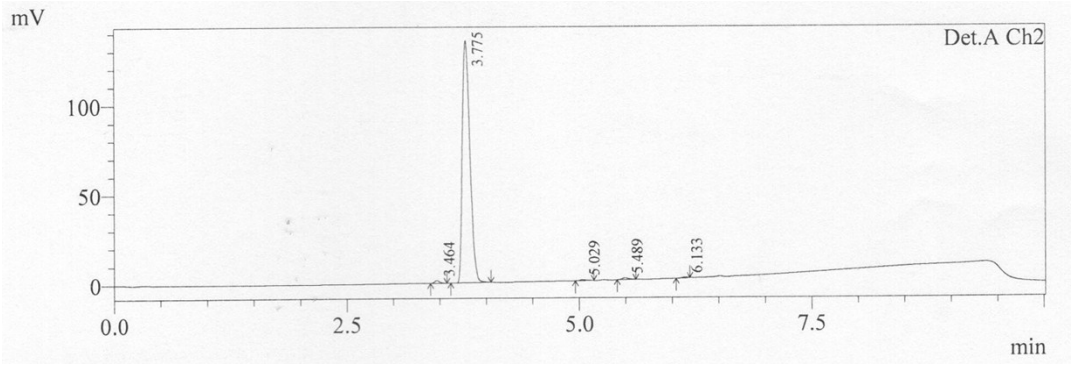


Figure S3. HPLC analysis of 2'-BDE 7 sulfate

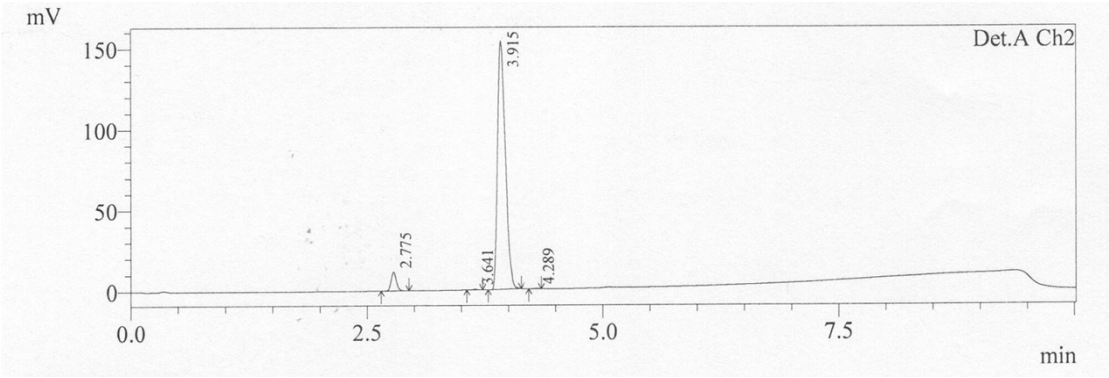


Figure S4. HPLC analysis of 3’-BDE 7 sulfate

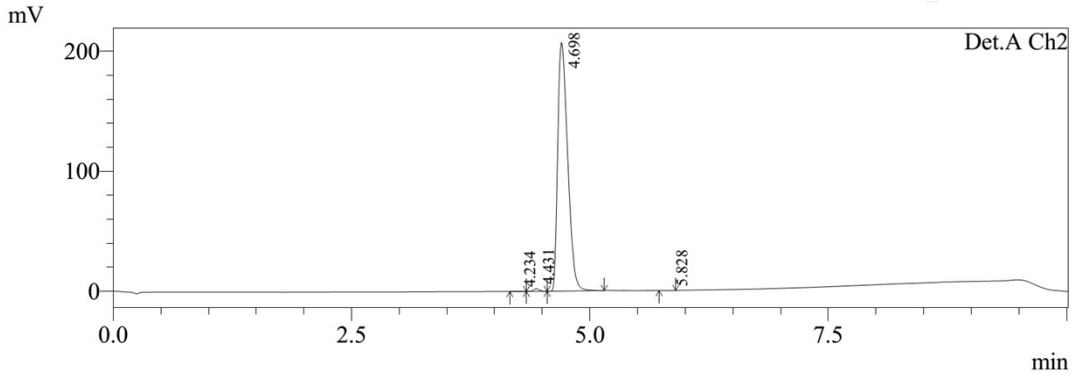


Figure S5. HPLC analysis of 2'-BDE 28 sulfate

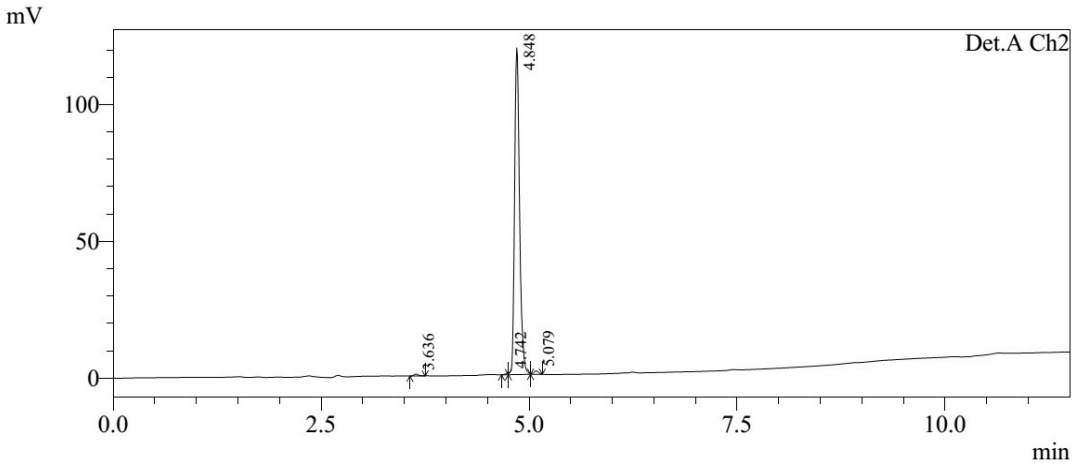


Figure S6. HPLC analysis of 2'-OH-BDE 3

