Supplementary Material (ESI) for Chemical Communications

Inhibition and Dispersion of Proteobacterial Biofilms

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Materials and Methods. All reagents including anhydrous solvents used for the chemical synthesis of the libraries were purchased from commercially available sources and used without further purification. Flash silica gel chromatography was performed with 60Å mesh standard grade silica gel from Sorbtech. ^{1}H and ^{13}C NMR spectra were obtained using Varian 300 MHz or 400 MHz spectrometers. NMR solvents were purchased from Cambridge Isotope Labs and used as is. Chemical shifts are given in parts per million relative to DMSO-d_6 (δ 2.50) for proton spectra and relative to DMSO-d_6 (δ 39.51) for carbon spectra with an internal TMS standard. High-resolution mass spectra were obtained at the North Carolina State Mass Spectrometry Laboratory for Biotechnology. FAB experiments were carried with a JOEL HX110HF mass spectrometer while ESI experiments were carried out on Agilent LC-TOF mass spectrometer.

P. aeruginosa strains PAO1, PA14, and PDO300 were supplied by the Wozniak group at WFU School of Medicine. *A. baumannii* was purchased from ATCC (strain # 19606).

B. bronchiseptica strain RB50 and the necessary media with supplement was graciously provided to us by the Deora group at WFU School of Medicine.

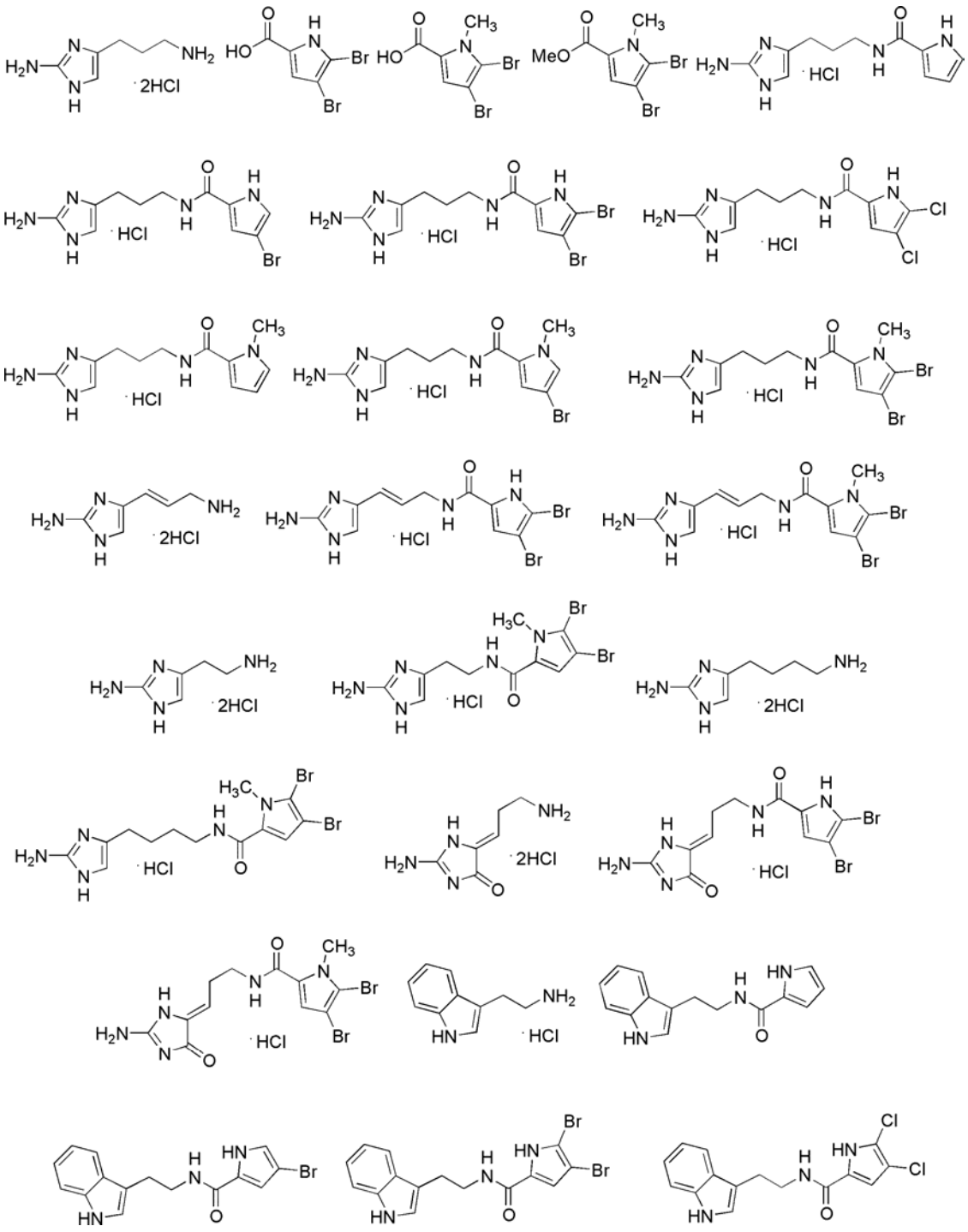
General Static Inhibition Assay Protocols for *Pseudomonas aeruginosa and Acinetobacter baumannii*. An overnight culture of the wild type strain was subcultured at an OD_{600} of 0.10 into either LBNS (*P. aeruginosa*) or LB (*A. Baumannii*) along with a predetermined concentration of the small molecule to be tested for biofilm inhibition. Samples were then aliquoted (100 μL) into the wells of a 96-well PVC microtiter plate. The microtiter dishes were covered and sealed before incubation under stationary conditions at 37 °C for 24 hours. After that time, the medium was discarded and the plates thoroughly washed with water. The wells were then inoculated with a 0.1% aqueous solution of crystal violet (100 μL) and allowed to stand at ambient temperature for 30 minutes. Following another thorough washing with water the remaining stain was solubilized with 200 μL of 95% ethanol. Biofilm inhibition was quantitated by measuring the OD_{540} for each well by transferring 125 μL of the ethanol solution into a fresh polystyrene microtiter dish for analysis.

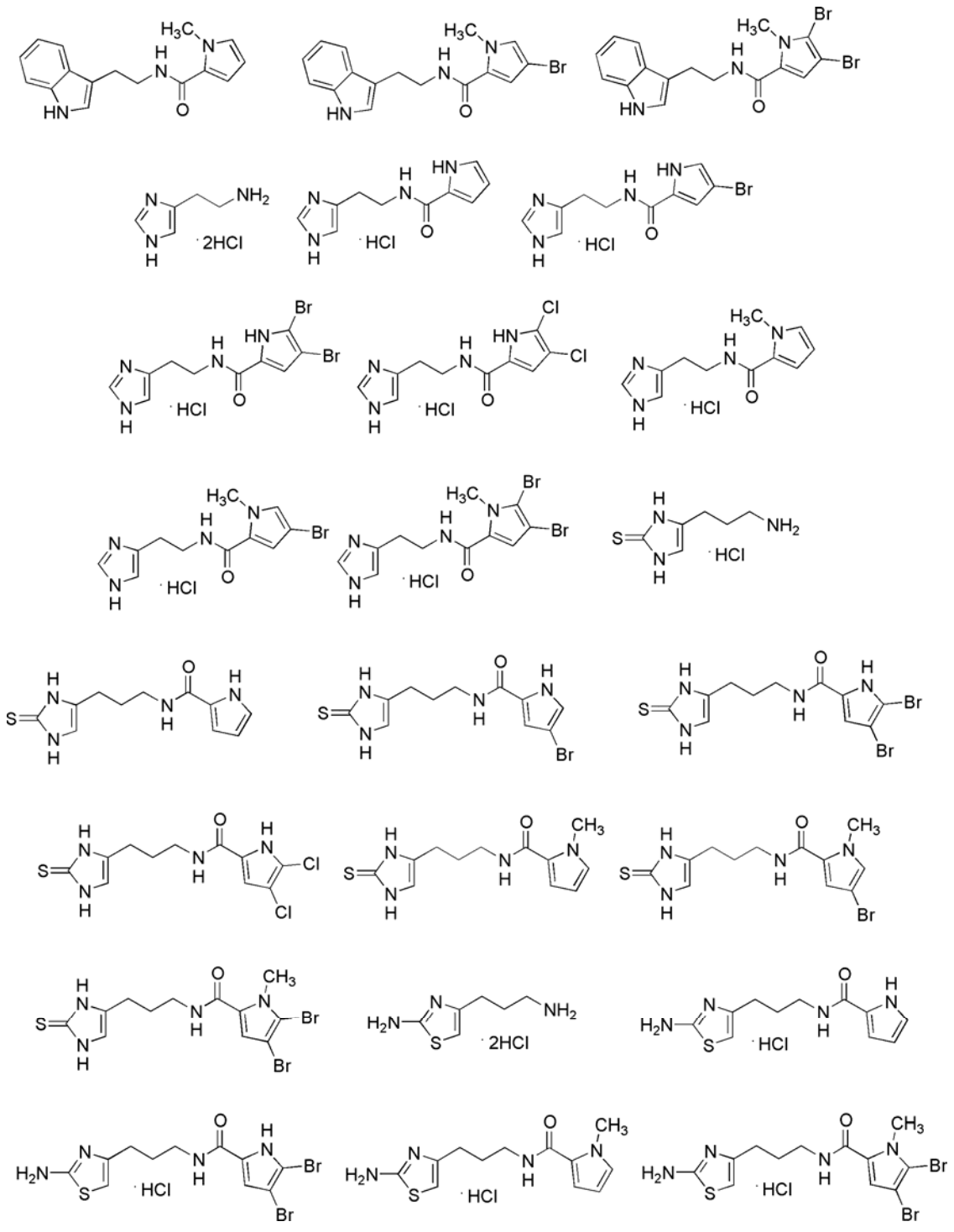
General Static Inhibition Assay Protocol for *Bordetella bronchiseptica* strain RB50. This procedure is identical to the above except that the overnight culture was subcultured at an OD_{600} of 0.50 into SS media containing 10 μL of 100X supplement per milliliter of media.

