Supporting Information

Synthesis and biological evaluation of gallidermin-siderophore conjugates

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Supporting Information Contents:

General information:

NMR spectra were recorded on an Inova 600, Inova 500, Unity 500 or Inova 400 spectrometer. For ^{1}H (400, 500 or 600 MHz) spectra, δ values were referenced to $CDCl_3$ (7.26 ppm), CD_3OD (3.30 ppm) or $(CD_3)_2CO$ (2.04 ppm) and for ^{13}C (100, 125 or 150 MHz) spectra, δ values were referenced to $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm) or $(CD_3)_2CO$ (29.8 ppm) as the solvents. Infrared spectra (IR) were recorded on a Nicolet Magna 750 or a 20SX FT-IR spectrometers. Mass spectra (MS) were recorded on a Kratos AEIMS-50 (high resolution, HRMS).

All commercially available reagents were purchased and used without further purification. All the solvents used for reactions were distilled over appropriate drying reagents prior to use. Commercially available ACS grade solvents (> 99.0% purity) were used for column chromatography without any further purification.

All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using glass plates with a UV fluorescent indicator (normal SiO_2, Merck 60 F_{254}). Flash chromatography was performed using Merck type 60, 230-400 mesh silica gel. The following visualization methods were used for monitoring reactions and column chromatography: UV absorption by fluorescence quenching; Ninhydrin spray (Ninhydrin: acetic acid: *n*-butanol/ 0.6g:6mL:200mL), PMA stain (phosphomolybdic acid, H_2SO_4)

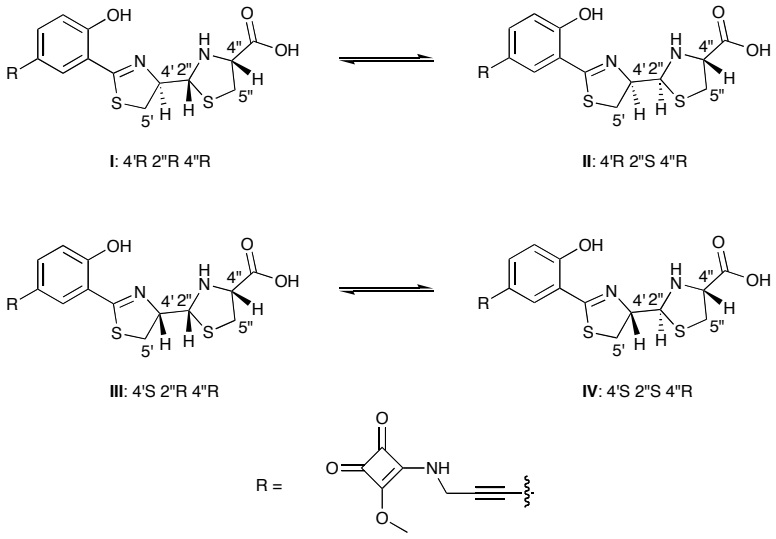
High performance liquid chromatography (HPLC) was performed on a Varian Prostar chromatograph equipped with a model 325 variable wavelengths UV detector and a Rheodyne 7725i injector. The columns used were Alltech ProSphere™ HP C_4 steel walled column (reverse phase, C_4 column, 5 mm, 300 Å, 4.6 x 250 mm) or a Vydac protein & peptide C_{18} (218TP510) steel walled column (reverse phase, C_{18} column, 5 μM, 10 x 250 mm).

Fluorescence imaging was done on a Nikon TE2000-E microscope, equipped with epifluorescence optic, using a 100x objective. Images were captured using a Fastcam Super 10K camera (Photron USA, Inc., San Diego, CA).

Experimental details:

1. Pyochelin analogues[1]

(4*R*)-2-((*R*)-2-(2-hydroxy-5-(3-(2-methoxy-3,4-dioxocyclobut-1-enylamino)prop-1-ynyl)phenyl)-4,5-dihydrothiazol-4-yl)-3-methylthiazolidine-4-carboxylic acid (**2**)



Pyochelin analogues were isolated as two sets of interconvertable diastereomers (4'*R*2"*R*4"*R*: 4'*R*2"*S*4"*R*:4'*S*2"*R*4"*R*:4'*S*2"*S*4"*R*/ 2:1:3:1 by ^{1}H NMR). Purified pyochelin I was analyzed by HPLC and the HPLC chromatogram shows pyochelin I (t_R = 15.5 min) and pyochelin II (t_R = 16.4 min) exist in equilibrium in solution. In addition, analysis shows that pyochelin III (t_R = 14.6 min) also exists in equilibrium with pyochelin IV (t_R = 16.6 min).

