Supporting Information

Amide Isosteres of Oroidin: Assessment of Antibiofilm Activity and *C. elegans* Toxicity

Justin J. Richards,[†] *Samuel Reyes*,[†] *Sean Stowe*,[‡] *Ashley T. Tucker*,[‡] *T. Eric Ballard*,[†§] *Laura D. Mathies*,[δ] *John Cavanagh*,[‡] *and Christian Melander*[†]*

[†] North Carolina State University, Department of Chemistry, Raleigh, NC, 27695
[‡] North Carolina State University, Department of Molecular and Structural Biochemistry, Raleigh, NC, 27695
[δ] North Carolina State University, Department of Genetics, Raleigh, NC, 27695
[§] Current address: University of Virginia, Department of Chemistry, Charlottesville, VA 22904

* christian_melander@ncsu.edu

Supporting Information

Table of Contents

Chemistry General Considerations: All reagents excluding Boc-guanidine used for the chemical synthesis of the library were purchased from commercially available sources and used as is without further purification. Boc-guanidine was prepared as described by Zapf et al.[1] Oroidin and DHS were synthesized as previously described.[2, 3] All reactions were run under a nitrogen or argon atmosphere unless otherwise noted. Flash silica gel chromatography was performed with 60Å mesh standard grade silica gel. ^{1}H and ^{13}C NMR spectra were obtained using 300 MHz or 400 MHz spectrometers and recorded at 23 °C. Chemical shifts (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, t = triplet, tt = triplet of triplets, q = quartet, m = multiplet) are given in parts per million relative to DMSO-d_6 (δ 2.50) and $CDCl_3$ (δ 7.27) for proton spectra and relative to DMSO-d_6 (δ 39.51) and $CDCl_3$ (δ 77.36) for carbon spectra. Compound purity for the analogues used for the antibiofilm assays and *C. elegans* toxicity assays was confirmed by LC/MS analysis.

Biofilm Inhibition and Dispersion Assay Considerations: Stock solutions (100 mM, 50 mM, 10 mM, 1mM) of all compounds were prepared in biological grade DMSO and stored at room temperature. The amount of DMSO used in both inhibition and dispersion screens did not exceed 1% (by volume). *P. aeruginosa* PA14 was supplied by the O'Toole Group at Dartmouth Medical School. *A. baumannii* was purchased from ATCC (ATCC 19606).

General Static Inhibition Assay Protocol for P. aeruginosa and A. baumannii. An overnight culture of the wild type strain was subcultured at an OD_{600} of 0.01 into LBNS (PA14) 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 Dispersion Assay Protocols for P. aeruginosa and A. baumannii. An overnight culture of the wild type strain was subcultured at an OD_{600} of 0.05 into LBNS (PA14) or LB (*A. baumannii*) 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 compound was then 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 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. Percent dispersion was calculated by comparison of the OD_{540} for established biofilm (untreated) versus treated established biofilm under identical conditions.

C. elegans Developmental/Reproductive Toxicity Assay Considerations: The nematode species employed in the toxicity assay was *Caenorhabditis elegans* N2 Bristol strain[4] while the two

Escherichia coli strains OP50 and HB101 were used in the maintenance and performance aspects of the bioassay. All worm and bacterial strains were provided by the Mathies Group at North Carolina State University. Stock solutions of the compounds used in the assay were identical to those used in the anti-biofilm activity assays.

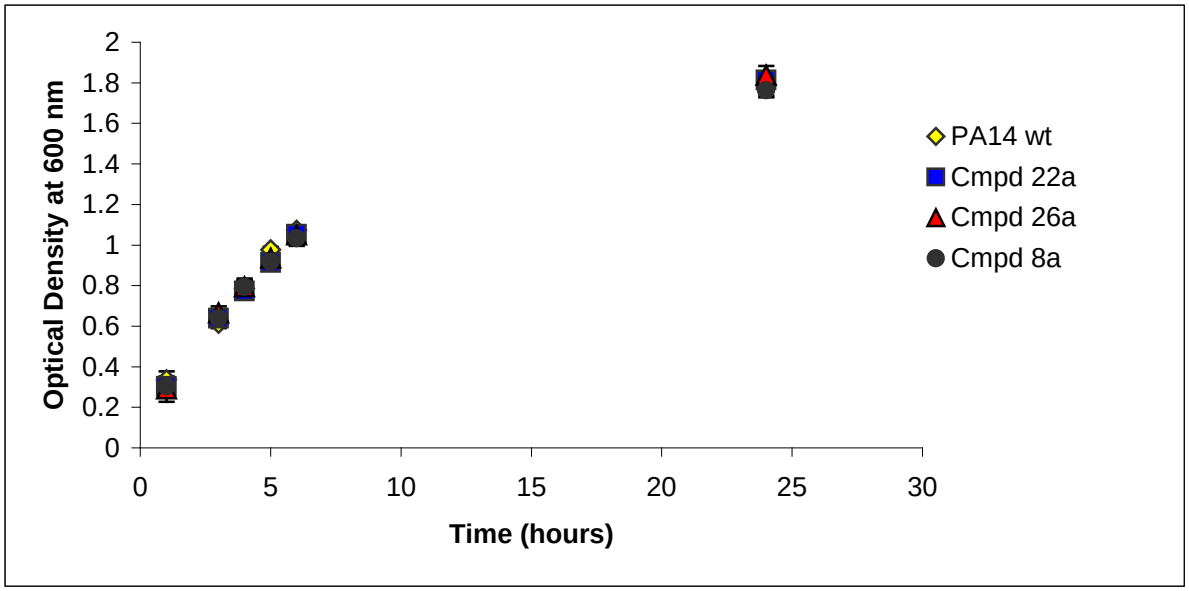
Complete Anti-biofilm Activities of Sulfonamide, Urea, and Thiourea Derivatives:

Compound	Actb Inhibition at 100 µM ± SE
8a	24 ± 2
9a	8 ± 3
10a	23 ± 2
11a	25 ± 5
12a	11 ± 2
13a	11 ± 4
14a	17 ± 2
15a	26 ± 3
16a	17 ± 5
17a	28 ± 4
18a	29 ± 4
19a	11 ± 3
20a	7 ± 3
21a	11 ± 3
22a	18 ± 3
23a	12 ± 3
24a	< 5
25a	8 ± 2
26a	14 ± 4

Compound	PA14 Inhibition at 100 µM ± SE	PA14 IC_{50} value (µM) ± SE	PA14 Dispersion at 100 uM ± SE
8a	80 ± 2	10.14 ± 1.96	42 ± 4
9a	45 ± 4	N/A	N/A
10a	92 ± 1	25.09 ± 3.44	N/A
11a	85 ± 3	19.45 ± 3.98	N/A
12a	43 ± 4	N/A	N/A
13a	14 ± 3	N/A	N/A
14a	87 ± 4	46.16 ± 3.62	N/A
15a	93 ± 1	15.60 ± 2.95	N/A
16a	50 ± 5	N/A	N/A
17a	94 ± 2	35.42 ± 3.34	N/A
18a	35 ± 4	N/A	N/A
19a	81 ± 4	50.40 ± 4.77	N/A
20a	45 ± 4	N/A	N/A
21a	69 ± 5	N/A	N/A
22a	90 ± 2	25.65 ± 2.19	55 ± 3
23a	81 ± 5	46.68 ± 4.13	N/A
24a	70 ± 3	N/A	N/A
25a	90 ± 3	26.80 ± 2.97	N/A
26a	89 ± 3	22.64 ± 3.24	25 ± 5

