Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Phenazine Antibiotic Inspired Discovery of Potent Bromophenazine Antibacterial Agents against *Staphylococcus aureus* and *Staphylococcus epidermidis*

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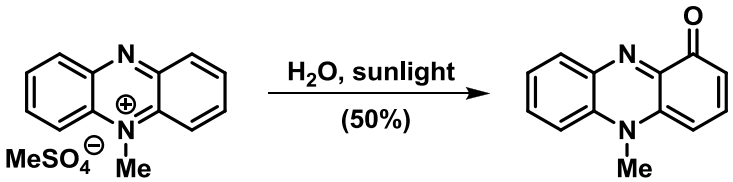
1) General Information:

All reactions were carried out under an atmosphere of argon unless otherwise specified. Anhydrous solvents were transferred via syringe to flame-dried glassware, which was cooled under a stream of dry argon. Anhydrous tetrahydrofuran, acetonitrile, diethyl ether, dichloromethane, toluene and all chemical reagents for synthesis were used without further purification. Analytical thin layer chromatography (TLC) was performed using 250 μm Silica Gel 60 F254 pre-coated plates (EMD Chemicals Inc.). Flash column chromatography was performed using 230-400 Mesh 60Å Silica Gel (Sorbent Technologies).

NMR experiments were recorded using broadband probes on a Varian Mercury-Plus-400 spectrometer via VNMR-J software (400 MHz for ^{1}H and 100 MHz for ^{13}C) and a Bruker Avance-III-500 spectrometer via TopSpin software (500 MHz for ^{1}H and 126 MHz for ^{13}C). All spectra have been formatted and presented using MestReNova (Mnova) software. Spectra were obtained in the following solvents (reference peaks also included for ^{1}H and ^{13}C NMRs): $CDCl_3$ (^{1}H NMR: 7.26 ppm; ^{13}C NMR: 77.23 ppm), d_6-DMSO (^{1}H NMR: 2.50 ppm; ^{13}C NMR: 39.52 ppm), CD_3OD (^{1}H NMR: 3.31 ppm; ^{13}C NMR: 49.00 ppm), d_6-benzene (^{1}H NMR: 7.16 ppm; ^{13}C NMR: 128.06 ppm). NMR samples where the respective solvent peaks were buried in the sample signals were referenced with TMS at 0.00 ppm for ^{1}H NMR experiments. NMR experiments were performed at room temperature unless otherwise indicated. Chemical shift values (δ) are reported in parts per million (ppm) for all ^{1}H NMR and ^{13}C NMR spectra. ^{1}H NMR multiplicities are reported as: s = singlet, d = doublet, t = triplet, q = quartet, hept = heptet, m = multiplet, br = broad.

Microdilution minimum inhibitory concentration (MIC) experiments were carried out according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI). Luria Broth was used in MIC experiments. Bacterial strains used during these investigations were: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228) and *Pseudomonas aeruginosa* (PAO1). All phenazine compounds were evaluated from 10 mM stock solutions in DMSO. A DMSO vehicle control was used in MIC experiments at the same volume as tested compounds in each 96-well plate. Kanamycin was used as a positive control against *S. aureus* and *S. epidermidis*.

2) Synthetic Procedures:



1

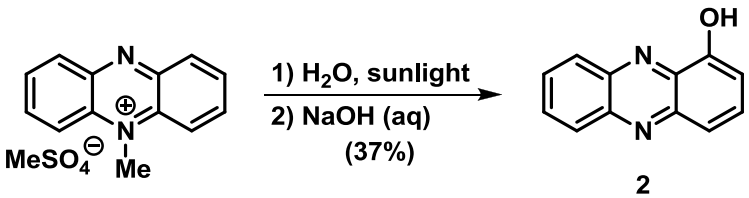
pyocyanin (1). Phenazine methosulfate (204 mg, 0.667 mmol) was added to a flask of 200 mL deionized water and subjected to direct sunlight for 30 minutes initially, followed by 5-10 minute periods of sunlight exposure during 8 hours whereby a dark green pigmentation was rapidly produced. The reaction was left stirring overnight, then quenched with 50 mL of 10% aqueous sodium carbonate. The deep blue solution was extracted exhaustively with 75 mL portions of chloroform. The organic layers were collected and dried with sodium sulfate, filtered and concentrated via rotavap. The crude reaction was then purified by column chromatography (sequential elution using 100% ethyl acetate, 19:1 ethyl acetate:methanol, 9:1 ethyl acetate:methanol) to furnish 59 mg (50% yield) of pyocyanin **1** as a blue solid. **NOTE:** Pyocyanin was synthesized using a known procedure[1] with a modified purification procedure.

^{1}H NMR (400 MHz, d_6-DMSO): δ 8.12 (d, J = 7.8 Hz, 1H), 7.95 (m, 1H), 7.89 (m, 1H) 7.66 (t, J = 8.7 Hz, 1H), 7.55 (t, 7.2 Hz, 1H), 6.26 (d, 9.3 Hz, 1H), 6.12 (d, 8.0 Hz, 1H), 3.91 (s, 3H); (500 MHz, CD_3OD): δ 8.18 (br s, 1H), 7.99 – 7.89 (m, 2H), 7.78 (br s, 1H), 7.61 (t, J = 7.7 Hz, 1H), 6.48 (d, J = 8.8 Hz, 1H), 6.38 (d, J = 7.6 Hz, 1H), 4.12 (br s, 3H).

^{13}C NMR (126 MHz, CD_3OD): δ 178.1, 146.8, 146.6, 137.9, 137.4, 136.2, 134.3, 133.8, 127.2, 116.5, 115.6, 94.4, 36.1.

HRMS (ESI): calc. for $C_{13}H_{11}N_2O$ $[M+H]^+$: 211.0866, found: 211.0857.

MP: 130-131 °C (lit. 130 °C).[2]



2

1-hydroxyphenazine (2). Phenazine methosulfate (604 mg, 1.97 mmol) was added to 600 mL deionized water and placed in direct sunlight for 30 minutes until a dark green color was observed. The flask was then position by a window receiving direct sunlight over the course of 58 hours. After this time, 11.5 grams sodium hydroxide dissolved in 35 mL water was slowly added to the reaction vessel, and stirring was continued for an additional 36 hours. The resulting purple solution was then transferred to a separatory funnel and washed with ether (removing phenazine as a side product in this reaction). The aqueous layer was then acidified with 30 mL glacial acetic acid and extracted with ether (2x). The organic layers were collected, dried with

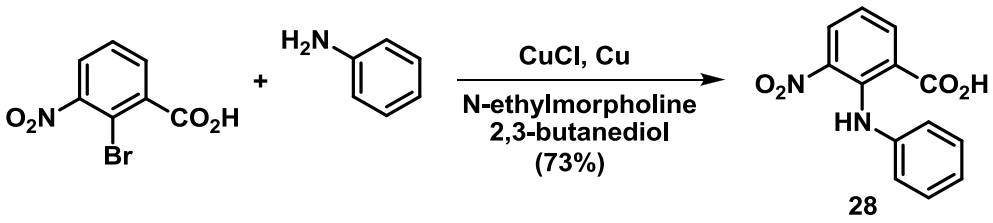
sodium sulfate, filtered and concentrated. The desired product was purified using flash chromatography (2:1 hexanes:ethyl acetate) to deliver 145 mg (37% yield) 1-hydroxyphenazine **2** as a bright yellow solid. **NOTE:** This reaction has been previously reported.[3]

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 – 8.17 (m, 3H), 7.89 – 7.80 (m, 2H), 7.80 – 7.73 (m, 2H), 7.24 (dd, *J* = 6.7, 1.8 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 151.9, 144.4, 144.0, 141.4, 134.9, 132.0, 131.0, 130.7, 129.9, 129.4, 120.1, 109.1.

HRMS (DART): calc. for $C_{12}H_8N_2O$ $[M+H]^+$: 197.0709, found: 197.0717.

MP: 155-157 °C (lit. 155-158 °C).[2]



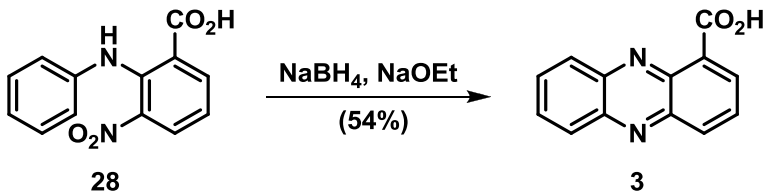
3-nitro-2-(phenylamino)benzoic acid (28). In a round bottom flask were sequentially added 2-bromo-3-nitrobenzoic acid (845 mg 3.43 mmol), copper(I) chloride (34 mg, 0.34 mmol), copper powder (11 mg, 0.17 mmol), aniline (0.470 mL, 5.15 mmol), *N*-ethylmorpholine (0.87 mL, 6.86 mmol) and 2,3-butanediol (2.14 mL). The reaction mixture was then heated to 70 ˚C and allowed to stir for 16 hours, then diluted with 18 mL 0.1N ammonium hydroxide solution and filtered over celite. The filtrate was poured slowly into 5 mL 2N aqueous hydrochloric acid, and the yellow precipitate filtered to yield the crude product which was recrystallized from toluene to afforded 886 mg (73% yield) of 3-nitro-2-(phenylamino)benzoic acid **28** as an amorphous yellow solid. **NOTE:** This reaction has been previously reported.[4]

^{1}H NMR (400 MHz, d_6-DMSO): δ 13.80 (br s, 1H), 9.88 (s, 1H), 8.21 (dd, *J* = 7.8, 1.7 Hz, 1H), 8.07 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.24 (t, *J* = 7.9 Hz, 2H), 7.10 (t, *J* = 8.0 Hz, 1H), 6.99 (t, *J* = 7.4 Hz, 1H), 6.91 (d, *J* = 7.8 Hz, 2 H).

^{13}C NMR (100 MHz, d_6-DMSO): δ 168.6, 141.4, 139.9, 138.8, 136.7, 130.8, 129.2, 123.1, 120.5, 119.0, 118.1.

HMRS (DART): calc. for $C_{13}H_{11}N_2O_4$ $[M+H]^+$: 259.0713, found: 259.0719.

