Cationic Oligo(thiophene ethynylene) with Broad-Spectrum and High Antibacterial Efficiency under White Light and Specific Biocidal Activity against *S. aureus* in Dark

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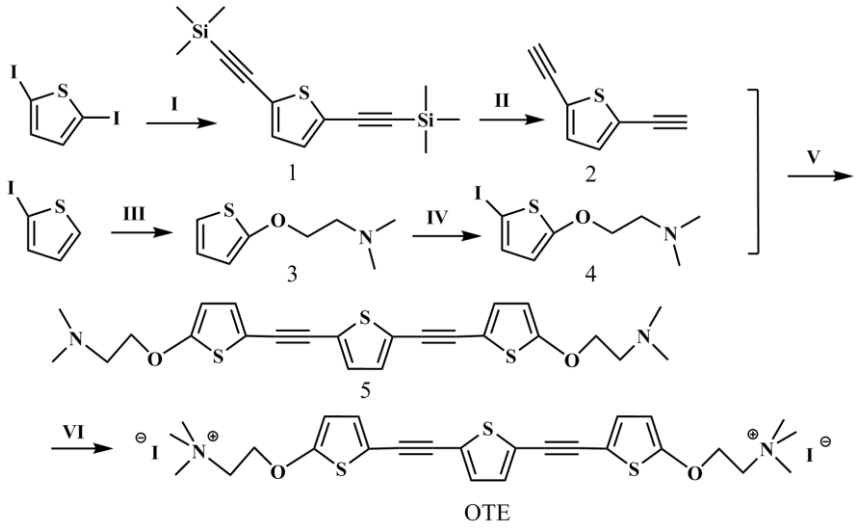
Supporting Information

Contents

1. Experiments

1.1 Synthesis



Scheme S1. Synthesis of OTE: (I) $Pd(PPh_3)_2Cl_2$/CuI, diethylamine/$CHCl_3$, r. t. 5 h; (II) K_2CO_3, MeOH/THF, r. t. 5 h; (III) Cu, DMAP, NaH, DMF, 75^{o}C, overnight; (IV) NIS, CH_2Cl_2/AcOH, r. t. 4 h; (V) $Pd(PPh_3)_2Cl_2$/CuI, diethylamine/$CHCl_3$, r. t. 24 h; (VI) CH_3I, $CHCl_3$, r. t. overnight.

Compound 1 and **compound 2** were synthesized according to the literature.[1]

Compound 3. Under nitrogen atmosphere, NaH (360 mg, 15 mmol) was added slowly to the solution of N,N-dimethylethanolamine (1.5 ml, 15 mmol) in DMF (30 mL) in the ice bath, the resulting solution was vigorously stirred for 30 min. Then, 2-iodothiophene (1 mL, 10 mmol) was drowsily added to the mixture. After adding of Cu (64 mg, 1 mmol) and DMAP (105 mg, 0.86 mmol), the mixture was heated to 75 ^{o}C overnight. The cooled solution was treated with methanol to remove excess NaH and the solid was removed through filtration. The filtrate was extracted with

DCM. Then the collected organic phase was washed with brine and water, dried over $MgSO_4$ and concentrated in vacuum. The residue was chromatographed on silica gel using DCM/methanol (v/v=50/1) to give **compound 3** (602 mg, 35%) as brown liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (q, *J* = 6.0 Hz, 1H), 6.79 (d, *J* = 6.0 Hz, 1H), 6.25 (d, *J* = 6.0 Hz, 1H), 4.05 (t, *J* = 6.0 Hz, 2H), 2.72 (t, *J* = 6.0 Hz, 2H), 2.33 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.73, 124.57, 119.63, 97.28, 68.07, 58.24, 45.80. HRMS (ESI): m/z: 172.0791 ($[M+H]^+$).

