Electronic Supplementary Information

ELISA detection of semicarbazide based on a fast sample pretreatment method

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1. Experiments

1.1. General Synthetic Procedures and Materials. Commercial grade reagents and solvents were used without further purification, except as indicated below. Anhydrous DMF and DCM were bought from Alfa Aesar. All reactions were stirred magnetically in flame- or oven-dried glassware under a positive pressure of N_2. The reactions were monitored using TLC, and visualized with UV light (254 nm). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV400 (400 and 100 MHz) or Bruker AV500 (500 and 125 MHz) instrument, and chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane (TMS). Coupling constants (J) are reported in Hz. Spin multiplicities are described as s (singlet), brs (broad singlet), t (triplet), q (quartet), and m (multiplet). High-resolution mass spectra were recorded on Kompact Axima-CFR MALDI mass spectrometers. Preparative HPLC was performed on a Waters Symmetry C18 column (19 mm×50 mm, 5 μM) using a gradient of 5-25% acetonitrile in water containing 0.05% trifluoacetic acid (TFA) over 20 min (25 min run time) at a flow rate of 30 mL/min.

1.2. Chemical Synthesis.

***N*-Boc-3,6-dioxaoctane-1,8-diamine 3.** A solution of $(Boc)_2O$ (1.0 g, 4.6 mmol) in 15 mL of DCM was added drop wisely to a stirred solution of 1,2-bis(2-aminoethoxy)ethane2 (4.0 g, 27.6 mmol) and DIPEA (0.8 mL, 4.6 mmol) in 25 mL of DCM at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. Solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography to give pure 3 as a colorless oil (0.97 g, 84%): ^{1}HNMR (400MHz, $CDCl_3$): δ5.22 (brs, 1H), 3.59 (s, 4H), 3.57-3.48 (m, 4H), 3.28 (dd, J=10.2, 5.2Hz, 2H), 2.85 (t, J=5.2Hz, 2H), 1.79 (s, 2H), 1.41 (s, 9H); ESI-MS *m/z* 249.1 $[M+H]^+$.

***N*-Boc-*N*'-biotinyl-3,6-dioxaoctane-1,8-diamine 4.** Biotin (548 mg, 2.4 mmol) was mixed with DIPEA (1.0 mL, 6 mmol) and HATU (0.87 g, 2.2 mmol) in 5 mL of DMF at room temperature. A solution of 3 (500 mg, 2 mmol) in 3 mL of DMF was added to the reaction mixture 0.5 h later. After being stirred at room temperature for 2 h, the reaction mixture was concentrated and roughly purified by silica gel column chromatography to give 4 as a

yellowish solid. Compound 4 was used for the next step without further purification (900 mg). ^{1}H NMR(400 MHz, CD_3OD): δ 4.49 (dd, J = 7.7, 4.9 Hz, 1H), 4.31 (dd, J = 7.7, 4.6 Hz, 1H), 3.64-3.59 (m, 4H), 3.58-3.50 (m, 4H), 3.39-3.30 (m, 2H), 3.24-3.19 (m, 3H), 2.92 (dd, J = 12.7, 5.0 Hz, 1H), 2.70 (d, J = 12.7 Hz, 1H), 2.21 (t, J = 7.2 Hz, 2H), 1.83-1.55 (m, 4H), 1.43 (s, 11H). ^{13}C NMR (125 MHz, CD_3OD): δ176.0, 166.1, 158.3, 80.0, 71.2, 71.0, 70.6, 63.3, 61.6, 57.0, 41.2, 41.1, 40.3, 36.7, 29.7, 29.5, 28.9, 26.8.ESI-MS *m/z* 475.2 $[M+H]^+$.

***N*-4-formylbenzoyl-*N'*-biotinyl-3,6-dioxaoctane-1,8-diamine 1.** To a cooled suspension of biotin derivative 4 (900 mg, 1.9 mmol) in 10 mL of DCM, 10 mL of 4N HCl/Dioxane was added. The resulting mixture was stirred at room temperature for 8 h, then concentrated under reduced pressure. The residue was dried overnight in vacuum to give an HCl salt of unprotected 5. ESI-MS *m/z* 375.2 $[M+H]^+$.

