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Supplemental Material

Binding and Activity of Tetrabromobisphenol A Mono-Ether Structural Analogs to Thyroid Hormone Transport Proteins and Receptors

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Figure S2. Competitive binding curves of the tetrabromobisphenol A (TBBPA) mono-ether structural analogs [TBBPA-mono(glycidyl ether) (TBBPA-MGE), TBBPA-mono(allyl ether) (TBBPA-MAE) and TBBPA-mono(2,3-dibromopropyl ether) (TBBPA-MDBPE)], the TBBPA bis-ether derivatives [TBBPA-bis(glycidyl ether) (TBBPA-BGE), TBBPA-bis(allyl ether) (TBBPA-BAE) and TBBPA-bis(2,3-dibromopropyl ether) (TBBPA-BDBPE)] and TBBPA with thyroxine-binding globulin (TBG). Three replicate wells were conducted for each group in a 96-well plate. Error bars represent the standard deviation of three replicates. *$p < 0.05$, compared with the control group (1% dimethyl sulfoxide). The p values of the experimental data were analyzed using one-way analysis of variance (ANOVA), followed by a least significant difference multiple comparisons test (IBM SPSS Statistics 20). See the summary data in Table S3.

Figure S3. The interactions of tetrabromobisphenol A (TBBPA) mono-ether structural analogs [TBBPA-mono(glycidyl ether) (TBBPA-MGE), TBBPA-mono(allyl ether) (TBBPA-MAE) and TBBPA-mono(2,3-dibromopropyl ether) (TBBPA-MDBPE)] and TBBPA with transthyretin (TTR).

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Figure S6. The interactions of tetrabromobisphenol A (TBBPA) mono-ether structural analogs [TBBPA-mono(glycidyl ether) (TBBPA-MGE), TBBPA-mono(allyl ether) (TBBPA-MAE) and TBBPA-mono(2,3-dibromopropyl ether) (TBBPA-MDBPE)] and TBBPA with thyroid hormone receptor α-ligand binding domain (TRα-LBD).

Figure S7. The interactions of tetrabromobisphenol A (TBBPA) mono-ether structural analogs [TBBPA-mono(glycidyl ether) (TBBPA-MGE), TBBPA-mono(allyl ether) (TBBPA-MAE) and TBBPA-mono(2,3-dibromopropyl ether) (TBBPA-MDBPE)] and TBBPA with thyroid hormone receptor β-ligand binding domain (TRβ-LBD).

Table S1. Summary data for Figure S1. “SD” means standard deviation of three replicates.

Table S2. Summary data for Figure 2. “RBP” means relative binding potency. “SD” means standard deviation of three replicates.

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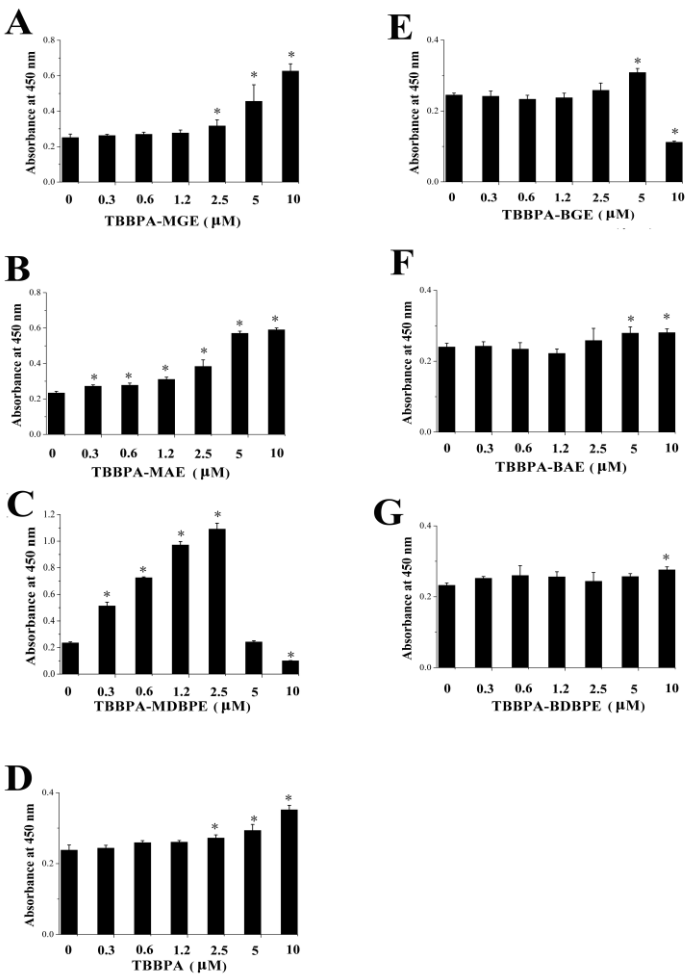
Table S9. Summary data for Figure 5. “RCP” means relative cell proliferation compared with dimethyl sulfoxide control group. “SD” means standard deviation of three replicates.

Table S10. Summary data for Figure 6. “RCP” means relative cell proliferation compared with dimethyl sulfoxide control group. “SD” means standard deviation of three replicates.