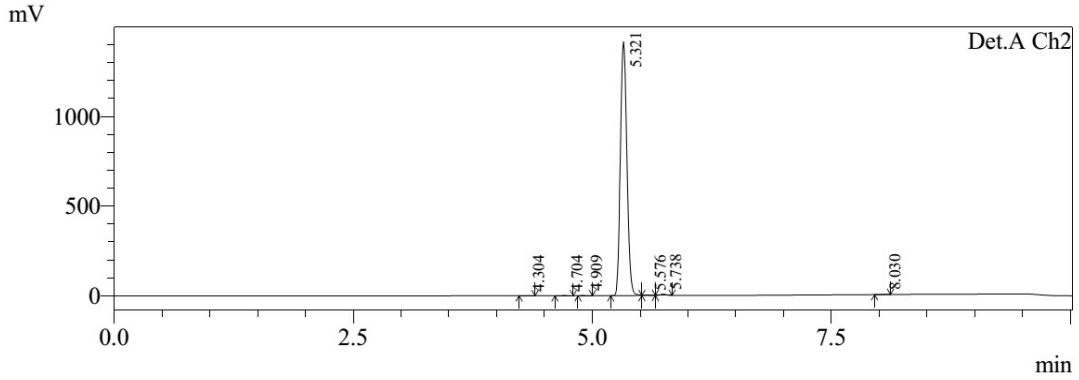


Figure S7. HPLC analysis of 2’-OH-BDE 7

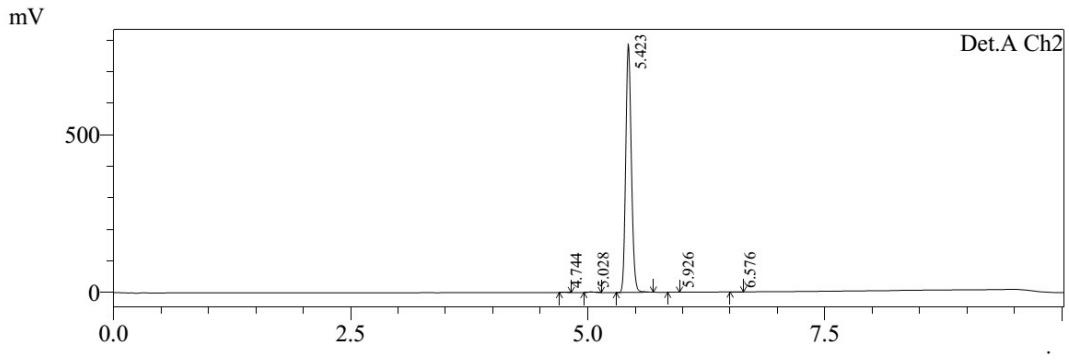


Figure S8. HPLC analysis of 3’-OH-BDE 7

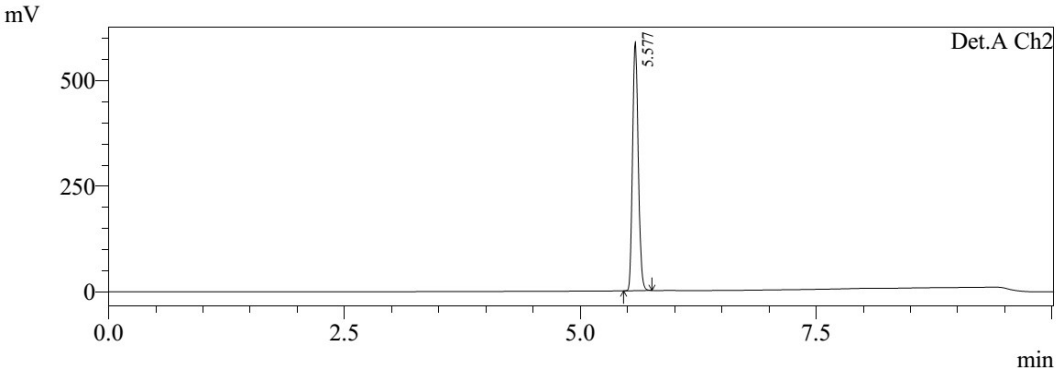


Figure S9. HPLC analysis of 2’-OH-BDE 28

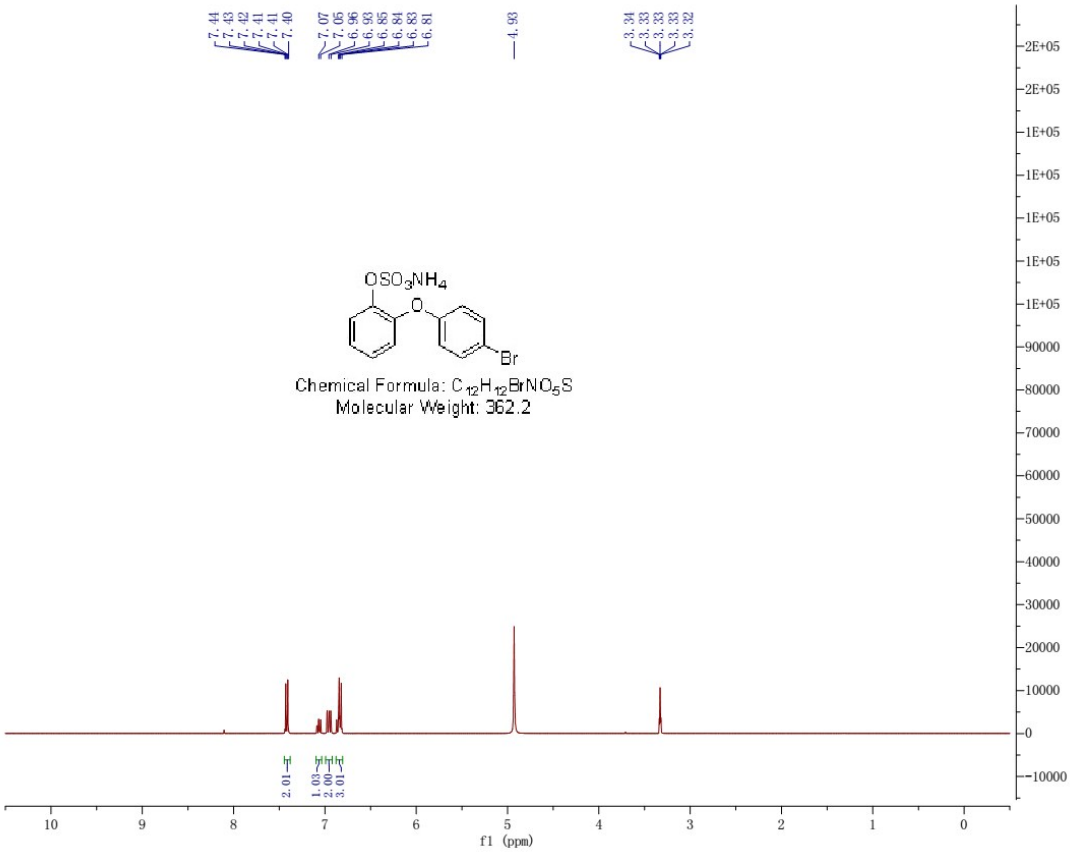