General Static Dispersion Assay Protocols for *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* An overnight culture of the wild type strain was subcultured at an OD_{600} of 0.50 into the appropriate medium for growth and then aliquoted (100 μL) into the wells of a 96-well PVC microtiter plate. The microtiter dishes were covered and sealed before incubation under stationary conditions at room temperature to allow formation of the biofilms. After 24 hours the medium was discarded and the plates thoroughly washed with water. Fresh medium containing the appropriate concentration of DHS were added to the wells. The plates were again sealed and this time incubated under stationary conditions at 37 °C. After 24 hours, the media was discarded from the wells and the plates washed thoroughly with water. The wells were then inoculated with a 0.1% aqueous solution of crystal violet (100 μL) and allowed to stand at ambient temperature for 30 minutes. Following another thorough washing with water the remaining stain was solubilized with 200 μL of 95% ethanol. Biofilm dispersion was quantitated by measuring the OD_{540} for each well by transferring 125 μL of the ethanol solution into a fresh polystyrene microtiter dish for analysis.

General Static Dispersion Assay Protocol for *Bordetella bronchiseptica* strain RB50. This procedure is identical to the dispersion protocol described above except that initial biofilm formation in the absence of DHS was first carried out at 37 °C due to the inability of the strain to form biofilms at ambient temperature.

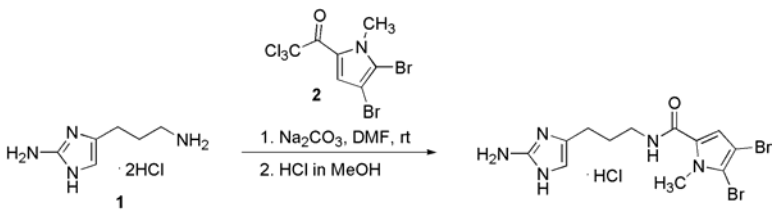
Chemical Library. The 50 member focused library of dihydrooroidin analogues were synthesized using solution phase methods and fully characterized (^{1}H NMR, ^{13}C NMR, and HRMS).





Synthesis of the natural products oroidin and sventrin. Oroidin was prepared as previously reported by the Horne group (*J. Org. Chem*. 1998, **63**, 1248). Sventrin was prepared utilizing the same synthetic pathway with the final step in the synthesis including the appropriate *N*-methyl derived trichloroacetyl ester.

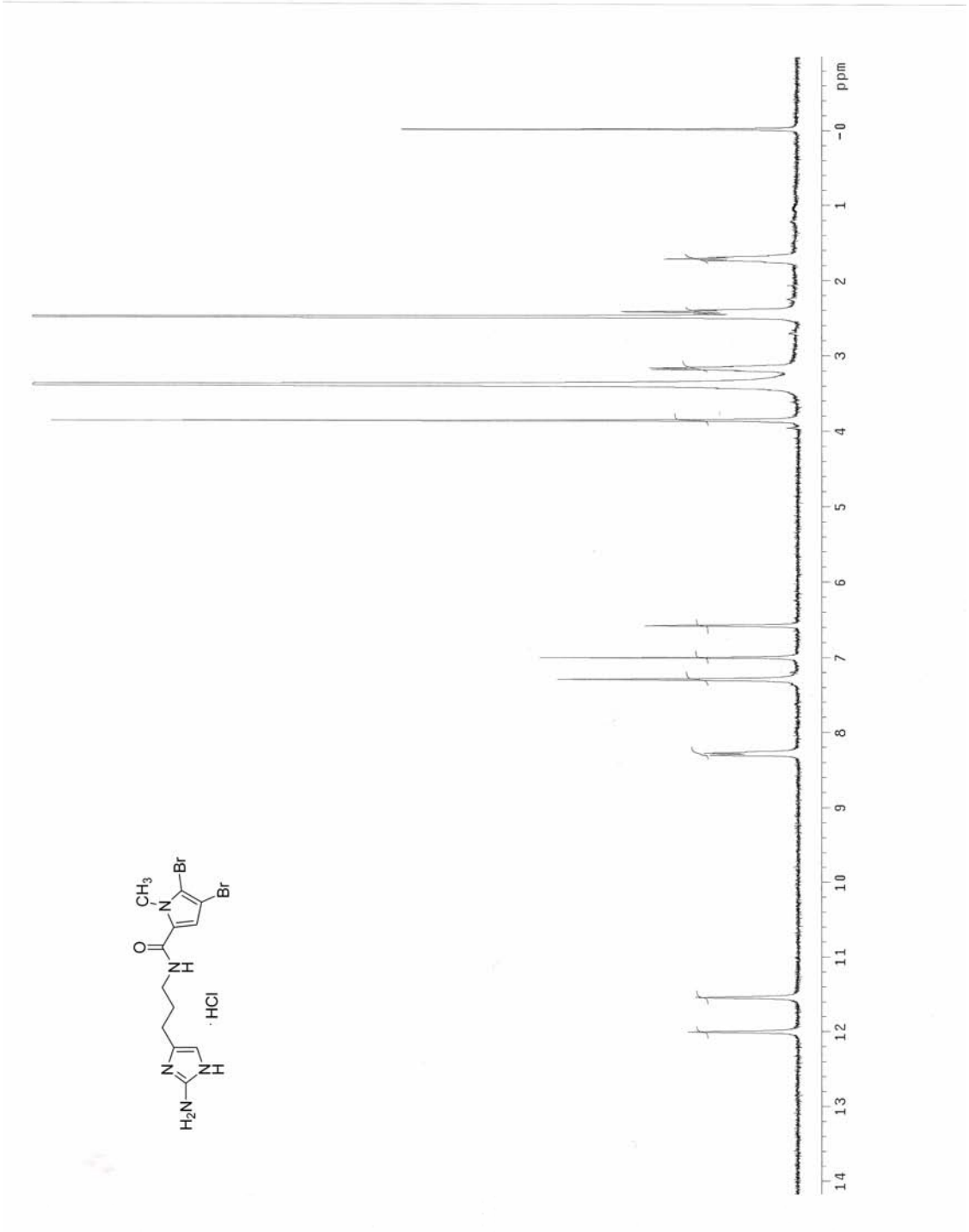
Synthesis of dihydrosventrin hydrochloride. 4-(3-amino-propyl)-1H-imidazol-2-ylamine dihydrochloride **1** was prepared as previously reported by the Horne group (*J. Org. Chem*. 1998, **63**, 1248). 2,2,2-trichloro-1-(4,5-dibromo-1-methyl-1H-pyrrol-2-yl)-