MP: 192-194 °C (lit. 196-198 °C).[5]



28 3

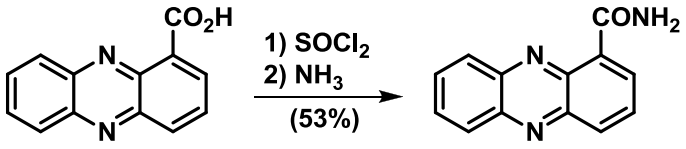
phenazine-1-carboxylic acid (3). 3-nitro-2-(phenylamino)benzoic acid (200 mg, 0.77 mmol) was dissolved in 20 mL 2N sodium ethoxide in ethanol and treated with sodium borohydride (176 mg, 4.65 mmol). The reaction was heated to 65 ˚C and allowed to stir for 24 hours. After this time, the reaction was poured into ice, quenched with 25 mL 2N aqueous hydrochloric acid, and extracted with dichloromethane 3x50 mL. The organic layers were collected and washed with 15 mL brine, dried with sodium sulfate, filtered and concentrated using a rotavap. The crude mixture was subjected to column chromatography using dichloromethane to elute to provide 100 mg (54% yield) of phenazine-1-carboxylic acid **3** as a yellow solid. **NOTE:** This reaction has been previously reported.[4] We also obtained highly pure PCA from column chromatography sequentially eluting with 100% hexanes, 3:1 hexanes:ethyl acetate, 49:49:2 hexanes:ethyl acetate:dichloromethane, 99:1 ethyl acetate:dichloromethane.

^{1}H NMR (400 MHz, $CDCl_3$): δ 15.59 (s, 1H), 9.00 (dd, *J* = 7.1, 1.5 Hz, 1H), 8.55 (dd, *J* = 8.8, 1.5 Hz, 1H), 8.36 (d, *J* = 8.2 Hz, 1H), 8.30 (d, *J* = 8.3 Hz, 1H), 8.09 – 7.96 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1, 144.2, 143.5, 140.1, 139.9, 137.6, 135.3, 133.4, 131.9, 130.5, 130.2, 128.1, 125.0.

HRMS (DART): calc. for $C_{13}H_9N_2O_2$ $[M+H]^+$: 225.0659, found: 225.0668 and calc. $C_{13}H_8N_2O_2Na$ $[M+Na]^+$: 247.0478, found: 247.0482.

MP: 238-240 °C (lit. 242 °C).[2]



3 4

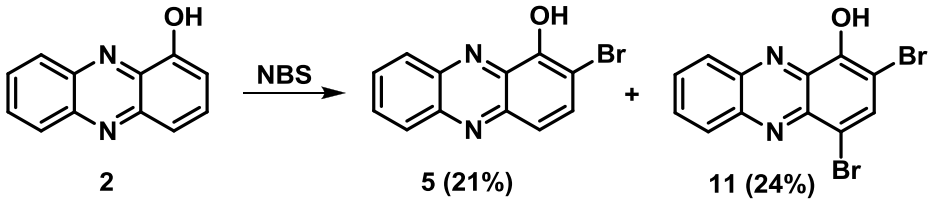
phenazine-1-carboxamide (4). To penazine-1-carboxylic acid (189 mg, 0.79 mmol) in 4 mL toluene was added thionyl chloride (0.295 mL, 3.94 mmol) dropwise. The solution was then heated to 65 ˚C for 4 hours. After this time, the volatiles were removed under vacuum, and the crude acid chloride was taken up in dichloromethane (5 mL). To this solution was added 0.5 mL 30% aqueous ammonia at ambient temperature resulting in an immediate precipitation of a yellow solid. Stirring was continued overnight, and the reaction was filtered and washed with a small amount of cold dichloromethane. The crude material was passed over a short silica plug, eluting with ethyl acetate to yield 100 mg (53% yield) of phenazine-1-carboxamide **4** as a green-yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.74 (br s, 1H), 9.02 (dd, *J* = 7.2, 1.5 Hz, 1H), 8.44 (dd, *J* = 8.7, 1.6, 1H), 8.30 (m, 1H), 8.24 (m, 1H), 8.01 – 7.89 (m, 3H), 6.27 (br s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8, 143.7, 143.3, 141.7, 141.0, 136.2, 134.5, 132.0, 131.3, 130.1, 130.0, 129.3, 129.1.

HRMS (DART): calc. for $C_{13}H_{10}N_3O$ $[M+H]^+$: 224.0818, found: 224.0817.

MP: 240 °C (decomposes) (lit. 242-243 °C).[6]



2 5 (21%) 11 (24%)

2-bromophenazin-1-ol (5) and 2,4-dibromophenazin-1-ol (11). 1-Hydroxyphenazine (120 mg, 0.612 mmol) and *N*-bromosuccinimide (120 mg, 0.730 mmol) were dissolved in 12 mL toluene, and heated at 50 °C for 5 hours. The reaction contents were then concentrated, taken up in dichloromethane and adsorbed onto silica for purification. Column chromatography eluting with dichloromethane furnished 35 mg (21% yield) of 2-bromophenazin-1-ol **5** as a yellow solid and 51 mg (24% yield) of 2,4-dibromophenazin-1-ol **11** as a yellow solid. **NOTE:** This reaction has been previously reported.[7] These two products were optimally separated on TLC and column chromatography using 85:15 hexanes:ethyl acetate.

2-bromophenazin-1-ol **(5):**
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (br s, 1H), 8.30 – 8.18 (m, 2H), 7.94 – 7.82 (m, 3H), 7.71 (d, *J* = 9.5 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 149.3, 144.3, 143.0, 141.5, 135.5, 134.5, 131.5, 131.3, 130.0, 129.3, 121.0, 103.7.

HRMS (DART): calc. for $C_{12}H_7N_2OBr$ $[M+H]^+$: 274.9815, found: 274.9824.

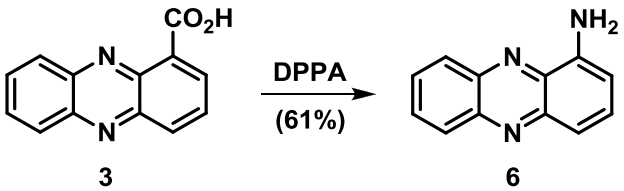
MP: 235-237 °C (lit. 233-235 °C).[7]

2,4-dibromophenazin-1-ol **(11):**
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.54 (br s, 1H), 8.43 – 8.38 (m, 1H), 8.30 – 8.24 (m, 2H), 7.98 – 7.90 (m, 2H); (400 MHz, d_6-DMSO) δ 11.58 (s, 1H), 8.44 (s, 1H), 8.40 – 8.31 (m, 2H), 8.11 – 8.03 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 149.3, 144.3, 141.6, 140.1, 137.4, 134.4, 132.3, 131.9, 130.4, 129.0, 113.1, 103.2.

HRMS (DART): calc. for $C_{12}H_6N_2OBr_2$ $[M+H]^+$: 354.8900, found: 354.8909.

MP: 198-199 °C (lit. 196-198 °C).[7]



3 6

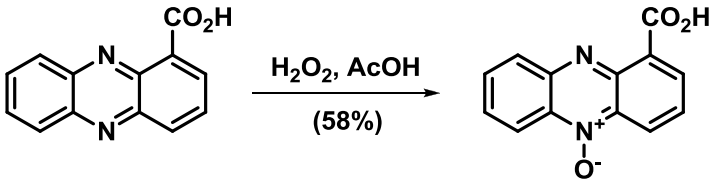
phenazin-1-amine (6). To phenazine-1-carboxylic acid (100 mg, 0.446 mmol) in a 10:1 solution of tetrahydrofuran:triethylamine (2.2 mL) was slowly added diphenylphosphoryl azide (105 µL, 0.491 mmol). The reaction contents were allowed to stir for 3 hours at ambient temperature. After this time, deionized water (0.5 mL) was added, and the reaction was refluxed for 2 hours resulting in a deep red solution. After cooling, the reaction was quenched with saturated solution of aqueous potassium carbonate and extracted with ethyl acetate. The organic layers were collected and subsequently washed with brine, dried with sodium sulfate, filtered and concentrated using a rotavap. Purification of the crude residue by flash chromatography using 9:1 hexane:ethyl acetate to elute afforded 53 mg (61%) phenazin-1-amine **6** as a red solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 – 8.17 (m, 2H), 7.86 – 7.72 (m, 2H), 7.64 (dd *J* = 8.8, 7.2 Hz, 1H), 7.57 (dd, *J* = 8.8, 1.4 Hz, 1H), 6.92 (dd, *J* = 7.2, 1.3 Hz, 1H), 5.10 (s, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 144.2, 144.2, 143.8, 141.4, 135.4, 132.3, 130.6, 129.9, 129.7, 129.5, 117.4, 107.9.

HRMS (DART): calc. for $C_{12}H_{10}N_3$ $[M+H]^+$: 196.0869, found: 196.0873.

MP: 183-184 °C (lit. 183-185 °C).[8]



3 **7**

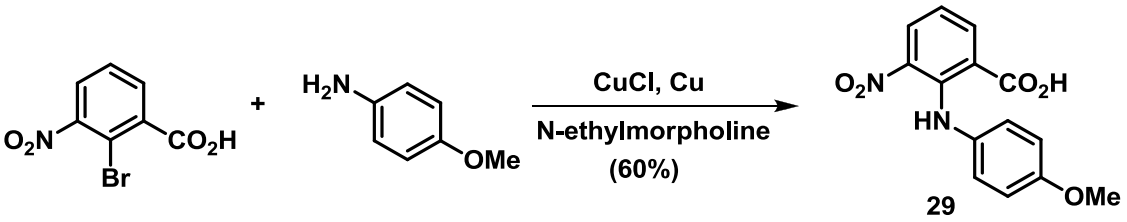
1-carboxyphenazine 5-oxide (7). Phenazine-1-carboxylic acid (34.5 mg, 0.154 mmol) was dissolved in 6 mL glacial acetic acid and treated with 30% hydrogen peroxide (0.70 mL, 6.12 mmol). The reaction was heated to 55 ˚C and allowed to stir for 17 hours. The reaction was then diluted with 200 mL of deionized water resulting in a yellow solid to precipitate. The yellow solid was filtered on a vacuum funnel. A second crop of crystals was obtained by storing the filtrate overnight at 2 ˚C. The combined solids were dried *in vacuo* to deliver 21.5 mg (58% yield) of 1-carboxyphenazine 5-oxide **7** as a yellow solid in high purity. **NOTE:** This reaction has been previously reported.[4]

^{1}H NMR (400 MHz, $CDCl_3$): δ 15.70 (s, 1H), 8.96 (d, *J* = 8.0 Hz, 1H), 8.91 (d, *J* = 8.0 Hz, 1H), 8.71 (d, *J* = 9.0 Hz, 1H), 8.26 (d, *J* = 8.9 Hz, 1H), 7.99 (t, *J* = 8.0 Hz, 1H), 7.95 – 7.82 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7, 143.0, 142.7, 138.0, 135.9, 135.8, 133.8, 131.2, 129.6, 129.0, 125.7, 124.4, 119.5.