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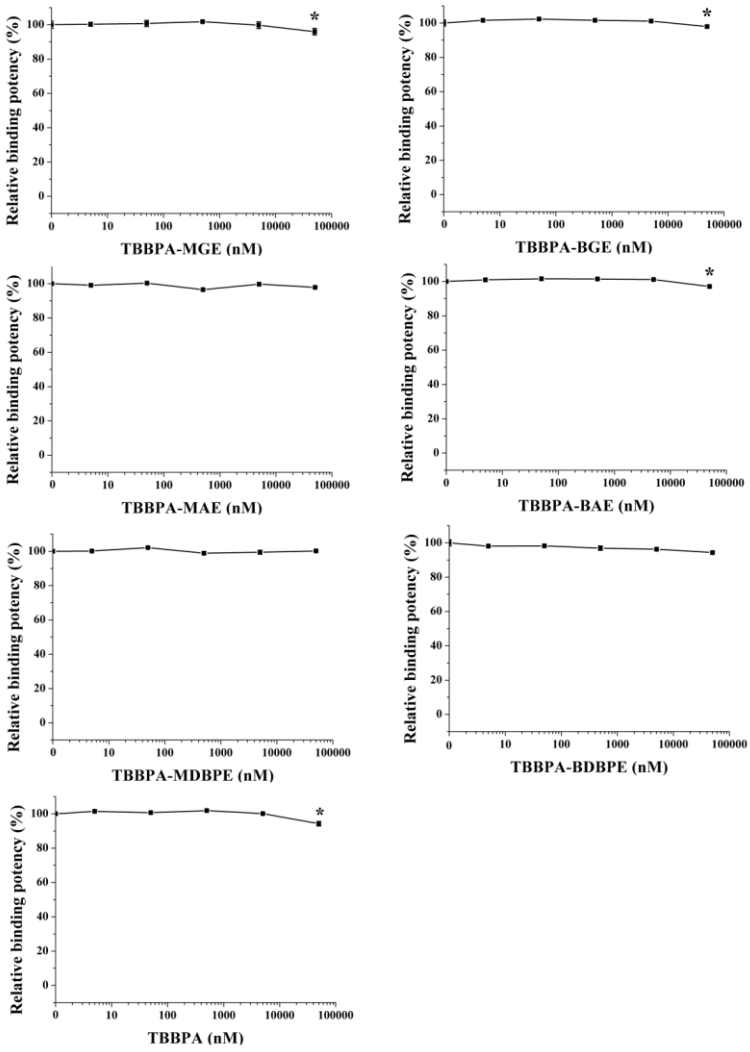

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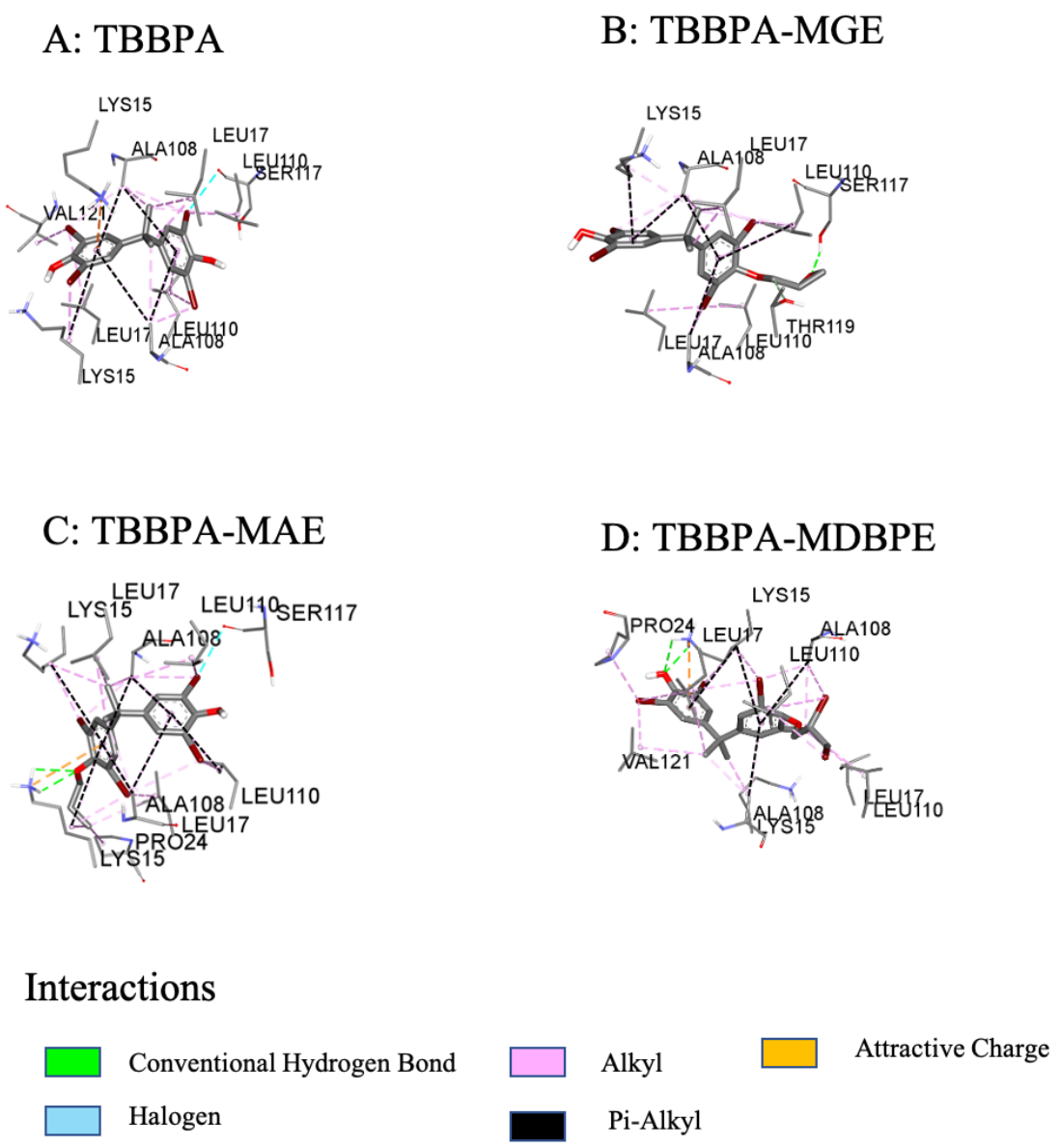