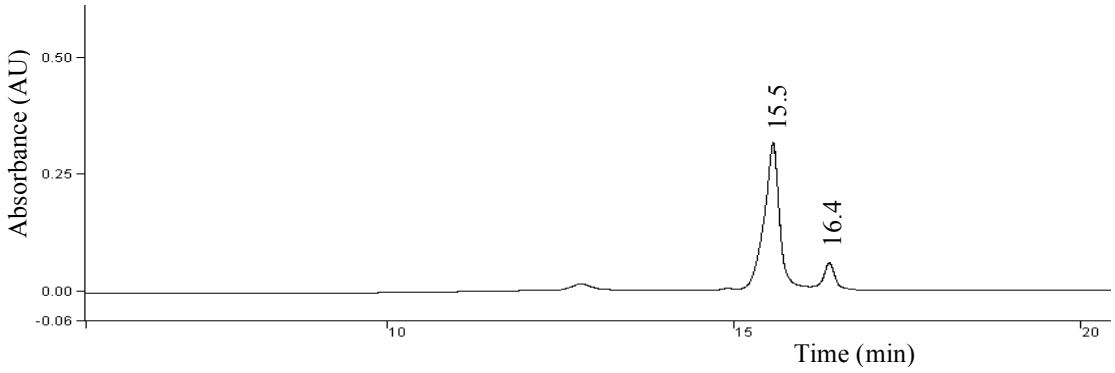


Figure 1: HPLC trace of purified pyochelin I

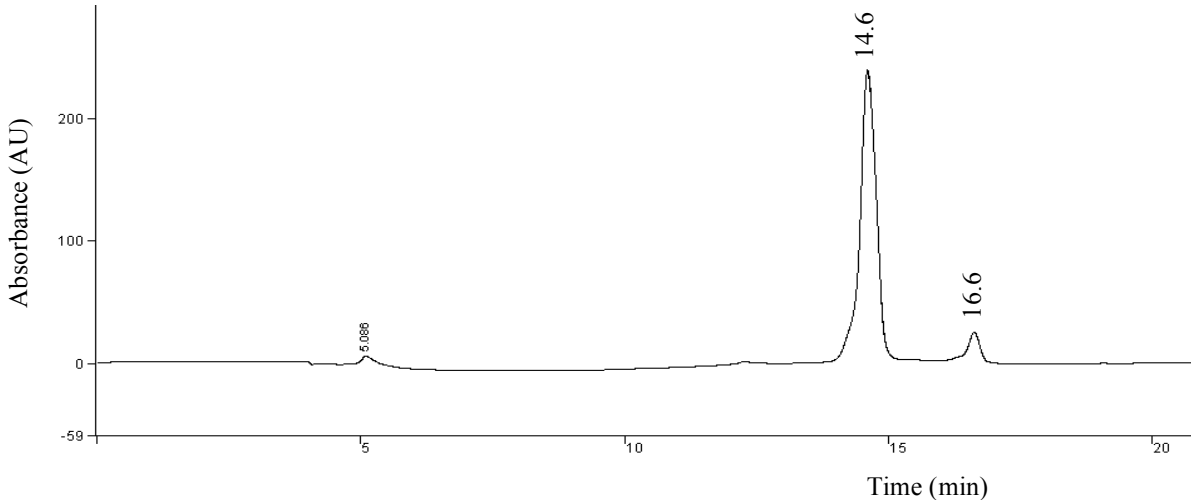


Figure 2: HPLC trace of purified pyochelin III

2. Growth promotion assay of selected Gram-negative bacteria under iron deficient condition[1, 2]

Growth promotion study was done using a 64-well plate assay, in iron deficient M9 minimal media. All three siderophores (**A**, **B**, **C**) were complexed to ferric ion to give a final concentration of 10 μM. The results from the evaluation of three strains are shown below.