Figure S10. ^{1}H-NMR analysis of 2'-BDE 3 sulfate

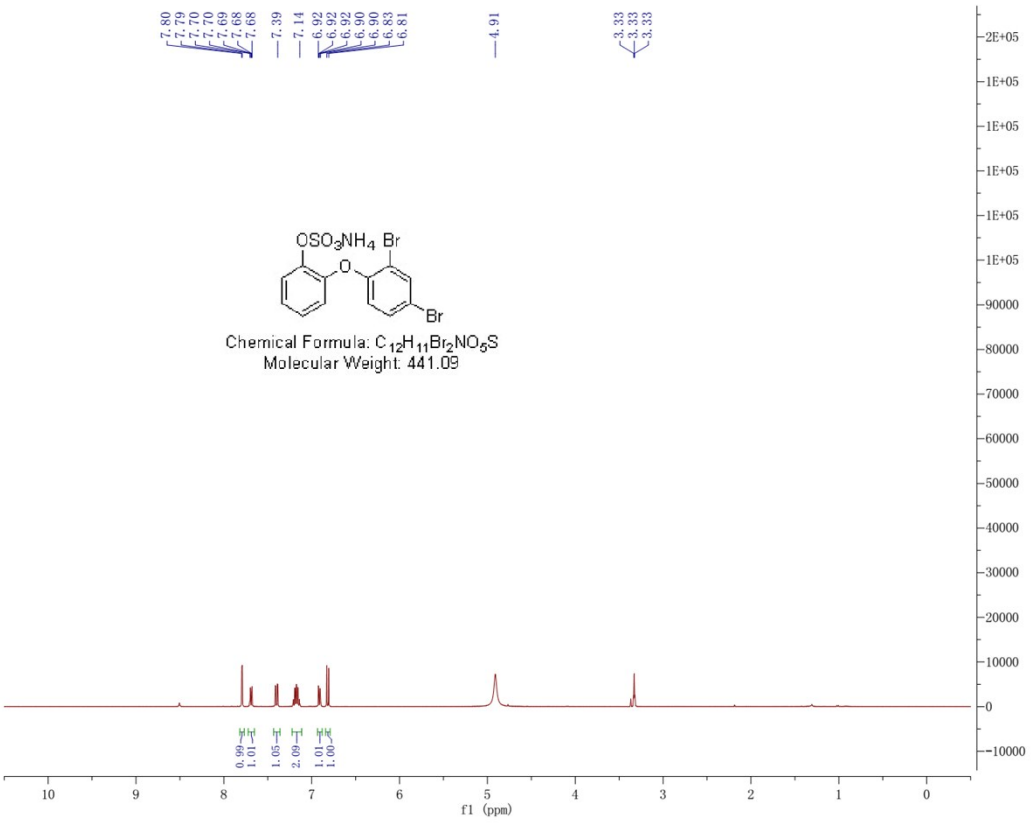


Figure S11. ^{1}H-NMR analysis of 2'-BDE 7 sulfate

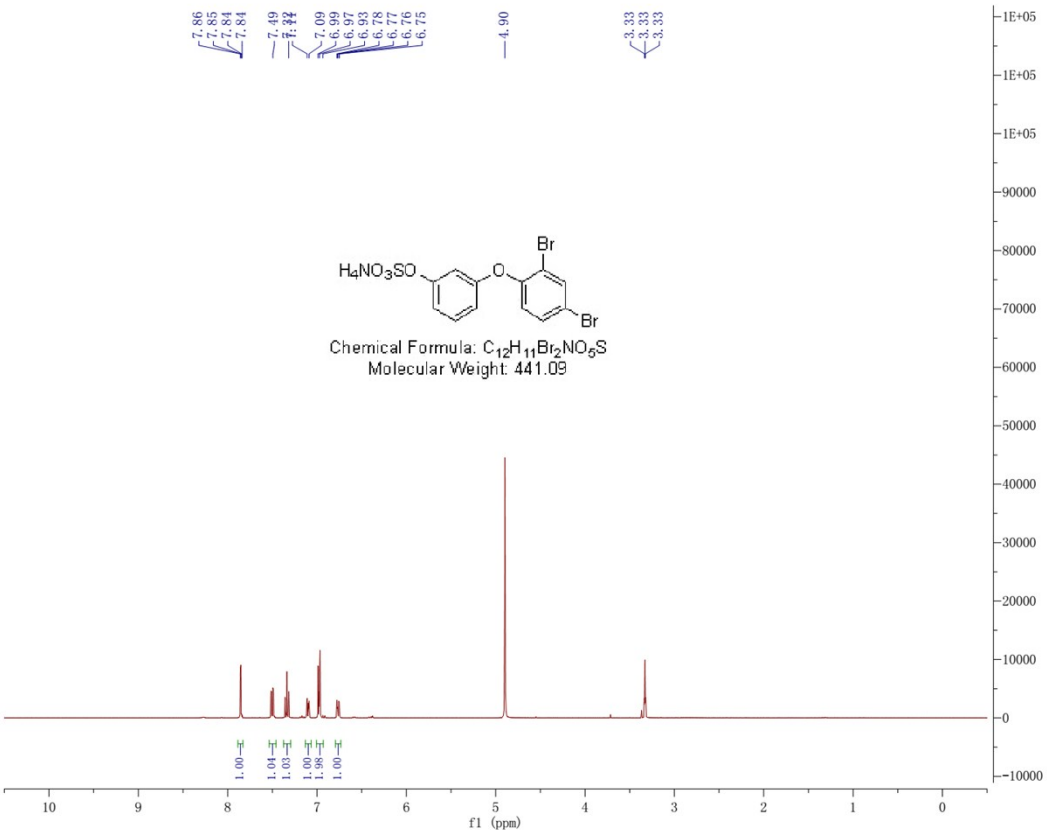


Figure S12. ^{1}H-NMR analysis of 3'-BDE 7 sulfate

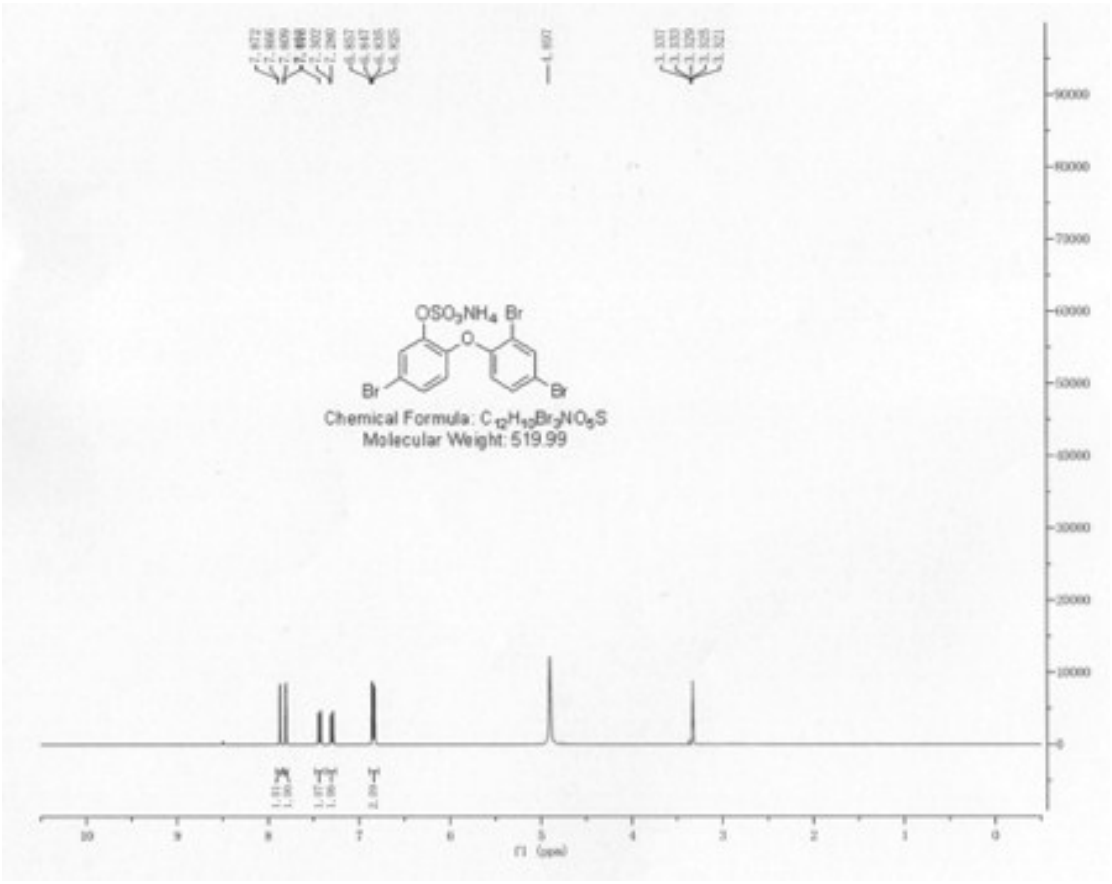


Figure S13. ^{1}H-NMR analysis of 2'-BDE 28 sulfate