4-carboxybenzaldehyde (570 mg, 3.8 mmol) was mixed with DIPEA (1.9 mL, 11.4 mmol) and HATU (1.5 g, 3.8 mmol) in 10 mL of DMF at room temperature. A solution of the HCl salt 5 in 5 mL of DMF was added to the reaction mixture 0.5 h later. After being stirred at room temperature for 2 h, the reaction mixture was concentrated and purified by preparative HPLC to give biotinylated aldehyde 1 as a white foam (410 mg, 40.5 % in three steps). ^{1}HNMR (400MHz, d_6-DMSO): δ10.07 (s, 1H), 8.74 (t, J = 5.4 Hz, 1H), 8.05-7.96 (m, 4H), 7.81 (t, J = 5.4 Hz, 1H), 6.42 (s, 1H), 6.36 (s, 1H), 4.35-4.25 (m, 1H), 4.17-4.08 (m, 1H), 3.60-3.48 (m, 6H), 3.47-3.41 (m, 2H), 3.41-3.37 (m, 2H), 3.21-3.13 (m, 2H), 3.12-3.04 (m, 1H), 2.81 (dd, J = 12.4, 5.0 Hz, 1H), 2.57 (d, J = 12.4 Hz, 1H), 2.05 (t, J = 7.4 Hz, 2H), 1.66-1.54 (m, 1H), 1.54-1.39 (m, 3H), 1.36-1.21 (m, 2H). ^{13}CNMR (125MHz, d_6-DMSO): δ 192.8, 172.1, 165.5, 162.7, 139.4, 137.7, 129.3, 127.9, 79.1, 69.5, 69.1, 68.7, 61.0, 59.2, 55.4, 38.4, 35.1, 28.1, 28.0, 25.2. ESI-MS *m/z* 507.1 $[M+H]^+$. HRMS-ESI Calcd for $C_{24}H_{35}N_4O_6S$ ($[M+H]^+$) 507.2277 found 507.2281.

1.3. ELISA Plate Preparation. Ascites was collected and purified by centrifugation at 12000 rpm. The purified ascites in sodium bicarbonate buffer (pH 9.6, 21 mg L^{-1}, 100 μL/well) was added into 96-well microtiter plate. The plate was incubated at 4°C overnight, and then washed three times with PBS containing 0.05% Tween-20 (PBST). After washing, the plate

was incubated with 200 μL of blocking solution (1% bovine serum albumin (BSA) in PBS) for 2 h at 37°C. The plate was washed three times with PBST, and stored at 4°C until use. Other plates were prepared with the same procedure using 2.5 mg L^{-1} of purified ascites for competitive ELISA detection.

1.4. Derivatisation and Detection of SEM. Ten microliters of derivatisation stock solution (10 g L^{-1} biotinylated aldehyde in methanol) and 15 μL of 0.1 M HCl were added into 1 mL of SEM standard solution. The mixture was incubated at 37°C for 4 h, a biotinylated SEM derivative solution was obtained (ESI-MS m/z 586.2 [M+Na]+, Figure **S5**). After incubation, 30 μL of this solution was mixed with 70 μL of PBS-Tris buffer (30 mM, PH=7.5, PBS buffer:Tris buffer=1:1, v:v) and added into the antibody coated plate. After washing, 100 μL of SA-HRP solution was added into each well. The plate was incubated for another 20 min at 37°C and then washed three times with PBST. Subsequently, 100 μL of HRP substrate was added into each well. The reaction was terminated with 50 μL/well of H_2SO_4 (2 M) in 15 minutes. Absorbance of each well was read at 450 nm with a microplate reader. The selectivity analysis was conducted in the same way. For the recovery analysis, derivatisation was carried out with a volume of 10 mL.

2. Results

SEM-CBA standard solutions (SEM-CBA spiked ultra-pure water) were tested using competitive ELISA. At the optimal condition, the log-linear range is 0.9-20 μg L^{-1}, IC50=3.93 μg L^{-1} (**Table S1, Figure S3**). The sensitive is 0.17 μg L^{-1}, which is much lower than 0.07 μg L^{-1} of the current assay.