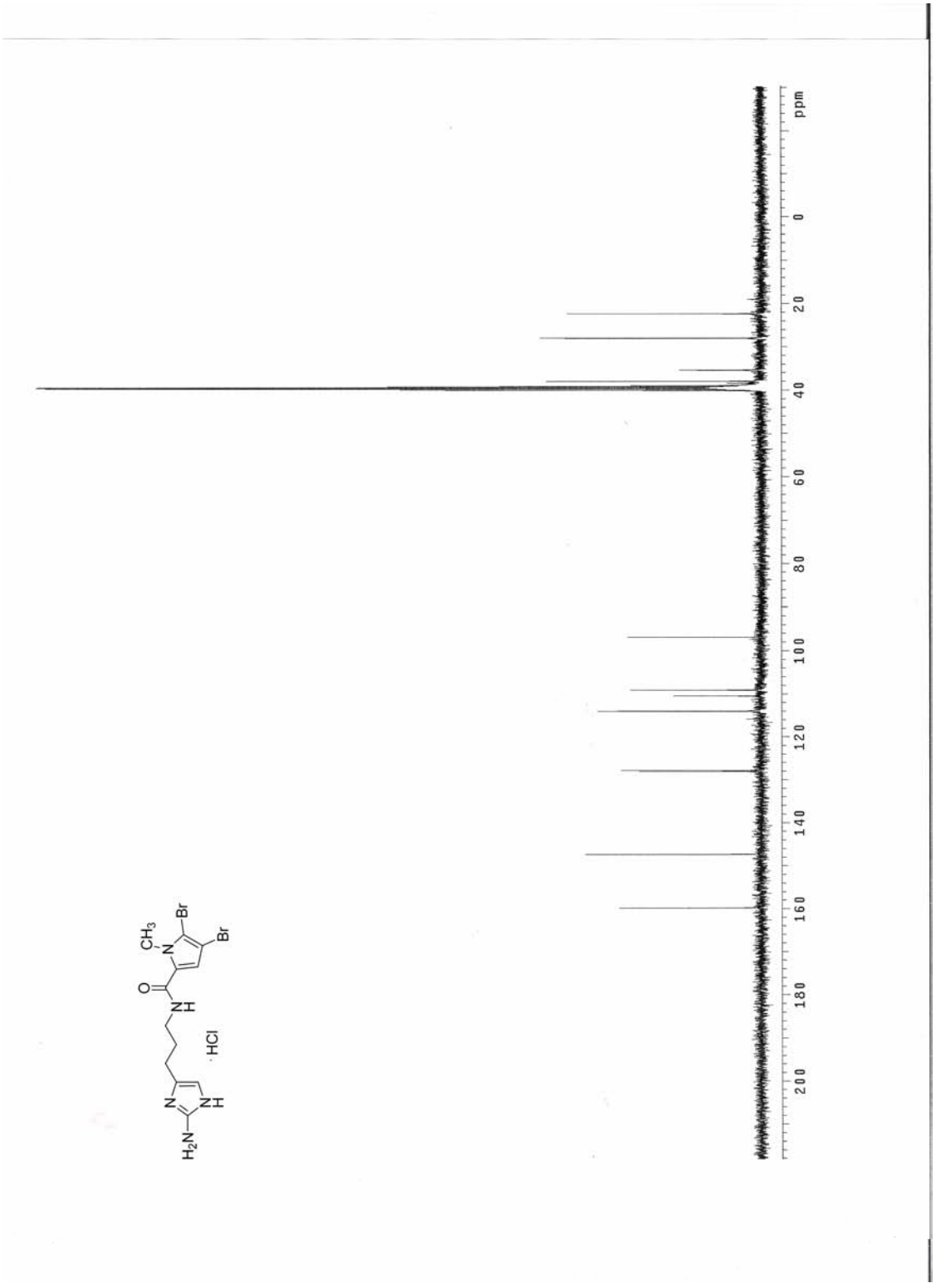


ethanone **2** was prepared using the procedure laid forth by Bailey (*J. Med. Chem*. 1973, **16**, 1300).

4-(3-amino-propyl)-1H-imidazol-2-ylamine dihydrochloride **1** (0.150 g, 0.698 mmol), 2,2,2-trichloro-1-(4,5-dibromo-1-methyl-1H-pyrrol-2-yl)-ethanone **2** (0.295 g, 0.767 mmol), and anhydrous sodium carbonate (0.221 g, 2.09 mmol), were dissolved in anhydrous *N,N*-dimethylformamide (8 mL). The reaction was stirred at ambient temperature for 16 h. Evaporation of the reaction under reduced pressure and purification of the residue by column chromatography (CH_2Cl_2/MeOH sat. NH_3 85:15) afforded the desired compound in its free base form. Addition of concentrated hydrochloric acid to a

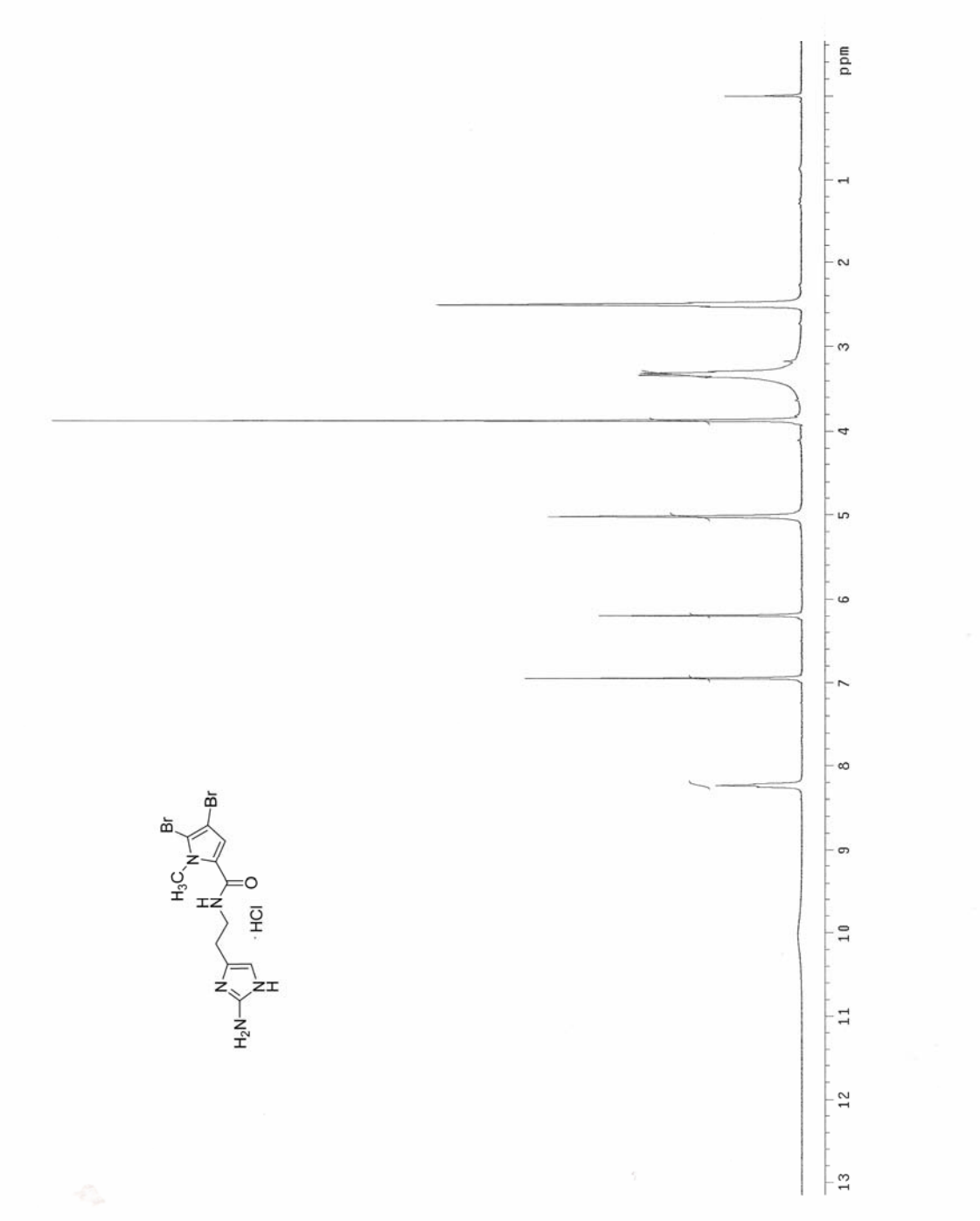
methanol solution (8 mL) of the freebase and evaporation under reduced pressure yielded 0.206 g (64%) of the title compound as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.06 (s, 1H), 11.59 (s, 1H), 8.31 (t, 1H, J = 5.4 Hz), 7.32 (s, 2H), 7.03 (s, 1H), 6.60 (s, 1H), 3.87 (s, 3H), 3.18 (m, 2H), 2.45 (t, 2H, J = 7.8 Hz), 1.73 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.77, 147.30, 127.99, 127.77, 114.00, 110.43, 109.06, 96.86; 37.94, 35.38, 27.96, 22.28; HRMS (FAB) calcd for $C_{12}H_{16}Br_2N_5O$ (MH^+) 403.9722, found 403.9728.