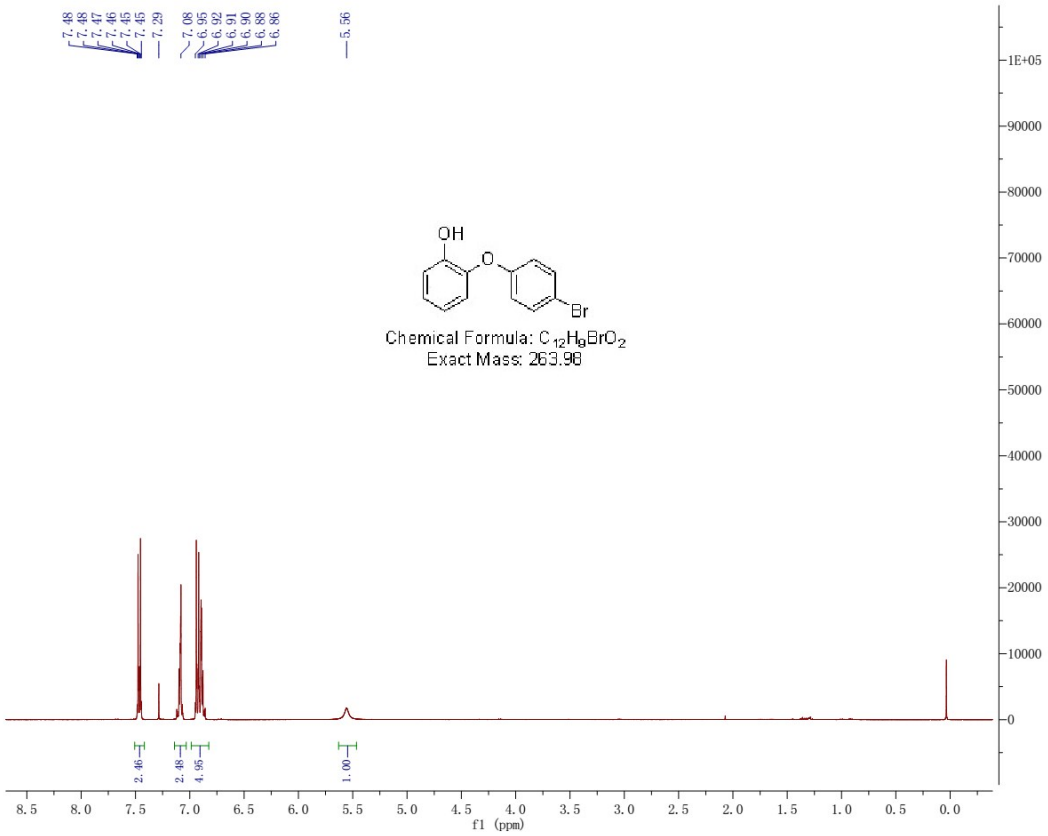


Figure S14. ^{1}H-NMR analysis of 2'-OH-BDE 3

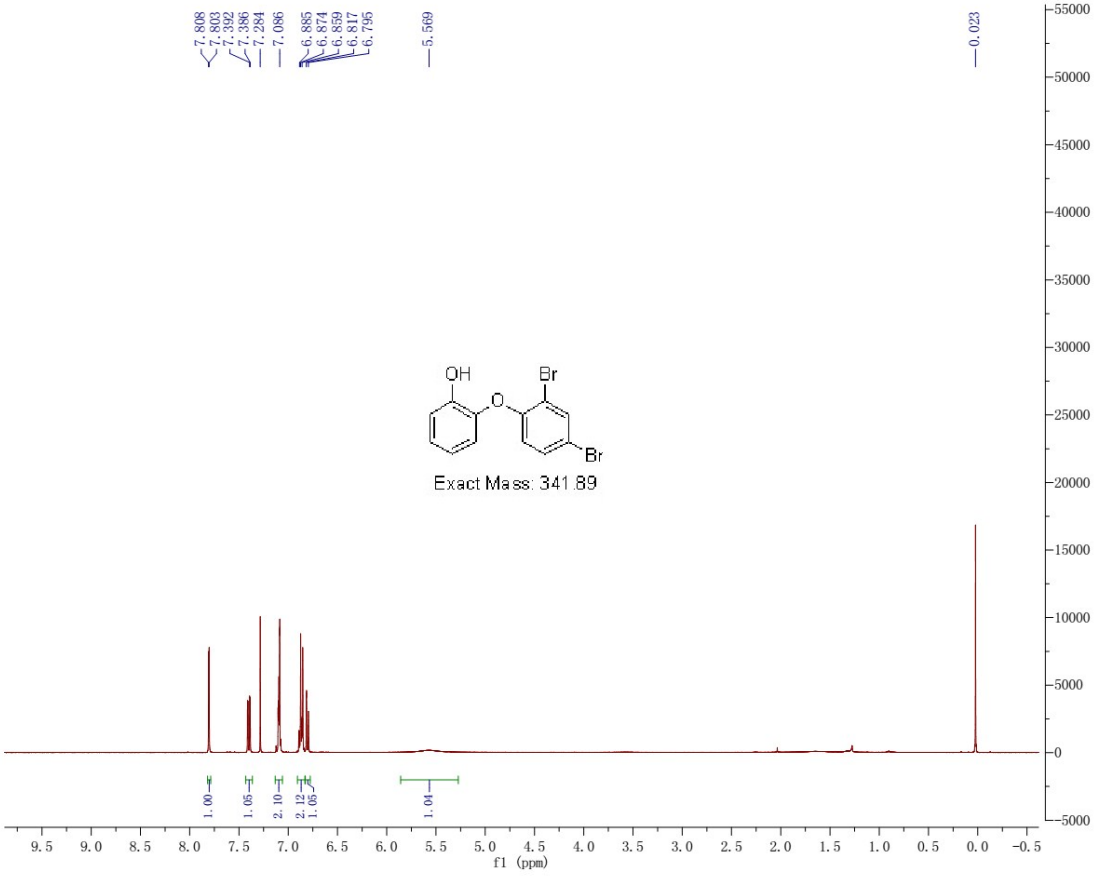


Figure S15. ^{1}H-NMR analysis of 2'-OH-BDE 7

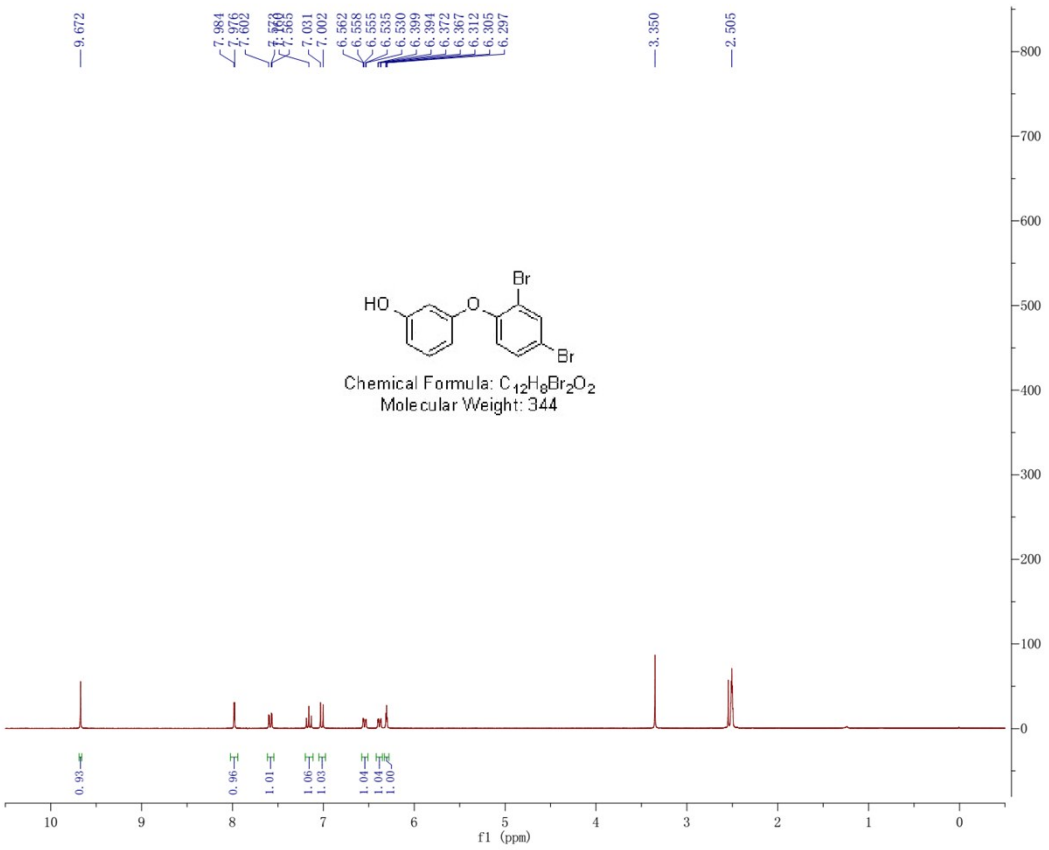


Figure S16. ^{1}H-NMR analysis of 3'-OH-BDE 7

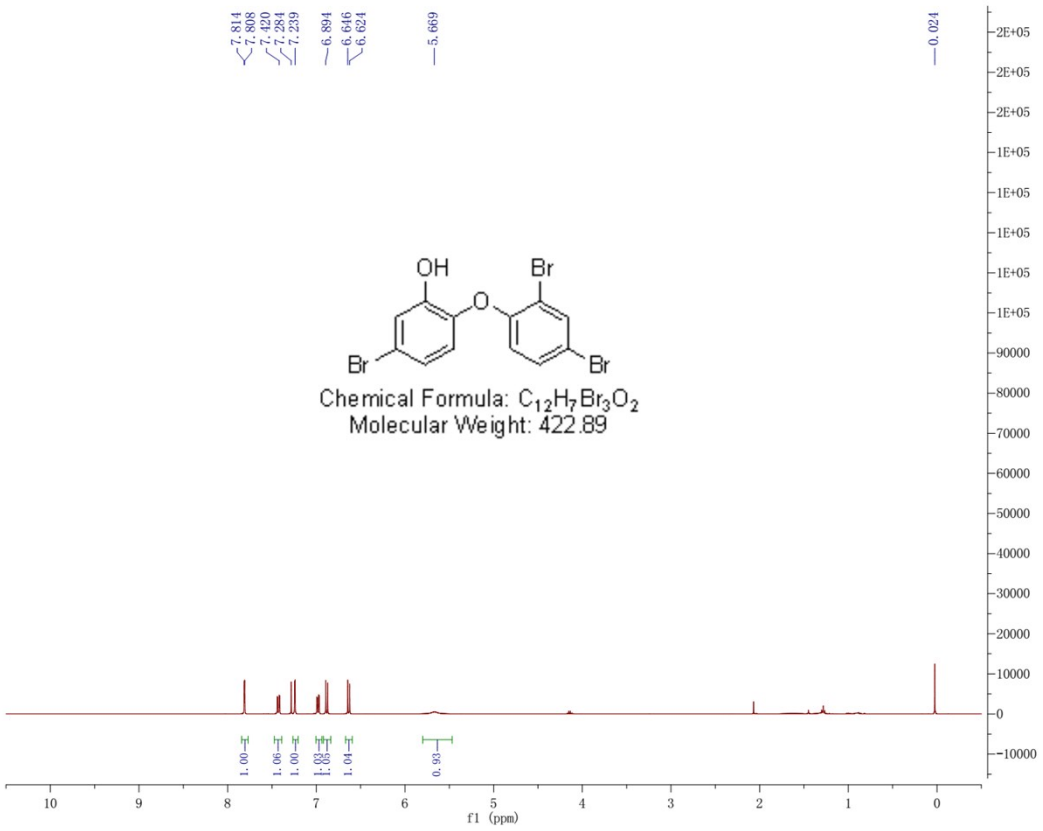


Figure S17. ^{1}H-NMR analysis of 2'-OH-BDE 28