Table S1. Optical densities of different concentration of SEM-CBA

SEM-CBA (μg L^{-1})	0	0.1	0.2	0.3	0.9	2.7	5	8.1	10	20
Optical density	1.351	1.345	1.331	1.228	1.252	0.881	0.715	0.588	0.476	0.293

3. Figures

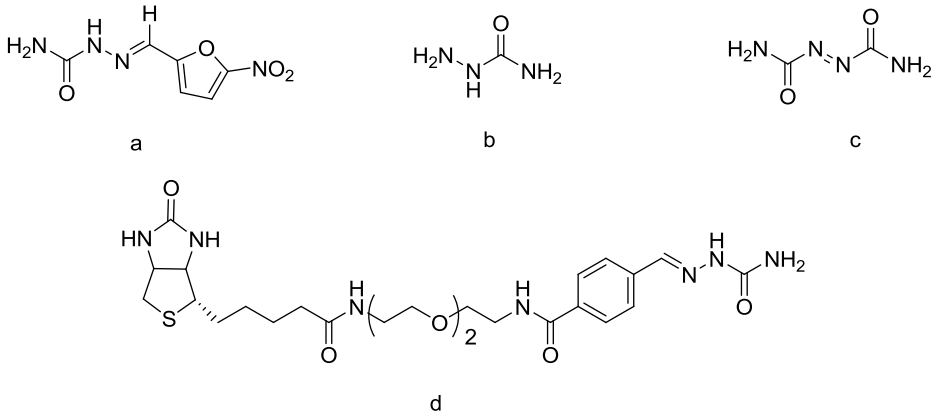

Figure S1. Molecular structures of nitrofurazone (a), semicarbazide (b), azodicarbonamide (c) and biotin-sem (d).

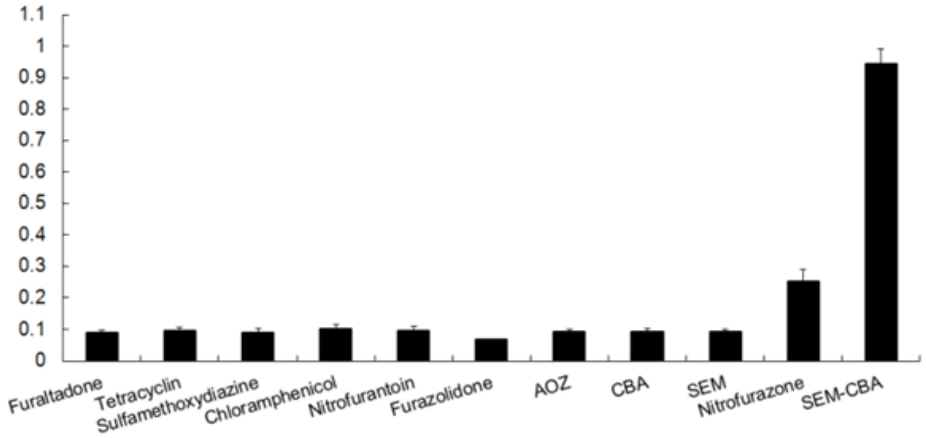

Figure S2. Optical density of various drugs and marker of residues at 37.5 μg L^{-1}. (From left to right are Furaltadone, Tetracyclin, Sulfamethoxydiazine, Chloramphenicol, Nitrofurantoin, Furazolidone, AOZ, CBA, SEM, Nitrofurazone, SEM-CBA).

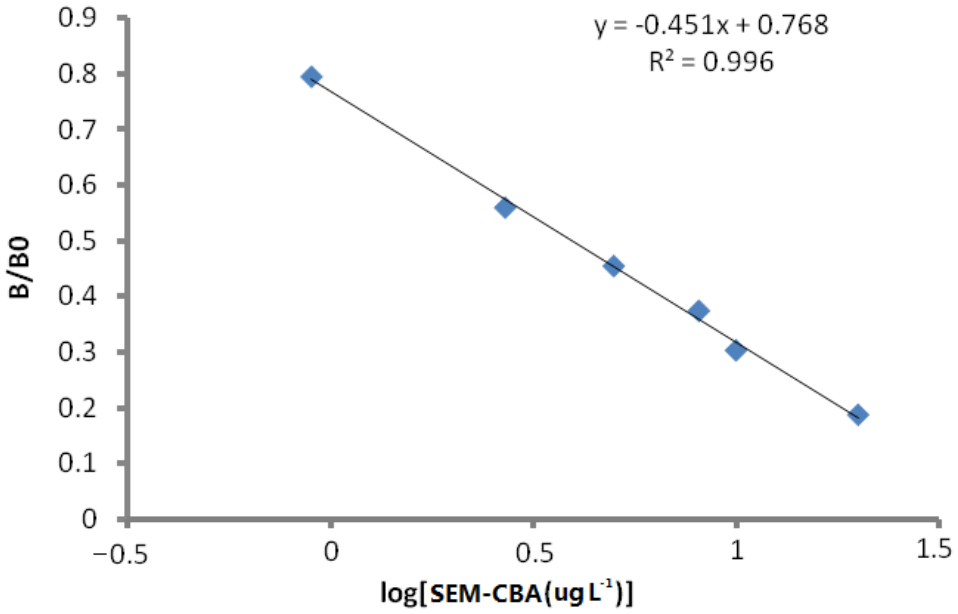

Figure S3. Standard curve of the assay. Optical densities are obtained from serially diluted SEM-CBA standard solutions. B/B0=optical density of sample/optical density of blank (μg L^{-1}).