***Bacterial Growth Curves performed at calculated IC_{50} values (at 600 nm)*:**

PA14 wt = PA14 wild type control with no compound
Cmpd **8a** = 10.14 μM
Cmpd **22a** = 25.65 μM
Cmpd **26a** = 22.64 μM



C. elegans Developmental/Reproductive Toxicity Assay General Procedure:

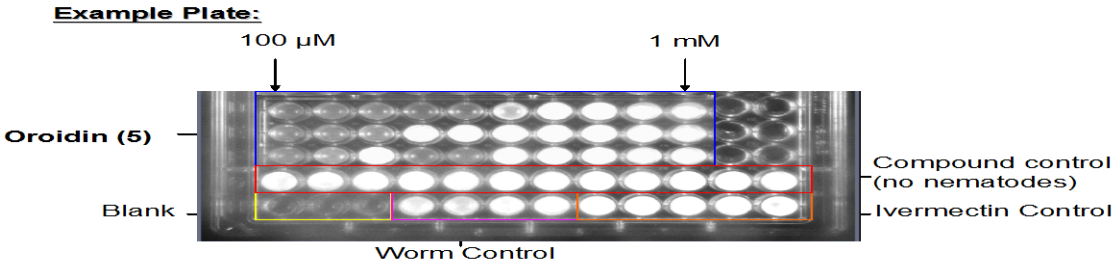
Overview: Dimethylsulfoxide (DMSO) has been reported to induce severe developmental and lethal effects on *C. elegans* at greater than 2% (v/v), therefore all test samples were diluted to have a final solvent concentration between 0.8 – 1.0%.[5, 6] Prior to use, all compounds were diluted from a 100 mM stock to 20x final assay volume (i.e. compound A was diluted to 20 mM for 1 mM assay volume) by using serial dilutions with ddH_2O and a small volume of DMSO to maintain consistency during the assay. Final assay concentrations ranged from 100 µM to 1 mM for all compounds assayed. Controls consisted of various compound concentrations with *E. coli* bacteria only, *E. coli* with compound only, *C. elegans* N2's with *E. coli* only, and a known nematocide (Ivermectin, Sigma I8898) at matching concentrations of compound and DMSO. DMSO controls were also employed at the volumes used in the assay and validated that the amounts used were not lethal to the worms.

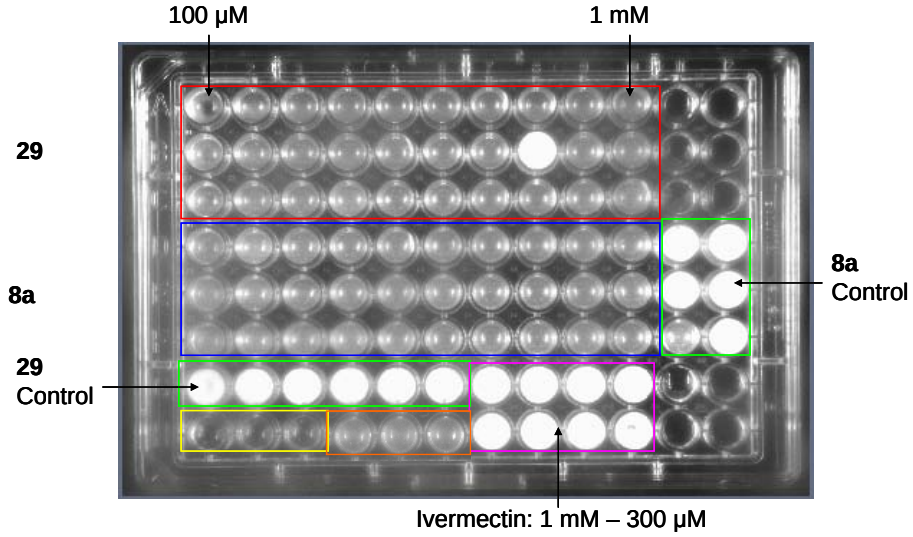
Maintenance and Synchronization: *C. elegans* N2 worms were maintained at 15º C on NGM agar plates with *E. coli* OP50 as a bacterial food source.[7] From these stock plates, six L4 to young adult stage worms were selected and transferred to separate NGM agar plates along with OP50 as a food source and incubated at 20°C for four days. Following incubation, the plates were rinsed off with M9 medium and spun down at 500x *g* for 2 minutes without braking. The excess M9 medium was decanted from the tube, and the pellet was resuspended in an alkaline bleach solution (1.5% v/v NaOCl, 0.12 M NaOH) for 4 to 6 minutes to harvest viable eggs for synchronization.[8] To correctly time the bleaching process, the release of eggs from the adults was observed carefully as prolonged exposure to the bleaching solution can have a damaging effect on the eggs. Once enough time had elapsed, the solution was spun down (500x *g* for 2 minutes) with braking and repeatedly washed with M9 medium until bleach was no longer detectable. The eggs were then resuspended into fresh M9 medium and incubated at 20°C for 24

to 36 hours on an orbital shaker to allow the eggs to hatch and for the L1 stage larva to arrest due to starvation, thereby providing a supply of synchronized L1's.

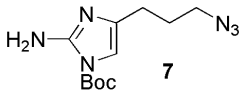
Fecundity and Development Assay: The synchronized L1's were recovered via centrifugation without braking and resuspended at a small volume (< 30 µL) to increase worm concentration. These worms were pipetted in 5 µL aliquots onto NGM agar with OP50 and allowed to evaporate before incubation at 20°C for at least 43 hours to allow the larva to reach the L4 stage. Using a 96-well plate, each assay was setup in triplicate with two compound ranges tested per plate along with blanks (100 µL S medium only) and controls. Two L4 stage larva were transferred from the plates to a single well containing 25 µL S medium, followed by 70 µL of a 5% (m/v) HB101 suspension in S medium and 5 µL of the compound to be tested at the appropriate dilution (see **Overview** section above), yielding a total assay volume of 100 µL/well. The plates were incubated at 22.5°C on an orbital shaker for 6 to 7 days to allow for maturation of the first generation of worms. Observations using a dissecting microscope were made on day 4 and again on day 6 or 7 (depending on growth evaluation on day 4), and the plates were scored based on the clarity of the wells. If the well was cleared or was observed to be in the process of clearing in addition to the presence of a large population of worms, it was scored as a non-toxic concentration; if the well was still opaque and/or did not have a large population of N2's, the particular concentration was scored as having possible developmental and/or reproductive toxic effects. Beginning at 100 µM with each compound and following incremental 100 µM steps, the assays were narrowed down based on the range of possible toxic concentrations found by the previous run until a comparable threshold concentration could be established.