Compound 4. NIS (545 mg, 2.4 mmol) was added in three portions over a period of 1.5 h to the solution of **Compound 3** (276 mg, 1.6 mmol) in DCM and AcOH (8 ml, 1:1) at 0 °C. Then, the mixture was warmed to room temperature and the reaction was stirred for 3 h. Water (3 ml) was added to the reaction mixture and the acid was neutralized with a saturated solution of $NaHCO_3$. The mixture was extracted with DCM, the combined organic layer organic phase was washed with brine and water, dried over $MgSO_4$ and concentrated in vacuum. The residue was chromatographed on silica gel using DCM/methanol (v/v=100/1, 0.5% triethylamine) to give **compound 4** (415 mg, 87%) as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, *J* = 6.0 Hz, 1H), 6.73 (d, *J* = 6.0 Hz, 1H), 4.49 (t, *J* = 6.0 Hz, 2H), 3.36 (t, *J* = 6.0 Hz, 2H), 2.88 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 130.88, 116.87, 66.17, 56.77, 46.55. HRMS (ESI): m/z: 297.9758 ($[M+H]^+$).

Compound 5. Under nitrogen atmosphere**, Compound 4** (595 mg, 2 mmol) was dissolved completely in degassed diethylamine and $CHCl_3$ (24 mL, 1:2). **Compound 2** (132 mg, 1 mmol), $Pd(PPh_3)_2Cl_2$ (70.2 mg, 0.1 mmol) and CuI(38.1 mg. 0.2 mmol)

then were added to the solution. The resulting mixture was stirred at room temperature for 24 h under nitrogen atmosphere. The solution was extracted with CH_2Cl_2. The collected organic phase was washed with brine and water, dried over $MgSO_4$ and concentrated in vacuum. The residue was chromatographed on silica gel using CH_2Cl_2/methanol (v/v=100/1, with 0.5% triethylamine) to give **compound 5** (58 mg, 12%) as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (d, *J* = 6.0 Hz, 2H), 7.08 (d, *J* = 6.0 Hz, 2H), 6.76 (d, *J* = 6.0 Hz, 2H), 4.42 (t, *J* = 6.0 Hz, 4H), 2.92 (t, *J* = 6.0 Hz, 4H), 2.51 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.18, 134.56, 131.58, 127.34, 126.23, 122.92, 117.18, 88.28, 86.08, 67.86, 57.25, 44.64. HRMS (ESI): m/z: 471.1227 ($[M+H]^+$).

OTE. (24 mg, 0.051 mmol) was dissolved in $CHCl_3$ (10 mL) and the reaction mixture was stirred vigorously to form a homogeneous solution. Then, CH_3I (213 mg, 1.53 mmol) was added and the mixture was stirred at room temperature overnight. The solid was collected by filtration, washed with $CHCl_3$ for three times and dried under vacuum to give OTE (30 mg, 88%) as brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72 (d, *J* = 6.0 Hz, 2H), 7.59 (d, *J* = 6.0 Hz, 2H), 7.38 (d, *J* = 6.0 Hz, 2H), 4.65 (b, 4H), 3.81 (b, 4H), 3.22 (s, 18H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.64, 136.48, 132.50, 131.50, 129.08, 128.63, 117.62, 87.34, 78.25, 65.00, 52.02, 44.52. HRMS (ESI): m/z: 250.0813 ($[M-2I]^{2+}$).

1.2. Bacterial growth conditions

Gram-positive bacteria (*S. aureus*, *S. epidermidis*) and Gram-negative bacteria (*E.*