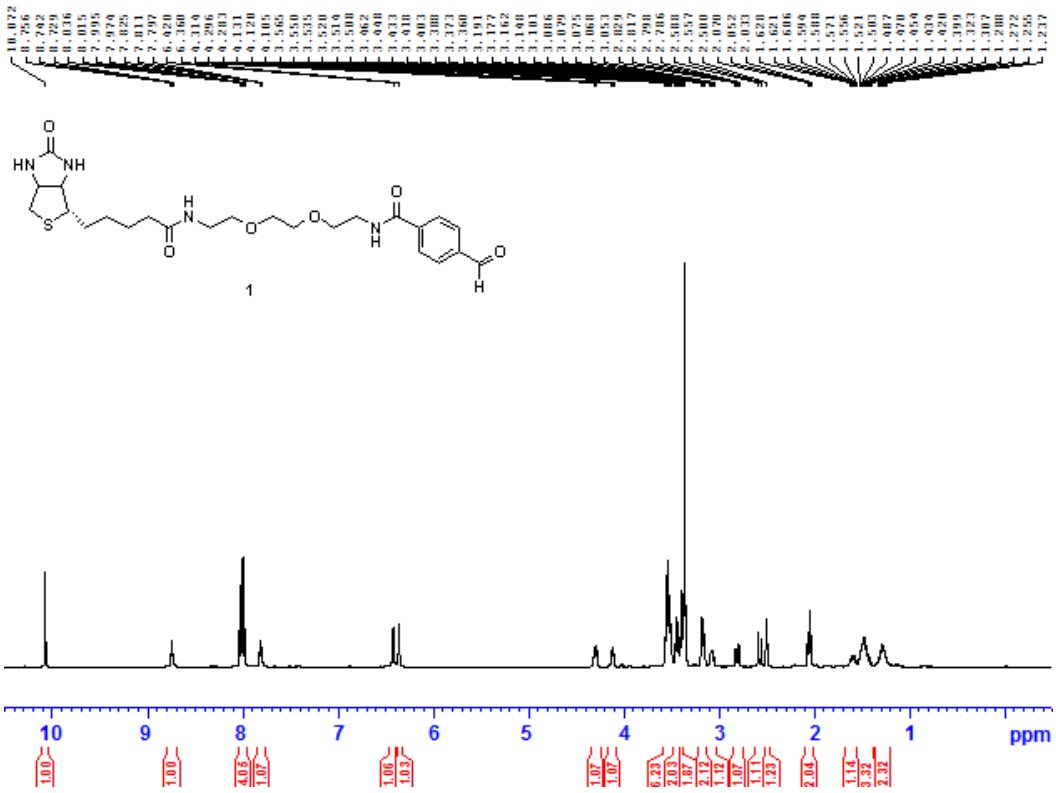

Figure S4. 1HNMR of biotinylated aldehyde 1.