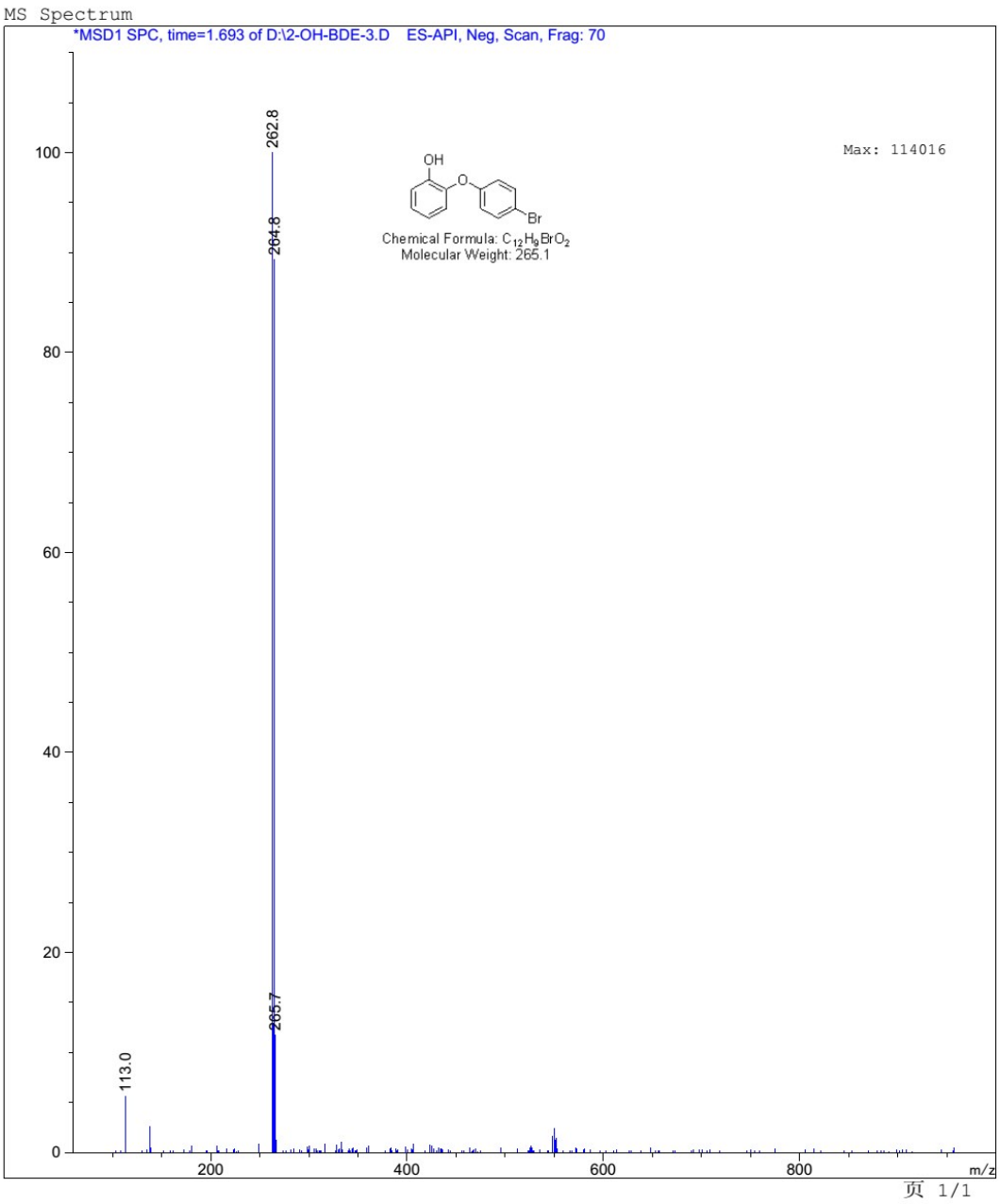


Figure S18. MS analysis of 2'-OH-BDE 3

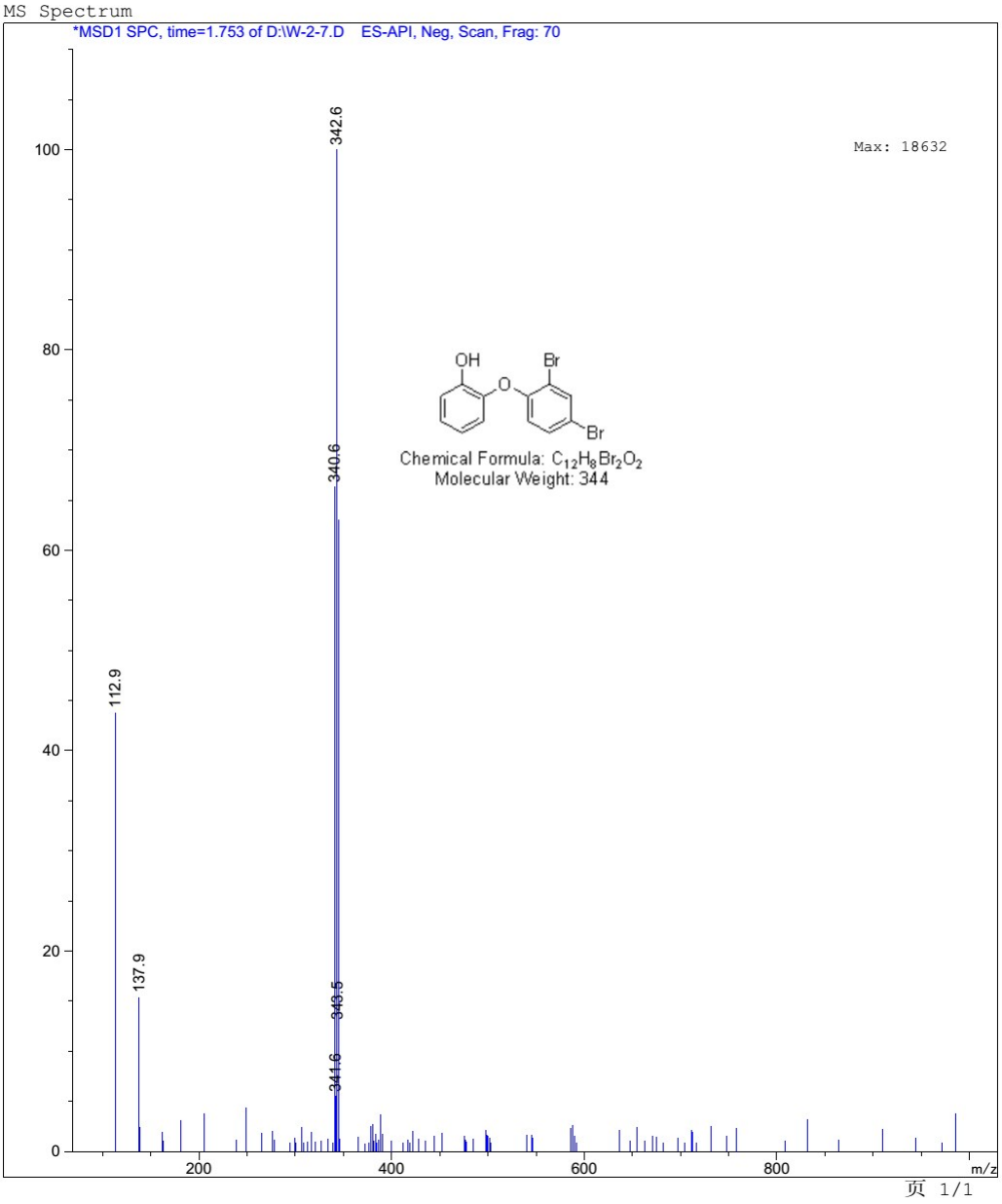


Figure S19. MS analysis of 2'-OH-BDE 7

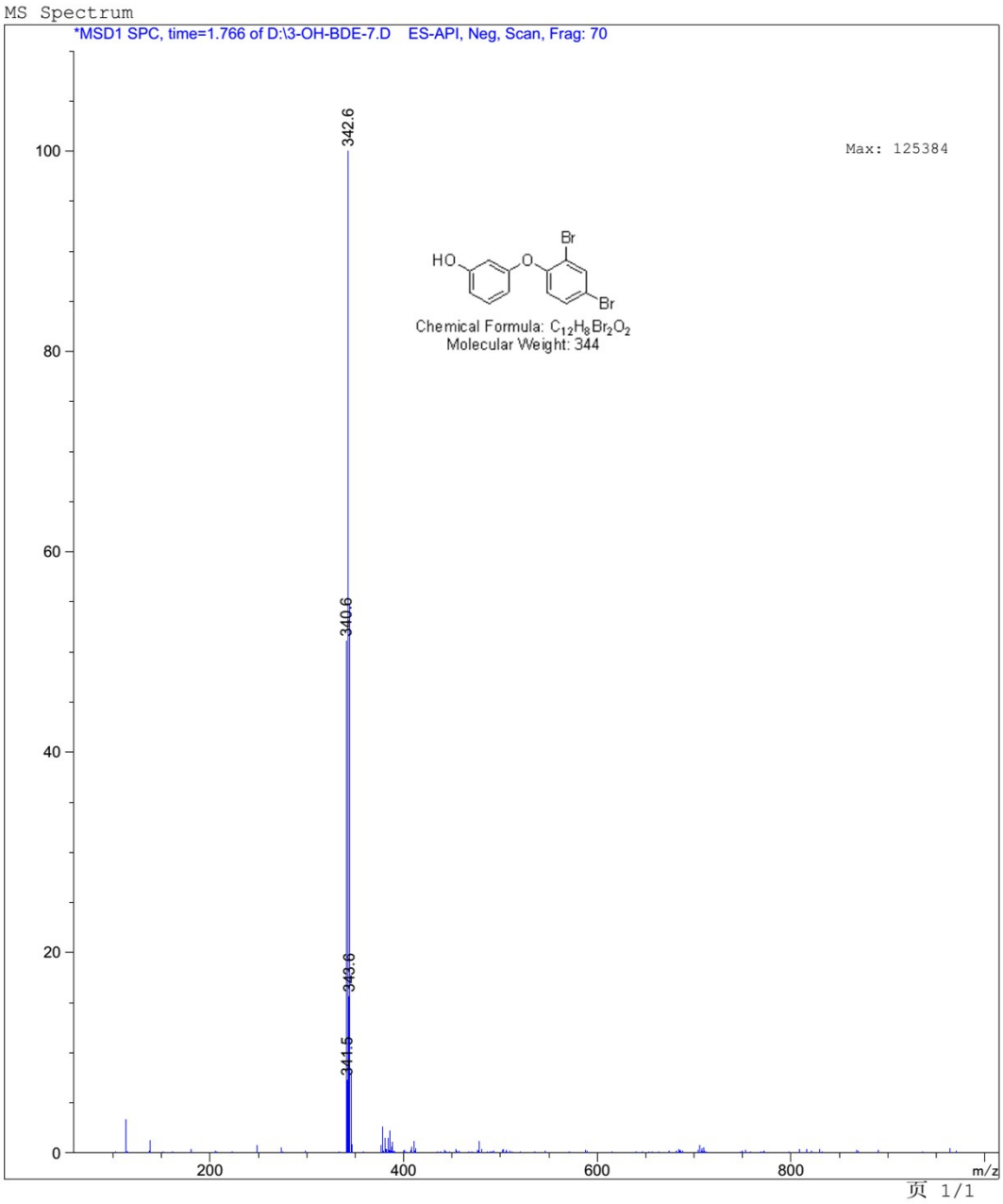


Figure S20. MS analysis of 3'-OH-BDE 7

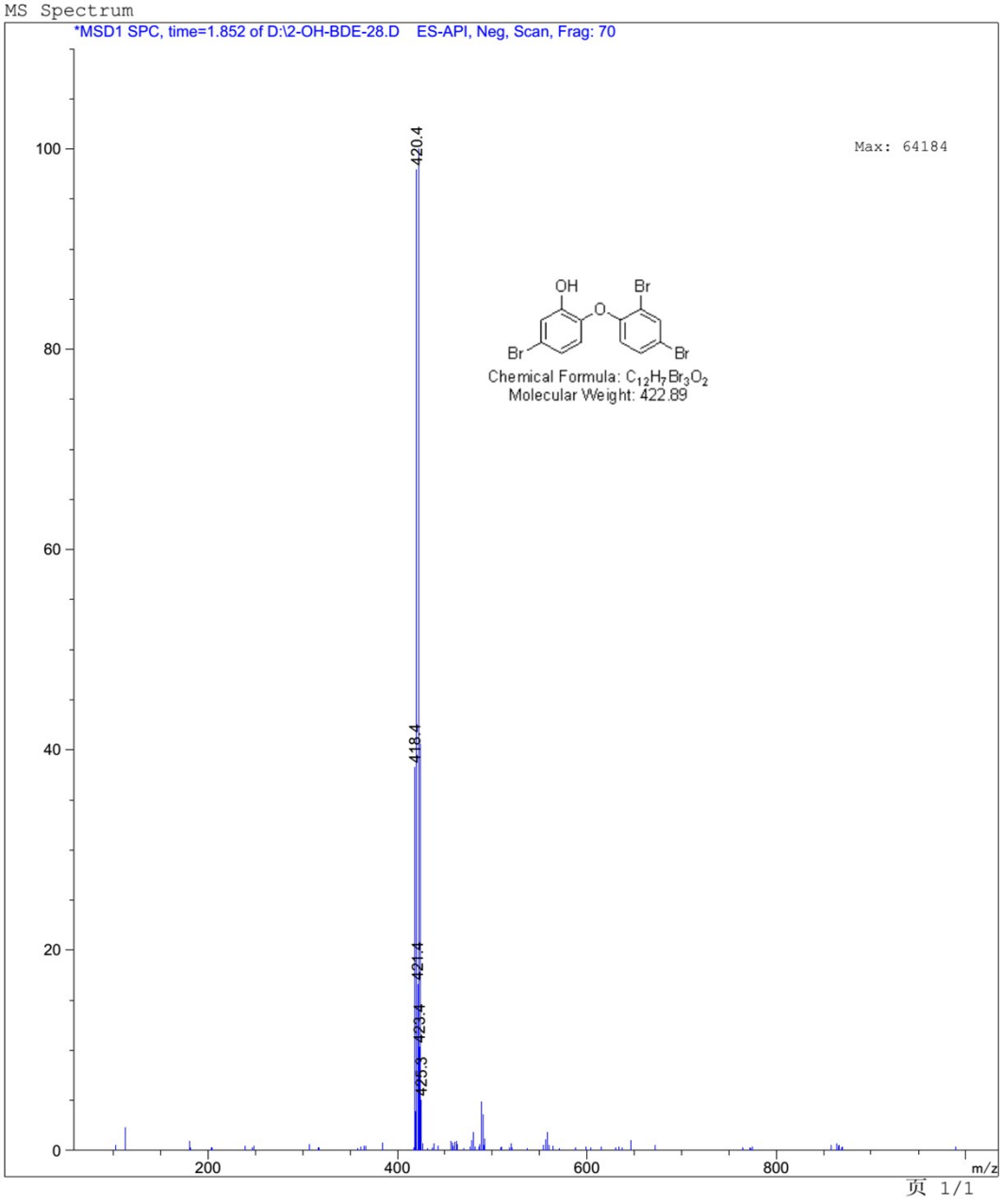


Figure S21. MS analysis of 2'-OH-BDE 28