HRMS (ESI): calc. for $C_{13}H_9N_2O_3$ $[M+H]^+$: 241.0608, found: 241.0613 and calc. $C_{13}H_8N_2O_3Na$ $[M+Na]^+$: 263.0427, found: 263.0427.

MP: 221-223 °C (lit. 223 °C).[4]



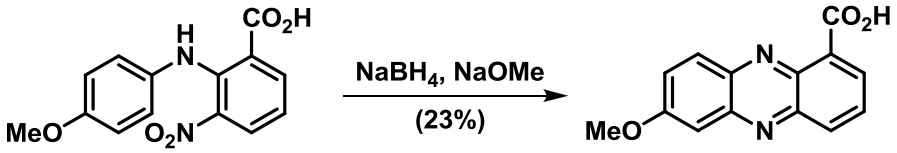
2-((4-methoxyphenyl)amino)-3-nitrobenzoic acid (29). In a round bottom flask were sequentially added 2-bromo-3-nitrobenzoic acid (1.00 g, 4.06 mmol), copper(I) chloride (40 mg, 0.40 mmol), copper powder (13 mg, 0.20 mmol), *p*-anisidine (0.687 mL, 6.09 mmol), *N*-ethylmorpholine (1.03 mL, 8.12 mmol) and 2,3-butanediol (2.5 mL). The reaction mixture was then heated to 70 ˚C with stirring for 16 hours, then diluted with 25 mL 0.1N aqueous ammonium hydroxide and filtered over celite. The filtrate was poured slowly into 5 mL 2N aqueous hydrochloric acid, and the yellow precipitate was filtered to yield 703 mg (60% yield) of 2-((4-methoxyphenyl)amino)-3-nitrobenzoic acid **29** as an orange solid. **NOTE:** The material from this reaction procedure appeared to be ≤80% pure by NMR. A small sample of this product was purified for spectral purposes via column chromatography eluting with 3:1 ethyl acetate:hexanes.

^{1}H NMR (400 MHz, CD_3OD): 8.23 (dd, J = 7.8, 1.8 Hz, 1H), 7.95 (dd, J = 7.8, 1.8 Hz, 1H), 6.93 – 6.85 (m, 3H), 6.83 – 6.77 (m, 2H), 3.75 (s, 3H).

^{13}C NMR (100 MHz, d_6-DMSO): δ 168.9, 155.8, 140.5, 138.8, 136.7, 134.1, 131.1, 121.0, 118.9, 117.5, 114.4, 55.2.

HRMS (DART): calc. for $C_{14}H_{13}N_2O_5$ $[M+H]^+$: 298.0819, found: 289.0812.

MP: 182-184 °C (lit. 182-185 °C).[4]



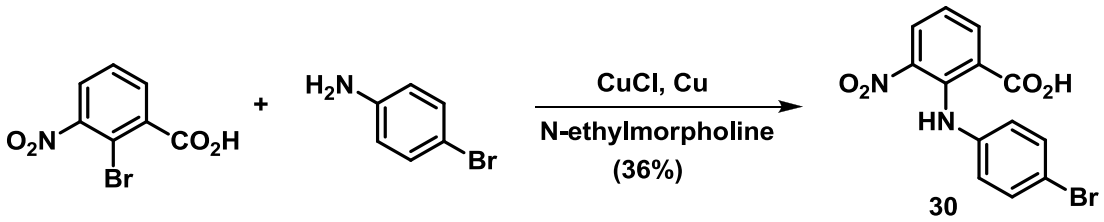
7-methoxyphenazine-1-carboxylic acid (8). 2-((4-Methoxyphenyl)amino)-3-nitrobenzoic acid (200 mg, 0.69 mmol) was dissolved in 17 mL 2N sodium methoxide in methanol and treated with sodium borohydride (261 mg, 6.90 mmol) and heated to 60 ˚C for 40 hours. After that time, the reaction contents were poured into ice, quenched with 2N aqueous hydrochloric acid, and extracted with dichloromethane 3x50 mL. The combined organic layers were then washed with brine, dried with sodium sulfate, filtered, and concentrated via rotavap. The crude reaction was adsorbed onto silica and chromatographed eluting with dichloromethane to deliver 37 mg (23% yield) of 7-methoxyphenazine-1-carboxylic acid **8** as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 15.50 (br s, 1H), 8.88 (dd, *J* = 7.2, 1.4 Hz, 1H), 8.43 (dd, *J* = 8.7, 1.5 Hz, 1H), 8.13 (d, *J* = 9.5 Hz, 1H), 8.00 (dd, *J* = 8.7, 7.1 Hz, 1H), 7.67 (dd, *J* = 9.5, 2.7 Hz, 1H), 7.48 (d, *J* = 2.7 Hz, 1H), 4.08 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.3, 162.4, 146.0, 143.5, 138.6, 137.4, 135.9, 134.5, 130.5, 129.6, 129.1, 125.2, 104.9, 56.5.

HRMS (ESI): calc. for $C_{14}H_{11}N_2O_3$ $[M+H]^+$: 255.0764, found: 255.0771 and calc. $C_{14}H_{10}N_2O_3Na$ $[M+Na]^+$: 277.0584, found: 277.0586.

MP: >250 °C (lit. 266-268 °C).[4]



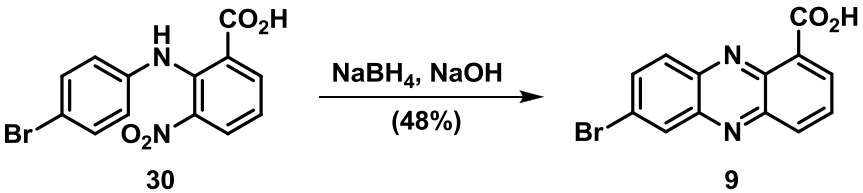
2-((4-bromophenyl)amino)-3-nitrobenzoic acid (30). In a round bottom flask were sequentially added 2-bromo-3-nitrobenzoic acid (1.00 g, 4.06 mmol), copper(I) chloride (40 mg, 0.40 mmol), copper powder (13 mg, 0.20 mmol), 4-bromoaniline (1.05 g, 6.09 mmol), *N*-ethylmorpholine (1.03 mL, 8.12 mmol) and 2,3-butanediol (2.5 mL). The reaction mixture was then heated to 70 ˚C with stirring for 16 hours before being diluted with 25 mL 0.1N aqueous ammonium hydroxide and filtered over celite. The filtrate was poured slowly into 5 mL 2N aqueous hydrochloric acid, and the yellow precipitate was filtered to give 491 mg (36% yield) of 2-((4-bromophenyl)amino)-3-nitrobenzoic acid **30** as an orange solid.

^{1}H NMR (400 MHz, d_6-DMSO): δ 8.17 (d, *J* = 8.0 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 7.1 Hz, 2H), 7.18 (t, *J* = 7.9 Hz, 1H), 6.85 (d, *J* = 7.1 Hz, 2H).

^{13}C NMR (100 MHz, d_6-DMSO): δ 168.1, 141.4, 140.6, 137.8, 136.4, 131.8, 130.3, 122.1, 120.0, 119.8, 114.0.

HRMS (DART): calc. for $C_{13}H_{10}N_2O_4Br$ $[M+H]^+$: 336.9818, found: 336.9821.

MP: 185-190 °C (lit. 194-196 °C).[9]



7-bromophenazine-1-carboxylic acid (9). 2-((4-Bromophenyl)amino)-3-nitrobenzoic acid (450 mg, 1.33 mmol) was dissolved in 22 mL 2N aqueous sodium hydroxide and treated with sodium borohydride (151 mg, 4.00 mmol). The reaction mixture was then refluxed for 2 hours. After

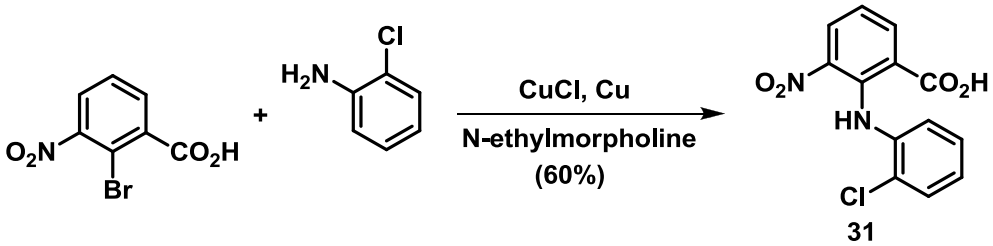
this time, the reaction was cooled on the ice bath, and filtered. The filter cake was washed with a small amount of cold 2N aqueous sodium hydroxide, taken up in 30 mL deionized water, acidified with glacial acetic acid and filtered to give 194 mg (48% yield) of 7-bromophenazine-1-carboxylic acid **9** as a yellow solid.

^{1}H NMR (500 MHz, d_6-DMSO): δ 14.19 (br s, 1H), 8.61 (d, J = 2.2 Hz, 1H), 8.54 – 8.44 (m, 2H), 8.34 (d, J = 9.2 Hz, 1H), 8.17 (dd, J = 9.2, 2.2 Hz, 1H), 8.11 (dd, J = 8.8, 6.9 Hz, 1H).

^{13}C NMR (126 MHz, d_6-DMSO): δ 166.5, 143.3, 142.8, 140.0, 139.8, 135.6, 133.7, 133.1, 131.2, 131.1, 130.8, 129.7, 125.5.

HRMS (DART): calc. for $C_{13}H_8N_2O_2Br$ $[M+H]^+$: 302.9764, found: 302.9778.

MP: >250 °C (lit. 268-269 °C).[9]



31

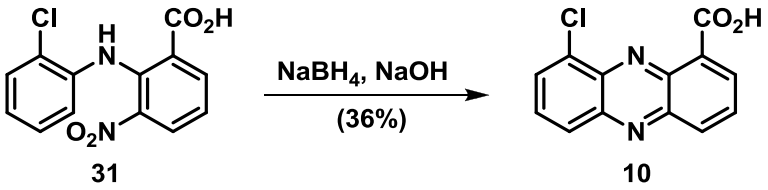
2-((2-chlorophenyl)amino)-3-nitrobenzoic acid (31). In a round bottom flask were sequentially added 2-bromo-3-nitrobenzoic acid (1.00 g, 4.06 mmol), copper(I) chloride (40 mg, 0.40 mmol), copper powder (13 mg, 0.20 mmol), 2-chloroaniline (0.640 mL, 6.09 mmol), *N*-ethylmorpholine (1.03 mL, 8.12 mmol) and 2,3-butanediol (2.5 mL). The mixture was heated to 70 ˚C with stirring for 16 hours, then diluted with 25 mL 0.1N aqueous ammonium hydroxide and filtered over a celite. The filtrate was poured slowly into 5 mL 2N aqueous hydrochloric acid, and the yellow precipitate was filtered to yield the crude product which was recrystallized from toluene to afforded 712 mg (60% yield) of 2-((2-chlorophenyl)amino)-3-nitrobenzoic acid **31** as a yellow solid. **NOTE:** The material from this reaction procedure appeared to be ≤80% pure by NMR. A small sample of this product was purified for spectral purposes via column chromatography eluting with 3:1 ethyl acetate:hexanes.