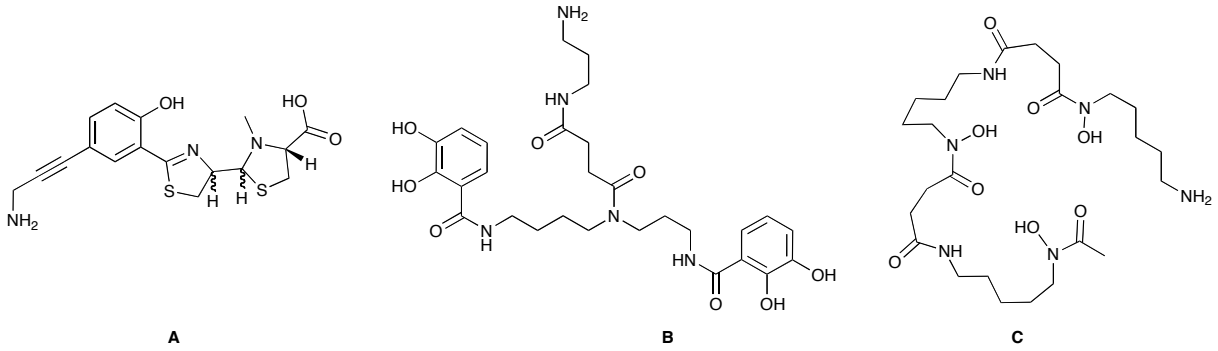


Figure 3: Siderophore analogues used in the growth promotion assay

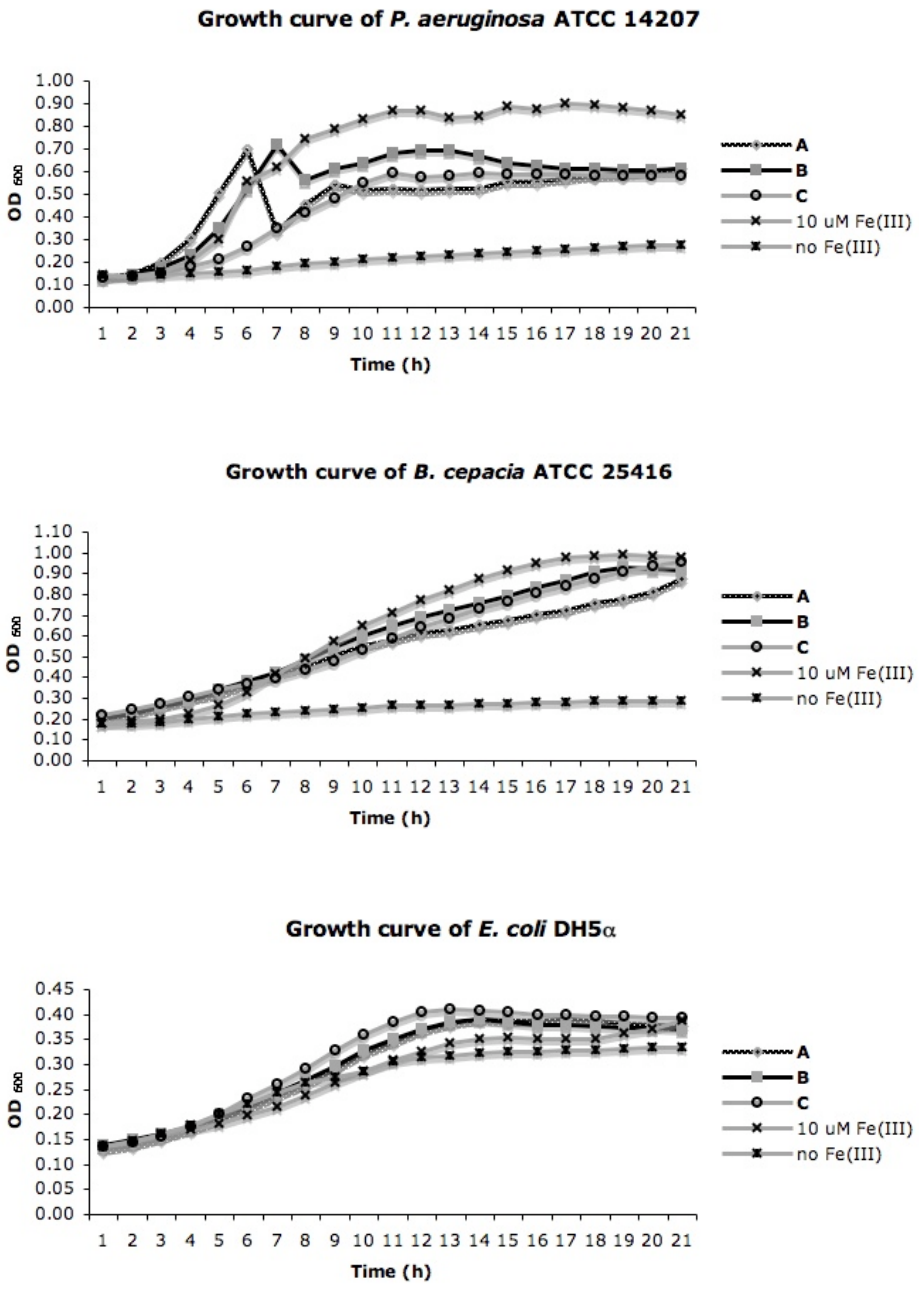
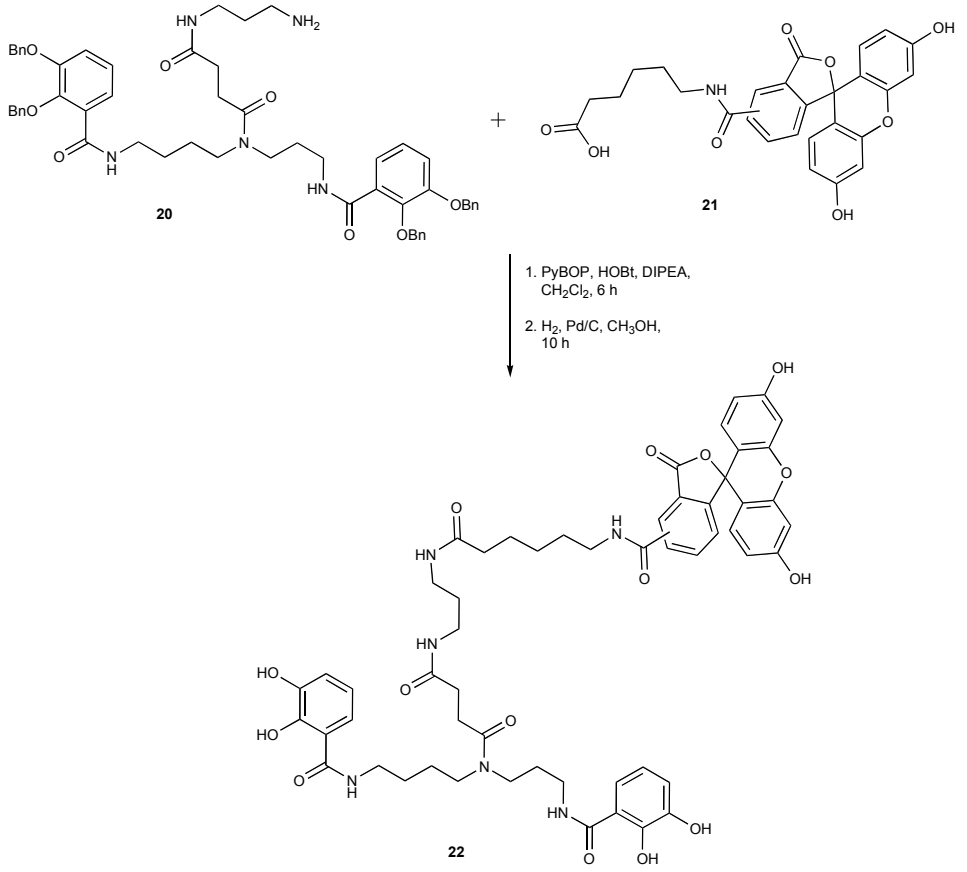