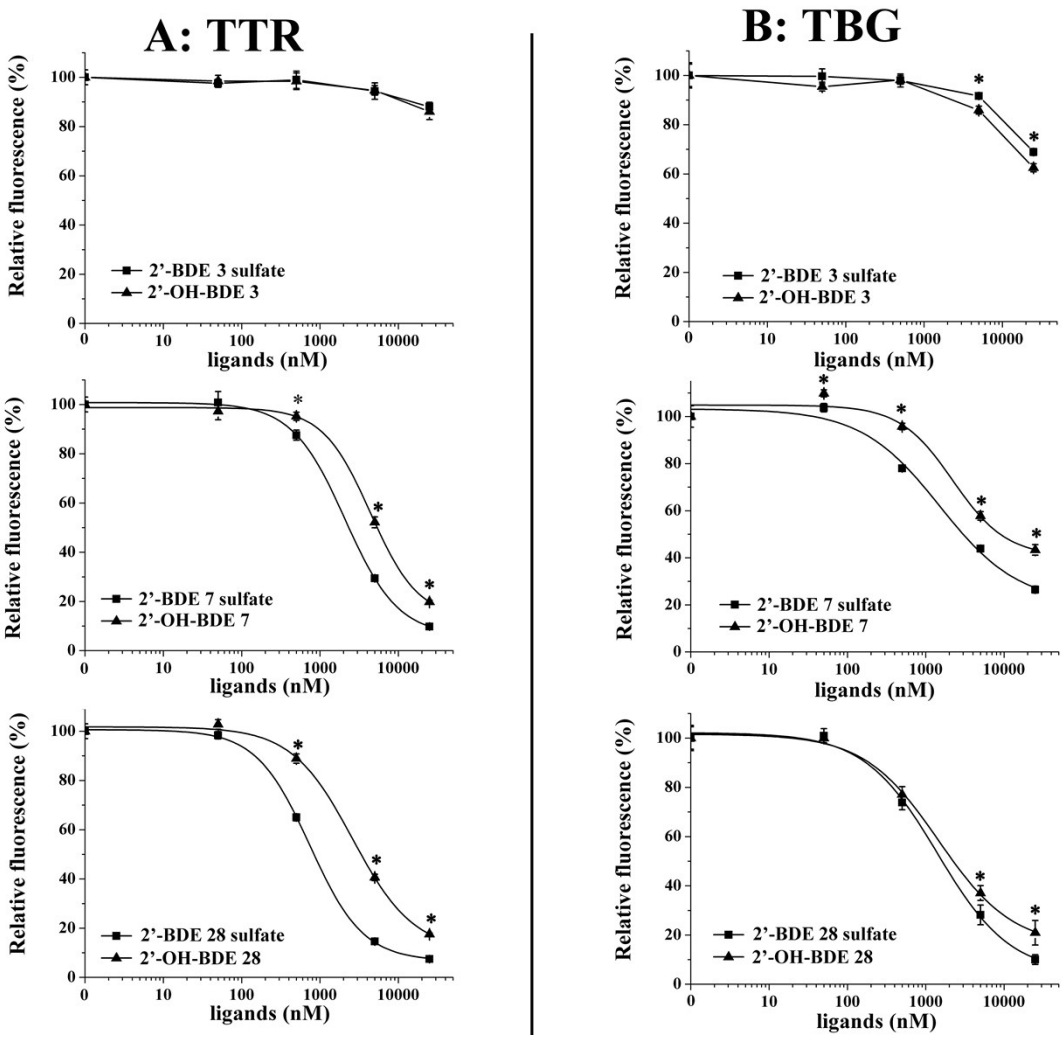


Figure S22. Fluorescence competitive binding curves of PBDE sulfates and OH-PBDEs with TBG (A) and TTR (B). The error bar represents the standard deviation of three independent measurements. *Means $p < 0.05$, PBDE sulfates compared with OH-PBDEs at the same test concentration.