^{1}H NMR (400 MHz, d_6-DMSO): δ 10.06 (br s, 1H), 8.26 (d, J = 7.7 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.19 – 7.07 (m, 2H), 7.01 (t, J = 7.3 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H).

^{13}C NMR (100 MHz, d_6-DMSO): δ 168.7, 139.6, 138.3, 138.1, 136.9, 131.0, 130.0, 127.6, 124.3 (2), 120.6, 119.7, 117.1.

HRMS (DART): calc. for $C_{13}H_{10}N_2O_4Cl$ $[M+H]^+$: 293.0324, found: 293.0332.

MP: 217-220 °C (lit. 219-221 °C).[4]



31 10

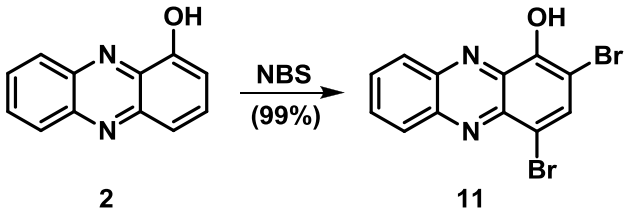
9-chlorophenazine-1-carboxylic acid (10). 2-((2-Chlorophenyl)amino)-3-nitrobenzoic acid (200 mg, 0.68 mmol) was dissolved in 17 mL 2N aqueous sodium hydroxide and treated with sodium borohydride (155 mg, 4.10 mmol) and heated to 70 ˚C for 5 hours. After this time, the reaction was poured into ice, quenched with 3 mL 2N aqueous hydrochloric acid, and extracted with dichloromethane 3x50 mL. The combined organic layers were washed with brine, dried with sodium sulfate, filtered, and concentrated via rotavap. The crude mixture was adsorbed onto silica and chromatographed using dichloromethane to deliver 63 mg (36% yield) of 9-chlorophenazine-1-carboxylic acid **10** as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 15.40 (s, 1H), 9.04 (dd, *J* = 7.0, 1.3 Hz, 1H), 8.56 (dd, *J* = 8.9, 1.2 Hz, 1H), 8.30 (dd, *J* = 8.9, 0.8 Hz, 1H), 8.15 – 8.07 (m, 2H), 7.92 (dd, *J* = 8.9, 7.3 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7, 144.6, 143.9, 140.0, 138.3, 137.1, 135.0, 132.2, 132.1, 131.4 (2), 129.2, 125.5.

HRMS (DART): calc. for $C_{13}H_8N_2O_2Cl$ $[M+H]^+$: 259.0269, found: 259.0271.

MP: >250 °C (lit. 272-273 °C).[4]



2 11

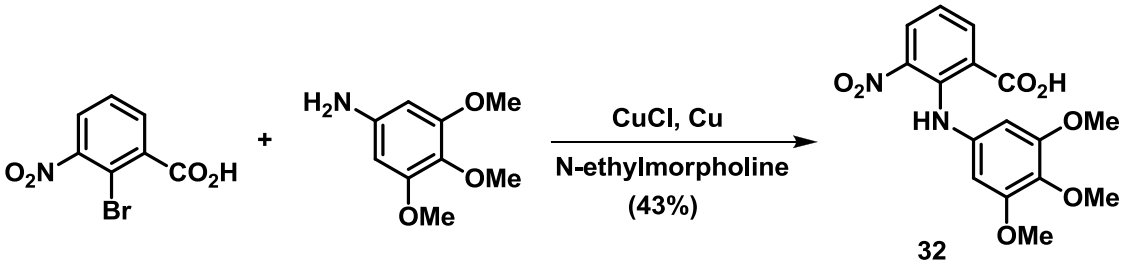
2,4-dibromophenazin-1-ol (11). 1-Hydroxyphenazine (81 mg, 0.412 mmol) was dissolved in 8 mL toluene and treated with *N*-bromosuccinimide (162 mg, 0.906 mmol). The reaction was heated to 50 °C for 5.5 hours. The reaction was then allowed to cool to room temperature before being concentrated via rotavap. The crude material was then adsorbed onto silica using dichloromethane and concentrated via rotavap before being applied to a column. Column chromatography using dichloromethane to elute delivered 145 mg (99% yield) 2,4-dibromophenazin-1-ol **11** as a yellow solid. **NOTE:** This reaction has been previously reported.[7] We have been able to synthesize 1.106 grams of 2,4-dibromophenazin-1-ol using this procedure starting from 795 milligrams of 1-hydroxyphenazine (77% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.54 (br s, 1H), 8.43 – 8.38 (m, 1H), 8.30 – 8.24 (m, 2H), 7.98 – 7.90 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 149.3, 144.3, 141.6, 140.1, 137.4, 134.4, 132.3, 131.9, 130.4, 129.0, 113.1, 103.2.

HRMS (DART): calc. for $C_{12}H_6N_2OBr_2$ $[M+H]^+$: 354.8900, found: 354.8909.

MP: 198-199 °C (lit. 196-198 °C).[7]



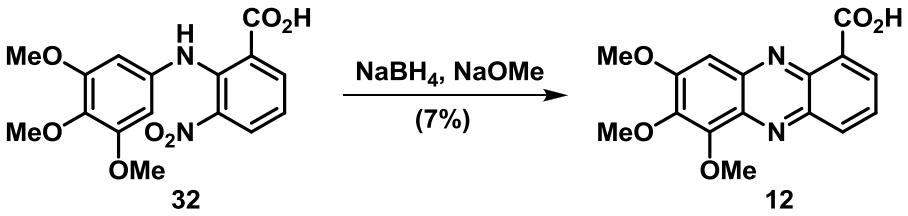
3-nitro-2-((3,4,5-trimethoxyphenyl)amino)benzoic acid (32). In a round bottom flask were sequentially added 2-bromo-3-nitrobenzoic acid (1.00 g, 4.06 mmol), copper(I) chloride (40 mg, 0.40 mmol), copper powder (13 mg, 0.20 mmol), 3,4,5-trimethoxyaniline (1.12 g, 6.09 mmol), *N*-ethylmorpholine (1.03 mL, 8.12 mmol) and 2,3-butanediol (2.5 mL). The reaction mixture was then heated to 70 ˚C and stirred for 16 hours, then diluted with 25 mL 0.1N aqueous ammonium hydroxide and filtered over celite. The filtrate was poured slowly into 5 mL 2N aqueous hydrochloric acid, and the resulting yellow precipitate was filtered to yield 605 mg (43% yield) 3-nitro-2-((3,4,5-trimethoxyphenyl)amino)benzoic acid **32** as an amorphous red solid. **NOTE:** The material from this reaction procedure appeared to be ≤80% pure by NMR. A small sample of this product was purified for spectral purposes via column chromatography sequentially eluting with 100% dichloromethane, 1:9 acetone:dichloromethane, 1:3 acetone:dichloromethane, 100% acetone.

^{1}H NMR (400 MHz, d_6-DMSO): δ 12.54 (br s, 1H), 8.25 (dd, J = 15.2, 1.6 Hz, 1H), 7.80 (dd, J = 8.0, 1.6 Hz, 1H), 6.87 (t, J = 8.0 Hz, 1H), 6.07 (s, 2H), 3.66 (s, 6H), 3.58 (s, 3H).

^{13}C NMR (100 MHz, d_6-DMSO): δ 169.1, 153.1, 139.2, 138.6, 138.5, 136.5, 132.5, 127.7, 127.5 117.0, 94.9, 60.1, 55.5.

HRMS (DART): calc. for $C_{16}H_{17}N_2O_7$ $[M+H]^+$: 349.1039, found: 349.1029.

MP: 183-184 °C.



6,7,8-trimethoxyphenazine-1-carboxylic acid (12). 3-Nitro-2-((3,4,5-trimethoxyphenyl)amino) benzoic acid (450 mg, 1.29 mmol) was dissolved in 32 mL of 2N sodium methoxide in methanol solution and treated with sodium borohydride (488 mg, 12.90 mmol) and heated to 60 ˚C for 16 hours. After this time, the reaction was poured into ice, quenched with 2N aqueous hydrochloric acid, and extracted with dichloromethane 3x75 mL. The combined organic layers were washed

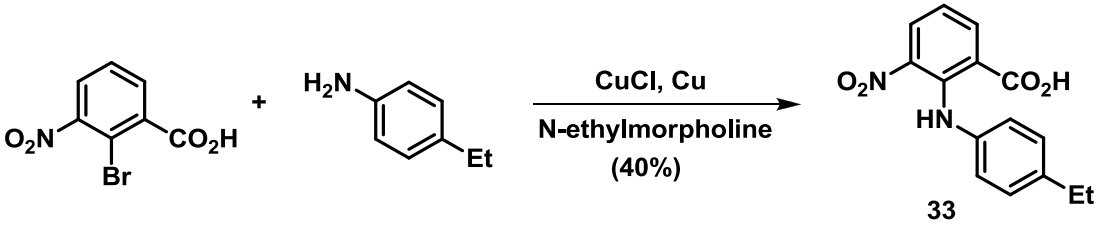
with brine, dried with sodium sulfate, filtered and concentrated via rotavap. The crude mixture was adsorbed onto silica and chromatographed using 95:5 dichloromethane:ethyl acetate to give 13 mg (7% yield) of 6,7,8-trimethoxyphenazine-1-carboxylic acid **12** as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 15.73 (br s, 1H), 8.85 (dd, *J* = 7.2, 1.5 Hz, 1H), 8.52 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.90 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.20 (s, 1H), 4.27 (s, 3H), 4.18 (s, 3H), 4.14 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 159.9, 146.1, 145.5, 140.9, 139.4, 139.1, 138.4, 136.5, 135.3, 128.9, 124.2, 99.8, 62.9, 62.1, 57.2.

HRMS (DART): calc. for $C_{16}H_{15}N_2O_5$ $[M+H]^+$: 315.0975, found: 315.0975.

MP: 229-230 °C.