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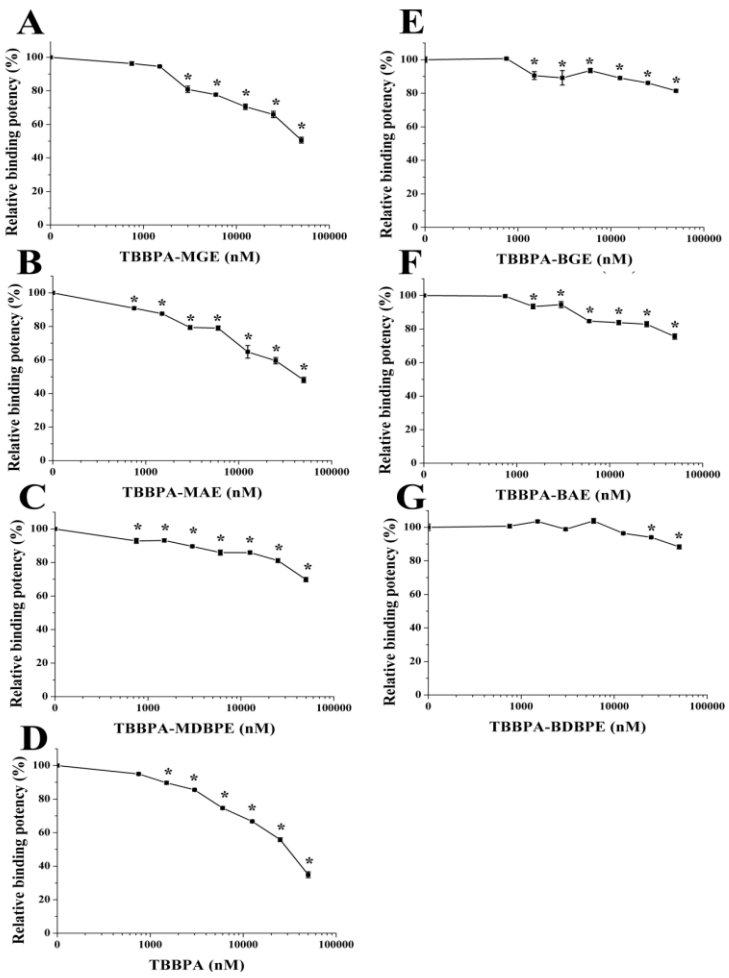

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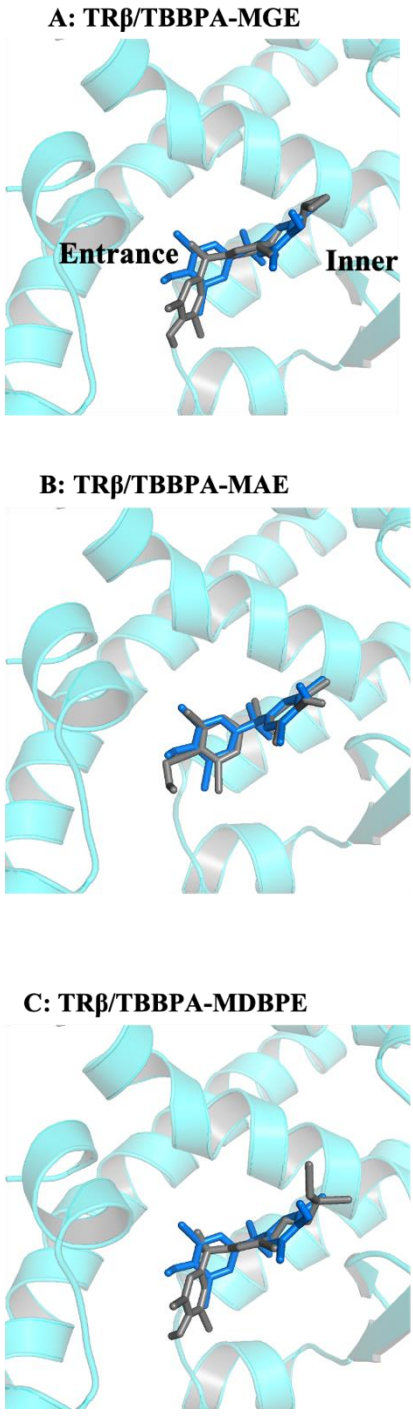