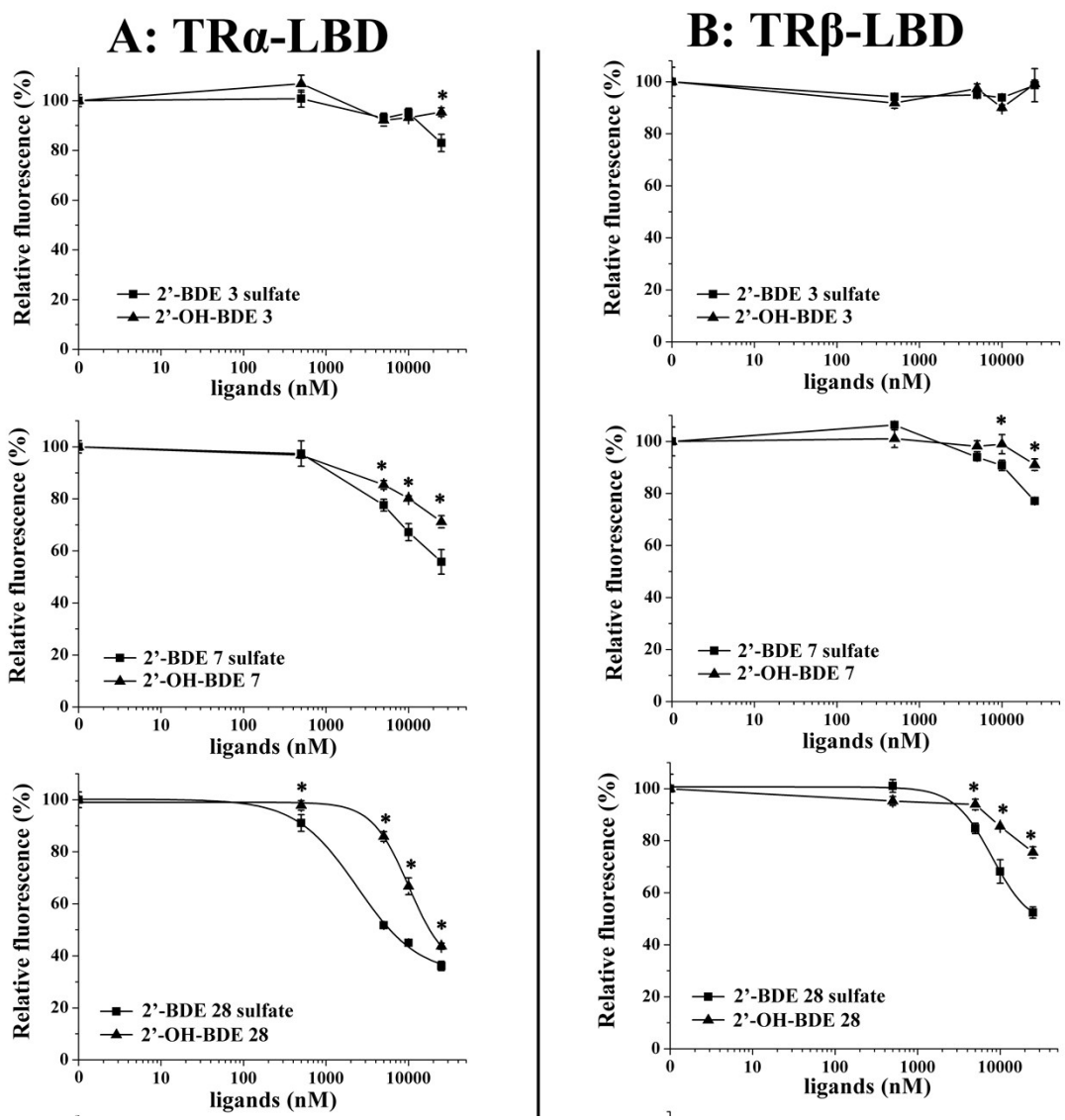


Figure S23. Fluorescence competitive binding curves of PBDE sulfates and OH-PBDEs with TRα-LBD (A) and TRβ-LBD (B). The error bar represents the standard deviation of three independent measurements. *Means $p < 0.05$, PBDE sulfates compared with OH-PBDEs at the same test concentration.

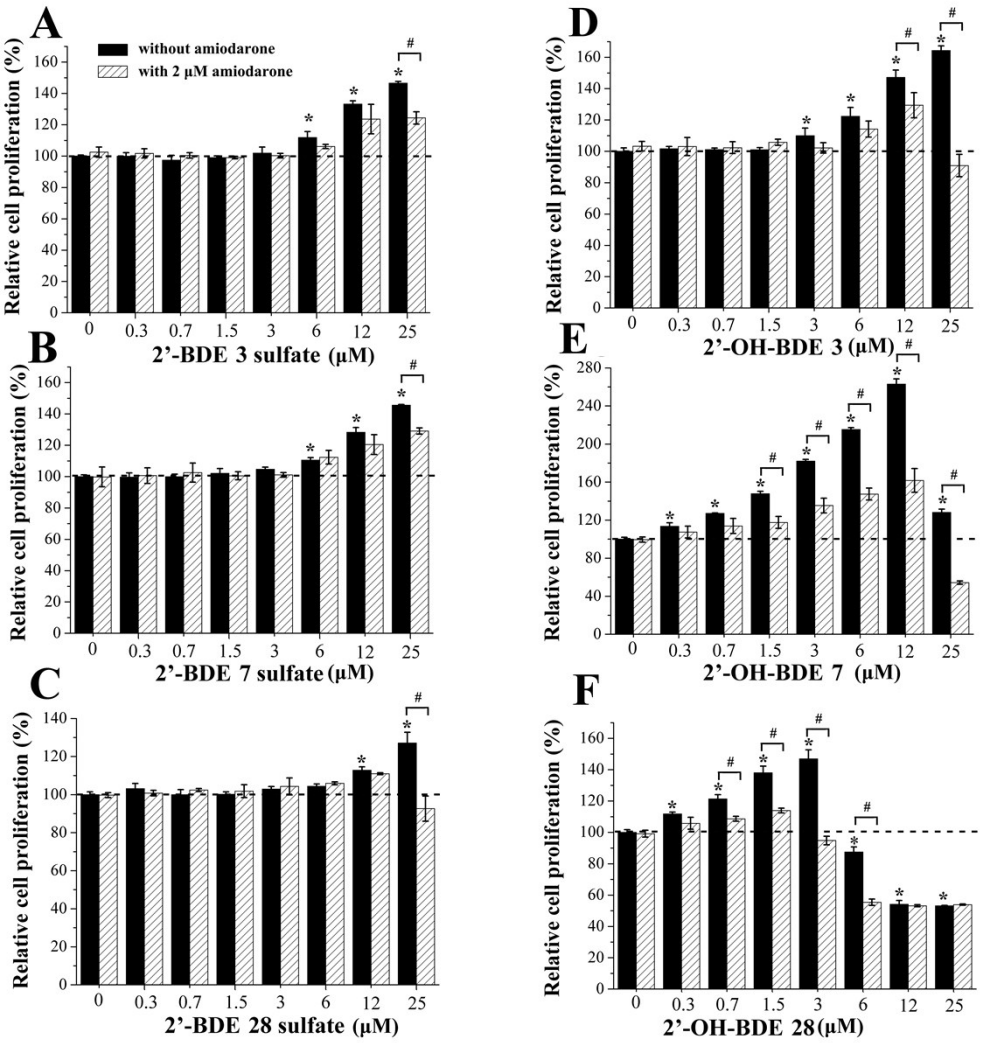


Figure S24. Agonistic activity of PBDE sulfates and OH-PBDEs determined by GH3 cell proliferation assay. GH3 cells were treated with different concentrations of PBDE sulfates and OH-PBDEs in the absence and presence of 2.0 μM amiodarone. The error bar represents the SD of three independent measurements. *Means $p < 0.05$, compared with cell samples treated with the control group (1% DMSO). #Means $p < 0.05$, compared with cell samples treated without 2.0 μM amiodarone.

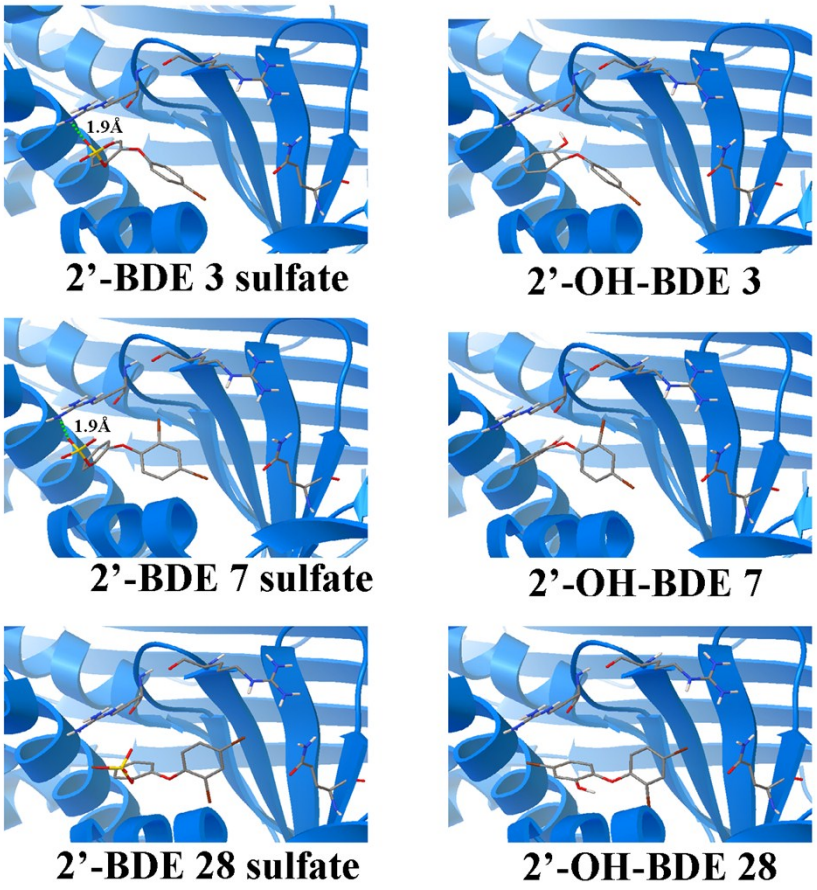


Figure S25. Molecular docking results of PBDE sulfates and OH-PBDEs with TBG.