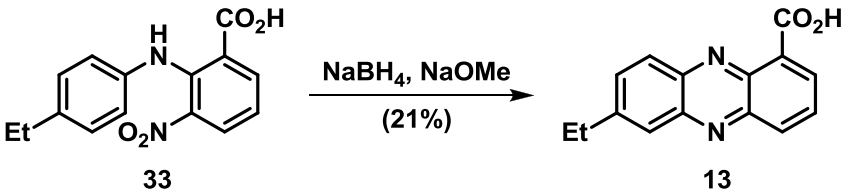
2-((4-ethylphenyl)amino)-3-nitrobenzoic acid (33). In a round bottom flask were sequentially added 2-bromo-3-nitrobenzoic acid (1.00 g, 4.06 mmol), copper(I) chloride (40 mg, 0.40 mmol), copper powder (13 mg, 0.20 mmol), 4-ethyl aniline (0.757 mL, 6.09 mmol), *N*-ethylmorpholine (1.03 mL, 8.12 mmol) and 2,3-butanediol (2.5 mL). The mixture was heated to 70 ˚C with stirring for 16 hours, then diluted with 25 mL 0.1N aqueous ammonium hydroxide and filtered over celite. The filtrate was then poured slowly into 5 mL 2N aqueous hydrochloric acid and the resulting yellow precipitate was filtered to yield 467 mg (40% yield) of 2-((4-ethylphenyl)amino)-3-nitrobenzoic acid **33** as a yellow solid.

^{1}H NMR (400 MHz, d_6-DMSO): δ 13.79 (br s, 1H), 9.91 (s, 1H), 8.20 (d, *J* = 7.7 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.14 – 6.99 (m, 3H), 6.84 (d, *J* = 7.9 Hz, 2H), 2.54 (q, *J* = 7.6 Hz, 2H, partially buried in d_6-DMSO), 1.15 (t, *J* = 7.6 Hz, 3H).

^{13}C NMR (100 MHz, d_6-DMSO): δ 168.8, 139.4 (2), 138.9, 138.8, 136.8, 131.0, 128.4, 119.8, 118.4, 118.3, 27.9, 16.0.

HRMS (ESI): calc. for $C_{15}H_{15}N_2O_4$ $[M+H]^+$: 287.1026, found: 287.1032 and calc. $C_{15}H_{14}N_2O_4Na$ $[M+Na]^+$: 309.0846, found: 309.0841.

MP: 191-192 °C.



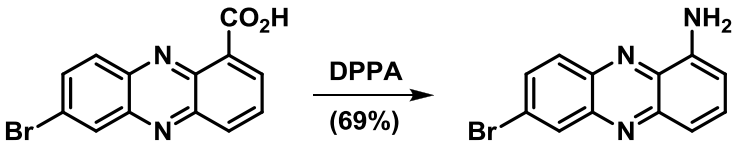
7-ethylphenazine-1-carboxylic acid (13). 2-((4-Ethylphenyl)amino)-3-nitrobenzoic acid (390 mg, 1.36 mmol) was dissolved in 34 mL of 2N sodium methoxide in methanol solution and treated with sodium borohydride (514 mg, 13.60 mmol) and heated to 60 ˚C for 40 hours. After this time, the reaction was poured into ice, quenched with 2N aqueous hydrochloric acid, and extracted with dichloromethane 3x75 mL. The combined organic layers were washed with brine, dried with sodium sulfate, filtered, and concentrated via rotavap. The crude mixture was adsorbed onto silica and chromatographed using dichloromethane to elute 70 mg (21% yield) 7-ethylphenazine-1-carboxylic acid **13** as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 15.52 (br s, 1H), 8.86 (dd, *J* = 7.0, 1.5 Hz, 1H), 8.41 (dd, *J* = 8.8, 1.5 Hz, 1H), 8.09 (d, *J* = 8.9 Hz, 1H), 8.02 – 7.89 (m, 2H), 7.82 (dd, *J* = 9.0, 1.9 Hz, 1H), 2.97 (q, *J* = 7.6 Hz, 2H), 1.42 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 148.9, 144.4, 143.3, 139.5, 138.9, 136.8, 135.6, 135.0, 130.2, 127.5, 126.5, 124.9, 29.4, 14.5.

HRMS (ESI): calc. for $C_{15}H_{13}N_2O_2$ $[M+H]^+$: 253.0972, found: 253.0981 and calc. $C_{15}H_{12}N_2O_2Na$ $[M+Na]^+$: 275.0791, found: 275.0788.

MP: 222 °C (decomposes).



9 **14**

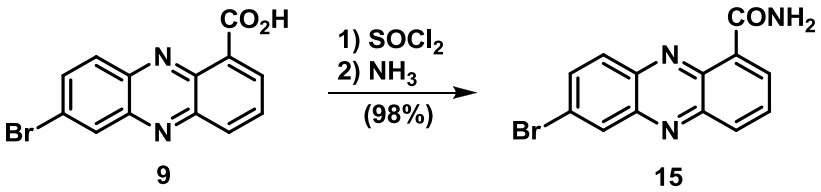
7-bromophenazin-1-amine (14). 7-Bromophenazine-1-carboxylic acid (200 mg, 0.66 mmol) was taken up in a 10:1 tetrahydrofuran:triethylamine solution (3.5 mL). Diphenylphosphoryl azide (0.17 mL, 0.79 mmol) was then slowly added to the reaction mixture which was allowed to stir for 3 hours at ambient temperature. Deionized water (1.5 mL) was then added to the reaction which was heated to reflux for an additional 2 h. After cooling, the reaction was quenched with saturated aqueous potassium carbonate and extracted with dichloromethane. The organic layer was dried with sodium sulfate, filtered and concentrated via rotavap. Purification of the crude residue by flash chromatography using 9:1 hexanes:ethyl acetate afforded 125 mg (69% yield) of 7-bromophenazin-1-amine **14** as a purple solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (d, *J* = 2.1 Hz, 1H), 8.06 (d, *J* = 9.2 Hz, 1H), 7.81 (dd, *J* = 9.3, 2.1 Hz, 1H), 7.65 (t, *J* = 8.1 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 6.93 (d, *J* = 7.8 Hz, 1H), 5.24 (br s, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 144.6, 144.3, 144.1, 139.9, 135.4, 133.4, 133.1, 131.6, 131.1, 125.0, 117.3, 108.2.

HRMS (DART): calc. for $C_{12}H_9N_3Br$ $[M+H]^+$: 273.9974, found: 273.9983.

MP: 202-205 °C.



9 15

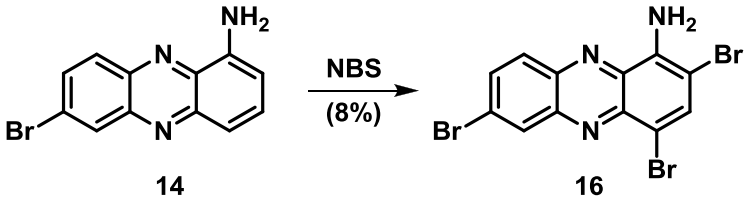
7-bromophenazine-1-carboxamide (15). 7-Bromophenazine-1-carboxylic acid (50 mg, 0.17 mmol) was dissolved in 1 mL toluene. Thionyl chloride (60 µL, 0.79 mmol) was added to the solution which was then heated at 70 ˚C for 3 hours. After this time, the volatiles were removed under vacuum, and the intermediate acid chloride was taken up in 1 mL of dichloromethane. Addition of 0.1 mL 30% aqueous ammonia at ambient temperature resulted in immediate precipitation of a yellow solid. Stirring was continued overnight, and the reaction was filtered and washed with a small amount of cold dichloromethane. The crude material was passed over a short silica plug, eluting with ethyl acetate, and concentrated to yield 49 mg (98% yield) of 7-bromophenazine-1-carboxamide **15** as a yellow solid.

^{1}H NMR (400 MHz, d_6-DMSO): δ 9.48 (br s, 1H), 8.67 (d, J = 8.0 Hz, 1H), 8.50 (d, J = 2.2 Hz, 1H), 8.38 (dd, J = 8.7, 1.6 Hz, 1H), 8.31 (d, J = 9.3 Hz, 1H), 8.13 – 8.02 (m, 2H), 7.80 (br s, 1H).

^{13}C NMR (100 MHz, d_6-DMSO): δ 165.7, 143.0, 142.7, 140.2, 140.1, 135.0, 134.3, 132.8, 131.2, 131.1, 131.0, 130.8, 125.1.

HRMS (DART): calc. for $C_{13}H_9N_3OBr$ $[M+H]^+$: 301.9923, found: 301.9923.

MP: 244-246 °C.



14 16

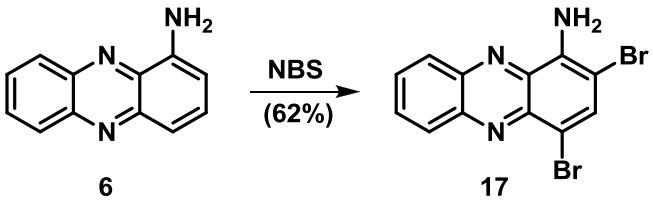
2,4,7-tribromophenazin-1-amine (16). 7-Bromophenazin-1-amine (70 mg, 0.359 mmol) was dissolved in 7.2 mL toluene, and treated with *N*-bromosuccinimide (134 mg, 0.753 mmol) at ambient temperature for 4 hours. The contents of the flask were then concentrated via rotavap, taken up in dichloromethane and adsorbed onto silica. Column chromatography was used eluting with 19:1 hexanes:ethyl acetate to yield 13 mg (8% yield) of 2,4,7-tribromophenazin-1-amine **16** as a purple solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (d, J = 2.1 Hz, 1H), 8.14 (s, 1H), 8.08 (d, J = 9.2 Hz, 1H), 7.89 (dd, J = 9.2, 2.1 Hz, 1H), 5.66 (br s, 2H).

^{13}C NMR (100 MHz $CDCl_3$): δ 143.9, 142.2, 140.6, 140.0, 138.0, 134.9, 134.6, 132.1, 130.6, 126.1, 108.8, 101.5.

HRMS (DART): calc. for $C_{12}H_7N_3Br_3$ $[M+H]^+$: 431.8164, found: 431.8184.

MP: 204-205 °C.