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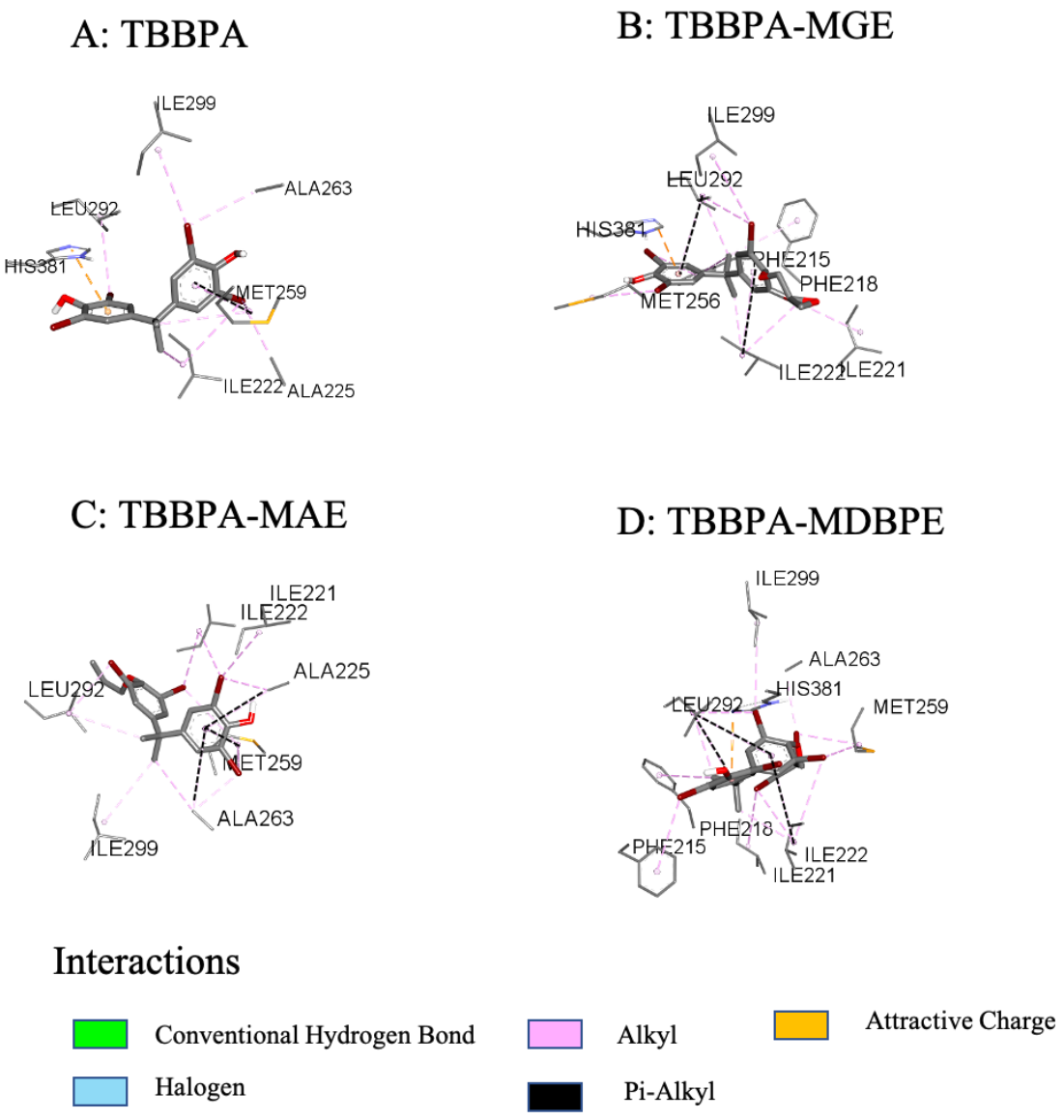

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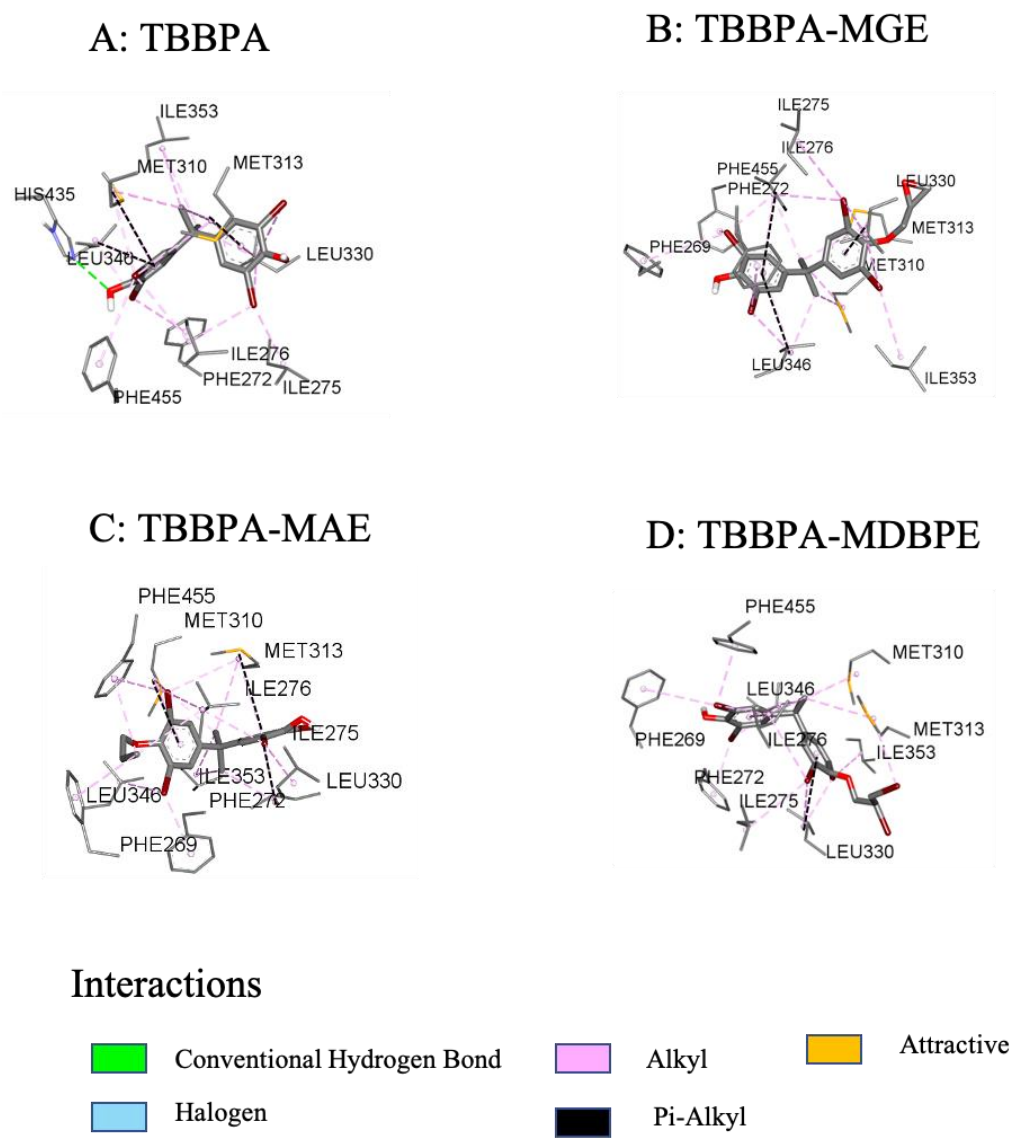