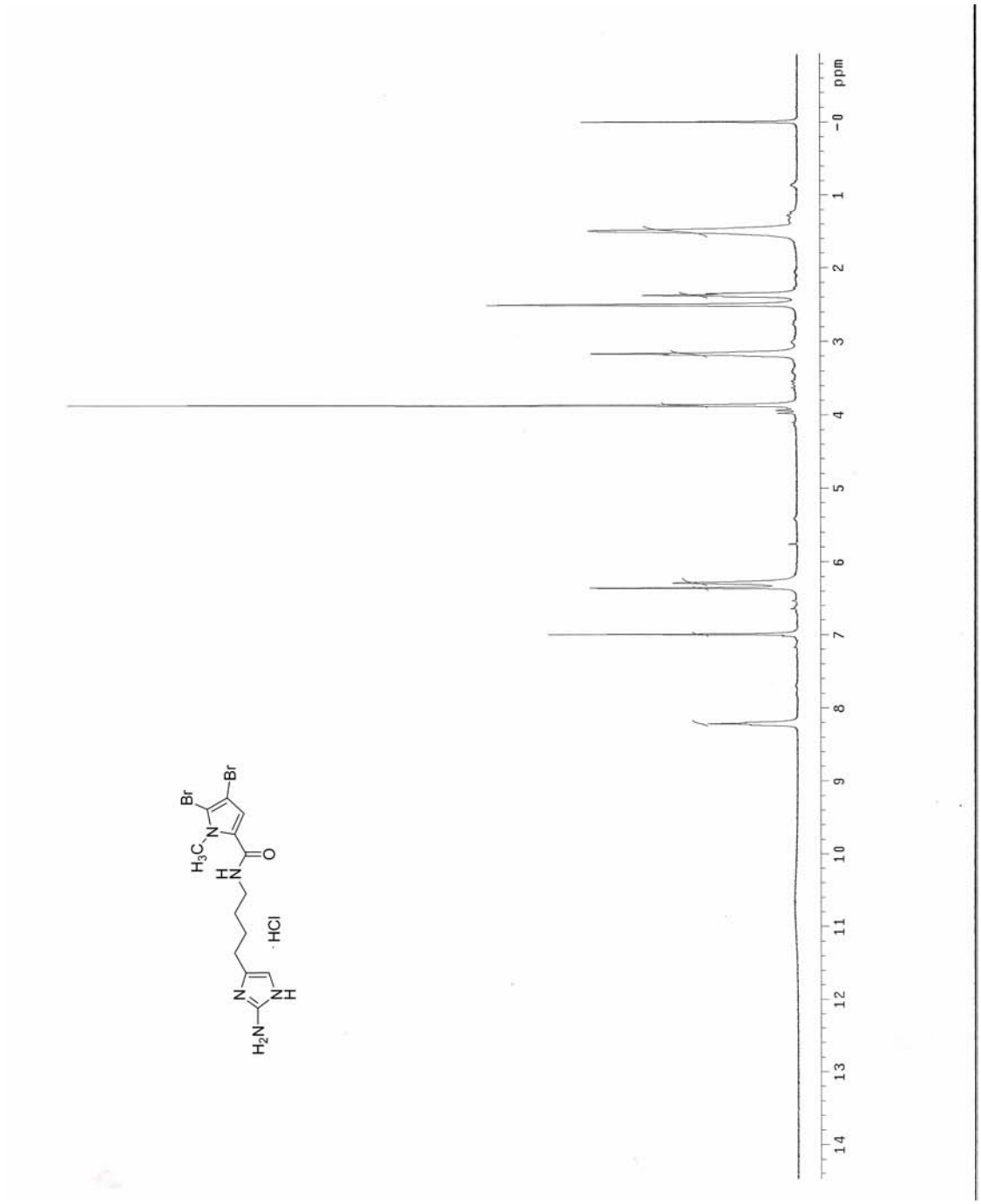


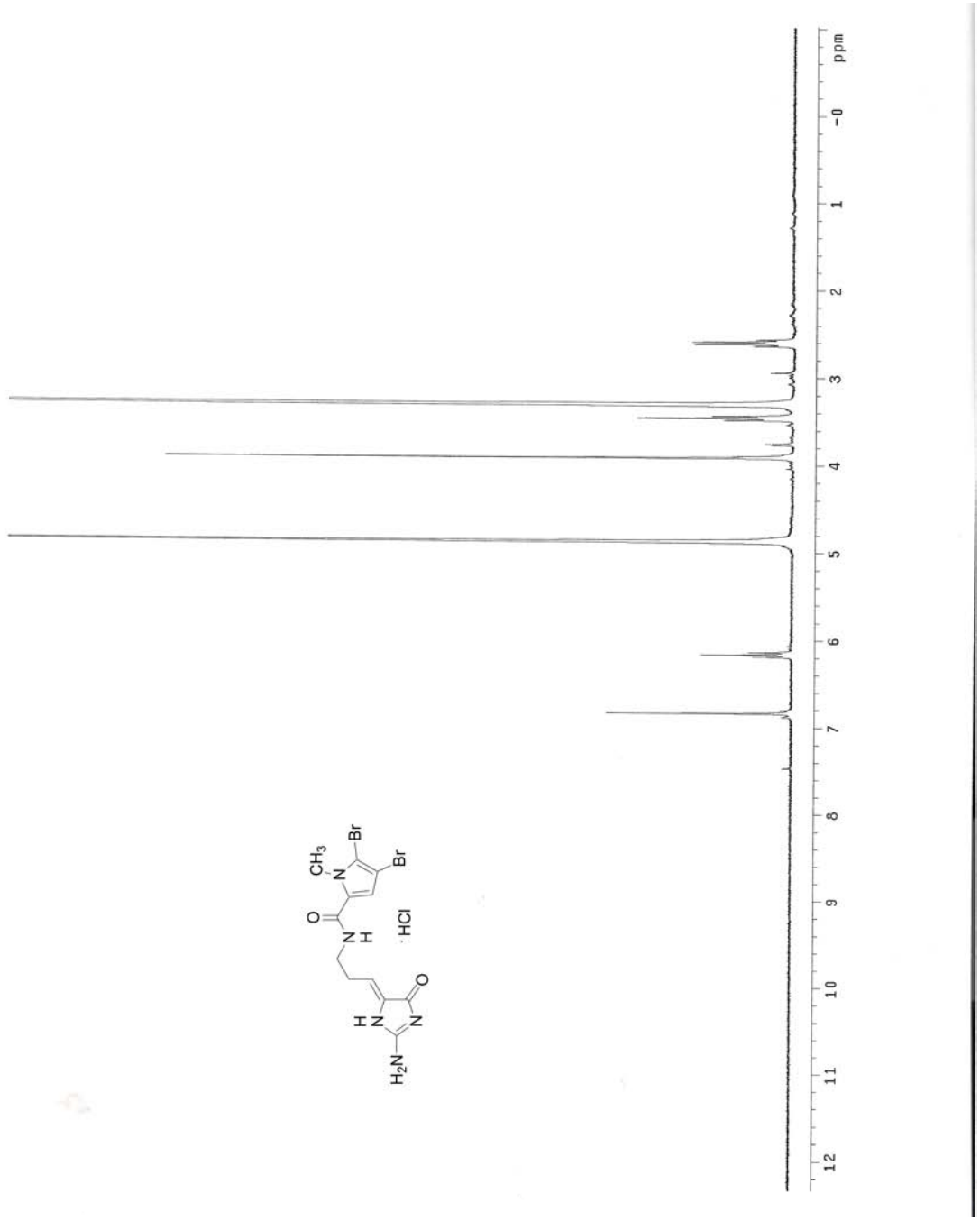


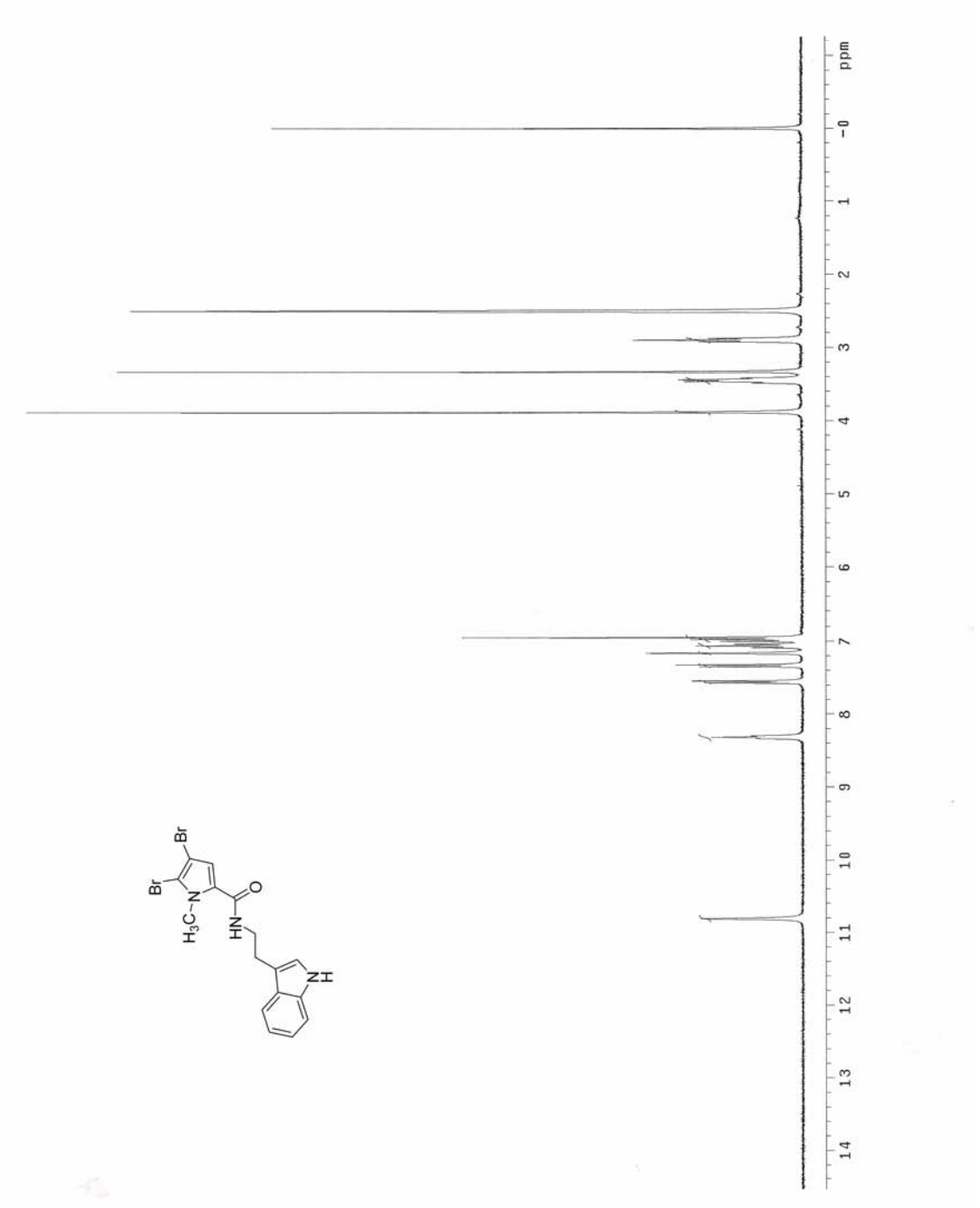
Representative ^{1}H NMR Spectra.

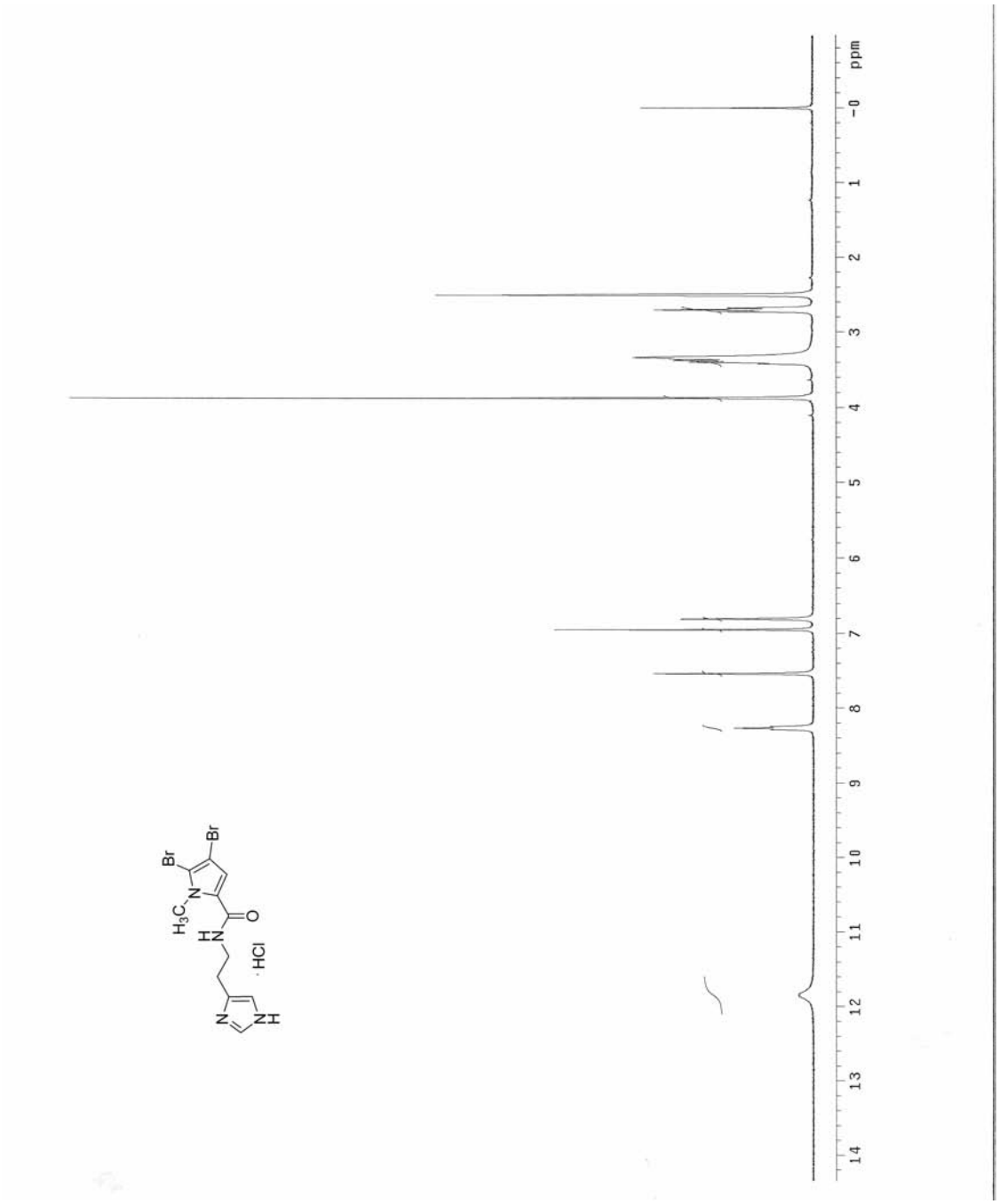
Full disclosure of the characterization data for all library members will be published in due course. Representative ^{1}H NMR spectra for target compounds from each scaffold are included below.