6 17

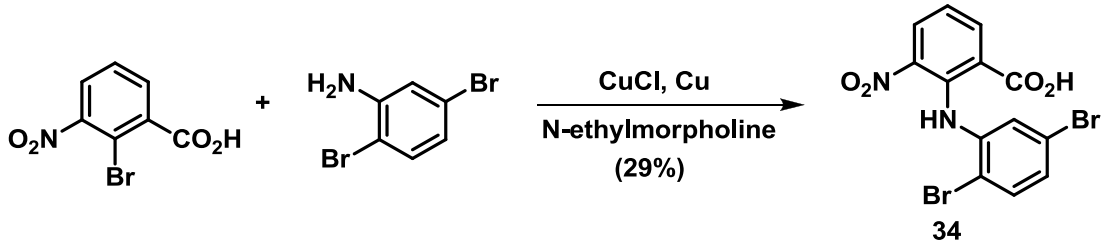
2,4-dibromophenazin-1-amine (17). Phenazin-1-amine (17 mg, 0.087 mmol) and *N*-bromosuccinimide (33 mg, 0.182 mmol) were dissolved in 1.7 mL toluene, and heated to 50 °C for 8 hours. The contents of the flask were then concentrated via rotavap, taken up in dichloromethane and adsorbed onto silica. Column chromatography was used to purify the desired compound eluting with 85:15 hexanes:ethyl acetate to yield 19 mg (62% yield) of 2,4-dibromophenazin-1-amine **17** as a red solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (dd, *J* = 8.5, 2.1 Hz, 1H), 8.19 (dd, *J* = 8.5, 2.4 Hz, 1H), 8.10 (s, 1H), 7.91 – 7.76 (m, 2H), 5.64 (br s, 2H).

^{13}C NMR (100 MHz $CDCl_3$): δ 143.8, 142.1, 141.4, 140.2, 137.2, 134.6, 131.4, 131.1, 130.1, 129.4, 108.9, 101.1.

HRMS (DART): calc. for $C_{12}H_8N_3Br_2$ $[M+H]^+$: 353.9059, found: 353.9066.

MP: 192-193 °C.



34

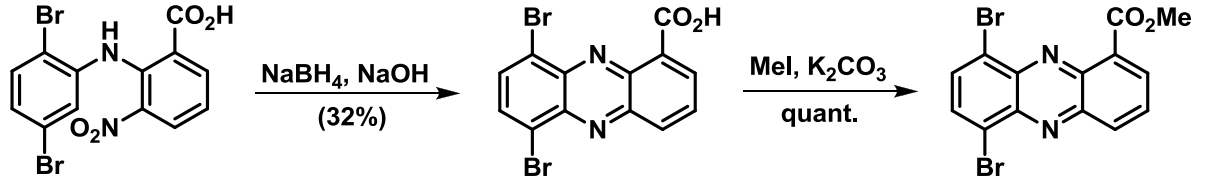
2-((2,5-dibromophenyl)amino)-3-nitrobenzoic acid (34). In a round bottom flask were sequentially added 2-bromo-3-nitrobenzoic acid (246 mg, 1.00 mmol), copper(I) chloride (10 mg, 0.05 mmol), copper powder (3 mg, 0.10 mmol), 2,5-dibromoaniline (376 mg, 1.50 mmol), *N*-ethylmorpholine (0.25 mL, 2.00 mmol) and 2,3-butanediol (0.75 mL). The mixture was then heated to 70 ˚C with stirring for 16 hours, then diluted with 25 mL 0.1N aqueous ammonium hydroxide and filtered over celite. The resulting filtrate was poured slowly into 5 mL 2N aqueous hydrochloric acid, and the resulting yellow precipitate was filtered to yield 200 mg (47% yield) of 2-((2,5-dibromophenyl)amino)-3-nitrobenzoic acid **34** as a yellow solid.

^{1}H NMR (400 MHz, d_6-DMSO): δ 14.02 (br s, 1H), 9.89 (s, 1H), 8.25 (dd, *J* = 7.8, 1.8 Hz, 1H), 8.15 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.60 (d, *J* = 8.6 Hz, 1H), 7.24 (t, *J* = 8.0 Hz, 1H), 7.12 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.94 (d, *J* = 2.3 Hz, 1H).

^{13}C NMR (100 MHz, d_6-DMSO): δ 168.5, 141.1, 140.3, 137.2, 136.8, 134.7, 130.8, 127.0, 121.6, 121.0 (2), 119.5, 113.6.

HRMS (DART): calc. for $C_{13}H_9N_2O_4Br_2$ $[M+H]^+$: 416.8904, found: 416.8922.

MP: 210-212 °C.



34 **18** **35**

Compound Screened in MIC Experiments **Compound Synthesized for Characterization**

6,9-dibromophenazine-1-carboxylic acid (18) and methyl-6,9-dibromophenazine-1-carboxylate (35). 2-((2,5-Dibromophenyl)amino)-3-nitrobenzoic acid (497 mg, 1.16 mmol) was dissolved in 19 mL 2N aqueous sodium hydroxide and treated with sodium borohydride (131 mg, 3.47 mmol). The resulting reaction mixture was then refluxed for 1.5 hours. After this time, the reaction was made acidic with 2N aqueous hydrochloric acid and the resulting precipitate was filtered. The filter cake was then washed with hot chloroform to afford 143 mg (32% yield) of 6,9-dibromophenazine-1-carboxylic acid **18** as a yellow-green solid. 6,9-Dibromophenazine-1-carboxylic acid displayed extremely low solubility and as a result, we were unable to obtain a ^{13}C NMR spectra. We then converted a small portion of this material to methyl ester **35** for characterization purposes using the following procedure: To a stirred suspension of 6,9-dibromophenazine-1-carboxylic acid (3.7 mg, 0.0097 mmol) in 0.4 mL dimethylformamide was sequentially added potassium carbonate (22 mg, 0.16 mmol) and one drop of iodomethane. The reaction was allowed to stir overnight at room temperature. The reaction was then transferred to a separatory funnel and partitioned between ethyl acetate and water. The organic layer was then washed with brine and collected. The organic layer was then dried with sodium sulfate, filtered and concentrated via rotavap. The crude material was taken up in chloroform and passed over a silica plug to furnish 3.8 mg (quantitative yield) of methyl-6,9-dibromophenazine-1-carboxylate **35** as a yellow solid.

***6,9-dibromophenazine-1-carboxylic acid* (18):**
^{1}H NMR (400 MHz, DMSO-d_6): δ 8.72 (d, J = 6.9 Hz, 1H), 8.62 (d, J = 8.6 Hz, 1H), 8.40 – 8.31 (m, 2H), 8.23 (t, J = 7.9 Hz, 1H).

HRMS (DART): calc. for $C_{13}H_7N_2O_2Br_2$ $[M+H]^+$: 382.8849, found: 382.8854.

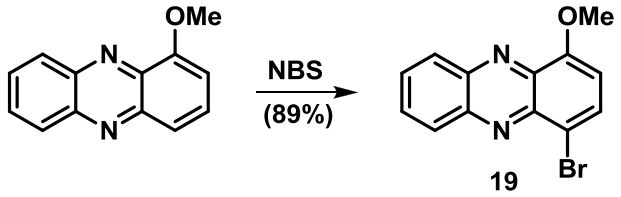
MP: >250 °C.

***methyl-6,9-dibromophenazine-1-carboxylate* (35):**
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (dd, J = 8.8, 1.4 Hz, 1H), 8.39 (dd, J = 7.0, 1.4 Hz, 1H), 8.11 – 8.06 (m, 2H), 7.97 (dd, J = 8.8, 7.0 Hz, 1H), 4.16 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 167.0, 143.3, 141.4, 141.3, 141.1, 134.2, 134.0 (2), 133.7, 131.6, 130.9, 125.2, 124.1, 53.1.

HRMS (DART): calc. for $C_{14}H_9N_2O_2Br_2$ $[M+H]^+$: 396.9005, found: 396.9015.

MP: 183-184 °C.



19

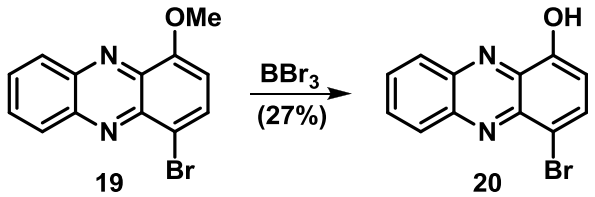
1-bromo-4-methoxyphenazine (19). 1-Methoxyphenazine (60 mg, 0.29 mmol) was dissolved in a 1:1 toluene:acetonitrile solution (10 mL) and treated with *N*-bromosuccinimide (53 mg, 0.30 mmol). The resulting reaction mixture was heated to 50 ˚C and allowed to stir for 14 hours. The reaction was then cooled, adsorbed onto silica gel and purified via column chromatography eluting with 3:1 hexanes:ethyl acetate to give 73 mg (89% yield) of 1-bromo-4-methoxyphenazine **19** as a yellow solid. **NOTE:** The synthesis of 1-methoxyphenazine and 1-bromo-4-methoxyphenazine have been previously described.[7]

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 – 8.25 (m, 2H), 7.96 (d, *J* = 8.2 Hz, 1H), 7.86 – 7.75 (m, 2H), 6.84 (d, *J* = 8.2 Hz, 1H), 4.07 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.1, 143.6, 142.3, 141.0, 137.1, 133.2, 131.4, 131.1, 129.9, 129.8, 114.2, 106.9, 56.7.

HRMS (DART): calc. for $C_{13}H_{10}N_2OBr$ $[M+H]^+$: 288.9971, found: 288.9979.

MP: 145-147 °C (lit. 148-150 °C).[7]



19 20

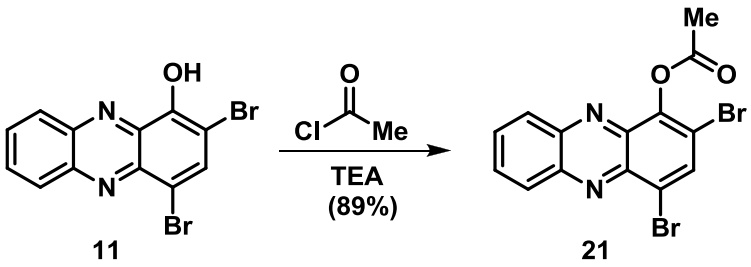
4-bromophenazin-1-ol (20). 1-Bromo-4-methoxyphenazine (25 mg, 0.086 mmol) was dissolved in 2 mL dichloromethane, and cooled to -78 °C. Boron tribromide (0.26 mL, 1.0 M in dichloromethane) was then added to the reaction and the mixture was allowed to warm to room temperature overnight. The reaction was then refluxed for 1 hour, then allowed to cool, and quenched with 3 mL of a saturated aqueous solution of sodium bicarbonate. The mixture was extracted with dichloromethane 3x20 mL. The combined organic layers were dried with sodium sulfate, filtered, and concentrated via rotavap. The crude material was subjected to column chromatography eluting with 3:1 hexanes:ethyl acetate to deliver 6.3 mg (27% yield) of 4-bromophenazin-1-ol **20** as a yellow solid. **NOTE:** This reaction has been previously reported.[7]

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (m, 1H), 8.26 (m, 1H), 8.22 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.96 – 7.86 (m, 2H), 7.15 (d, *J* = 8.1 Hz, 1H).