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Table S1. Summary data for Figure S1. “SD” means standard deviation of three replicates.

A: TBBPA-MGE	mean absorbance at 450 nm	SD
0 μM	0.251	0.020
0.3 μM	0.263	0.006
0.6 μM	0.270	0.011
1.2 μM	0.277	0.017
2.5 μM	0.317	0.034
5 μM	0.456	0.093
10 μM	0.626	0.040

B: TBBPA-MAE	mean absorbance at 450 nm	SD
0 μM	0.234	0.009
0.3 μM	0.272	0.007
0.6 μM	0.278	0.012
1.2 μM	0.311	0.012
2.5 μM	0.384	0.037
5 μM	0.571	0.011
10 μM	0.590	0.010

C: TBBPA-MDBPE	mean absorbance at 450 nm	SD
0 μM	0.235	0.007
0.3 μM	0.513	0.027
0.6 μM	0.725	0.006
1.2 μM	0.971	0.025
2.5 μM	1.091	0.044
5 μM	0.242	0.009
10 μM	0.101	0.001

D: TBBPA	mean absorbance at 450 nm	SD
0 μM	0.238	0.015
0.3 μM	0.244	0.008
0.6 μM	0.259	0.006
1.2 μM	0.261	0.005
2.5 μM	0.272	0.009
5 μM	0.294	0.017
10 μM	0.352	0.012

E: TBBPA-BGE	mean absorbance at 450 nm	SD
0 μM	0.245	0.005
0.3 μM	0.242	0.014
0.6 μM	0.233	0.011
1.2 μM	0.238	0.013
2.5 μM	0.258	0.020
5 μM	0.308	0.011
10 μM	0.112	0.003

F: TBBPA-BAE	mean absorbance at 450 nm	SD
0 μM	0.240	0.010
0.3 μM	0.243	0.012
0.6 μM	0.234	0.018
1.2 μM	0.222	0.012
2.5 μM	0.258	0.034
5 μM	0.279	0.017
10 μM	0.281	0.010

G: TBBPA-BDBPE	mean absorbance at 450 nm	SD
0 μM	0.232	0.006
0.3 μM	0.252	0.005
0.6 μM	0.260	0.028
1.2 μM	0.256	0.014
2.5 μM	0.244	0.024
5 μM	0.257	0.008
10 μM	0.276	0.008

Table S2. Summary data for Figure 2. “RBP” means relative binding potency. “SD” means standard deviation of three replicates.

A: TBBPA-MGE	mean RBP	SD		**E: TBBPA-BGE**	mean RBP	SD
0 nM	100.0	0.3		0 nM	100.0	0.8
5 nM	99.0	0.3		5 nM	98.5	0.9
50 nM	97.6	0.5		50 nM	94.9	1.6
500 nM	57.3	2.2		500 nM	88.2	1.7
5000 nM	13.7	0.5		5000 nM	72.2	1.4
50000 nM	25.5	3.0		50000 nM	63.5	1.3
B: TBBPA-MAE	mean RBP	SD		**F: TBBPA-BAE**	mean RBP	SD
0 nM	100.0	0.3		0 nM	100.0	0.6
5 nM	95.5	0.9		5 nM	98.4	0.5
50 nM	91.8	0.3		50 nM	97.6	0.5
500 nM	1.8	1.7		500 nM	97.1	0.3
5000 nM	16.8	0.7		5000 nM	97.3	0.6
50000 nM	16.6	3.7		50000 nM	88.2	0.5
C: TBBPA-MDBPE	mean RBP	SD		**G: TBBPA-BDBPE**	mean RBP	SD
0 nM	100.0	0.7		0 nM	100.0	0.5
5 nM	97.9	0.7		5 nM	99.3	0.4
50 nM	97.4	0.4		50 nM	99.0	0.2
500 nM	85.8	0.8		500 nM	99.6	0.2
5000 nM	13.2	1.5		5000 nM	98.6	0.6
50000 nM	12.1	0.9		50000 nM	90.3	0.4
D: TBBPA	mean RBP	SD				
0 nM	100.0	0.5				
5 nM	97.8	0.2				
50 nM	93.0	0.2				
500 nM	29.2	0.7				
5000 nM	4.8	0.9				
50000 nM	10.7	1.7				

Table S3. Summary data for Figure S2. “RBP” means relative binding potency. “SD” means standard deviation of three replicates.