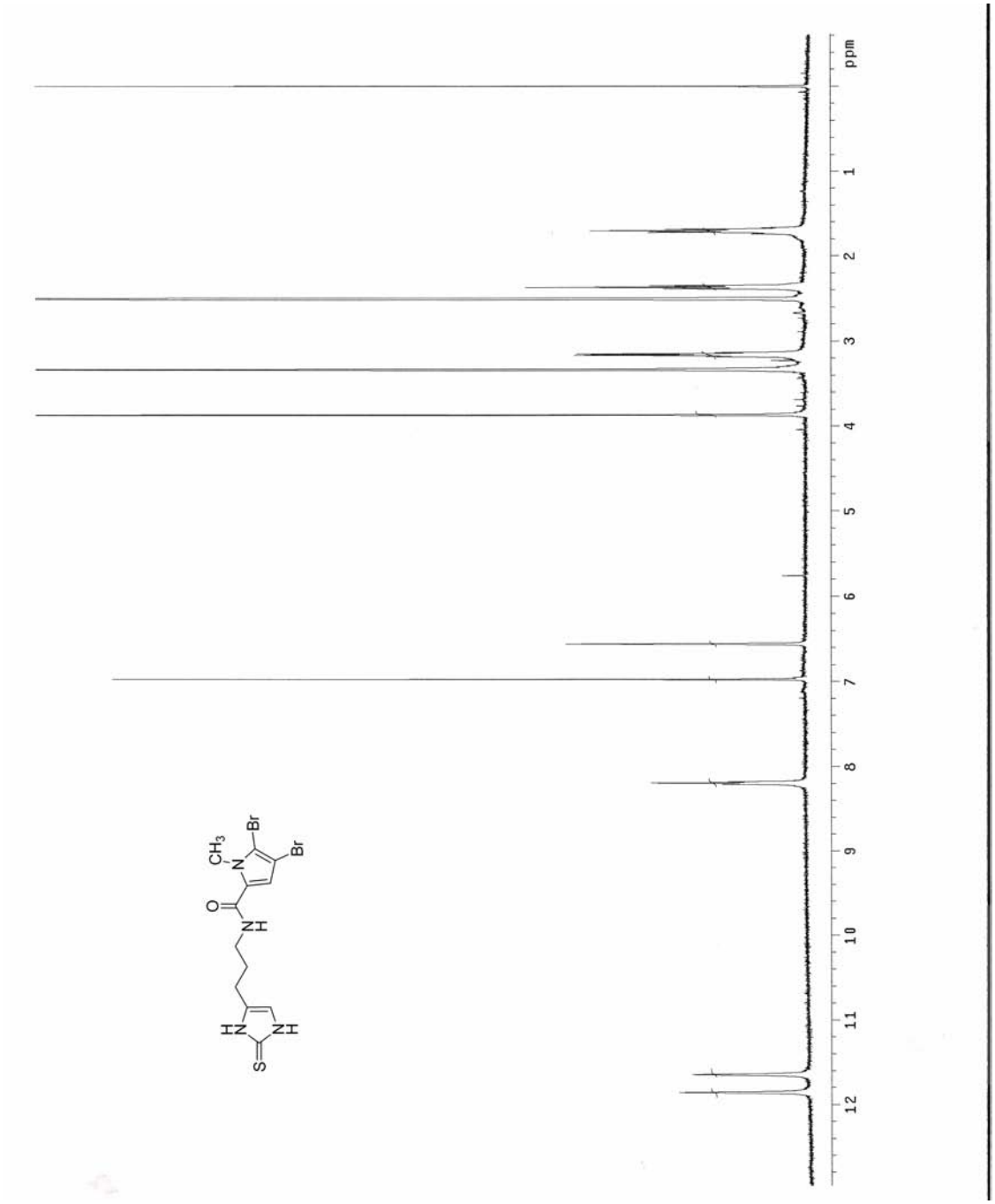


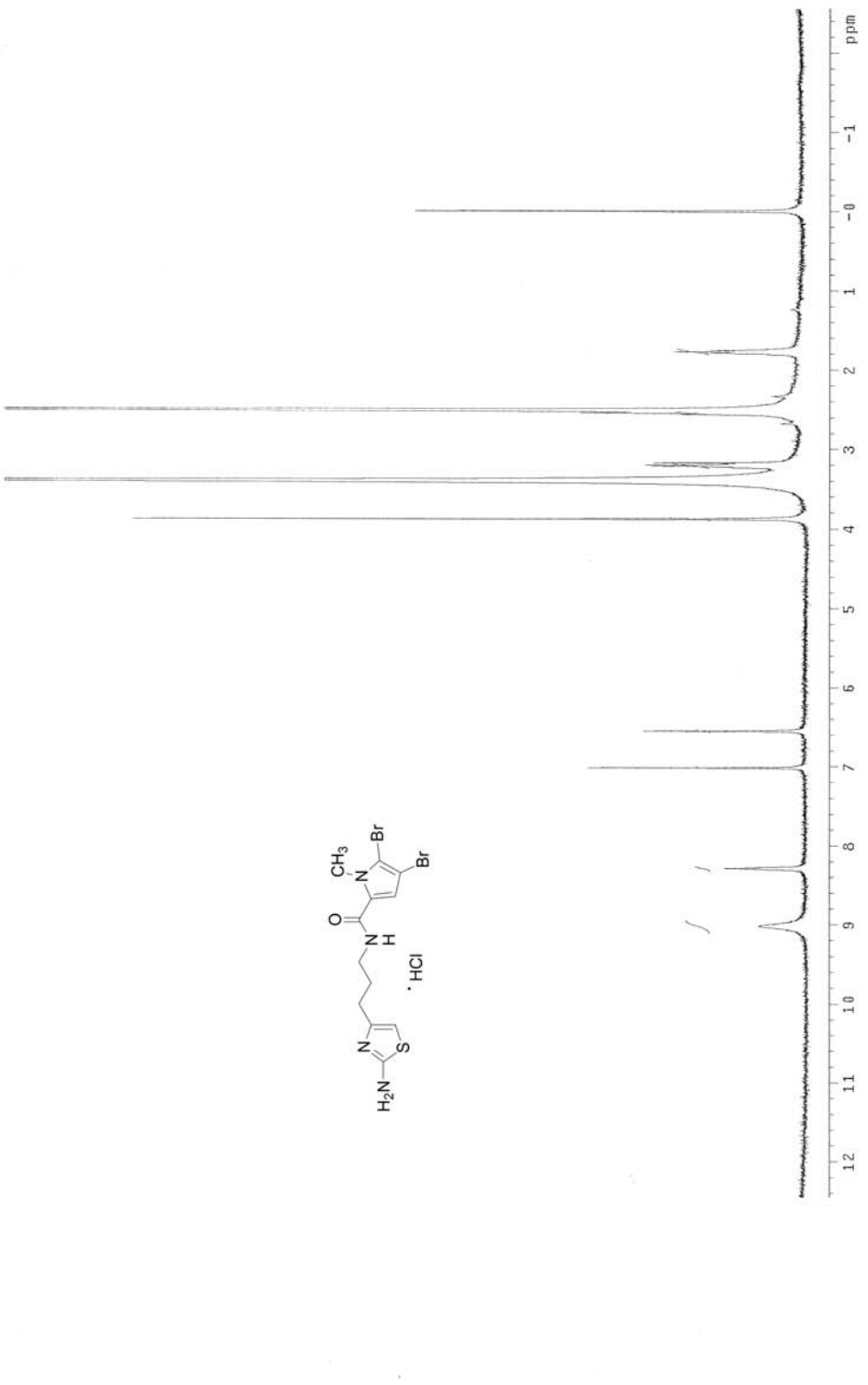












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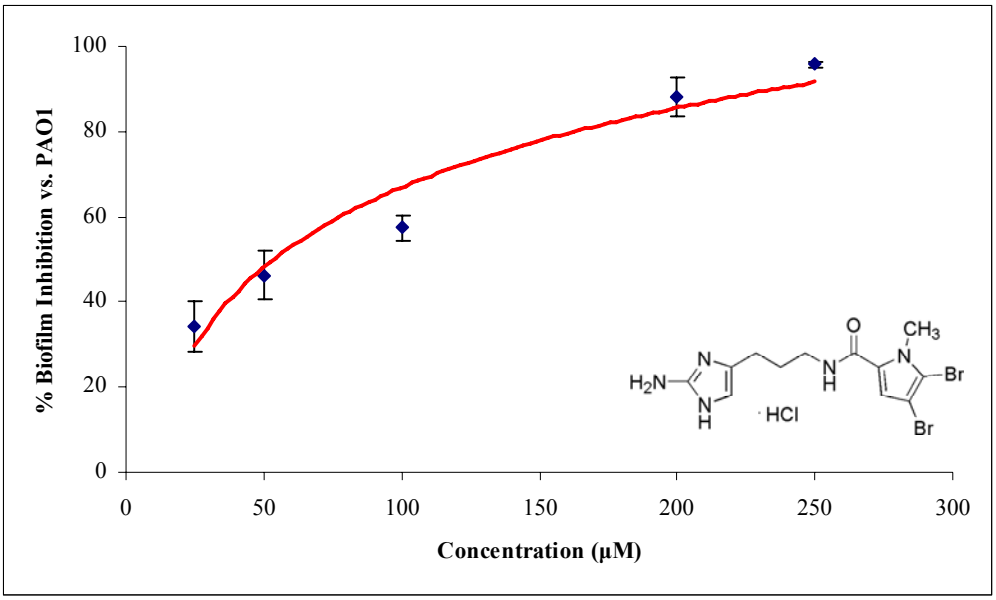