Representative Plates from C. elegans Toxicity Assays:



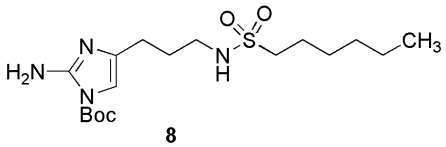


***Experimental Procedures and Characterization Data*:**



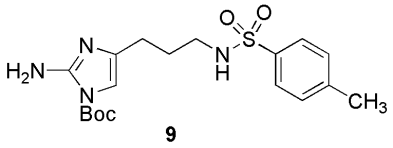
7

4-Azido-butyric acid[9] (2.15 g, 16.7 mmol) was dissolved in anhydrous dichloromethane (80 mL) at 0 ºC and a catalytic amount of DMF was added. To this solution was added oxalyl chloride (4.40 mL, 50 mmol) drop-wise and the solution was then warmed to room temperature. After 1 h, the solvent and excess oxalyl chloride were removed under reduced pressure. The resulting oil was dissolved into anhydrous dichloromethane (10 mL) and added drop-wise to a 0 ºC solution of CH_2N_2 (50 mmol generated from Diazald®/KOH) in diethyl ether (125 mL). This solution was stirred at 0 ºC for 1.5 h at which time the reaction was quenched via the drop-wise addition of 48% HBr (6.0 mL). The reaction mixture was diluted with dichloromethane (25 mL) and immediately washed with sat. $NaHCO_3$ (3 x 25 mL) and brine (2 x 25 mL) before being dried ($MgSO_4$), filtered and concentrated. The resulting oil representing the α-bromoketone obtained upon concentration was pure (3.1 g, 90%) and used in the following steps without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.35 (s, 2H), 3.33 (t, 2H, J = 6.9 Hz), 2.66 (t, 2H, J = 7.2 Hz), 1.75 (quint, 2H, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.3, 50.6, 36.6, 34.3, 23.3; HRMS (ESI) calcd for $C_5H_8BrN_3O$ (M^+) 204.9851, found 204.9850. The α-bromoketone (0.870 g, 4.22 mmol) and Boc-guanidine (2.00 g, 12.7 mmol) were dissolved in DMF (15 mL) and allowed to stir at room temperature. After 24 h the DMF was removed under reduced pressure and the residue taken up in ethyl acetate (50 mL) and washed with water (3 x 25 mL) and brine (25 mL) before being dried (Na_2SO_4), filtered and evaporated to dryness. The resulting oil was purified by flash column chromatography (10-100% EtOAc/Hexanes) to obtain the title compound **7** (0.700 g, 63%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.53 (s, 1H), 5.98 (s, 2H), 3.30 (t, 2H, J = 6.9 Hz), 2.43 (t, 2H, J = 7.2 Hz), 1.87 (tt, 2H, J = 6.9, 12.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.6, 149.6, 137.6, 107.1, 84.8, 50.9, 28.1, 27.8, 25.3; HRMS (ESI) calcd for $C_{11}H_{18}N_6O_2$ (M^+) 266.1491, found 266.1499.



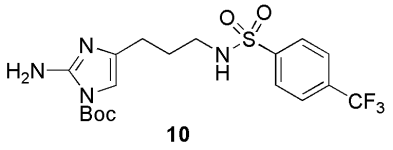
8

To a solution of anhydrous THF (5.0 mL) and 10% Pd/C (0.010 g) was charged compound **7** (0.100 g, 0.375 mmol). Air was removed from the system and the reaction was back flushed with hydrogen. This process was repeated three times before setting the reaction under a hydrogen balloon at atmospheric pressure and temperature for 12 h. After that time, the reaction was filtered to remove the catalyst. The filtrate was cooled to -78 °C and triethylamine (0.052 mL, 0.375 mmol) was added. 1-hexanesulfonyl chloride (0.069 g, 0.375 mmol) diluted in anhydrous dichloromethane (0.50 mL) was subsequently added drop-wise to this solution. The reaction was stirred at -78 °C for 30 mins and then quenched with methanol. Evaporation of the crude reaction to dryness and purification by flash column chromatography (0-10% MeOH/CH_2Cl_2) gave the title compound **8** (0.093 g, 64%) as a colorless oil: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.00 (t, 1H, J = 5.7 Hz), 6.54 (s, 1H), 6.38 (br s, 2H), 2.92 (m, 4H), 2.80 (t, 2H, J = 7.5 Hz), 1.56 – 1.71 (m, 4H), 1.53 (s, 9H), 1.23 – 1.40 (m, 6H), 0.86 (t, 1H, J = 6.6 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 150.6, 149.6, 138.8, 106.6, 84.8, 51.6, 42.6, 31.4, 29.1, 28.2, 27.9, 25.5, 23.8, 22.5, 14.5; HRMS (ESI) calcd for $C_{17}H_{32}N_4O_4S$ (M^+) 388.2144, found 388.2128.



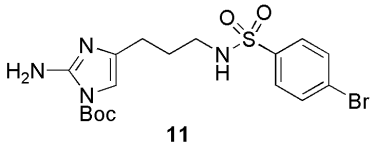
9

In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.121 g (82%) of compound **9** as a white foam: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65 (d, 2H, J = 8.4 Hz), 7.53 (t, 1H, J = 5.7 Hz), 7.37 (d, 2H, J = 8.1 Hz), 6.48 (s, 1H), 6.38 (br s, 2H), 2.70 (dt, 2H, J = 6.6, 13.2 Hz), 2.37 (s, 3H), 2.20 (t, 2H, J = 6.9 Hz), 1.58 (m, 2H), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.9, 148.9, 142.4, 137.9, 137.7, 129.5, 126.4, 105.8, 84.1, 42.1, 27.8, 27.5, 25.5, 24.8, 20.9; HRMS (ESI) calcd for $C_{18}H_{26}N_4O_4S$ (M^+) 394.1675, found 394.1665.



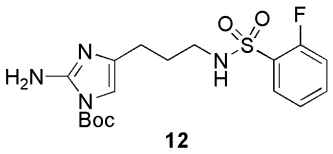
10

In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.140 g (83%) of compound **10** as a white foam: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.99 (m, 4H), 7.90 (t, 1H, J = 5.7 Hz), 6.47 (s, 1H), 6.36 (br s, 2H), 2.80 (dt, 2H, J = 6.9, 13.2 Hz), 2.20 (t, 2H, J = 7.2 Hz), 1.58 (tt, 2H, J = 7.2, 14.4 Hz), 1.52 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.9, 148.8, 144.6, 137.8, 131.8, 127.4, 126.4, 125.3, 121.7, 105.8, 84.0, 42.1, 27.9, 27.5, 24.8; HRMS (ESI) calcd for $C_{18}H_{23}F_3N_4O_4S$ (M^+) 448.1392, found 448.1383.



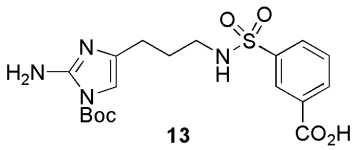
11

In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.146 g (85%) of compound **11** as a white foam: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.67 – 7.82 (m, 5H), 6.47 (s, 1H), 6.38 (br s, 2H), 2.74 (dt, 2H, J = 6.6, 12.3 Hz), 2.20 (t, 2H, J = 7.2 Hz), 1.58 (m, 2H), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.9, 148.8, 139.9, 137.9, 132.2, 128.5, 126.0, 105.9, 84.1, 42.1, 27.8, 27.5, 24.7; HRMS (ESI) calcd for $C_{17}H_{23}BrN_4O_4S$ (M^+) 458.0623, found 458.0625.



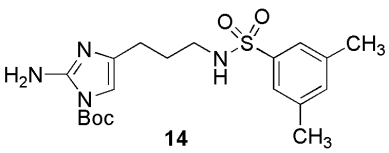
12

In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.125 g (84%) of compound **12** as a white foam: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (t, 1H, J = 5.7 Hz), 7.65 – 7.78 (m, 2H), 7.37 (m, 2H), 6.46 (s, 1H), 6.36 (br s, 2H), 2.85 (dt, 2H, J = 6.9, 13.2 Hz), 2.20 (t, 2H, J = 7.2 Hz), 1.60 (tt, 2H, J = 7.2, 14.4 Hz), 1.52 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.8, 156.5, 149.8, 148.8, 137.8, 135.1, 129.6, 128.5, 117.3, 105.8, 84.1, 42.1, 27.9, 27.5, 24.7; HRMS (ESI) calcd for $C_{17}H_{23}FN_4O_4S$ (M^+) 398.1424, found 398.1430.