A: TBBPA-MGE	mean RBP	SD		**E: TBBPA-BGE**	mean RBP	SD
0 nM	100.0	2.1		0 nM	100.0	1.8
5 nM	100.2	1.1		5 nM	101.6	0.6
50 nM	100.7	1.8		50 nM	102.3	0.4
500 nM	101.8	0.5		500 nM	101.6	0.5
5000 nM	99.7	1.9		5000 nM	101.1	0.6
50000 nM	95.9	1.9		50000 nM	97.9	0.9
B: TBBPA-MAE	mean RBP	SD		**F: TBBPA-BAE**	mean RBP	SD
0 nM	100.0	0.5		0 nM	100.0	0.4
5 nM	99.0	0.3		5 nM	100.9	1.0
50 nM	100.3	0.4		50 nM	101.5	0.9
500 nM	96.5	0.5		500 nM	101.4	0.3
5000 nM	99.7	0.8		5000 nM	101.1	0.3
50000 nM	97.9	0.4		50000 nM	97.1	1.0
C: TBBPA-MDBPE	mean RBP	SD		**G: TBBPA-BDBPE**	mean RBP	SD
0 nM	100.0	1.0		0 nM	100.0	1.8
5 nM	100.1	0.4		5 nM	98.1	0.6
50 nM	102.1	0.5		50 nM	98.2	0.9
500 nM	98.9	0.9		500 nM	96.9	1.3
5000 nM	99.4	1.1		5000 nM	96.3	0.9
50000 nM	100.2	0.5		50000 nM	94.3	0.9
D: TBBPA	mean RBP	SD				
0 nM	100.0	0.8				
5 nM	101.5	0.6				
50 nM	100.7	0.6				
500 nM	101.9	0.3				
5000 nM	100.1	0.3				
50000 nM	94.2	1.3				

Table S4. The results of docking scores and interactions of tetrabromobisphenol A (TBBPA) mono-ether structural analogs [TBBPA-mono(glycidyl ether) (TBBPA-MGE), TBBPA-mono(allyl ether) (TBBPA-MAE) and TBBPA-mono(2,3-dibromopropyl ether) (TBBPA-MDBPE)] and TBBPA with transthyretin (TTR).

	TBBPA	**TBBPA-MGE**	**TBBPA-MAE**	**TBBPA-MDBPE**
Docking score	8.9	8.7	9.1	8.6
Hydrophobic interaction	(A): Leu 17, Ala 108, Leu 110, Val 121 (C): Lys 15, Leu 17, Ala 108, Leu 110	(A): Leu 17, Ala 108, Leu 110 (C): Leu 17, Pro 24, Ala 108, Leu 110	(A): Leu 17, Pro 24, Ala 108, Leu 110 (C): Lys 15, Leu 17, Ala 108, Leu 110	(A): Lys 15, Leu 17, Ala 108, Leu 110 (C): Leu 17, Ala 108, Leu 110, Thr 119
Hydrogen bond interaction		(C): Lys 15	(A): Lys 15	(A): Ser 117
π-cation interaction	(A): Lys 15			
Halogen interaction	(A): Ser 117	(A): Ser 117		

Note: The docking scores and the interactions between chemicals and TTR were obtained by flexible docking with AutoDock Vina. The higher docking score means higher potential binding potency between a chemical and TTR. (A): means the monomer A of TTR. (C): means the monomer C of TTR.

Table S5. Summary data for Figure 4. “RBP” means relative binding potency. “SD” means standard deviation of three replicates.

A: TBBPA-MGE	mean RBP	SD		**E: TBBPA-BGE**	mean RBP	SD
0 nM	100.0	1.0		0 nM	100.0	2.7
750 nM	98.2	2.4		750 nM	107.8	0.9
1500 nM	93.7	2.7		1500 nM	105.7	1.6
3000 nM	84.0	2.3		3000 nM	106.2	1.3
6000 nM	80.4	0.9		6000 nM	101.9	0.3
12500 nM	79.1	1.1		12500 nM	101.9	1.9
25000 nM	73.2	1.3		25000 nM	99.3	0.9
50000 nM	65.5	1.6		50000 nM	95.3	1.2
B: TBBPA-MAE	mean RBP	SD		**F: TBBPA-BAE**	mean RBP	SD
0 nM	100.0	0.5		0 nM	100.0	0.6
750 nM	89.5	0.3		750 nM	98.4	0.6
1500 nM	89.1	0.4		1500 nM	98.8	0.2
3000 nM	84.6	0.8		3000 nM	98.9	0.2
6000 nM	79.3	1.9		6000 nM	94.7	0.1
12500 nM	78.5	1.2		12500 nM	94.6	0.7
25000 nM	71.4	1.5		25000 nM	92.8	0.2
50000 nM	58.6	2.2		50000 nM	87.7	0.9
C: TBBPA-MDBPE	mean RBP	SD		**G: TBBPA-BDBPE**	mean RBP	SD
0 nM	100.0	1.3		0 nM	100.0	0.2
750 nM	96.7	0.3		750 nM	99.2	0.5
1500 nM	94.4	1.5		1500 nM	99.6	0.8
3000 nM	92.8	0.8		3000 nM	99.8	0.5
6000 nM	89.3	1.1		6000 nM	96.4	0.3
12500 nM	89.5	1.2		12500 nM	95.4	0.3
25000 nM	88.9	1.0		25000 nM	94.0	1.1
50000 nM	80.0	1.5		50000 nM	86.2	0.5
D: TBBPA	mean RBP	SD				
0 nM	100.0	2.9				
750 nM	85.8	0.3				
1500 nM	81.5	0.4				
3000 nM	70.4	0.7				
6000 nM	60.5	1.0				
12500 nM	50.8	0.3				
25000 nM	43.2	1.1				
50000 nM	33.9	1.3				

Table S6. Summary data for Figure S4. “RBP” means relative binding potency. “SD” means standard deviation of three replicates.