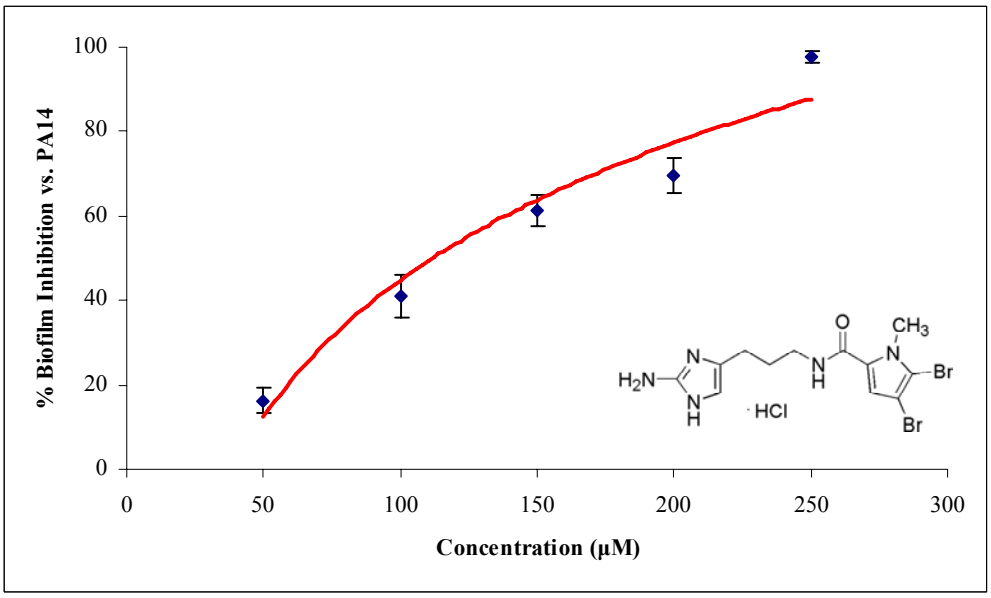
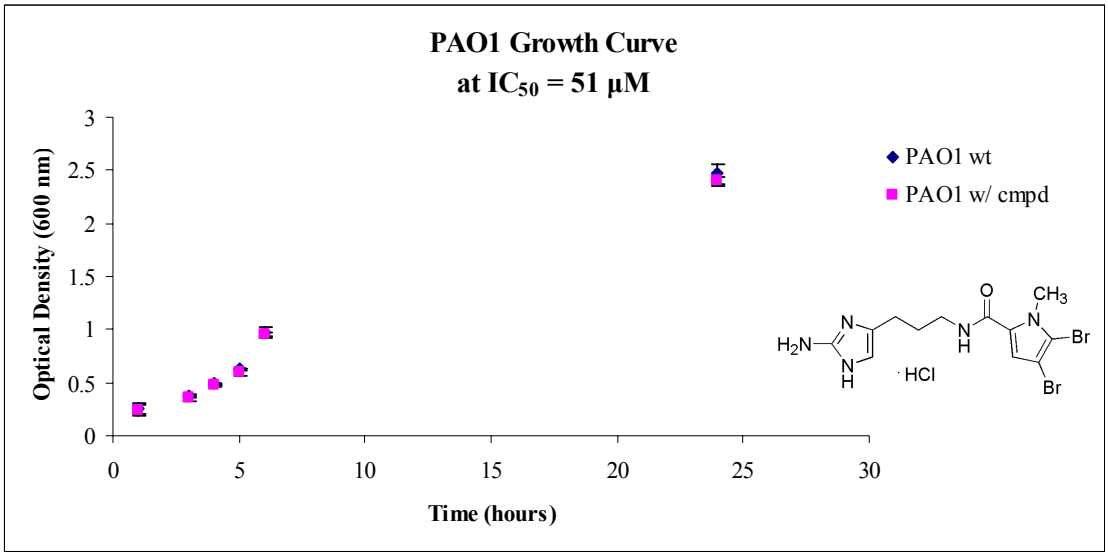
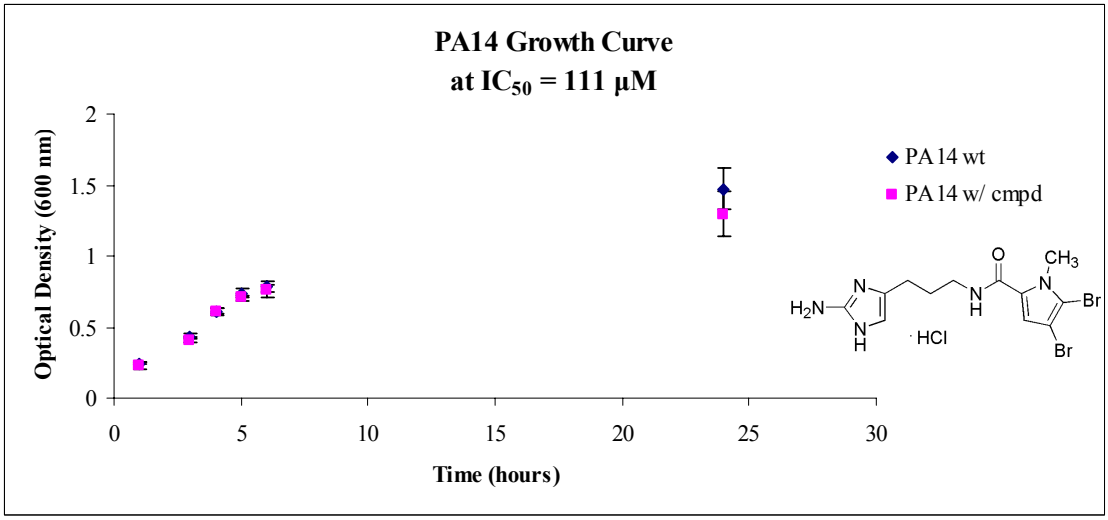
Representative Dose Response Curves for DHS against *Pseudomonas aeruginosa* strains PAO1 and PA14.