^{13}C NMR (100 MHz $CDCl_3$): δ 151.8, 144.6, 141.5, 141.0, 135.2, 134.7, 131.6 (2), 130.4, 129.0, 112.3, 109.6.

HRMS (DART): calc. for $C_{12}H_8N_2OBr$ $[M+H]^+$: 274.9815, found: 274.9819.

MP: 199-200 °C (lit. 199-201 °C).[7]



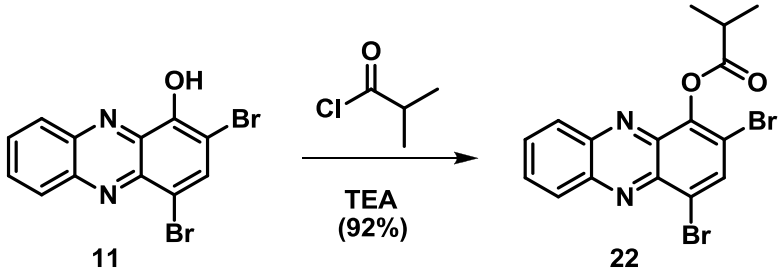
2,4-dibromophenazin-1-yl acetate (21). 2,4-Dibromophenazin-1-ol (25 mg, 0.071 mmol) was dissolved in 2 mL dichloromethane. Triethylamine (50 µL, 0.35 mmol) was then added followed by the addition of acetyl chloride (15 µL, 0.21 mmol) at room temperature. The reaction mixture was allowed to stir for 30 minutes before being quenched with a saturated aqueous solution of sodium bicarbonate. The contents were then transferred to a separatory funnel and dichloromethane was used for extraction. The combined organic layers were dried with sodium sulfate, filtered and concentrated via rotavap. Purification via column chromatography using 9:1 hexanes:ethyl acetate afforded 25 mg (89% yield) of 2,4-dibromophenazin-1-yl acetate **21** as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 – 8.29 (m, 1H), 8.34 (s, 1H, partially buried in multiplet), 8.27 – 8.20 (m, 1H), 7.95 – 7.85 (m, 2H), 2.61 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 145.2, 143.7, 143.6, 140.3, 137.8, 135.8, 132.3, 132.0, 130.2, 130.0, 122.3, 117.2, 20.9.

HRMS (DART): calc. for $C_{14}H_9N_2O_2Br_2$ $[M+H]^+$: 396.9005, found: 396.9014.

MP: 167-168 °C.



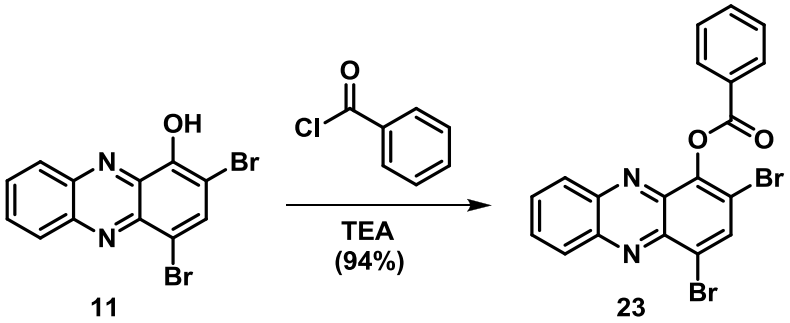
2,4-dibromophenazin-1-yl isobutyrate (22). 2,4-Dibromophenazin-1-ol (25 mg, 0.071 mmol) was dissolved in 2 mL dichloromethane. Triethylamine (50 µL, 0.35 mmol) was then added followed by the addition of isobutyryl chloride (22 µL, 0.21 mmol) at room temperature. The reaction mixture was allowed to stir for 1 hour before being quenched with a saturated aqueous solution of sodium bicarbonate. The contents were then transferred to a separatory funnel and dichloromethane was used for extraction. The combined organic layers were dried with sodium sulfate, filtered and concentrated via rotavap. Purification via column chromatography using 19:1 hexanes:ethyl acetate afforded 28 mg (92% yield) of 2,4-dibromophenazin-1-yl isobutyrate **22** as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 – 8.30 (m, 2H), 8.19 (m, 1H), 7.94 – 7.83 (m, 2H), 3.16 (hept, *J* = 7.0 Hz, 1H), 1.55 (d, *J* = 7.0 Hz, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.5, 145.3, 143.6, 143.5, 140.3, 137.7, 135.9, 132.2, 131.9, 130.2, 129.9, 122.0, 117.0, 34.5, 19.4.

HRMS (DART): calc. for $C_{16}H_{13}N_2O_2Br_2$ $[M+H]^+$: 424.9319, found: 424.9327.

MP: 122-123 °C.



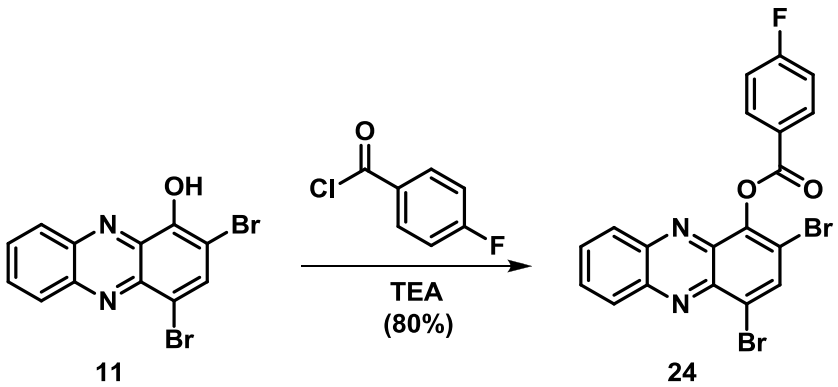
2,4-dibromophenazin-1-yl benzoate (23). 2,4-Dibromophenazin-1-ol (25 mg, 0.071 mmol) was dissolved in 2 mL dichloromethane. Triethylamine (50 µL, 0.35 mmol) was then added followed by the addition of benzoyl chloride (25 µL, 0.21 mmol) at room temperature. The reaction mixture was allowed to stir for 1 hour before being quenched with a saturated aqueous solution of sodium bicarbonate. The contents were then transferred to a separatory funnel and dichloromethane was used for extraction. The combined organic layers were dried with sodium sulfate, filtered and concentrated via rotavap. Purification via column chromatography using 19:1 hexanes:ethyl acetate afforded 30 mg (94% yield) of 2,4-dibromophenazin-1-yl benzoate **23** as a yellow solid. **NOTE:** ^{1}H and ^{13}C NMR spectra are reported in $CDCl_3$ and d_6-benzene. Two carbon signals overlap in the ^{13}C NMR spectra obtained in $CDCl_3$ resulting in 16 signals. All expected 17 carbon signals are observed in the ^{13}C NMR spectra in d_6-benzene.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 – 8.34 (m, 4H), 8.15 (m, 1H), 7.91 (ddd, *J* = 8.6, 6.7, 1.5 Hz, 1H), 7.84 (ddd, *J* = 8.3, 6.6, 1.5 Hz, 1H), 7.74 (m, 1H), 7.65 – 7.57 (m, 2H); (400 MHz, d_6-benzene) δ 8.46 (d, *J* = 7.3 Hz, 2H), 8.06 (d, *J* = 8.6 Hz, 1H), 7.92 (s, 1H), 7.77 (d, *J* = 8.9 Hz, 1H), 7.21 – 7.03 (m, 4H), 6.99 (ddd, *J* = 8.4, 6.7, 1.5 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 164.2, 145.5, 143.7, 143.7, 140.4, 138.0, 135.9, 134.3, 132.2, 132.0, 131.0, 130.2, 129.0, 128.9, 122.4, 117.3; (100 MHz, d_6-benzene) δ 164.0, 145.9, 143.7, 143.5, 140.6, 138.2, 135.6, 133.9, 131.7, 131.4, 131.1, 130.1, 129.9, 129.5, 128.9, 123.0, 117.6.

HRMS (DART): calc. for $C_{19}H_{11}N_2O_2Br_2$ $[M+H]^+$: 458.9162, found: 458.9173.

MP: 204-205 °C.



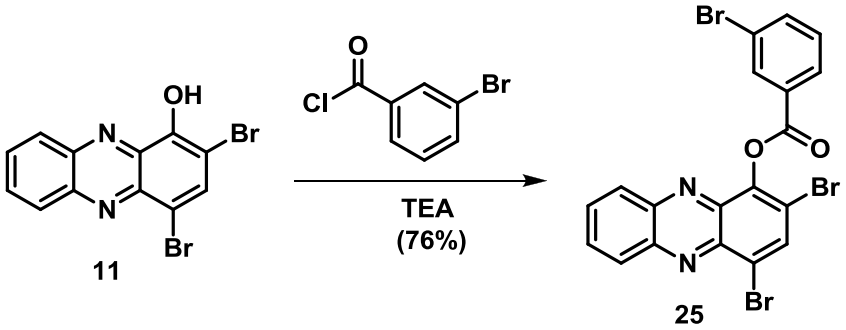
2,4-dibromophenazin-1-yl 4-fluorobenzoate (24). 2,4-Dibromophenazin-1-ol (25 mg, 0.071 mmol) was dissolved in 2 mL dichloromethane. Triethylamine (50 µL, 0.35 mmol) was then added followed by the addition of 4-fluorobenzoyl chloride (25 µL, 0.21 mmol) at room temperature. The reaction mixture was allowed to stir for 1 hour before being quenched with a saturated aqueous solution of sodium bicarbonate. The contents were then transferred to a separatory funnel and dichloromethane was used for extraction. The combined organic layers were dried with sodium sulfate, filtered and concentrated via rotavap. Purification via column chromatography using 19:1 hexanes:ethyl acetate afforded 27 mg (80% yield) of 2,4-dibromophenazin-1-yl 4-fluorobenzoate **24** as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 – 8.37 (m, 3H), 8.34 (d, J = 8.8 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H), 7.89 (ddd, J = 8.6, 6.7, 1.6 Hz, 1H), 7.83 (ddd, J = 8.4, 6.7, 1.5 Hz, 1H), 7.32 – 7.22 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7 (d, J = 254.2 Hz, 1J C-F coupling), 163.2, 145.3, 143.7, 143.6, 140.3, 137.9, 135.8, 133.7, 133.6, 132.2, 132.0, 130.1 (d, J = 9.1 Hz, 3J C-F coupling) 125.1 (d, J = 2.9 Hz, 4J C-F coupling), 122.5, 117.3, 116.2 (d, J = 21.9 Hz, 2J C-F coupling).