A: TBBPA-MGE	mean RBP	SD		**E: TBBPA-BGE**	mean RBP	SD
0 nM	100.0	0.6		0 nM	100.0	1.6
750 nM	96.2	1.1		750 nM	100.7	0.5
1500 nM	94.5	0.2		1500 nM	90.5	2.4
3000 nM	80.9	1.9		3000 nM	89.2	4.3
6000 nM	77.7	0.9		6000 nM	93.5	1.3
12500 nM	70.5	1.7		12500 nM	89.1	1.0
25000 nM	65.9	1.9		25000 nM	86.1	0.8
50000 nM	50.6	1.8		50000 nM	81.4	0.8
B: TBBPA-MAE	mean RBP	SD		**F: TBBPA-BAE**	mean RBP	SD
0 nM	100.0	0.8		0 nM	100.0	0.6
750 nM	90.9	0.7		750 nM	99.6	0.8
1500 nM	87.6	0.1		1500 nM	93.5	1.4
3000 nM	79.4	1.2		3000 nM	94.5	1.8
6000 nM	79.0	1.3		6000 nM	84.7	0.7
12500 nM	64.8	3.7		12500 nM	83.8	1.3
25000 nM	59.5	1.9		25000 nM	82.9	1.6
50000 nM	48.0	1.6		50000 nM	75.5	1.6
C: TBBPA-MDBPE	mean RBP	SD		**G: TBBPA-BDBPE**	mean RBP	SD
0 nM	100.0	0.6		0 nM	100.0	2.0
750 nM	92.9	1.4		750 nM	100.7	1.1
1500 nM	93.1	1.0		1500 nM	103.5	0.7
3000 nM	89.6	0.5		3000 nM	98.8	0.7
6000 nM	85.9	1.5		6000 nM	103.8	1.4
12500 nM	85.9	1.0		12500 nM	96.4	0.4
25000 nM	81.1	1.2		25000 nM	94.0	0.2
50000 nM	69.8	1.3		50000 nM	88.3	1.2
D: TBBPA	mean RBP	SD				
0 nM	100.0	0.9				
750 nM	95.0	0.5				
1500 nM	89.7	0.9				
3000 nM	85.5	0.1				
6000 nM	74.7	0.9				
12500 nM	66.7	0.2				
25000 nM	55.8	1.1				
50000 nM	34.9	1.7				

Table S7. The results of docking scores and interactions of tetrabromobisphenol A (TBBPA) mono-ether structural analogs [TBBPA-mono(glycidyl ether) (TBBPA-MGE), TBBPA-mono(allyl ether) (TBBPA-MAE) and TBBPA-mono(2,3-dibromopropyl ether) (TBBPA-MDBPE)] and TBBPA with thyroid hormone receptor α-ligand binding domain (TRα-LBD).

	TBBPA	**TBBPA-MGE**	**TBBPA-MAE**	**TBBPA-MDBPE**
Docking score	10.1	9.7	9.9	9.4
Hydrophobic interaction	Ile 222, Ala 225, Met 259, Ala 263, Leu 292, Ile 299	Phe 215, Phe 218, Ile 221, Ile 222, Met 256, Leu 292, Ile 299	Ile 221, Ile 222, Ala 225, Met 259 Ala 263, Leu 292, Ile 299;	Phe 215, Phe 218, Ile 221, Ile 222, Met 259 Ala 263, Leu 292, Ile 299, His 381
π-cation interaction	His 381	His 381		

Note: The docking scores and the interactions between chemicals and TRα-LBD were obtained by flexible docking with AutoDock Vina. The higher docking score means higher potential binding potency between a chemical and TRα-LBD.

Table S8. The results of docking scores and interactions of tetrabromobisphenol A (TBBPA) mono-ether structural analogs [TBBPA-mono(glycidyl ether) (TBBPA-MGE), TBBPA-mono(allyl ether) (TBBPA-MAE) and TBBPA-mono(2,3-dibromopropyl ether) (TBBPA-MDBPE)] and TBBPA with thyroid hormone receptor β-ligand binding domain (TRβ-LBD).

	TBBPA	**TBBPA-MGE**	**TBBPA-MAE**	**TBBPA-MDBPE**
Docking score	9.8	9.0	9.8	8.9
Hydrophobic interaction	Phe 272, Ile 275, Ile 276, Met 310, Met 313, Leu 330, Leu 346, Ile 353	Phe 269, Phe 272, Ile 275, Ile 276, Met 310, Met 313, Leu 330, Leu 346, Ile 353, Phe 455	Phe 269, Phe 272, Ile 275, Ile 276, Met 310, Met 313, Leu 330, Leu 346, Ile 353, Phe 455	Phe 269, Phe 272, Ile 275, Ile 276, Met 310, Met 313, Leu 330, Leu 346, Ile 353, Phe 455
Hydrogen bond interaction	His 435			

Note: The docking scores and the interactions between chemicals and TRβ-LBD were obtained by flexible docking with AutoDock Vina. The higher docking score means higher potential binding potency between a chemical and TRβ-LBD.

Table S9. Summary data for Figure 5. “RCP” means relative cell proliferation compared with dimethyl sulfoxide control group. “SD” means standard deviation of three replicates.

	Without amiodarone		With 2 μM amiodarone	
A: TBBPA-MGE	mean RCP	SD	mean RCP	SD
0 μM	100.0	7.9	93.3	4.0
0.3 μM	104.6	2.5	105.4	2.7
0.6 μM	107.4	4.2	103.3	1.1
1.2 μM	110.2	6.0	105.0	8.4
2.5 μM	126.1	10.9	109.2	7.6
5 μM	181.6	20.3	131.0	11.7
10 μM	249.4	6.4	160.8	8.3

	Without amiodarone		With 2 μM amiodarone	
B: TBBPA-MAE	mean RCP	SD	mean RCP	SD
0 μM	100.0	3.9	93.0	4.1
0.3 μM	116.5	2.7	107.0	1.5
0.6 μM	118.9	4.3	103.9	7.1
1.2 μM	132.9	4.0	114.1	4.6
2.5 μM	164.2	9.6	116.7	10.0
5 μM	244.1	2.0	146.6	2.1
10 μM	252.6	1.7	60.2	1.9

	Without amiodarone		With 2 μM amiodarone	
C: TBBPA-MDBPE	mean RCP	SD	mean RCP	SD
0 μM	100.0	2.9	93.4	7.1
0.3 μM	218.2	5.3	145.2	7.1
0.6 μM	308.5	0.8	190.0	6.9
1.2 μM	413.1	2.6	267.3	3.2
2.5 μM	464.1	4.0	188.6	10.3
5 μM	102.9	3.6	53.1	1.9
10 μM	42.8	1.0	42.5	1.9

	Without amiodarone		With 2 μM amiodarone	
D: TBBPA	mean RCP	SD	mean RCP	SD
0 μM	100.0	6.1	94.6	5.6
0.3 μM	102.5	3.1	100.6	3.6
0.6 μM	108.9	2.1	102.4	6.9
1.2 μM	109.5	1.7	109.3	4.6
2.5 μM	114.4	3.2	111.8	6.6
5 μM	123.3	5.8	122.8	2.8
10 μM	147.9	3.5	129.6	5.9

	Without amiodarone		With 2 μM amiodarone	
E: TBBPA-BGE	mean RCP	SD	mean RCP	SD
0 μM	100.0	2.2	94.5	5.6
0.3 μM	98.7	6.0	97.1	5.1
0.6 μM	95.2	4.6	97.9	0.1
1.2 μM	97.0	5.3	101.0	6.6
2.5 μM	105.5	7.8	101.7	3.3
5 μM	125.9	3.6	89.3	3.8
10 μM	45.7	2.8	43.9	3.0

	Without amiodarone		With 2 μM amiodarone	
F: TBBPA-BAE	mean RCP	SD	mean RCP	SD
0 μM	100.0	4.2	98.2	1.8
0.3 μM	101.0	5.0	102.4	2.6
0.6 μM	97.6	7.6	92.9	7.7
1.2 μM	92.4	5.6	99.6	10.4
2.5 μM	107.5	13.3	95.7	10.4
5 μM	116.3	6.2	105.9	12.4
10 μM	116.9	3.7	105.7	6.9

	Without amiodarone		With 2 μM amiodarone	
G: TBBPA-BDBPE	mean RCP	SD	mean RCP	SD
0 μM	100.0	2.7	99.0	8.5
0.3 μM	108.5	1.9	108.7	8.8
0.6 μM	111.8	10.6	105.0	5.3
1.2 μM	110.2	5.5	110.8	7.3
2.5 μM	104.9	10.0	100.2	1.7
5 μM	110.6	3.1	105.3	7.9
10 μM	118.8	2.9	119.8	3.3

Table S10. Summary data for Figure 6. “RCP” means relative cell proliferation compared with dimethyl sulfoxide control group. “SD” means standard deviation of three replicates.

A: TBBPA-MGE	mean RCP	SD		**E: TBBPA-BGE**	mean RCP	SD
0 μM	100.0	0.8		0 μM	100.0	9.9
0.3 μM	98.9	2.2		0.3 μM	102.5	10.2
0.6 μM	106.0	6.1		0.6 μM	101.0	5.2
1.2 μM	117.7	3.2		1.2 μM	104.1	6.4
2.5 μM	118.9	4.0		2.5 μM	95.8	3.2
5 μM	126.8	2.1		5 μM	56.4	2.6
10 μM	142.3	7.5		10 μM	15.0	0.5
B: TBBPA-MAE	mean RCP	SD		**F: TBBPA-BAE**	mean RCP	SD
0 μM	100.0	0.6		0 μM	100.0	3.9
0.3 μM	109.3	2.3		0.3 μM	101.6	7.5
0.6 μM	115.1	2.2		0.6 μM	105.6	2.2
1.2 μM	123.2	4.4		1.2 μM	91.2	0.9
2.5 μM	126.6	2.4		2.5 μM	73.0	2.8
5 μM	131.6	4.1		5 μM	46.3	1.3
10 μM	81.3	5.8		10 μM	43.9	0.7
C: TBBPA-MDBPE	mean RCP	SD		**G: TBBPA-BDBPE**	mean RCP	SD
0 μM	100.0	1.3		0 μM	100.0	1.9
0.3 μM	112.9	1.5		0.3 μM	100.6	1.9
0.6 μM	123.3	1.9		0.6 μM	105.9	2.2
1.2 μM	143.6	7.2		1.2 μM	101.7	3.8
2.5 μM	120.6	5.5		2.5 μM	104.1	5.2
5 μM	21.5	1.2		5 μM	109.9	9.8
10 μM	12.4	0.1		10 μM	116.5	1.1
D: TBBPA	mean RCP	SD				
0 μM	100.0	2.2				
0.3 μM	101.9	2.8				
0.6 μM	103.5	2.7				
1.2 μM	105.9	3.9				
2.5 μM	111.6	4.5				
5 μM	112.6	2.1				
10 μM	120.8	10.0				