coli, R. solanacearum) were chosen for this study. Bacterial samples were transferred from the frozen state onto agar slants (1.8% agar + triphenyl tetrazolium chloride medium (TTC)) for *R. solanacearum*, 1.2% agar + Lysogeny Broth (LB) for *E. coli*, 1.5% agar + Nutrient Broth (NB) for *S. epidermidis* and 1.2% agar + brain heart infusion (BHI) for *S. aureus*) incubated at 37 ^{o}C overnight and then held at 4 ^{o}C for up to 2 weeks. A single colony from the slants was incubated in 50 mL of NB (*R. solanacearum*) for 18 h with shaking at 30 ^{o}C. The culture for *E. coli, S. epidermidis, S. aureus* is LB, NB and BHI, respectively, and the incubation was kept overnight with shaking at 37 ^{o}C. After growth, the bacterial culture was centrifuged at 2000 rpm for 20 min (*R. solanacearum*) or 6000 rpm (*S. aureus*, *S. epidermidis* and *E. coli*) for 2 min at 4 ^{o}C and the pellet was suspended in 0.9% NaCl solution. This washing procedure was repeated twice. The final concentration of bacteria was around 2×10^{7}/mL.

1.3. Bacterial killing experiments

The antibacterial activities of EO-OPE-1(Th), EO-OPE-1(C3) and OTE were determined by incubation with bacterial cells suspensions for 10 min in the dark at room temperature. Then the mixture solutions were exposed to 90 mW/cm^2 white light for 30 or 60 min, or incubated in the dark for 30 or 60 min. After bacteria were incubated with the oligomers in the light or dark, a 1:1 ratio of dyes were added to the samples and kept in the dark for 15 min. The used bacteria stains were SYTO 9 (green fluorescence) for Gram-negative bacteria, SYTO 24 (green fluorescence) for

Gram-positive bacteria and propidium iodide (PI, red fluorescence). The final concentrations of SYTO 9, SYTO 24 and PI were 1.25 μM, 0.25 μM and 7.5 μM respectively. Finally, the bacteria were examined using an Accuri C6 flow cytometry and Olympus IX73 fluorescence microscope.

1.4. Reactive oxygen species (ROS) measurements

In the experiments, 1.0 mL of the activated DCFH solution (40 μM) was added EO-OPE-1(Th), EO-OPE-1(C3) and OTE (final concentration is 1.0 μM). The fluorescence spectra were measured after the specimens were irradiated under white light (5 $mW \cdot cm^{-2}$) for 5 min. Fluorescence spectra of DCF solution was recorded in 500–700 nm emission range with the excitation wavelength of 488 nm.

1.5. Cytotoxicity

The cytotoxicity of OTE in the dark was evaluated by MTT assay with HeLa cells. HeLa cells were seeded into a 96-well plate at a density of 1.0×10^4 cells per well in 100 of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and incubated for 24 h at 37 °C in 5% CO_2. The solution of OTE was diluted with DMEM to obtain predetermined concentrations (10, 30, 60, 90, 120, 500 and 1200 ng/mL) of OTE. The medium in the well was then replaced with the solution of OTE (200 μL) and the cells were incubated for 24 h and 48 h in the dark to evaluate the cytotoxicity. A 100 μL aliquot of MTT was then added into each well after removing the solution of OTE and incubated for a further 4 h. Finally, 100 μL of DMSO were added to each well to dissolve the formazan crystals. The plate was kept

at 37 °C for 30 min and then the optical density (OD) was read on a microplate reader at 595 nm (OD595). The cells without OTE were used as a control and their cell viability was set at 100%. Each sample was tested in six replicates per plate.

1.6. Observation of cell morphology

2 mL of bacterial suspension (~10^9 colony forming units (CFU)/mL) was mixed with OTE followed by incubation under visible light irradiation or in the dark for 1h. The mixture of bacteria cells and OTE was centrifuged at 5000 rpm for 5 minutes. The cell pellets were resuspended with 2.5% glutaraldehyde and incubated at 4 °C overnight, followed by washing with 0.01 M PBS buffer for three times. Then, the fixed cells were dehydrated by sequential treatment with increasing concentrations of ethanol for 20 min at 4 ^{o}C and dehydrated with absolute ethanol twice. The dehydrated samples were resuspended with *tert*-butanol and transferred into freezing dryer (ALPHA1-2, CHRIST). The totally dried samples were sputter-coated with 10 nm thick gold/palladium. Morphologies of the bacterial cells were observed by SEM (Quanta 200, FEI).

2. Supporting Figures and Tables

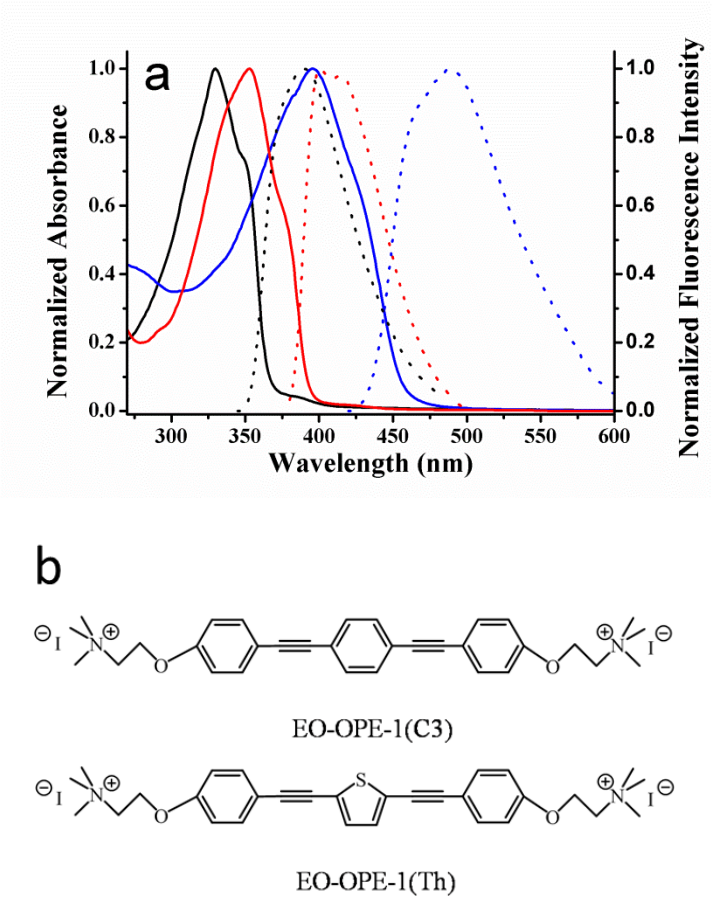


Figure S1. (a) Absorption and fluorescence spectra of OTE (blue), EO-OPE-1(C3) (black) and EO-OPE-1(Th) (red) in water. Solid lines indicate absorbance spectra and dot lines indicate fluorescence spectra. (b) The structure of EO-OPE-1(C3) and EO-OPE-1(Th).

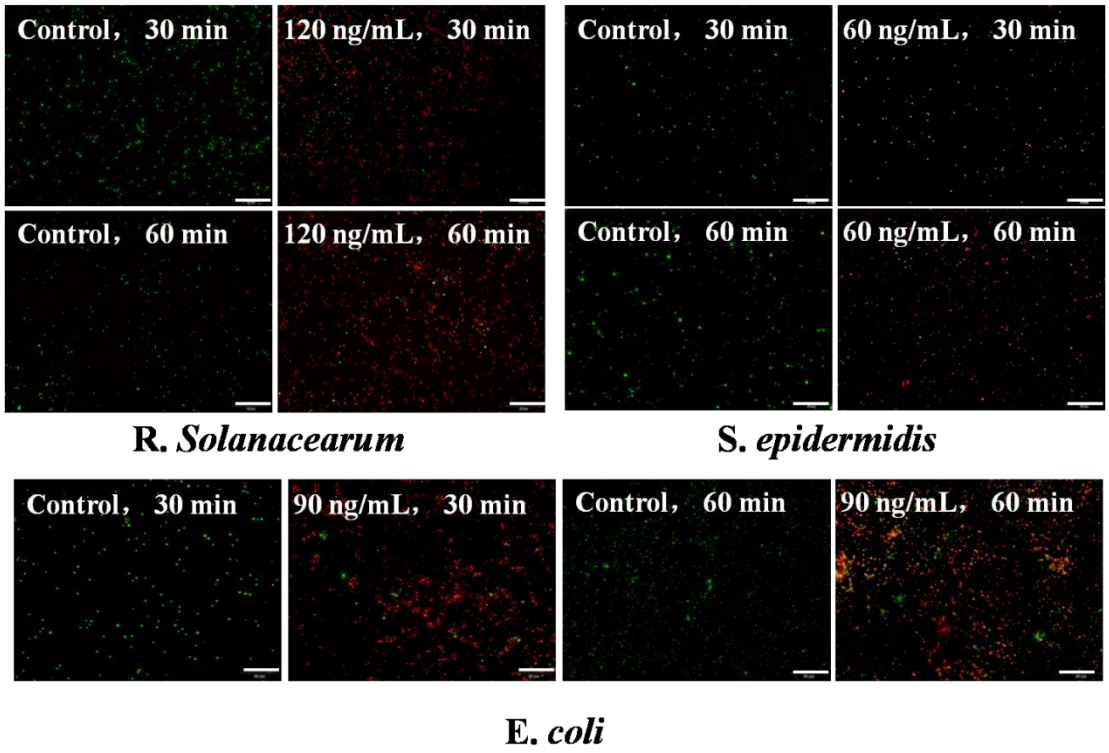


Figure S2. Fluorescence microscope images of OTE with *R. solanacearum*, *E. coli* and *S. epidermidis* after 30 and 60 min of irradiation with visible light. Green staining indicates live bacteria and red staining indicates dead bacteria. Scale bar, 50μm.

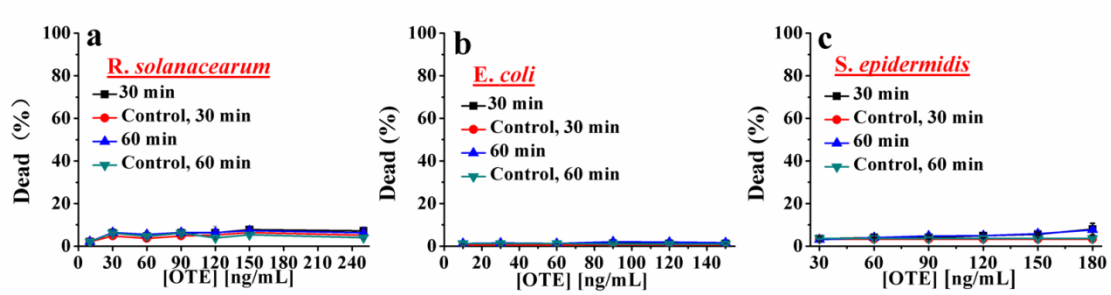


Figure S3. *R. solanacearum* (a), *E. coli* (b) and *S. epidermidis* (c) viabilities against OTE at various concentrations for 30 and 60 min in the dark. The error bars represent the standard deviations of three parallel measurements.

Table S1. The half inhibitory concentrations (IC_{50}) of *R. solanacearum*, *E. coli*, *S. aureus* and *S. epidermidis* upon exposure to visible light.

Strains	Half Inhibitory Concentrations (IC_{50}) (ng/mL)	
	30 min	**60 min**
S. aureus	8	7
S. epidermidis	13	10
E. coli	24	17
R. solanacearum	52	28

Reference:

1. Zhou, Z. J.; Corbitt, T. S.; Parthasarathy, A.; Tang, Y. L.; Ista, L. F.; Schanze, K. S.; Whitten, D. G., "End-Only" Functionalized Oligo(phenylene ethynylene)s: Synthesis, Photophysical and Biocidal Activity. *J. Phys. Chem. Lett.* 2010, 1, 3207-3212.