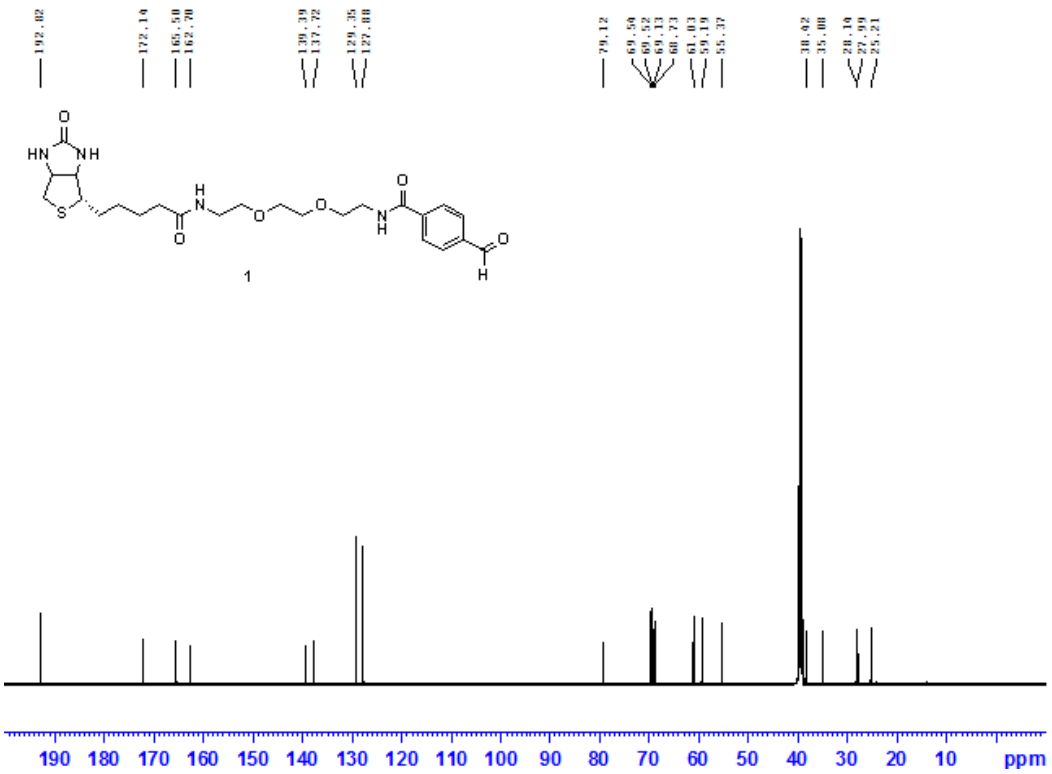

Figure S5. ^{13}CNMR of biotinylated aldehyde **1.**

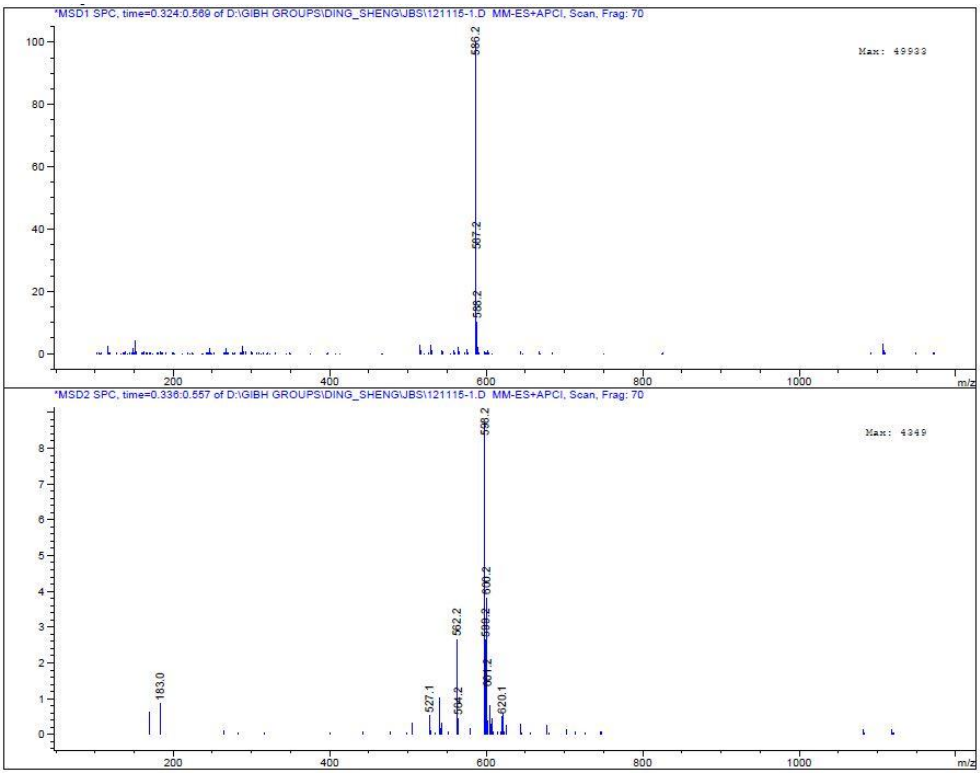

Figure S6. ESI-MS of Biotin-SEM.