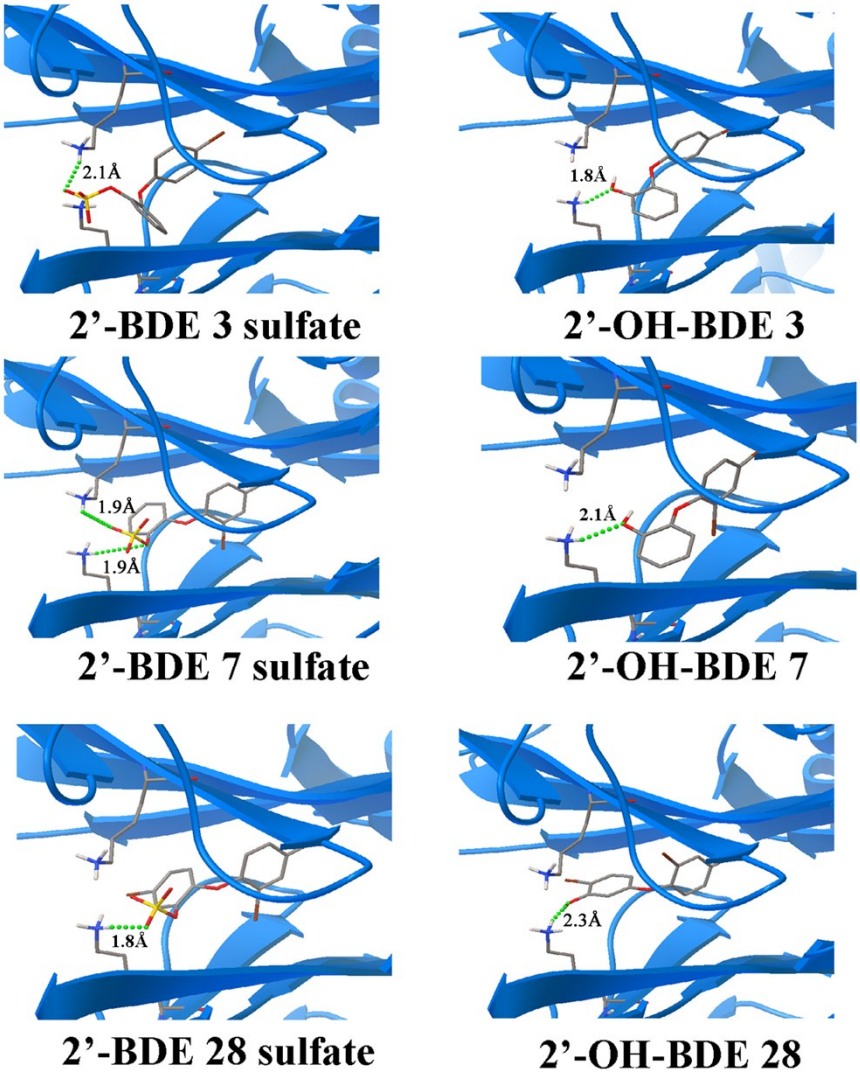


Figure S26. Molecular docking results of PBDE sulfates and OH-PBDEs with TTR.

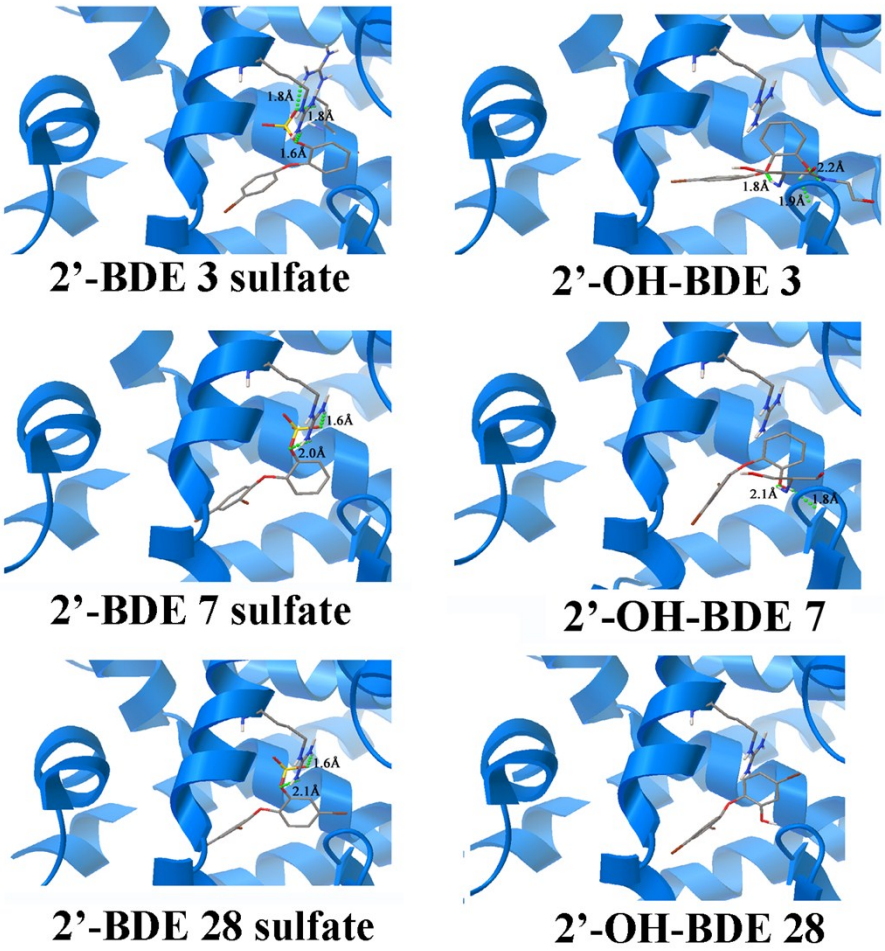


Figure S27. Molecular docking results of PBDE sulfates and OH-PBDEs with TRα.

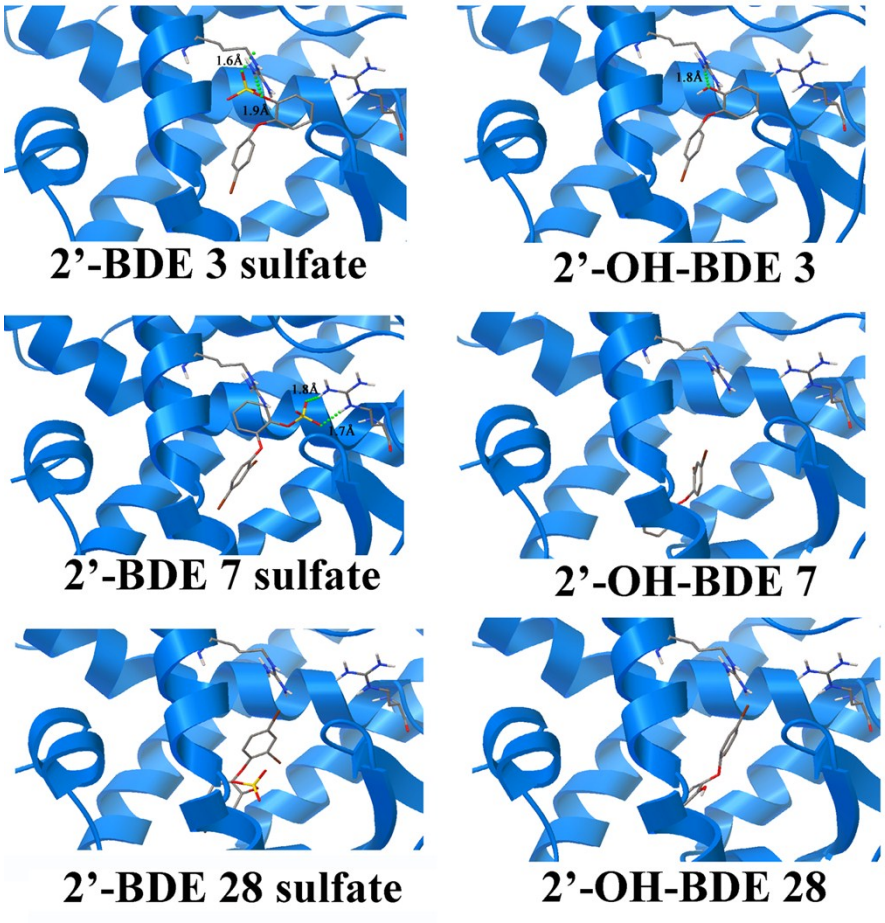


Figure S28. Molecular docking results of PBDE sulfates and OH-PBDEs with TRβ.