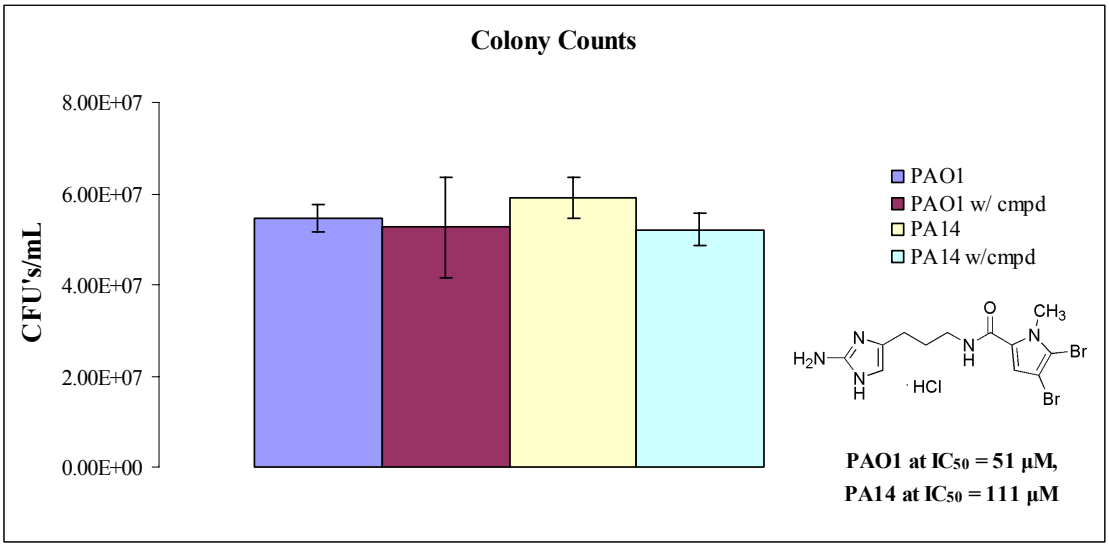


Growth Curves for DHS against *Pseudomonas aeruginosa* strains PAO1 and PA14.

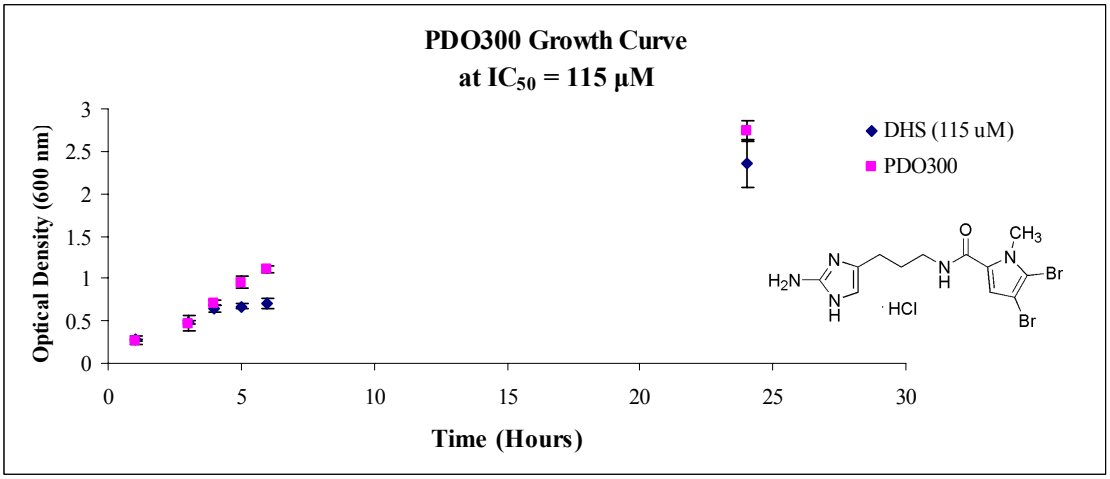




Colony Counts of *Pseudomonas aeruginosa* strains PAO1 and PA14 in the presence and absence of DHS.

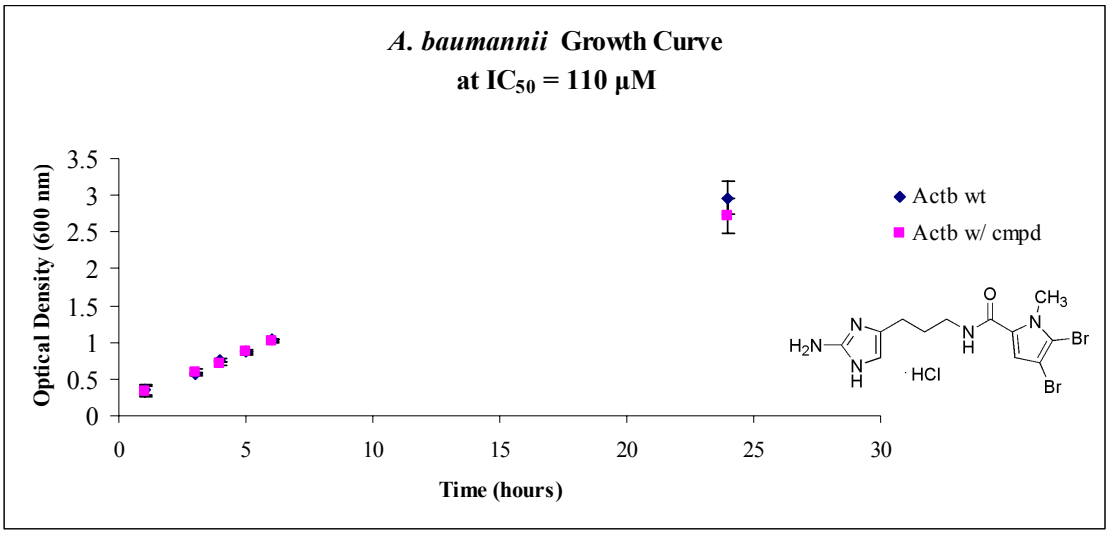


Growth Curve of *Pseudomonas aeruginosa* mucoid strain PDO300 in the presence and absence of DHS.

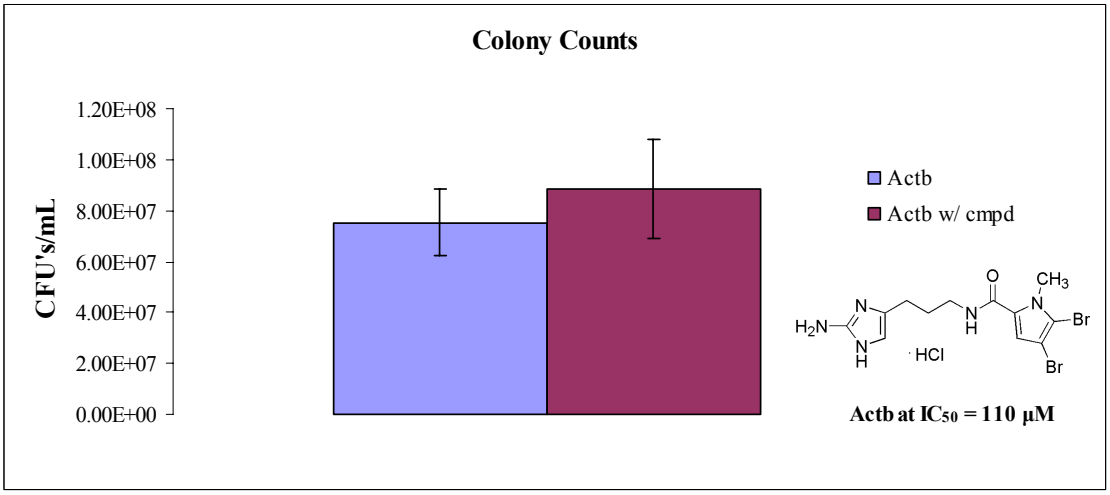


*Note: Although we observe a lag in growth, optical density of the bacteria was identical for both treated and untreated samples (within error) at the time point (24 hours) where biofilm development was measured for inhibition assays.

Growth curve of *Acinetobacter baumannii* in the presence and absence of DHS.



Colony Count of *Acinetobacter baumannii* in the presence or absence of DHS.



Growth Curve of *Bordetella bronchiseptica* strain RB50 in the presence or absence of DHS.