Figure 4: Bacterial growth promoted by siderophore analogues used in this study

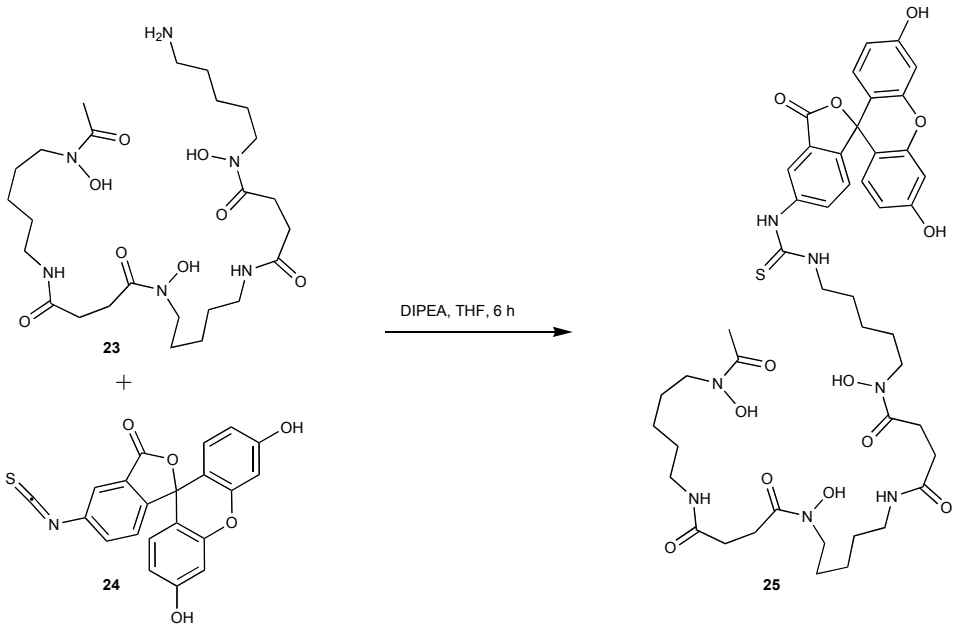
3. Fluorescence imaging of bacterial cells

3.1 Synthesis of agrobactin analogue-fluorescein conjugate (22)



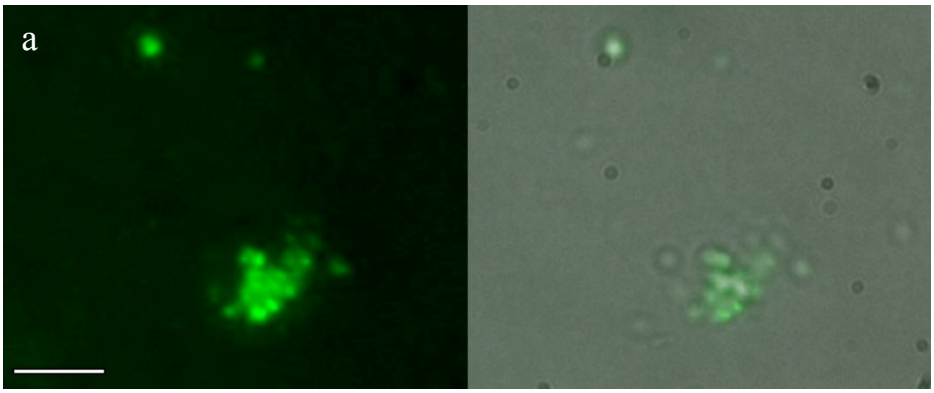
A solution of fluorescein derivative **21** (17 mg, 0.035 mmol), PyBOP (18 mg, 0.035 mmol), HOBt (5.0 mg, 0.037 mmol) and DIPEA (12 μL, 0.07 mmol) in CH_2Cl_2 (3 mL) was stirred at 0 ˚C for 5 minutes. Then the solution was added with agrobactin analogue **20** (20 mg, 0.035 mmol) and allowed to stir at 23 ˚C for another 6 h. The reaction mixture was concentrated, re-dissolved in EtOAc (20 mL) and washed with water (2 x 10 mL). The organic layer was dried over Na_2SO_4 and concentrated to yield the product. The crude product was used in the next step without further purification. The product was dissolved in MeOH (10 mL) and to the solution was added Pd/C (7.5 mg, 7.0 μmol). The reaction mixture was stirred under a hydrogen atmosphere for 10 h, filtered through a pad of celite and concentrated to give the crude product. The product was purified by HPLC (eluent: H_2O/CH_3CN with 0.1% TFA) using a C18 preparative column (Phenomenex Luna, 10 μm, 21.2 mm × 250 mm). Flow rate: 10.0 mL/min. Gradient: Starting from 10% CH_3CN for 3 minutes and 1st ramp to 40% over 27 minutes, 2nd ramp to 90% CH_3CN over 3 minutes and then 90% CH_3CN for 5 minutes, followed by ramping down to 10 % CH_3CN over 1 minutes, then 10% CH_3CN for 4 minutes. Fractions containing the product were combined and lyophilized to yield the product (10 mg, 27%) as a pale yellow residue. HPLC retention time: 19.5 min. *m/z*(MALDI-TOF) calcd for $C_{55}H_{61}N_6O_{15}$ 1045.4, found 1045.3 [MH^+].

3.2 Synthesis of desferrioxamine B-fluorescein conjugate (25)



A solution of fluorescein derivative **24** (29 mg, 0.076 mmol) and DIPEA (26 μL, 0.15 mmol) in THF (5 mL) at 0 ˚C was added with desferrioxamine B • mesylate salt (50 mg, 0.076 mmol) and allowed to stir at 23 ˚C for 8 h. The reaction mixture was concentrated, re-dissolved in H_2O: CH_3OH (1:1) and purified by HPLC (eluent: H_2O/CH_3CN with 0.1% TFA) using a C8 preparative column (ZORBAX Rx 7 μm, 21.2 mm × 250 mm). Flow rate: 10.0 mL/min. Gradient: Starting from 10% CH_3CN for 3 minutes and 1st ramp to 35% over 4 minutes, 2nd ramp to 90% CH_3CN over 23 minutes and then 90% CH_3CN for 5 minutes, followed by ramping down to 10 % CH_3CN over 1 minutes, then 10% CH_3CN for 4 minutes.. Fractions containing the product were combined and lyophilized to yield the product (50 mg, 70%) as a yellow residue. HPLC retention time: 15.9 min. *m/z*(ES+) calcd for $C_{46}H_{60}N_7O_{13}S$ 949.4, found 950.3 [MH^+].

3.3 Fluorescence images of bacterial cells treated with compound 22



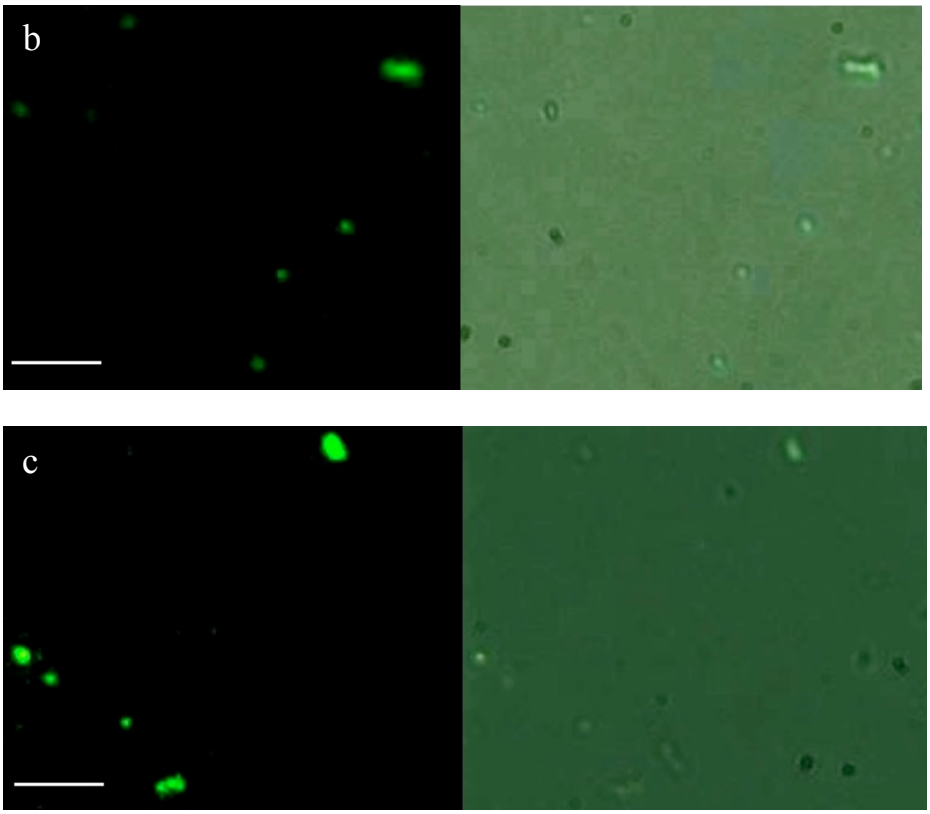
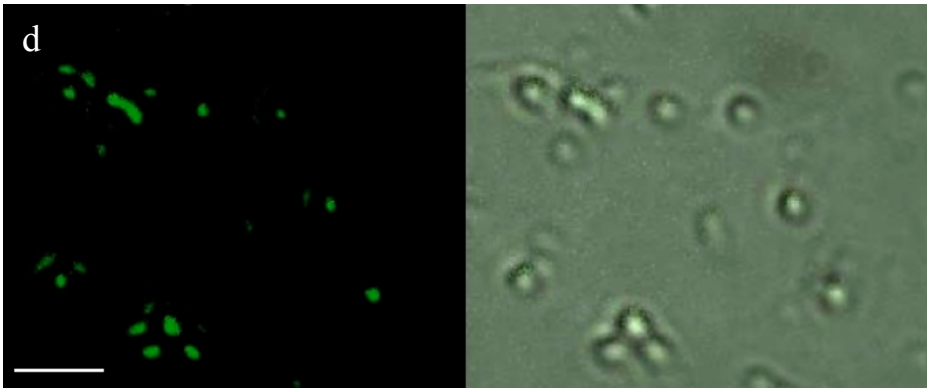


Figure 5: Fluorescence images of (a) *P. aeruginosa* ATCC 14207, (b) *B. cepacia* ATCC 25416 and (c) *E. coli* DH5α (scale bar, 10 μm)

3.4 Fluorescence images of bacterial cells treated with compound 25



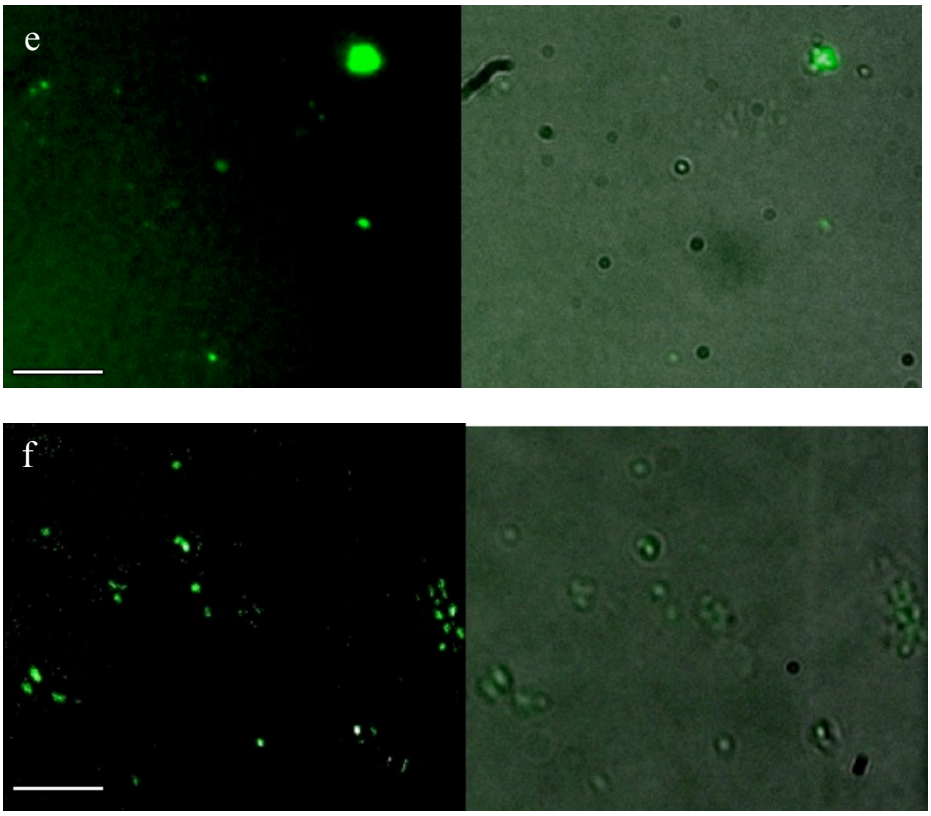
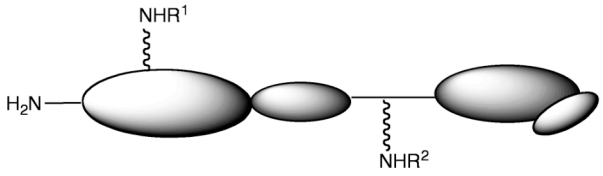
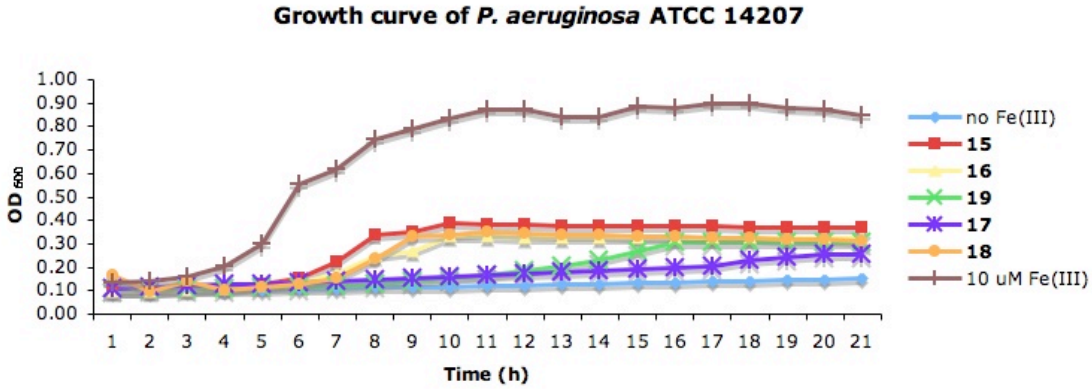


Figure 6: Fluorescence images of (d) *P. aeruginosa* ATCC 14207, (e) *B. cepacia* ATCC 25416 and (f) *E. coli* DH5α (scale bar, 10 μm)

4. Bacterial testing of gallidermin-siderophore conjugates against Gram-negative bacteria



15 : R^1 = H, R^2 = pyochelin derivative;
16 : R^1 = pyochelin derivative, R^2 = H;
17 : R^1 = H, R^2 = biscatechol derivative;
18 : R^1 = biscatechol derivative, R^2 = H;
19 : R^1 = R^2 = desferrioxamine B



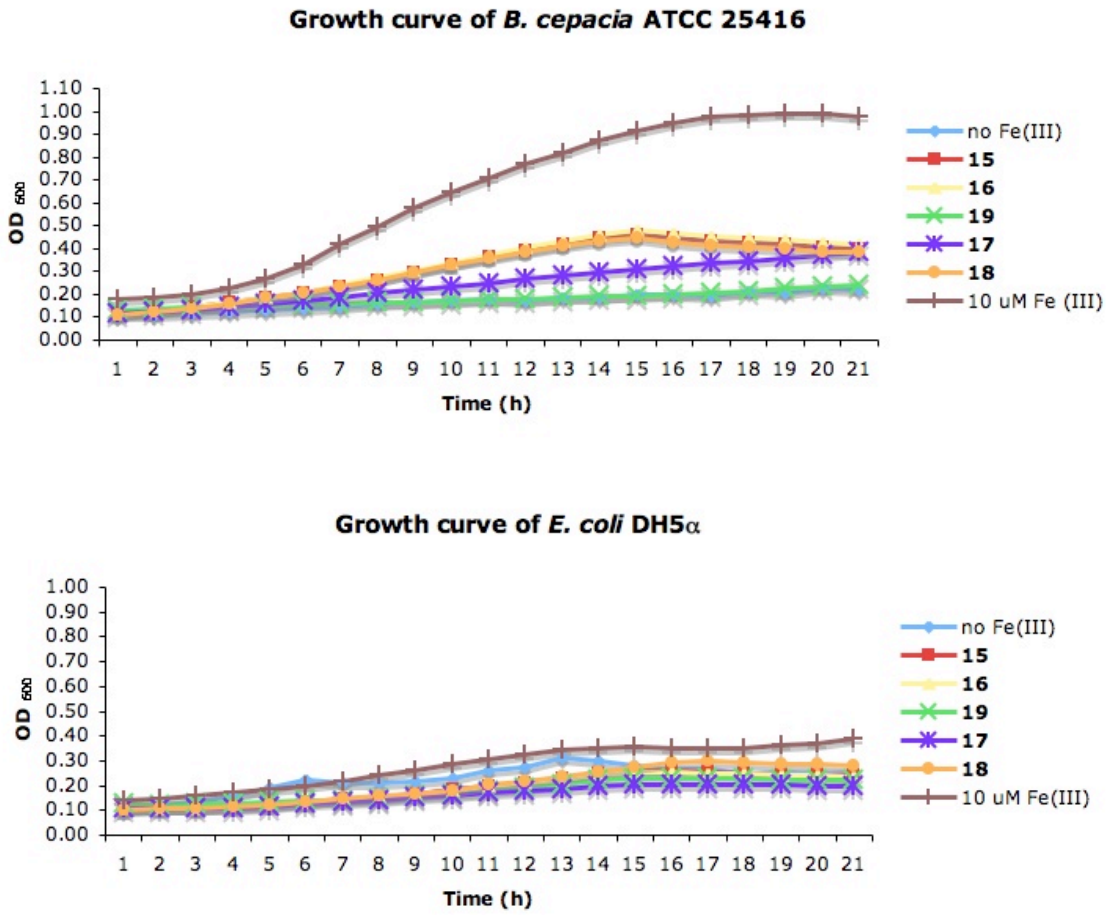


Figure 7. Evaluation of gallidermin-siderophore conjugates against Gram-negative bacteria

References:

1. Rivault, F.; Schons, V.; Liebert, C.; Burger, A.; Salcr, E.; Abdallah, M. A.; Schalk, I. J.; Mislin, G. L. A. *Tetrahedron* **2006**, *62*, 2247-2254.
2. Sharma, S. K.; Miller, M. J.; Payne, S. M. *J. Med. Chem.* **1989**, *32*, 357-367.