HRMS (DART): calc. for $C_{19}H_{10}N_2O_2Br_2F$ $[M+H]^+$: 476.9068, found: 476.9077.

MP: 198-199 °C.



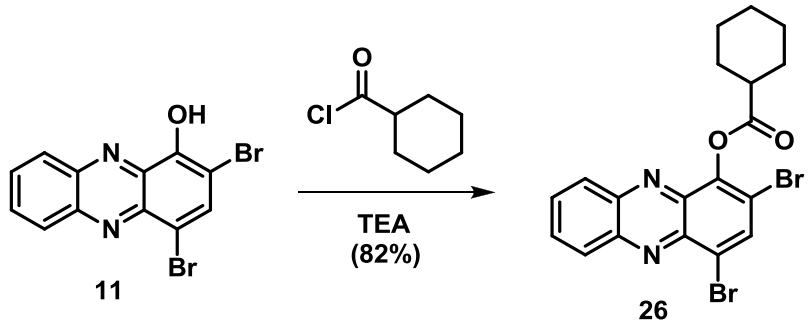
2,4-dibromophenazin-1-yl 3-bromobenzoate (25). 2,4-Dibromophenazin-1-ol (25 mg, 0.071 mmol) was dissolved in 2 mL dichloromethane. Triethylamine (50 µL, 0.35 mmol) was then added followed by the addition of 3-bromobenzoyl chloride (28 µL, 0.21 mmol) at room temperature. The reaction mixture was allowed to stir for 1 hour before being quenched with a saturated aqueous solution of sodium bicarbonate. The contents were then transferred to a separatory funnel and dichloromethane was used for extraction. The combined organic layers were dried with sodium sulfate, filtered and concentrated via rotavap. Purification via column chromatography using 19:1 hexanes:ethyl acetate afforded 29 mg (76% yield) of 2,4-dibromophenazin-1-yl 3-bromobenzoate **25** as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (t, *J* = 1.7 Hz, 1H), 8.39 (s, 1H), 8.36 (dd, *J* = 8.8, 1.6 Hz, 1H), 8.31 (dt, *J* = 8.0, 1.3 Hz, 1H), 8.15 (m, 1H), 7.91 (ddd, *J* = 8.6, 6.6, 1.6 Hz, 1H), 7.88 – 7.81 (m, 2H), 7.49 (t, *J* = 7.9 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 163.0, 145.1, 143.8, 143.7, 140.4, 137.8, 137.3, 135.8, 133.8, 132.3, 132.1, 130.8, 130.6, 130.2, 130.1, 129.5, 123.1, 122.7, 117.3.

HRMS (DART): calc. for $C_{19}H_{10}N_2O_2Br_3$ $[M+H]^+$: 536.8267, found: 536.8267.

MP: 180-181 °C.



2,4-dibromophenazin-1-yl cyclohexanecarboxylate (26). 2,4-Dibromophenazin-1-ol (25 mg, 0.071 mmol) was dissolved in 2 mL dichloromethane. Triethylamine (50 µL, 0.35 mmol) was then added followed by the addition of cyclohexanecarbonyl chloride (28 µL, 0.21 mmol) at room temperature. The reaction mixture was allowed to stir for 1 hour before being quenched with a saturated aqueous solution of sodium bicarbonate. The contents were then transferred to a separatory funnel and dichloromethane was used for extraction. The combined organic layers

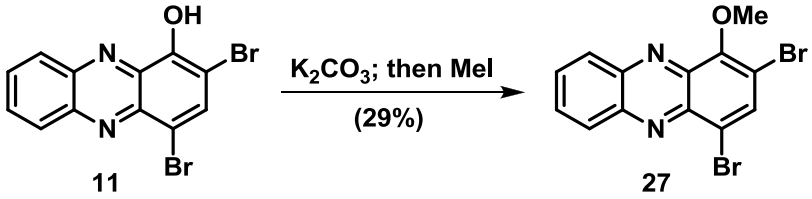
were dried with sodium sulfate, filtered and concentrated via rotavap. Purification via column chromatography using 19:1 hexanes:ethyl acetate afforded 27 mg (82% yield) of 2,4-dibromophenazin-1-yl cyclohexanecarboxylate **26** as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): 8.37 – 8.29 (m, 2H), 8.19 (m, 1H), 7.95 – 7.82 (m, 2H), 2.93 (tt, *J* = 11.0, 3.7 Hz, 1H), 2.38 – 2.23 (m, 2H), 2.00 – 1.70 (m, 5H), 1.55 – 1.34 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 173.4, 145.3, 143.6, 143.5, 140.3, 137.8, 135.9, 132.2, 131.9, 130.1, 130.0, 121.9, 117.1, 43.3, 29.4, 26.0, 25.6.

HRMS (DART): calc. for $C_{19}H_{17}N_2O_2Br_2$ $[M+H]^+$: 464.9632, found: 464.9647.

MP: 180-182 °C.



2,4-dibromo-1-methoxyphenazine (27). Potassium carbonate (39 mg, 0.28 mmol) was added to a stirring solution of 2,4-dibromo-1-hydroxyphenazine (20 mg, 0.056 mmol) in 1 mL anhydrous acetone. The resulting mixture was allowed to stir at room temperature for 30 minutes before iodomethane (35 µL, 0.57 mmol) was added to the reaction. The resulting reaction mixture was then allowed to stir for an additional 6 hours. The reaction contents were then partitioned between deionized water and chloroform. The organic contents were then extracted with chloroform, dried with sodium sulfate, filtered and concentrated via rotavap. The crude residue was purified by column chromatography using 8:1 hexanes:ethyl acetate to afford 5.9 mg (29% yield) of 2,4-dibromo-1-methoxyphenazine **27** as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 – 8.33 (m, 2H), 8.32 (s, 1H), 7.96 – 7.87 (m, 2H), 4.31 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 153.3, 143.4, 143.0, 140.9, 139.2, 136.7, 132.0, 131.8, 130.1, 130.0, 119.3, 116.1, 62.9.

HRMS (ESI): calc. for $C_{13}H_9N_2OBr_2$ $[M+H]^+$: 368.9056, found: 368.9068.

MP: 173-174 °C (lit. 171-173 °C).[7]

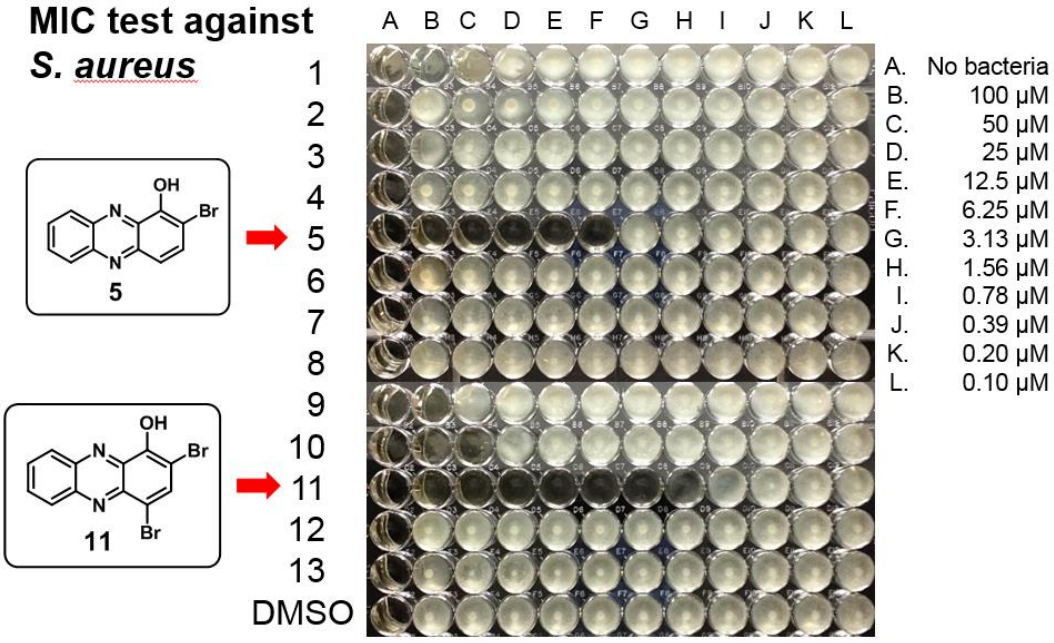
3) Biological Evaluation of Phenazine Small Molecules:

Compound Storage:

Solid stocks of phenazine compounds were stored at -80 ºC in vials sealed with parafilm. Between 2.0 and 4.5 milligrams of each phenazine was weighed out and a 10 mM DMSO stock solutions was prepared. DMSO stocks were stored at room temperature in the dark (i.e., wrapped in aluminum foil). At this concentration, a few phenazines required gentle heating to completely solubilize. DMSO stocks were not subjected to any freeze-thaw cycles during this study to prevent compound breakdown. The phenazine compounds described herein were stable in DMSO for several weeks at a time under these mild storage conditions, with the exception of pyocyanin. **NOTE:** Pyocyanin is unstable in DMSO at room temperature after one day. In NMR experiments, we observed significant decomposition of pyocyanin in d_6-DMSO after 8 hours at room temperature. DMSO stock solutions of ~2 milligrams of pyocyanin were made fresh each day.

Antibiotic Susceptibility Tests (MIC assay protocol):

The minimum inhibitory concentration (MIC) for each phenazine was determined by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI).[10] In a 96-well plate, eleven two-fold serial dilutions of each compound were made in a final volume of 100 µL Luria Broth (one column served as a blank; see MIC assay below). Each well was inoculated with 10^5 bacterial cells at the initial time of incubation, prepared from a fresh log phase culture (OD_{600} of 0.5). The MIC was defined as the lowest concentration of compound that prevented bacterial growth after incubating 16 to 20 hours at 37 °C. The concentration range tested for each phenazine/antibiotic during this study was 0.10 to 100 µM. DMSO served as our vehicle and negative control in each microdilution MIC assay. DMSO was serially diluted at the same concentration as the phenazine compounds with a top concentration of 1% v/v. Bacterial strains used: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228) and *Pseudomonas aeruginosa* (PAO1).



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5) ^{1}H and ^{13}C NMR Spectra: