Supporting Information

An Immunoassay to Evaluate Human/Environmental Exposure to the Antimicrobial Triclocarban

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Number of Tables: 4

Hapten Syntheses

Chemicals and Instruments. All reagents were analytical grade (Sigma-Aldrich, St. Louis, MO). Precoated silica gel 60 F254 glass plates (0.25 mm, EMD Chemicals, Gibbstown, NJ) were used for thin layer chromatography (TLC) analysis. Silica gel was used for column chromatography. Proton NMR (^{1}H NMR) spectra were measured with a Bruker Avance 500 NMR or a General Electric QE-300 spectrometer (Bruker NMR, Billerica, ME) using tetramethylsilane as an internal standard. Electrospray mass spectra of haptens in negative (MS-ESI$^-$) mode were recorded by a Micromass Quattro Ultima triple quadrupole tandem mass spectrometer (Micromass, Manchester, UK). R_f values refer to TLC on the silica gel plates with visualization under exposure to either ultraviolet light (254 nm) or iodine vapor.

Nomenclature. The nomenclature of haptens was designated with the aid of Chemdraw Ultra 11.0 (CambridgeSoft, Cambridge, MA).

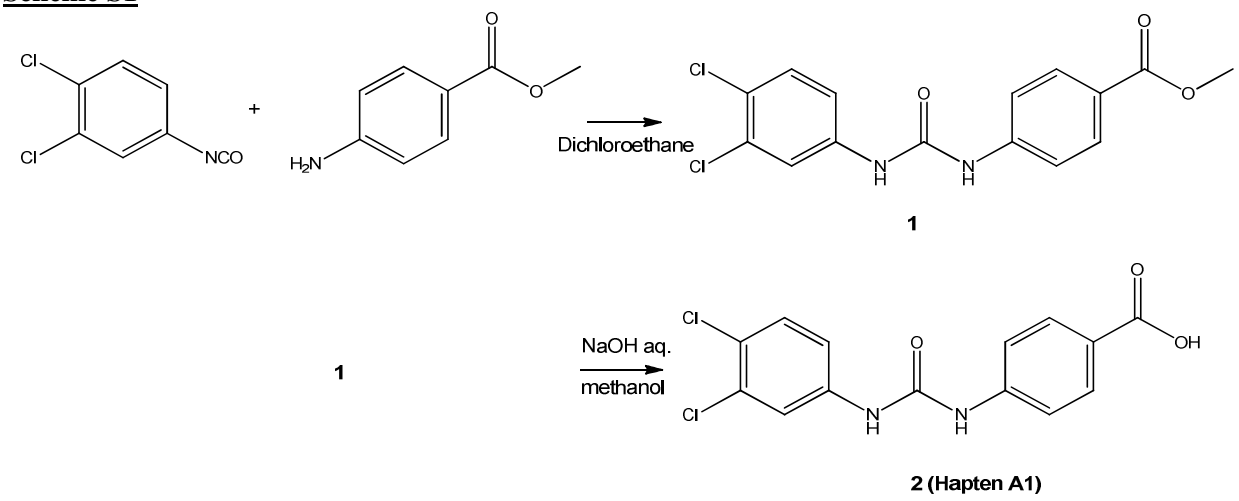
Preparation of Haptens. Hapten types A-E are shown in Figure 1 of the main article.

Type A Haptens

Synthesis of Hapten A1 (Scheme S1). The mixture of methyl 4-aminobenzoate (100 mg, 0.66 mmol) and 3,4-dichlorophenyl isocyanate (124 mg, 0.66 mmol) in 1,2-dichloroethane (6 mL) was stirred at 70 ^{o}C for 24 h, and then the solvent was removed under reduced pressure. The residues was purified by silica gel chromatography with a mixture of ethyl acetate and hexanes (1:1, v/v) to give methyl 4-(3-(3,4-dichlorophenyl)ureido)benzoate (166 mg, yield 73%, **1**) as a white solid. TLC [hexanes/ethyl acetate (1:1, v/v)] R_f, 0.50. ^{1}H NMR (DMSO-d_6) δ 3.89 (CH_3, s, 3H), 7.11-7.98 (2Ar, m, 7H), 8.42 (NH, s, 1H), 8.45 (NH, s, 1H).

The hydrolysis reaction of ester compound **1** (156 mg, 0.46 mmol) was conducted in a mixture of methanol (15 mL) and 1N NaOH (15 mL) at 60 ^{o}C overnight. The resulting mixture was acidified to pH 4 with 6 N HCl and extracted twice with a mixture of ethyl acetate and hexane (1:1, v/v, 50 mL). The combined organic phase was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was recrystallized from a mixture of ethyl acetate and hexane to give 125 mg (yield 84%) of a white solid 4-(3-(3,4-dichlorophenyl)ureido)benzoic acid (**2**) designated as **Hapten Al**; TLC [methanol/methylene chloride/acetic acid (0.5:9.5:0.1, v/v)] R_f, 0.30. ^{1}H NMR (D_2O/ DMSO-d_6) δ 7.31-8.19 (2Ar, m, 7H), 8.99 (NH, s, 1H), 9.04 (NH, s, 1H), 12.45 (COOH, s, 1H). MS-ESI *m/z* calcd for [M - H]$^-$ = $C_{14}H_{10}Cl_2N_2O_3$, 324.01; observed, 323.1.

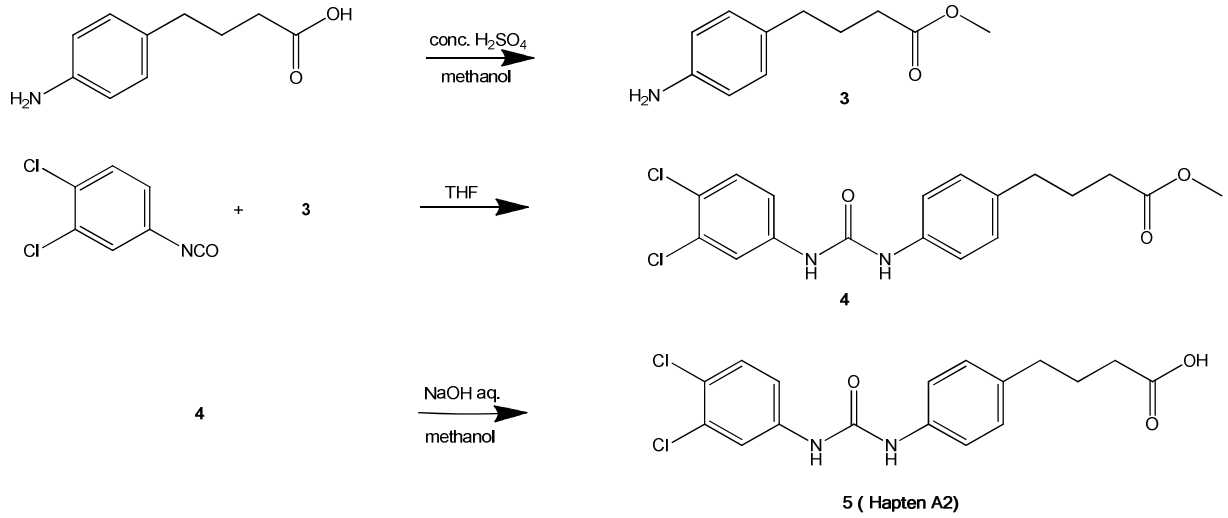
Scheme S1



Synthesis of Hapten A2 (Scheme S2). Methyl 4-(4-aminophenyl)butyrate (**3**) was synthesized according to the previous method (Kasagami et al., 2009). A mixture of 3,4-dichlorophenyl isocyanate (0.1 mmol) and methyl 4-(4-aminophenyl)butyrate (**3**, 0.1 mmol) in 8 mL of anhydrous tetrahydrofuran (THF) was stirred at room temperature for 1 h under an atmosphere of nitrogen. After evaporation, the residue was washed with hexane-ethyl acetate (1:1, 10 mL). The solid as crude product was purified by silica gel column chromatography eluting with hexane-ethyl acetate (1:1) then ethyl acetate only to afford the desired urea (**4**, methyl 4-[3-(3,4-dichlorophenyl)ureido]phenylbutanoate, yield 49%) as a solid. ^{1}H NMR ($CDCl_3$) δ 1.90 ($CH_2CH_2CH_2COO$, m, 2H), 2.33 ($CH_2CH_2CH_2COO$, m, 2H), 2.57 ($CH_2CH_2CH_2COO$, m, 2H), 3.68 ($COOCH_3$, s, 3H), 7.06-7.39 (2Ar, m, 7H), 7.31 (NH, s, 1H), 7.16 (NH, s, 1H).

4-[3-(3,4-Dichlorophenyl)ureido]phenylbutanoic acid (yield 80%, **5**) of a white solid designated as **Hapten A2** was obtained by alkaline hydrolysis as described above. ^{1}H NMR (DMSO-d_6) δ 1.77 ($CH_2CH_2CH_2COO$, m, 2H), 2.20 ($CH_2CH_2CH_2COO$, m, 2H), 2.53 ($CH_2CH_2CH_2COO$, m, 2H), 7.10-7.88 (2Ar, m, 7H), 8.70 (NH, s, 1H), 8.94 (NH, s, 1H), 12.02 (COOH, s, 1H). MS-ESI *m*/*z* calcd for $[M - H]^-$ = $C_{17}H_{16}Cl_2N_2O_3$, 366.05; observed, 365.4.

Scheme S2



Type B Haptens

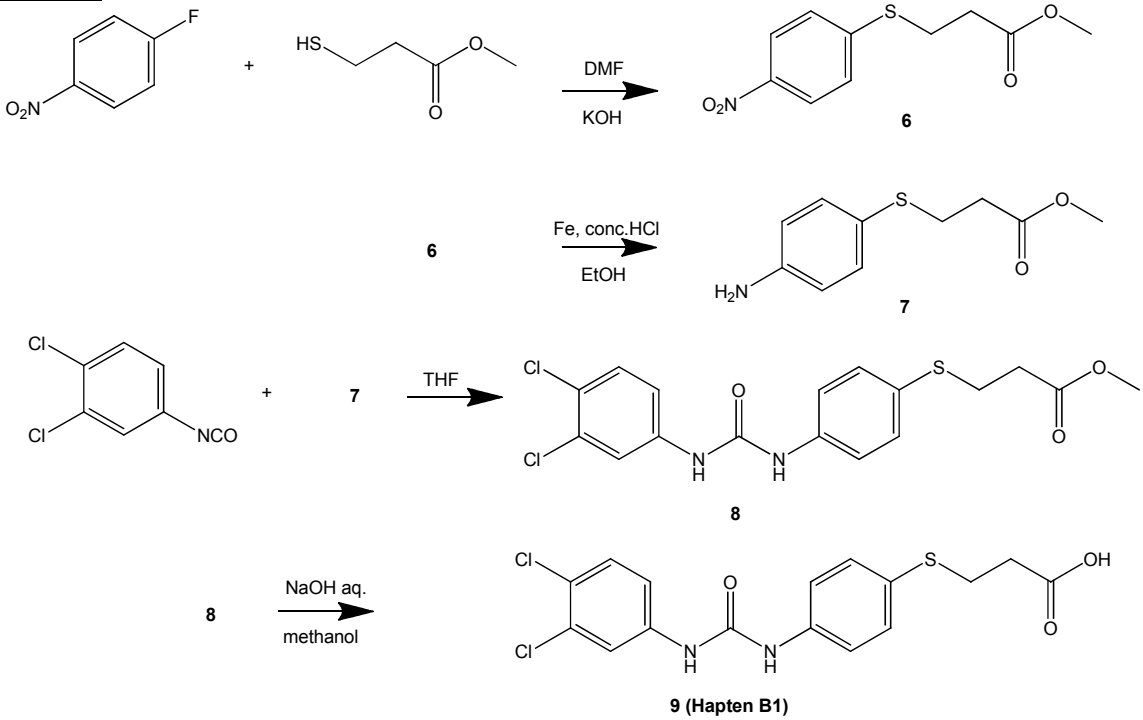
Synthesis of Hapten B1 (Scheme S3). To a mixture of 1-fluoro-4-nitrobenzene (0.877 g, 6.22 mmol) and methyl 3-mercaptopropionate (1.31 g, 10.9 mmol) in 10 mL of DMF was added KOH (0.48 g, 8.56 mmol, 1.40 eq, dissolved in 3 mL of aqueous dimethylformamide; DMF) at 0 °C with stirring. This mixture was stirred at 80 °C for 1 h followed by pouring into ice-water (ca. 60 mL). A yellow-colored solid was obtained by vacuum filtration, and then recrystallized from methanol-water. Methyl 3-[(4-nitrophenyl)-thio]propionate (**6**, yield 1.23 g, 82%) was used for the next reaction.

A mixture of compound **6** (1.10 g, 4.56 mmol), iron powder (0.764 g, 13.7 mmol) and conc. HCl (100 μL) in 10 mL of ethanol was refluxed for 2 h. This hot solution was filtered. The filtrate was poured into water (25 mL). The mixture was extracted with ethyl acetate (25 mL x 3) then the organic layer was dried over sodium sulfate and evaporated. The crude product was purified by silica gel column chromatography eluting with hexane-ethyl acetate (2:1) to afford methyl 3-[(4-aminophenyl)thio]-propionate (**7**) as a red oil (yield 78%). ^{1}H NMR ($CDCl_3$) δ 2.56 (t, J_{H-H}=7.5 Hz, 2H), 2.99 (t, J_{H-H}=7.5 Hz, 2H), 3.66 (s, 3H), 3.71 (s br, 2H), 6.62 (d, J_{H-H}=8.5 Hz, 2H), 7.26 (d, J_{H-H}=8.5 Hz, 2H).

Methyl 4-[3-(3,4-dichlorophenyl)ureido]phenylthiopropionate (**8**) was obtained after reaction of 3,4-dichlorophenyl isocyanate and the amine **7** as described above**.** ^{1}H NMR ($CDCl_3$-DMSO) δ 2.57 (SCH_2CH_2COO, t, J_{H-H}=7.0 Hz, 2H), 3.06 (SCH_2CH_2COO, t, J_{H-H}=7.5 Hz, 2H), 3.65 ($COOCH_3$, s, 3H), 7.23 (d, J_{H-H}=9.0 Hz, 1H), 7.31-7.34 (m, 3H), 7.42 (d, J_{H-H}=8.5 Hz, 2H), 7.81 (s, 1H), 8.50 (s br, 1H), 8.66 (s br, 1H).

4-[3-(3,4-Dichlorophenyl)ureido]phenylthiopropionic acid a white solid (yield 69%, **9**) designated as **Hapten B1** was obtained by alkaline hydrolysis as described above. MS-ESI *m/z* calcd for $[M - H]^- = C_{16}H_{14}Cl_2N_2O_3S$, 384.01; observed, 383.3.

Scheme S3



Type C Haptens

Synthesis of Hapten C1 (Scheme S4). To a solution of 3-chloro-4-fluoronitrobenzene (1.05 g, 6 mmol) and methyl 3-mercaptopropionate (1.26 g, 10.5 mmol) in DMF (10 mL) was added dropwise a DMF solution (5 mL) containing 0.46 g of KOH (8.28 mmol) dissolved in H_2O (250 μL). The reaction mixture was stirred at 80 °C for 1 h. Ice water (60 mL) was added after the reaction mixture was cooled to room temperature. The yellow-colored solid was precipitated and then filtered. The yellow solid was recrystallized from methanol to give methyl 3-(4-nitrophenylthio)propanoate (950 mg, yield 58%, **10**). TLC [hexanes/acetone (2:1, v/v)] R_f, 0.25.

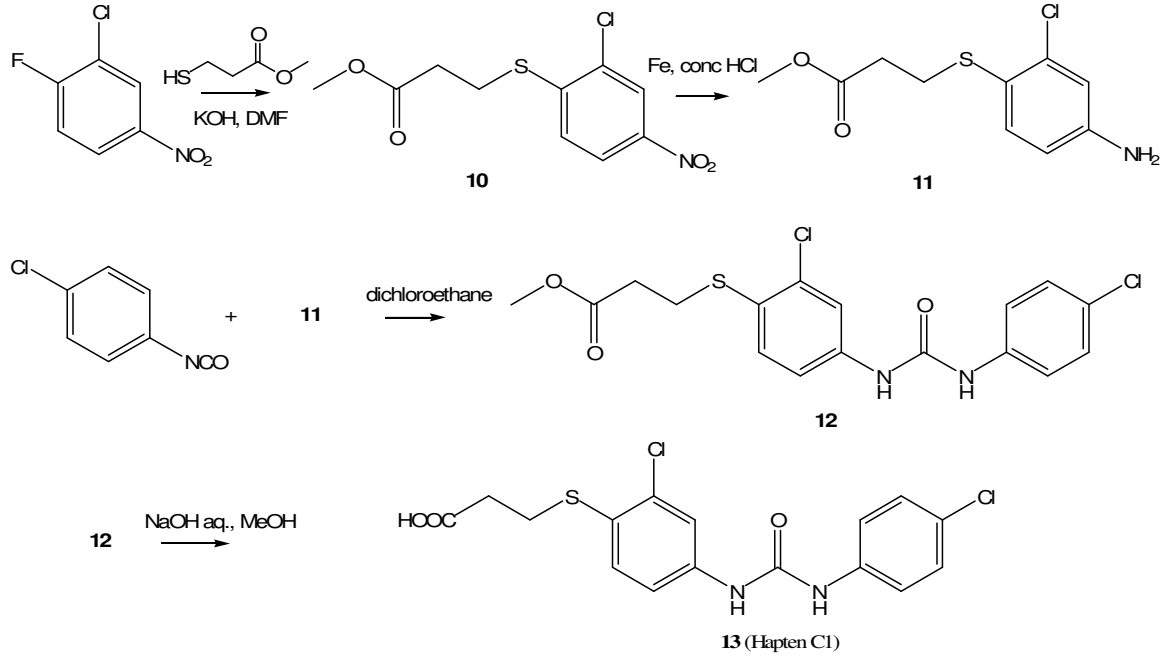
The mixture of the compound **10** (900 mg, 3.27 mmol) and Fe (0.669 g) in 8 mL of ethanol and 4 mL of H_2O and conc. HCl (50 uL) was overnight stirred at 70 °C in an oil bath. The mixture was cooled and poured into water (25 mL). The mixture was filtered, and the solids were washed with water. The yellow solid was dried to give methyl 3-(4-aminophenylthio)propanoate (650 mg, yield 81%, **11**). TLC [hexanes/ethyl acetate (4:1, v/v)] R_f, 0.13.

The mixture of the compound **11** (0.4 g, 1.63 mmol) and 4-chlorophenyl isocyanate (250 mg, 1.63 mmol) in 1,2-dichloroethane (6 mL) was stirred at 70 °C for 24 h, and then the solvent was removed under reduced pressure. The residue was recrystallized from a mixture of hexanes and ethyl acetate to give methyl 3-((2-chloro-4-(3-(4-chlorophenyl)ureido)phenyl)thio)propanoate (630 mg, yield 97%, **12**). TLC hexanes/ethyl acetate (1:1, v/v)] R_f, 0.44. ^{1}H NMR ($CDCl_3$-DMSO) δ 2.62 (SCH_2CH_2COO, t, J_{H-H}=7.0 Hz, 2H), 3.13 (SCH_2CH_2COO, t, J_{H-H}=7.5 Hz, 2H), 3.60 ($COOCH_3$, s, 3H), 7.30-7.76 (2Ar, m, 7H), 8.94 (NH, s, 1H), 9.90 (NH, s, 1H).

The hydrolysis reaction of the ester compound **12** (220 mg, 0.5 mmol) was conducted in a mixture of THF (5 mL) and 1N NaOH (5 mL) overnight. The resulting mixture was acidified to pH 4 with 6 N HCl and extracted twice with ethyl acetate (30 mL). The combined organic phase was washed with distilled water (30 mL) and dried over anhydrous sodium sulfate. The solvent was removed under

reduced pressure. The residue was purified by using silica gel chromatography with the mixture of methanol/methylene chloride/acetic acid (1:19:0.02, v/v/v) as an eluant to give 3-((2-chloro-4-(3-(4-chlorophenyl)ureido)phenyl)thio)propanoic acid (150 mg, yield 78%, **13**) white solid designated as **Hapten C1.** TLC [methanol/methylene chloride/acetic acid (1:19:0.02, v/v/v)] R_f, 0.33. MS-ESI *m/z* calcd for $[M - H]^- = C_{16}H_{14}Cl_2N_2O_3S$, 383.01; observed, 383.07.

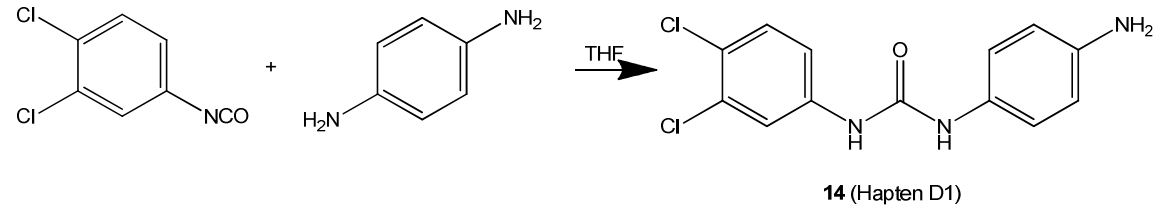
Scheme S4



Type D Hapten

Synthesis of Hapten D1 (Scheme S5). A mixture of 3,4-dichlorophenyl isocyanate (0.127 g, 0.675 mmol) and 1,4-phenylenediamine (0.073 g, 0.675 mmol) in 8 mL of anhydrous THF was stirred at room temperature for 1 h under an atmosphere of nitrogen. After evaporation, the residue was washed with hexane-ethyl acetate (1:1, 10 mL). The solid as crude product was purified by silica gel column chromatography eluting with hexane-ethyl acetate (1:1) then ethyl acetate only to afford 3-(4-aminophenyl)-1-(3,4-dichlorophenyl)urea as a solid (yield 52%, **14**) designated as **Hapten D1**. ^{1}H NMR (DMSO-d_6) δ 4.11 (NH_2, s, br, 2H), 7.31-8.19 (2Ar, m, 7H), 8.99 (NH, s, 1H), 9.04 (NH, s, 1H). MS-ESI *m/z* calcd for $[M - H]^- = C_{13}H_{11}Cl_2N_3O$, 295.03; observed, 294.3.

Scheme S5

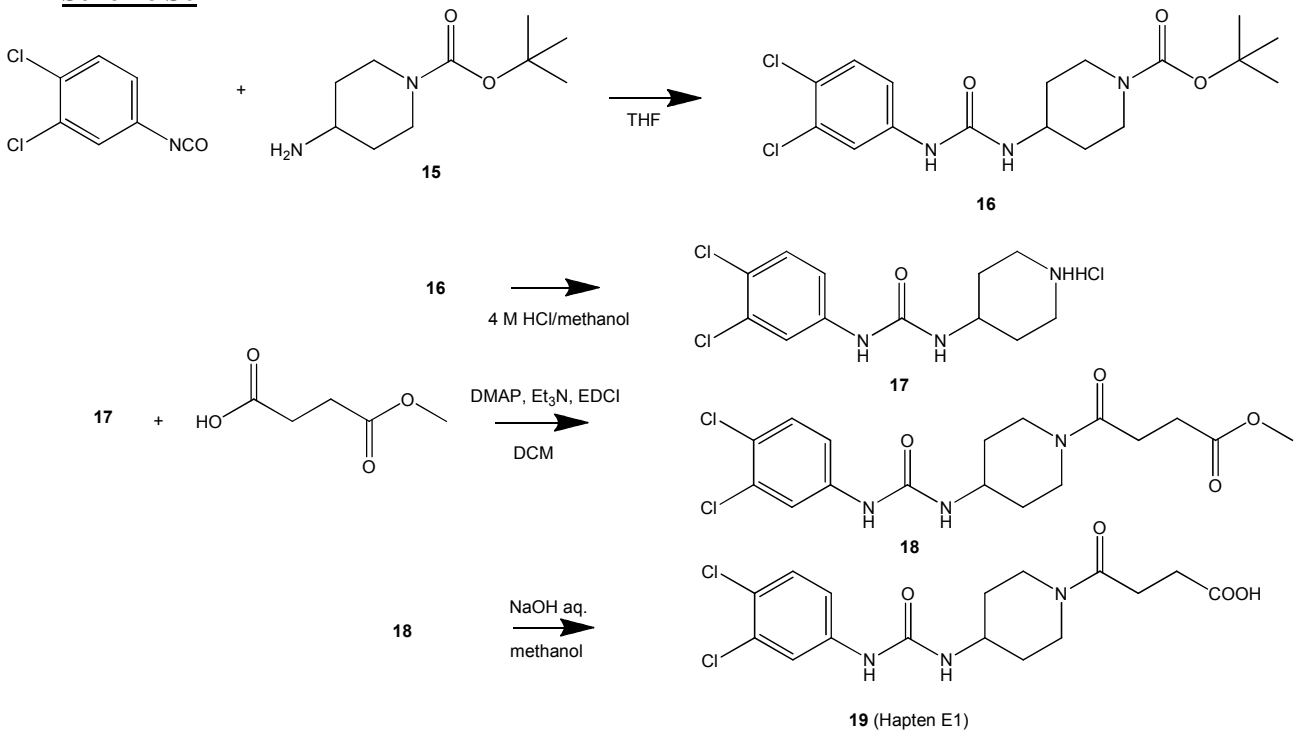


Type E Haptens

Synthesis of Hapten E1 (Scheme S6). 3-(1-*Tert*-butoxycarbonylpiperidin-4-yl)-1-(3,4-dichlorophenyl) urea (**16**) was synthesized using 3,4-dichlorophenyl isocyanate and a protected piperidine compound (*tert*-butyl 4-aminopiperidine-1-carboxylate **15**, Jones et al., 2006)**.** ^{1}H NMR ($CDCl_3$-DMSO) δ 1.33 (q, J_{H-H}=10.5 Hz, 2H), 1.45 (s, 9H), 1.99 (d, J_{H-H}=12.5 Hz, 2H), 3.20 (d, J_{H-H}=15.0 Hz, 2H), 3.72-3.74 (m, 1H), 3.93 (d, J_{H-H}=11.0 Hz, 2H), 6.04 (s br, 1H), 7.14 (d, J_{H-H}=8.5 Hz, 1H), 7.27 (d, J_{H-H}=8.0 Hz, 1H), 7.78 (s, 1H), 8.38 (s br, 1H).

Compound **18** was synthesized through compounds **16** and **17** according to the previous method (Jones et al., 2006). 4-[4-(3-(3,4-Dichlorophenyl)ureido)piperidin-1-yl]-4-oxobutanoic acid (yield 50%, **19**) of a white solid designated as **Hapten E1** was obtained by alkaline hydrolysis of the ester **18**. ^{1}H NMR (DMSO-d_6): δ 1.23 (Py, m, 1H), 1.35 (Py, m, 1H), 1.78 (Py, d, 1H), 1.84 (Py, d, 1H), 2.43 (Py, t, 2H), 2.53 (Py, m, 2H), 2.81 (C*H*$_2$CH$_2$, , t, 1H), 3.14 (C*H*$_2$CH$_2$, t, 1H), 3.71 (Py, m, 1H), 3.78 (CH$_2$C*H*$_2$COO, d, 1H), 4.14 (CH$_2$C*H*$_2$COO, d, 1H), 6.41 (NH, d, 1H), 7.45-7.83 (Ar, m, 3H), 8.77 (NH, s, 1H), 12.01 (COOH, s, 1H). MS-ESI *m*/*z* calcd for [M - H]$^-$ = $C_{16}H_{19}Cl_2N_3O_4$, 387.08; observed, 386.4.

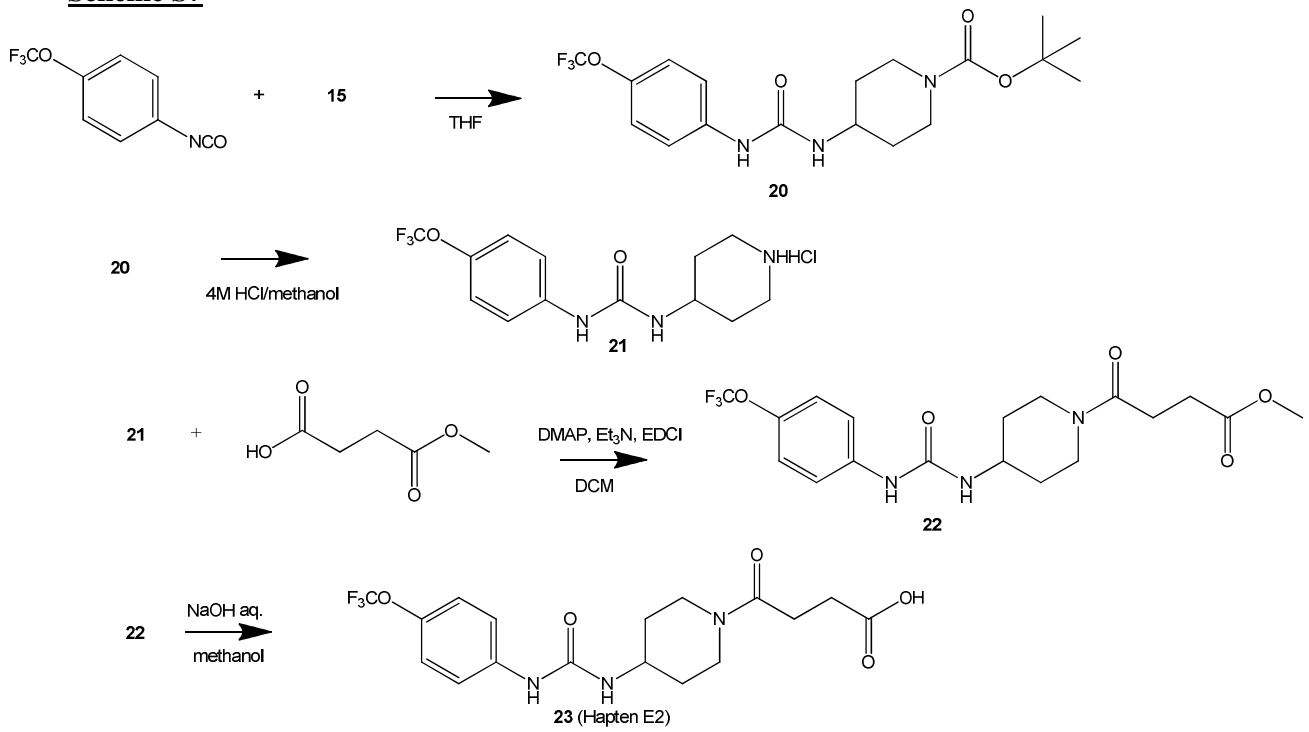
Scheme S6



Synthesis of Hapten E2 (Scheme S7). Compound **22** was synthesized through compounds **20** and **21** using 4-trifluoromethoxyphenyl isocyanate instead of 3,4-dichlorophenyl isocyanate in a similar fashion as described above. ^{1}H NMR ($CDCl_3$) δ 1.22-1.32 (Py, m, 1H), 1.93 (Py, d, 1H), 2.11 (Py, d, 1H), 2.56-2.64 (Py, m, 3H), 2.81 (C*H*$_2$CH$_2$, t, 2H), 3.83 (Py, d, 1H), 3.92-3.94 (Py, m, 1H), 4.42 (Py, d, 1H), 5.18 (NH, d, 1H), 7.14 (Ar, d, 2H), 7.27 (NH, s, 1H), 7.39 (Ar, m, 2H).

3-(4-(3-(4-(Trifluoromethoxy)phenyl)ureido)piperidin-1-ylsulfinyl)propanoic acid a white solid (yield 60%, **23**) designated as **Hapten E2** was obtained by alkaline hydrolysis of the ester **22** as described above. MS-ESI *m*/*z* calcd for [M - H]$^-$ = $C_{17}H_{20}F_3N_3O_5$, 403.14; observed, 402.5.

Scheme S7



LC/MS/MS Analysis.

For blood and serum samples, 200 µL of ethyl acetate, and 10 µL of surrogate ^{13}C-TCC (50 ng/mL dissolved in methanol) were added. The mixture was vigorously shaken for about 30 seconds on a Vortex® mixer and then centrifuged at 10,000 rpm at room temperature for 5 min. The ethyl acetate layer was removed and the aqueous layer was again extracted with 200 µL of ethyl acetate. The combined ethyl acetate layers were evaporated to dryness by a vacuum centrifugal concentrator, and dissolved in 50 µL of an internal standard (136 ng/mL of 12-(3-cyclohexyl-1-yl-ureido) dodecanoic acid, CUDA in methanol) prior to the instrumental analysis. This procedure recovered >95% of the TCC in the ethyl acetate phase.

Chromatographic separation was performed using a Shimadzu LC-10A separation module (Shimadzu, Columbia, MD) equipped with a 50 × 2.1 mm Agilent 5 µm C8 ZORBAX column (Agilent, Folsom, CA) held at 40°C. A solvent system consisting of water (solvent A) and acetonitrile (solvent B) was used. The target analytes were separated using a gradient program (0.6 mL/min) starting with a solvent composition of 40% solvent B, ramped using a linear gradient for 5 min to 100 % solvent B, and then held for 3 min. The injection volume was 10 µL. The samples were kept at 10 °C in the autosampler.

TCC was detected by electrospray ionization in negative mode, tandem quadrupole mass spectrometry in multiple reaction monitoring mode (MRM) using a Quattro Ultima tandem quadrupole mass spectrometer (Waters, Milford, MA). Nitrogen gas flow rates were fixed with a cone gas flow of 50 L/h and a desolvation gas flow of 650 L/h. Electrospray ionization was performed in negative mode with a capillary voltage fixed at 1.5kV using a source temperature of 125 °C and a desolvation temperature of 400 °C. Argon was used as collision gas (2.3×10^{-3} Torr). An optimal transition of 313.1 > 160.0 *m/z* for TCC, 319.0 > 160.0 *m/z* for ^{13}C-TCC and 339.2 > 214.0 *m/z* for CUDA was monitored using a cone voltage of 42V for TCC and ^{13}C-TCC, and 30V for CUDA, and a collision voltage of 12V for TCC and ^{13}C-TCC, and 20V for CUDA. A limit of detection (LOD, S/N > 3/1) of 0.56 ng/mL and a limit of quantification (LOQ) of 2.8 ng/mL in whole blood were achieved, respectively. The average of recovery of ^{13}C-TCC in whole blood added to the final concentration of 10 ng/mL was 82 ± 10%.

Table S1. Screening antibodies and coating antigens in competitive indirect ELISA for TCC

Immunogen	Antibody	Coating antigen	A_{max} (A)	Slope	IC_{50} (μg/L)	A_{min} (D)	A/D
Hapten A1-Thy	Ab #1641	Hapten E2-BSA	1.28	0.58	4.49	0.03	43
Hapten A2-Thy	Ab #1645	Hapten A2-BSA	0.82	0.61	32.9	-0.01	82
		Hapten C1-BSA	1.07	0.72	42.8	-0.03	36
		Hapten D1-BSA	0.65	0.75	8.36	0.01	65
	Ab #1646	Hapten A2-BSA	1.11	0.67	28.9	0.05	22
		Hapten D1-BSA	0.57	0.9	7.54	0.01	57
	Ab #1647	Hapten A1-BSA	1.42	0.7	19.5	0.01	142
		Hapten A2-BSA	0.24	1.09	22.8	0.04	6
		Hapten B1-BSA	0.99	0.75	23.9	0.01	99
		Hapten C1-BSA	1.14	0.83	4.85	-0.01	114
		Hapten D1-BSA	0.45	0.63	1.21	0.01	45
		Hapten E2-BSA	1.17	0.68	3.48	0.02	59
Hapten C1-Thy	Ab #1648	Hapten A1-BSA	1.07	0.59	5.61	-0.02	54
		Hapten A2-BSA	0.92	0.60	3.69	-0.02	46
		Hapten B1-BSA	0.91	0.61	3.10	-0.01	91
		Hapten C1-BSA	0.55	0.62	7.67	0.01	55
		Hapten E1-BSA	1.07	0.58	7.59	-0.01	107
		Hapten E2-BSA	1.13	0.60	3.44	-0.02	57
	Ab #1649	Hapten A1-BSA	1.25	0.49	23.4	-0.04	31
		Hapten A2-BSA	1.27	0.53	12.4	-0.03	42
		Hapten B1-BSA	1.29	0.48	33.3	-0.01	129
		Hapten C1-BSA	1.08	0.62	11.1	0.01	108
		Hapten D1-BSA	0.40	0.53	1.9	0.01	40
		Hapten A1-BSA	1.4	0.75	8.8	-0.01	140

Table S1 (continued).

Immunogen	Antibody	Coating antigen	A_{max} (A)	Slope	IC_{50} (µg/L)	A_{min} (D)	A/D
	Ab #1650	Hapten A2-BSA	1.3	0.71	5.5	-0.01	130
		Hapten B1-BSA	1.2	0.69	6.69	-0.02	60
		Hapten C1-BSA	0.7	0.59	4.57	-0.01	70
		Hapten D1-BSA	0.3	0.81	1.05	0.02	15
		Hapten E2-BSA	1.4	0.60	24.9	-0.04	35
Hapten A1 -Thy	Ab #1641	Hapten E2-CON	1.66	0.58	15.1	-0.04	42
Hapten A2 -Thy	Ab #1647	Hapten A2-CON	0.44	0.74	16.4	0.00	8
		Hapten D1-CON	0.69	0.35	1.44	-0.01	69
		Hapten E2-CON	1.27	0.61	4.66	-0.03	42
Hapten B1 -Thy	Ab #1642	Hapten E2-CON	1.30	0.61	7.5	0.03	43
Hapten C1 -Thy	Ab #1648	Hapten B1-CON	0.71	0.60	2.06	-0.01	71
		Hapten E1-CON	1.16	0.5	9.24	-0.05	23
		Hapten E2-CON	1.04	0.61	4.78	-0.04	26
	Ab #1649	Hapten D1-CON	0.52	0.67	0.78	0.02	26
	Ab #1650	Hapten B1-CON	0.91	0.66	3.13	-0.04	23
		Hapten D1-CON	0.75	0.59	0.88	0.06	13

Assay conditions: for homologous assays, coating antigen was 0.1 µg/ml, antibody was diluted 1/25,600 (final dilution in well); for heterologous assays, coating antigen was 1 µg/mL , antibody was diluted 1/6400 (final dilution in well). TCC standards were prepared in 40% MeOH in phosphate buffered saline (PBS), goat-anti-rabbit IgG-horseradish peroxidase conjugate was diluted 1/5000 in PBS containing 0.05% Tween 20

Table S2. The effects of solvent, pH and ionic strength on the assay sensitivity

Assay parameter	A_{max}	Slope	IC_{50} (µg/L)	A_{min}	A_{max}/A_{min}
MeOH content (%)					
10	1.31	1.03	2.49	0.13	10
20	1.16	0.85	2.72	0.11	11
40	0.91	0.69	2.88	0.09	10
60	0.80	0.77	2.80	0.10	8
DMSO content (%)					
10	1.51	0.88	1.77	0.13	12
20	1.36	0.83	1.00	0.12	11
40	1.10	0.83	0.65	0.11	10
60	0.68	0.78	0.62	0.12	6
pH					
5.5	0.86	0.79	0.88	0.14	6
7.5	0.74	0.84	0.54	0.13	6
8.5	0.74	0.80	0.57	0.12	6
11	0.94	0.76	0.41	0.13	7
Ionic strength					
1x	0.82	1.00	1.98	0.12	7
2x	0.69	1.13	1.53	0.12	6
4x	0.64	1.15	1.36	0.12	5
6x	0.591	1.07	1.42	0.12	5

Assay conditions: coating antigen hapten-E2-BSA was 1 µg/ml, antibody #1648 was diluted 1/6,000. TCC standards were prepared in 40% DMSO in phosphate buffered saline (PBS), goat-anti-rabbit IgG-horseradish peroxidase conjugate was diluted 1/3000 in PBS containing 0.05% Tween 20.

Table S3. Recoveries of TCC in Serum Samples (0.5 mL)

Spiked concentration (ng/mL)	Calf serum	Human serum
3.0	4.0 ± 0.2	2.8 ± 0.1
5.0	5.4 ± 0.7	5.8 ± 0.5
10.0	9.4 ± 0.8	12 ± 0.8
50.0	41 ± 2.0	58 ± 3.5

Assay conditions: coating antigen hapten-E2-BSA was 1 µg/ml, antibody #1648 was diluted 1/6,000. TCC standards were prepared in 40% DMSO in phosphate buffered saline (PBS), goat-anti-rabbit IgG-horseradish peroxidase conjugate was diluted 1/3000 in PBS containing 0.05% Tween 20.

Table S4. Analysis of Urine Samples Before and After Acid Hydrolysis

Time	LC-MS/MS		Immunoassay		Creatinine
h	nM	SD	nM	SD	....g/L
After Hydrolysis					
-20	3.4	1.3	0	3.8	1.5
0	2.2	0.9	0	5	1.3
2	200	8.4	310	9.8	1.5
5	630	23	940	52	1.1
10	1010	14	1000	39	1.1
18	370	15	270	6.9	0.7
25	590	41	650	41	1.6
117	3.6	0.10	0	3.8	0.4
Before Hydrolysis					
-20	0.22	0.07	6.6	2.9	
0	0.18	0.12	7.0	2.0	
5	0.31	0.07	8.3	2.0	
2	0.46	0.03	12	3.2	
10	0.74	0.08	12	1.3	
18	0.77	0.16	16	2.7	
25	0.4	0.21	8	1.2	
117	0.21	0.04	6.4	2.2	

Assay conditions: coating antigen hapten-E2-BSA was 1 µg/ml, antibody #1648 was diluted 1/6,000. TCC standards were prepared in 40% DMSO in phosphate buffered saline (PBS), goat-anti-rabbit IgG-horseradish peroxidase conjugate was diluted 1/3000 in PBS containing 0.05% Tween 20.

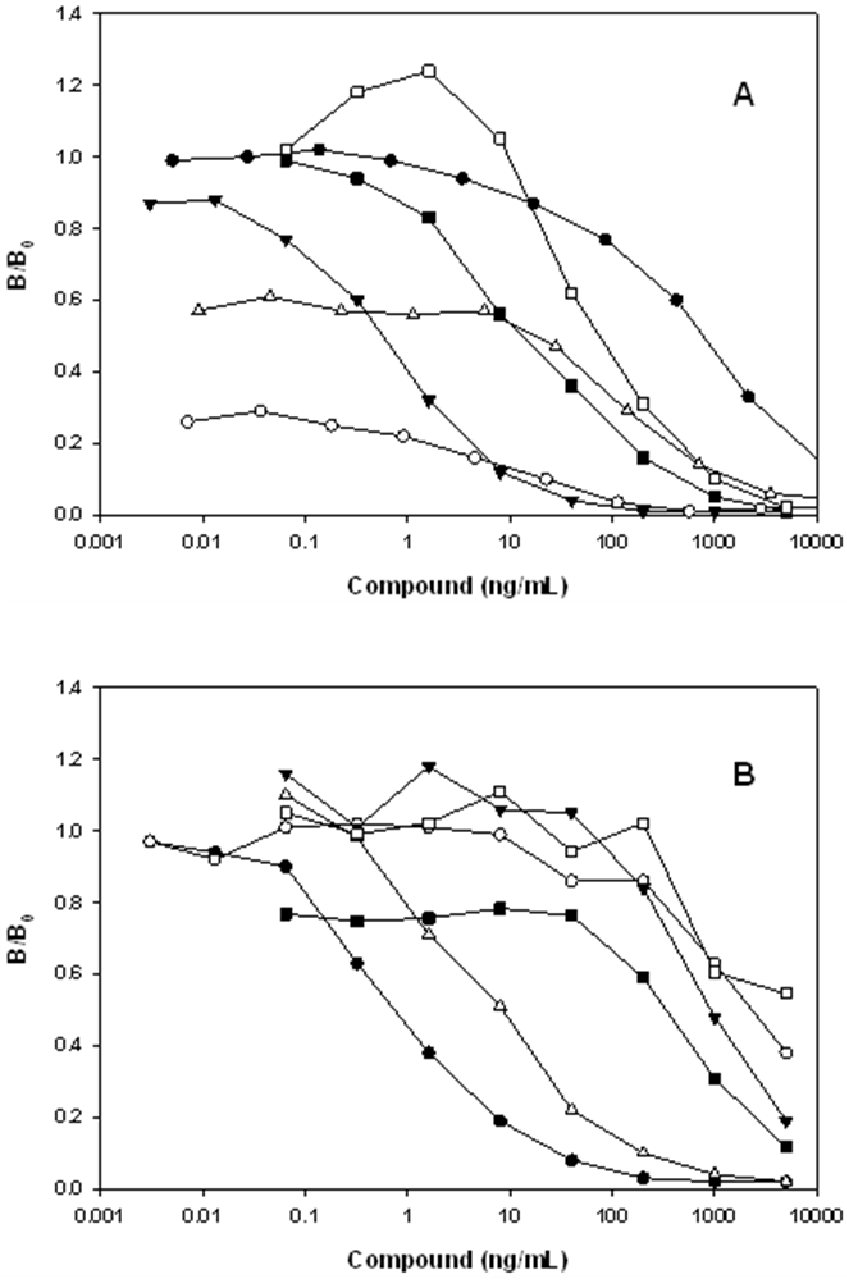


Figure S1. Cross reactivity profiles of compounds described in Table 1 of main text. Assay conditions: coating antigen hapten-E2-BSA was 1 µg/ml, antibody #1648 was diluted 1/6,000. TCC standards were prepared in 40% DMSO in phosphate buffered saline (PBS), goat-anti-rabbit IgG-horseradish peroxidase conjugate was diluted 1/3000 in PBS containing 0.05% Tween 20. Panel A: Carbanilide (●), dichlorocarbanilide (○), trichlorocarbanilide (TCC) (▼), tetrachlorocarbanilide (Δ), the sulfate conjugate of 2'-hydroxy-TCC (■), 2'-hydroxy-TCC (□). Panel B: Trichlorocarbanilide (TCC) (●), triclosan (○), sEHi 1555 (▼), TFC (Δ), sEHi 1709 (■), diuron (□).

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