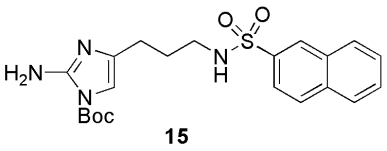
13

In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.091 g (57%) of compound **13** as a colorless film: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.33 (m, 2H), 8.12 (d, 2H, J = 7.5 Hz), 7.78 (m, 1H), 7.68 (t, 1H, J = 5.7 Hz), 7.57 (t, 1H, J = 7.8 Hz), 6.46 (s, 1H), 6.36 (br s, 2H), 2.74 (dt, 2H, J = 6.9, 13.2 Hz), 2.20 (t, 2H, J = 7.5 Hz), 1.60 (tt, 2H, J = 7.2, 14.4 Hz), 1.52 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.5, 149.8, 148.8, 140.1, 137.9, 132.6, 128.6, 127.9, 127.0, 105.6, 84.0, 70.5, 42.1, 27.9, 27.5, 24.7; HRMS (ESI) calcd for $C_{18}H_{24}N_4O_6S$ (M^+) 424.1417, found 424.1417.



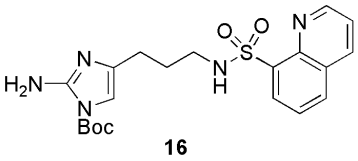
14

In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.121 g (79%) of compound **14** as a white foam: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.50 (t, 1H, J = 5.4 Hz), 7.37 (s, 2H), 7.25 (s, 1H), 6.48 (s, 1H), 6.37 (br s, 2H), 2.72 (dt, 2H, J = 6.9, 13.5 Hz), 2.34 (s, 6H), 2.20 (t, 2H, J = 7.5 Hz), 1.61 (tt, 2H, J = 7.2, 14.4 Hz), 1.52 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.8, 148.9, 140.5, 138.6, 137.9, 133.5, 128.6, 123.9, 127.0, 105.8, 84.1, 70.5, 42.1, 27.9, 27.5, 24.7, 20.7; HRMS (ESI) calcd for $C_{19}H_{28}N_4O_4S$ (M^+) 408.1831, found 408.1836.



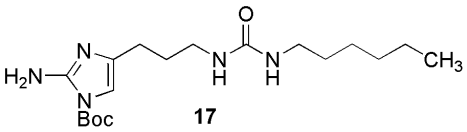
15

In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.122 g (76%) of compound **15** as a white foam: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (m, 1H), 8.14 (m, 2H), 8.05 (dd, 1H, J = 9.0, 10.5 Hz), 7.82 (dd, 1H, J = 9.0, 10.5 Hz), 7.65 – 7.79 (m, 3H), 6.45 (s, 1H), 6.35 (br s, 2H), 2.78 (dt, 2H, J = 6.3, 12.6 Hz), 2.20 (t, 2H, J = 7.2 Hz), 1.61 (tt, 2H, J = 7.2, 14.4 Hz), 1.52 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.8, 148.8, 137.9, 137.6, 134.0, 131.7, 129.3, 129.1, 128.6, 127.8, 127.5, 127.2, 122.2, 105.8, 84.0, 42.1, 27.9, 27.5, 24.8; HRMS (ESI) calcd for $C_{21}H_{26}N_4O_4S$ (M^+) 430.1675, found 430.1677.



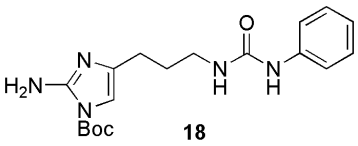
16

In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.143 g (88%) of compound **16** as a white foam: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.08 (m, 1H), 8.55 (dd, 1H, J = 1.5, 8.4 Hz), 8.28 (m, 2H), 7.37 (m, 2H), 7.22 (t, 1H, J = 6.0 Hz), 6.34 (br s, 2H), 6.32 (s, 1H), 2.80 (dt, 2H, J = 6.6, 13.2 Hz), 2.10 (t, 2H, J = 7.2 Hz), 1.51 (m, 11H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 151.3, 149.7, 148.8, 142.7, 137.9, 137.0, 136.5, 133.4, 130.5, 128.4, 125.7, 122.5, 105.7, 84.0, 42.5, 27.9, 27.5, 24.7; HRMS (ESI) calcd for $C_{20}H_{25}N_5O_4S$ (M^+) 431.1627, found 431.1621.



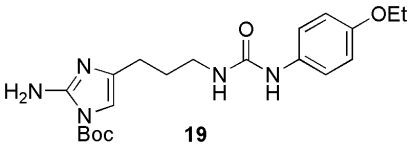
17

In a similar manner, 0.050 g (0.187 mmol) of **7** afforded 0.023 g (33%) of compound **17** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.54 (s, 1H), 5.58 (br s, 2H), 4.55 (br s, 1H), 3.22 (t, 2H, J = 6.9 Hz), 3.14 (dt, 2H, J = 6.6, 12.6 Hz), 2.42 (t, 2H, J = 7.2 Hz), 1.79 (tt, 2H, J = 6.9, 14.4 Hz), 1.59 (s, 9H), 1.49 (m, 2H), 1.30 (m, 4H), 0.88 (t, 3H, J = 6.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.5, 149.9, 149.4, 138.2, 107.0, 84.8, 40.7, 40.1, 31.5, 30.2, 29.0, 28.0, 26.6, 25.2, 22.6, 14.0; HRMS (ESI) calcd for $C_{18}H_{33}N_5O_3$ (M^+) 367.2583, found 367.2586.

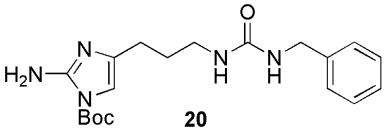


18

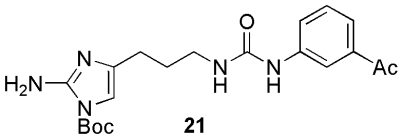
In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.094 g (70%) of compound **18** as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.37 (s, 1H), 7.37 (d, 2H, J = 8.8 Hz), 7.20, (t, 2H, J = 7.6 Hz), 6.87 (t, 2H, J = 7.6 Hz), 6.56 (s, 1H), 6.37 (s, 2H), 6.17 (t, 1H, J = 5.9 Hz), 3.08 (dt, 2H, J = 6.3, 12.3 Hz), 2.28 (t, 2H, J = 7.4 Hz), 1.65 (tt, 2H, J = 7.4, 14.8 Hz), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.5, 150.2, 149.3, 140.9, 138.7, 128.9, 121.2, 117.9, 106.1, 84.4, 28.8, 27.9, 25.5; HRMS (ESI) calcd for $C_{18}H_{25}N_5O_3$ (M^+) 359.1957, found 359.1951.



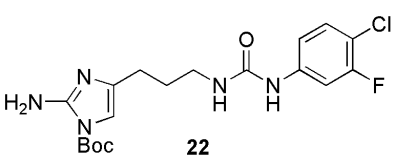
In a similar manner, 0.050 g (0.187 mmol) of **7** afforded 0.055 g (73%) of compound **19** as a white solid: ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.15 (s, 1H), 7.25 (d, 2H, *J* = 8.9 Hz), 6.78 (d, 2H *J* = 8.9 Hz), 6.55 (s, 1H), 6.37 (s, 2H), 6.05 (t, 1H, *J* = 5.6 Hz), 3.94 (q, 2H, *J* = 7.0 Hz), 3.07 (dt, 2H, *J* = 6.3, 12.6 Hz), 2.27 (t, 2H, *J* = 7.4 Hz), 1.64 (tt, 2H, *J* = 7.4, 14.4 Hz), 1.53 (s, 9H), 1.29 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 155.8, 153.4, 150.2, 149.3, 138.8, 134.0, 119.7, 114.8, 106.1, 84.4, 63.4, 39.1, 28.9, 27.9, 25.5, 15.1; HRMS (ESI) calcd for $C_{20}H_{29}N_5O_4$ (M^+) 403.2219, found 403.2213.



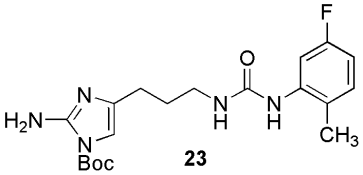
In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.100 g (71%) of compound **20** as a white solid: ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 7.29 (m, 2H), 7.23 (m, 3H), 6.53 (s, 1H), 6.37 (s, 2H), 6.27 (t, 1H, *J* = 6.0 Hz), 5.97 (t, 1H, *J* = 5.7 Hz), 4.19 (d, 2H, *J* = 6.0 Hz), 3.01 (dt, 2H, *J* = 6.5, 12.6 Hz), 2.24 (t, 2H, *J* = 7.6 Hz), 1.60 (m, 2H), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 158.4, 150.2, 149.3, 141.4, 138.8, 128.5, 127.3, 126.8, 106.1, 84.4, 43.2, 29.1, 27.9, 25.5; HRMS (ESI) calcd for $C_{19}H_{27}N_5O_3$ (M^+) 373.2114, found 373.2108.



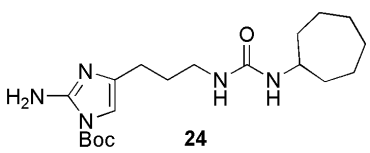
In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.119 g (79%) of compound **21** as a white foam: ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.70 (s, 1H), 7.99 (m, 1H), 7.62 (dd, 1H, *J* = 2.1, 8.1 Hz), 7.48 (d, 1H, *J* = 8.1 Hz), 7.36 (t, 1H, *J* = 8.1 Hz), 6.59 (s, 1H), 6.49 (br s, 2H), 6.28 (t, 1H, *J* = 5.7 Hz), 3.08 (dt, 2H, *J* = 6.6, 12.6 Hz), 2.53 (s, 3H), 2.29 (t, 2H, *J* = 7.5 Hz), 1.66 (tt, 2H, *J* = 7.5, 14.4 Hz), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 197.8, 155.1, 149.8, 148.8, 141.0, 137.3, 128.9, 122.1, 120.9, 116.8, 105.9, 84.2, 38.7, 28.4, 27.5, 26.7, 24.9; HRMS (ESI) calcd for $C_{20}H_{27}N_5O_4$ (M^+) 401.2063, found 401.2063.



In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.068 g (44%) of compound **22** as a white solid: ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.67 (s, 1H), 7.76 (dd, 1H, *J* = 2.4, 6.6 Hz), 7.21 (m, 3H), 6.59 (s, 1H), 6.55 (br s, 2H), 6.30 (t, 1H, *J* = 5.7 Hz), 3.08 (dt, 2H, *J* = 6.6, 12.6 Hz), 2.28 (t, 2H, *J* = 7.5 Hz), 1.66 (tt, 2H, *J* = 7.5, 14.4 Hz), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 155.0, 153.3, 150.1, 149.8, 148.8, 137.9, 118.7, 117.6, 116.8, 116.5, 105.9, 84.2, 38.4, 28.3, 27.5, 24.9; HRMS (ESI) calcd for $C_{28}H_{23}ClFN_5O_3$ (M^+) 411.1474, found 411.1476.

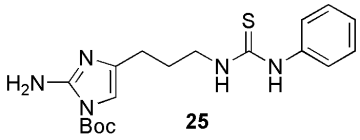


In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.115 g (78%) of compound **23** as a white solid: ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 7.85 (dd, 1H, *J* = 2.8, 12.5 Hz), 7.71 (s, 1H), 7.10 (m, 1H), 6.78 (t, 1H, *J* = 5.5 Hz), 6.63 (dt, 1H, *J* = 2.9, 8.5 Hz), 6.57 (s, 1H), 6.37 (s, 2H), 3.11 (dt, 2H, *J* = 6.4, 12.4 Hz), 2.30 (t, 2H, *J* = 7.6 Hz), 2.14 (s, 3H), 1.67 (tt, 2H, *J* = 7.4, 14.4 Hz), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 162.5, 159.4, 155.4, 150.2, 149.3, 140.2, 138.7, 131.2, 121.3, 107.5, 106.0, 28.7, 27.9, 25.5, 17.5; HRMS (ESI) calcd for $C_{19}H_{26}FN_5O_3$ (M^+) 391.2020, found 391.2021.



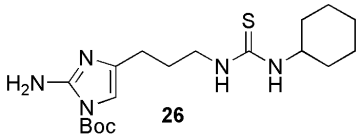
24

In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.100 g (70%) of compound **24** as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.52 (s, 1H), 6.36 (s, 2H) 5.68 (m, 2H), 3.55 (m, 1H), 2.96 (dt, 2H, J = 6.5, 12.6 Hz), 2.22 (t, 2H, J = 7.6 Hz), 1.69 – 1.79 (m, 2H), 1.46 – 1.62 (m, 14H), 1.35 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.6, 150.1, 149.2, 138.8, 106.0, 84.4, 50.2, 39.1, 35.4, 29.1, 28.0, 27.8, 25.5, 23.9; HRMS (ESI) calcd for $C_{19}H_{33}N_5O_3$ (M^+) 379.2583, found 379.2573.



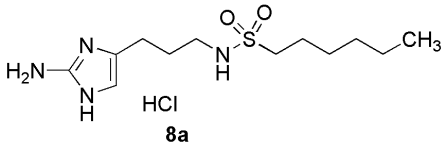
25

In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.130 g (92%) of compound **25** as a pale yellow solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.44 (s, 1H), 7.80 (br s, 1H), 7.40 (m, 2H), 7.31 (m, 2H), 7.09 (t, 1H, J = 7.2 Hz), 6.60, (s, 1H), 6.37 (s, 1H), 3.47 (m, 2H), 2.29 (t, 2H, J = 7.3 Hz), 1.78 (m, 2H), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 180.3, 149.8, 148.9, 139.3, 138.2, 128.5, 123.9, 122.9, 105.9, 84.0, 43.5, 27.5, 27.2, 25.2; HRMS (ESI) calcd for $C_{18}H_{25}N_5O_2S$ (M^+) 375.1729, found 375.1727.



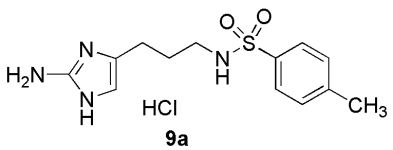
26

In a similar manner, 0.100 g (0.375 mmol) of **7** afforded 0.111 g (78%) of compound **26** as a white foam: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.23 (br s, 1H), 7.19 (d, 1H, J = 7.6 Hz), 6.57 (s, 1H), 6.39 (br s, 2H), 3.91 (br s, 1H), 2.25 (t, 2H, J = 7.2 Hz), 1.81 (m, 2H), 1.63 – 1.73 (m, 4H), 1.53 (m, 13H), 1.10 – 1.30 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 180.9, 149.8, 148.8, 138.3, 105.8, 84.0, 51.6, 43.0, 32.3, 27.5, 25.1, 24.9; HRMS (ESI) calcd for $C_{18}H_{31}N_5O_2S$ (M^+) 381.2199, found 381.2205.



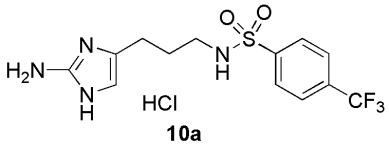
8a

Compound **8** (0.070 g, 0.180 mmol) was dissolved in anhydrous dichloromethane (5 mL) and cooled to 0 ºC. Trifluoroacetic acid (0.50 mL) was added drop-wise while the reaction continued to stir at 0 ºC. Upon completion, the reaction was allowed to warm to room temperature over the course of 12 h. Toluene (2 mL) was added and the reaction was evaporated to dryness. The crude TFA salt was then dissolved in dichloromethane (3 mL) and a 2M solution of HCl in diethyl ether (0.10 mL) was added. The solution was again concentrated under reduced pressure and the resulting product triturated with cold diethyl ether (5 mL) to afford 0.057 g (98%) of compound **8a** in its corresponding hydrochloride salt form as an amber oil: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.07 (s, 1H), 11.62 (s, 1H), 7.34 (s, 2H), 7.09 (t, 1H, J = 6.0 Hz), 6.57 (s, 1H), 2.93 (m, 4H), 2.45 (t, 2H, J = 7.5 Hz), 1.57 – 1.71 (m, 4H), 1.24 – 1.35 (m, 6H), 0.86 (t, 2H, J = 6.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 146.7, 126.1, 108.7, 50.8, 41.3, 30.7, 28.2, 27.2, 23.1, 21.8, 21.2, 13.8; HRMS (ESI) calcd for $C_{12}H_{24}N_4O_2S$ (M^+) 288.1620, found 288.1626.

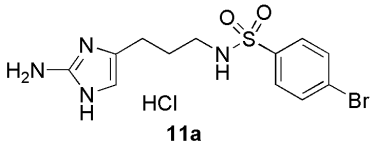


9a

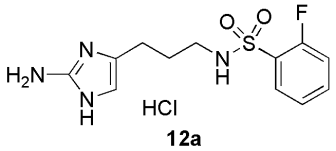
In a similar manner, 0.050 g (0.127 mmol) of **9** afforded 0.042 g (99%) of compound **9a** in its corresponding hydrochloride salt form as an amber oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.02 (s, 1H), 11.60 (s, 1H), 7.67 (d, 2H, J = 8.1 Hz), 7.61 (t, 1H, J = 6.0 Hz), 7.37 (d, 2H, J = 8.1 Hz), 7.32 (br s, 2H), 6.49 (s, 1H), 2.70 (dt, 2H, J = 6.6, 12.6 Hz), 2.40 (m, 5H), 1.59 (tt, 2H, J = 6.6, 14.1 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 146.7, 142.5, 137.5, 129.6, 126.4, 125.9, 108.7, 41.5, 27.5, 21.1, 20.9; HRMS (ESI) calcd for $C_{13}H_{18}N_4O_2S$ (M^+) 294.1151, found 294.1158.



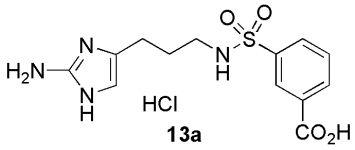
In a similar manner, 0.064 g (0.143 mmol) of **10** afforded 0.054 g (98%) of compound **10a** in its corresponding hydrochloride salt form as a tan amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.07 (s, 1H), 11.63 (s, 1H), 8.02 (m, 4H), 7.34 (s, 2H), 6.51 (s, 1H), 2.78 (dt, 2H, J = 6.6, 12.6 Hz), 2.41 (t, 2H, J = 7.5 Hz), 1.65 (tt, 2H, J = 6.9, 14.1 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 146.7, 144.4, 132.0, 127.4, 126.4, 125.9, 121.6, 108.7, 41.5, 27.6, 21.1; HRMS (ESI) calcd for $C_{13}H_{15}F_3N_4O_2S$ (M^+) 348.0868, found 348.0871.



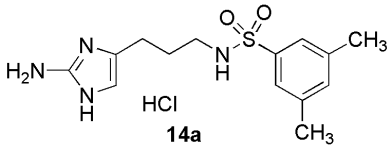
In a similar manner, 0.056 g (0.122 mmol) of **11** afforded 0.048 g (99%) of compound **11a** in its corresponding hydrochloride salt form as a colorless film. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.97 (s, 1H), 11.56 (s, 1H), 7.81 (m, 3H), 7.70 (d, 2H, J = 8.4 Hz), 7.33 (br s, 2H), 6.52 (s, 1H), 2.75 (m, 2H), 2.39 (t, 2H, J = 7.2 Hz), 1.65 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 147.4, 140.4, 133.0, 129.2, 126.8, 126.7, 109.5, 41.5, 28.3, 21.8; HRMS (ESI) calcd for $C_{12}H_{15}BrN_4O_2S$ (M^+) 358.0099, found 358.0102.



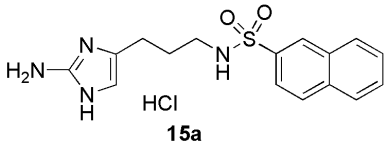
In a similar manner, 0.043 g (0.108 mmol) of **12** afforded 0.034 g (94%) of compound **12a** in its corresponding hydrochloride salt form as an amber oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.03 (s, 1H), 11.61 (s, 1H), 8.00 (t, 2H, J = 5.7 Hz), 7.66 – 7.81 (m, 2H), 7.33 – 7.47 (m, 4H), 6.50 (s, 1H), 2.85 (dt, 2H, J = 6.6, 12.6 Hz), 2.40 (t, 2H, J = 7.5 Hz), 1.64 (tt, 2H, J = 6.9, 14.1 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.8, 156.4, 146.7, 135.2, 129.6, 126.0, 124.8, 117.2, 108.7, 41.4, 27.7, 21.0; HRMS (ESI) calcd for $C_{12}H_{15}FN_4O_2S$ (M^+) 298.0900, found 298.0891.



In a similar manner, 0.034 g (0.080 mmol) of **13** afforded 0.028 g (99%) of compound **13a** in its corresponding hydrochloride salt form as a tan amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.08 (s, 1H), 11.67 (s, 1H), 8.32 (s, 1H), 8.17 (d, 1H, J = 6.3 Hz), 8.02 (d, 1H, J = 7.5 Hz), 7.89 (t, 1H, J = 5.7 Hz), 7.74 (t, 1H, J = 7.5 Hz), 7.36 (br s, 2H), 6.51 (s, 1H), 2.76 (m, 2H), 2.40 (t, 2H, J = 7.5 Hz), 1.63 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.9, 146.7, 140.9, 132.8, 131.7, 130.4, 129.8, 127.0, 125.9, 108.6, 41.4, 27.6, 21.0; HRMS (ESI) calcd for $C_{13}H_{16}N_4O_4S$ (M^+) 324.0892, found 324.0890.

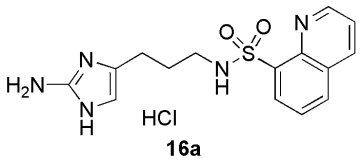


In a similar manner, 0.046 g (0.113 mmol) of **14** afforded 0.038 g (99%) of compound **14a** in its corresponding hydrochloride salt form as a tan film. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.96 (s, 1H), 11.55 (s, 1H), 7.56 (t, 1H, J = 5.7 Hz), 7.32 (m, 5H), 6.51 (s, 1H), 2.72 (dt, 2H, J = 6.9, 12.9 Hz), 2.37 (m, 8H), 1.63 (tt, 2H, J = 7.5, 14.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 146.7, 140.3, 138.7, 133.6, 126.1, 123.9, 108.6, 41.5, 27.6, 21.1, 20.7; HRMS (ESI) calcd for $C_{14}H_{20}N_4O_2S$ (M^+) 308.1307, found 308.1309.



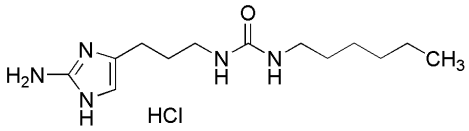
15a

In a similar manner, 0.050 g (0.116 mmol) of **15** afforded 0.041 g (98%) of compound **15a** in its corresponding hydrochloride salt form as a tan amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.05 (s, 1H), 11.61 (s, 1H), 8.42 (s, 1H), 8.15 (m, 2H), 8.04 (d, 2H, J = 8.4 Hz), 7.82 (m, 2H), 7.69 (m, 2H), 7.33 (br s, 2H), 6.50 (s, 1H), 2.78 (dt, 2H, J = 6.3, 12.9 Hz), 2.40 (t, 2H, J = 7.2 Hz), 1.65 (tt, 2H, J = 6.9, 14.1 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 146.7, 137.4, 134.1, 131.7, 129.3, 129.1, 128.6, 127.8, 127.5, 127.2, 125.9, 122.2, 108.6, 41.6, 27.7, 21.1; HRMS (ESI) calcd for $C_{16}H_{18}N_4O_2S$ (M^+) 330.1151, found 330.1146.



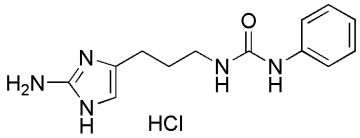
16a

In a similar manner, 0.060 g (0.139 mmol) of **16** afforded 0.050 g (98%) of compound **16a** in its corresponding mono hydrochloride salt form as an amber oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.97 (s, 1H), 11.57 (s, 1H), 9.07 (dd, 1H, J = 1.8, 4.5 Hz), 8.55 (dd, 1H, J = 1.8, 4.5 Hz), 8.30 (d, 2H, J = 7.5 Hz), 7.49 (m, 2H), 7.32 (m, 2H), 6.41 (s, 1H), 2.78 (dt, 2H, J = 6.3, 12.9 Hz), 2.34 (t, 2H, J = 7.5 Hz), 1.59 (tt, 2H, J = 6.6, 14.1 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 151.3, 146.6, 142.6, 137.0, 136.3, 133.6, 130.7, 128.4, 126.0, 125.7, 122.5, 108.6, 41.9, 27.7, 21.1; HRMS (ESI) calcd for $C_{15}H_{17}N_5O_2S$ (M^+) 331.1103, found 331.1109.



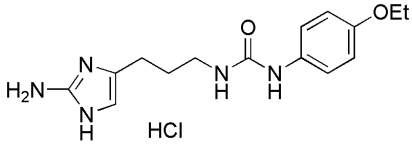
17a

In a similar manner, 0.052 g (0.141 mmol) of **17** afforded 0.042 g (97%) of compound **17a** in its corresponding hydrochloride salt form as a colorless film. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.05 (s, 1H), 11.54 (s, 1H), 7.31 (s, 2H), 6.58 (s, 1H), 5.93 (m, 2H), 2.96 (m, 4H), 2.38 (t, 2H, J = 7.5 Hz), 1.59 (tt, 2H, J = 6.6, 14.1 Hz), 1.31 (m, 2H), 1.23 (m, 6H), 0.85 (t, 3H, J = 6.6 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.3, 146.6, 126.5, 108.6, 38.2, 30.9, 29.9, 28.9, 26.0, 22.0, 21.3, 13.9; HRMS (ESI) calcd for $C_{13}H_{25}N_5O$ (M^+) 267.2059, found 267.2062.



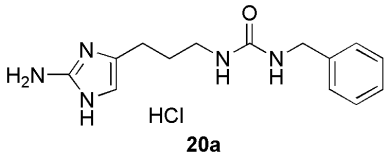
18a

In a similar manner, 0.041 g (0.114 mmol) of **18** afforded 0.031 g (94%) of compound **18a** in its corresponding hydrochloride salt form as a tan oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.03 (s, 1H), 11.58 (s, 1H), 8.75 (s, 1H), 7.37 (m, 4H), 7.19 (t, 2H, J = 7.2 Hz), 6.86 (t, 1H, J = 6.9 Hz), 6.61 (s, 1H), 6.47 (br s, 1H), 3.08 (m, 2H), 2.43 (t, 2H, J = 7.5 Hz), 1.67 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.4, 146.7, 140.6, 128.5, 126.4, 120.8, 117.5, 108.6, 38.1, 28.4, 21.4; HRMS (ESI) calcd for $C_{13}H_{17}N_5O$ (M^+) 259.1433, found 259.1432.



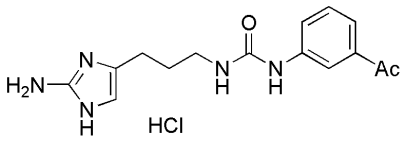
19a

In a similar manner, 0.055 g (0.136 mmol) of **19** afforded 0.045 g (97%) of compound **19a** in its corresponding hydrochloride salt form as a colorless film. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.00 (s, 1H), 11.55 (s, 1H), 8.40 (s, 1H), 7.31 (s, 2H), 7.27 (d, 2H, J = 9.0 Hz), 6.77 (d, 2H, J = 9.0 Hz), 6.61 (s, 1H), 6.24 (br s, 1H), 3.93 (q, 2H, J = 6.6 Hz), 3.08 (m, 2H), 2.43 (t, 2H, J = 7.2 Hz), 1.67 (tt, 2H, J = 7.2, 14.1 Hz), 1.28 (t, 3H, J = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.6, 153.1, 146.6, 133.6, 126.5, 119.3, 114.4, 108.7, 63.0, 38.2, 28.5, 21.4, 14.7; HRMS (ESI) calcd for $C_{15}H_{21}N_5O_2$ (M^+) 303.1695, found 303.1693.



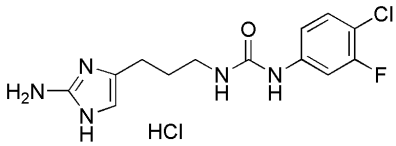
20a

In a similar manner, 0.042 g (0.112 mmol) of **20** afforded 0.030 g (88%) of compound **20a** in its corresponding hydrochloride salt form as a tan oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H), 11.63 (s, 1H), 7.18 – 7.32 (m, 7H), 6.58 (s, 1H), 4.20 (s, 2H), 3.02 (m, 2H), 2.40 (t, 2H, J = 7.6 Hz), 1.61 (tt, 2H, J = 6.8, 14.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.3, 146.7, 140.9, 128.2, 126.9, 126.5, 126.4, 108.6, 42.8, 38.3, 28.7, 21.3; HRMS (ESI) calcd for $C_{14}H_{19}N_5O$ (M^+) 273.1590, found 273.1584.



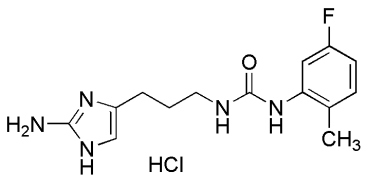
21a

In a similar manner, 0.036 g (0.089 mmol) of **21** afforded 0.030 g (99%) of compound **21a** in its corresponding hydrochloride salt form as a tan oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.05 (s, 1H), 11.59 (s, 1H), 9.13 (s, 1H), 8.01 (s, 1H), 7.62 (d, 1H, J = 7.8 Hz), 7.50 (d, 1H, J = 7.8 Hz), 7.32 (m, 3H), 6.63 (s, 2H), 3.10 (m, 2H), 2.52 (s, 3H), 2.45 (t, 2H, J = 7.5 Hz), 1.68 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 197.8, 155.4, 146.7, 141.1, 137.3, 128.9, 126.4, 122.0, 121.0, 116.6, 108.7, 38.1, 28.3, 26.7, 21.5; HRMS (ESI) calcd for $C_{15}H_{19}N_5O_2$ (M^+) 301.1539, found 301.1574.



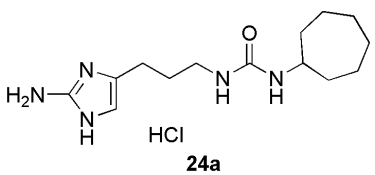
22a

In a similar manner, 0.037 g (0.090 mmol) of **22** afforded 0.030 g (96%) of compound **22a** in its corresponding hydrochloride salt form as a tan oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H), 11.59 (s, 1H), 8.99 (s, 1H), 7.76 (d, 1H, J = 6.8 Hz), 7.26 (m, 4H), 6.61 (s, 1H), 6.54 (m, 1H), 3.10 (m, 2H), 2.43 (t, 2H, J = 7.5 Hz), 1.67 (tt, 2H, J = 6.8, 14.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.2, 153.3, 150.2, 146.7, 137.9, 126.4, 118.6, 117.6, 116.6, 108.7, 38.2, 28.3, 21.5; HRMS (ESI) calcd for $C_{13}H_{15}ClFN_5O$ (M^+) 311.0949, found 311.0956.



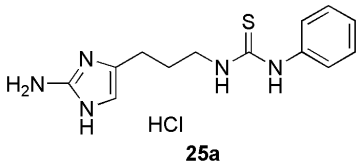
23a

In a similar manner, 0.059 g (0.151 mmol) of **23** afforded 0.047 g (96%) of compound **23a** in its corresponding hydrochloride salt form as a tan amorphous solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.07 (s, 1H), 11.59 (s, 1H), 8.03 (s, 1H), 7.82 (dd, 1H, J = 2.8, 12.6 Hz), 7.30 (br s, 3H), 7.08 (t, 1H, J = 7.7 Hz), 6.61 (dt, 2H, J = 2.8, 8.4 Hz), 3.09 (dt, 2H, J = 6.5, 12.6 Hz), 2.45 (m, 2H), 2.15 (s, 3H), 1.67 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.5, 155.7, 147.1, 140.2, 131.2, 126.7, 121.6, 109.1, 107.5, 106.1, 28.6, 21.9, 17.9; HRMS (ESI) calcd for $C_{14}H_{19}FN_5O$ (M^+) 291.1495, found 291.1496.



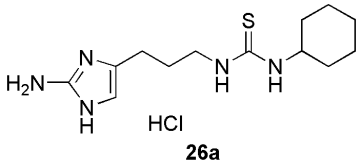
24a

In a similar manner, 0.050 g (0.134 mmol)of **24** afforded 0.042 g (99%) of compound **24a** in its corresponding hydrochloride salt form as a tan amorphous solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.10 (s, 1H), 11.58 (s, 1H), 7.31 (br s, 2H), 6.58 (s, 1H), 3.52 – 3.62 (m, 1H), 2.98 (dt, 2H, J = 6.8, 12.6 Hz), 2.38 (t, 2H, J = 7.5 Hz), 1.73 (m, 2H), 1.47 – 1.63 (m, 6H), 1.32 – 1.42 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.9, 147.0, 126.9, 109.0, 50.3, 38.5, 35.4, 29.3, 28.1, 23.9, 21.7; HRMS (ESI) calcd for $C_{14}H_{25}N_5O$ (M^+) 279.2059, found 279.2057.



25a

In a similar manner, 0.039 g (0.104 mmol) of **25** afforded 0.032 g (99%) of compound **25a** in its corresponding hydrochloride salt form as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.05 (s, 1H), 11.60 (s, 1H), 9.90 (s, 1H), 8.17 (t, 1H, J = 5.4 Hz), 7.27 – 7.46 (m, 6H), 7.08 (t, 1H, J = 7.2 Hz), 6.64 (s, 1H), 3.50 (m, 2H), 2.46 (t, 2H, J = 7.2 Hz), 1.80 (tt, 2H, J = 6.9, 14.1 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 180.5, 146.7, 139.5, 128.4, 126.3, 123.8, 122.7, 108.7, 42.8, 26.7, 21.6; HRMS (ESI) calcd for $C_{13}H_{17}N_5S$ (M^+) 275.1205, found 275.1207.



26a

In a similar manner, 0.047 g (0.123 mmol) of **26** afforded 0.037 g (95%) of compound **26a** in its corresponding hydrochloride salt form as a tan oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.01 (s, 1H), 11.56 (s, 1H), 7.31 – 7.50 (m, 4H), 6.61 (s, 1H), 3.37 (m, 2H), 2.42 (t, 2H, J = 7.2 Hz), 1.52 – 1.82 (m, 6H), 1.10 – 1.27 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 180.6, 146.7, 126.4, 108.7, 51.7, 42.5, 32.3, 27.4, 25.2, 24.5, 21.6; HRMS (ESI) calcd for $C_{13}H_{23}N_5S$ (M^+) 281.1674, found 281.1677.

References:

(1) Zapf, C. W.; Creighton, C. J.; Tomioka, M.; Goodman, M., A novel traceless resin-bound guanidinylating reagent for secondary amines to prepare N,N-disubstituted guanidines. *Org. Lett.* **2001**, 3, (8), 1133-1136.

(2) Olofson, A.; Yakushijin, K.; Horne, D. A., Synthesis of marine sponge alkaloids oroidin, clathrodin, and dispacamides. Preparation and transformation of 2-amino-4,5-dialkoxy-4,5-dihydroimidazolines from 2-aminoimidazoles. *J. Org. Chem.* **1998**, 63, (4), 1248-1253.

(3) Richards, J. J.; Ballard, T. E.; Huigens, R. W.; Melander, C., Synthesis and screening of an oroidin library against Pseudomonas aeruginosa biofilms. *ChemBioChem* **2008**, 9, (8), 1267-1279.

(4) Brenner, S., Genetics of Caenorhabditis elegans. *Genetics* **1974**, 77, (1), 71-94.

(5) Burns, A. R.; Kwok, T. C. Y.; Howard, A.; Houston, E.; Johanson, K.; Chan, A.; Cutler, S. R.; McCourt, P.; Roy, P. J., High-throughput screening of small molecules for bioactivity and target identification in Caenorhabditis elegans. *Nat. Protoc.* **2006**, 1, (4), 1906-1914.

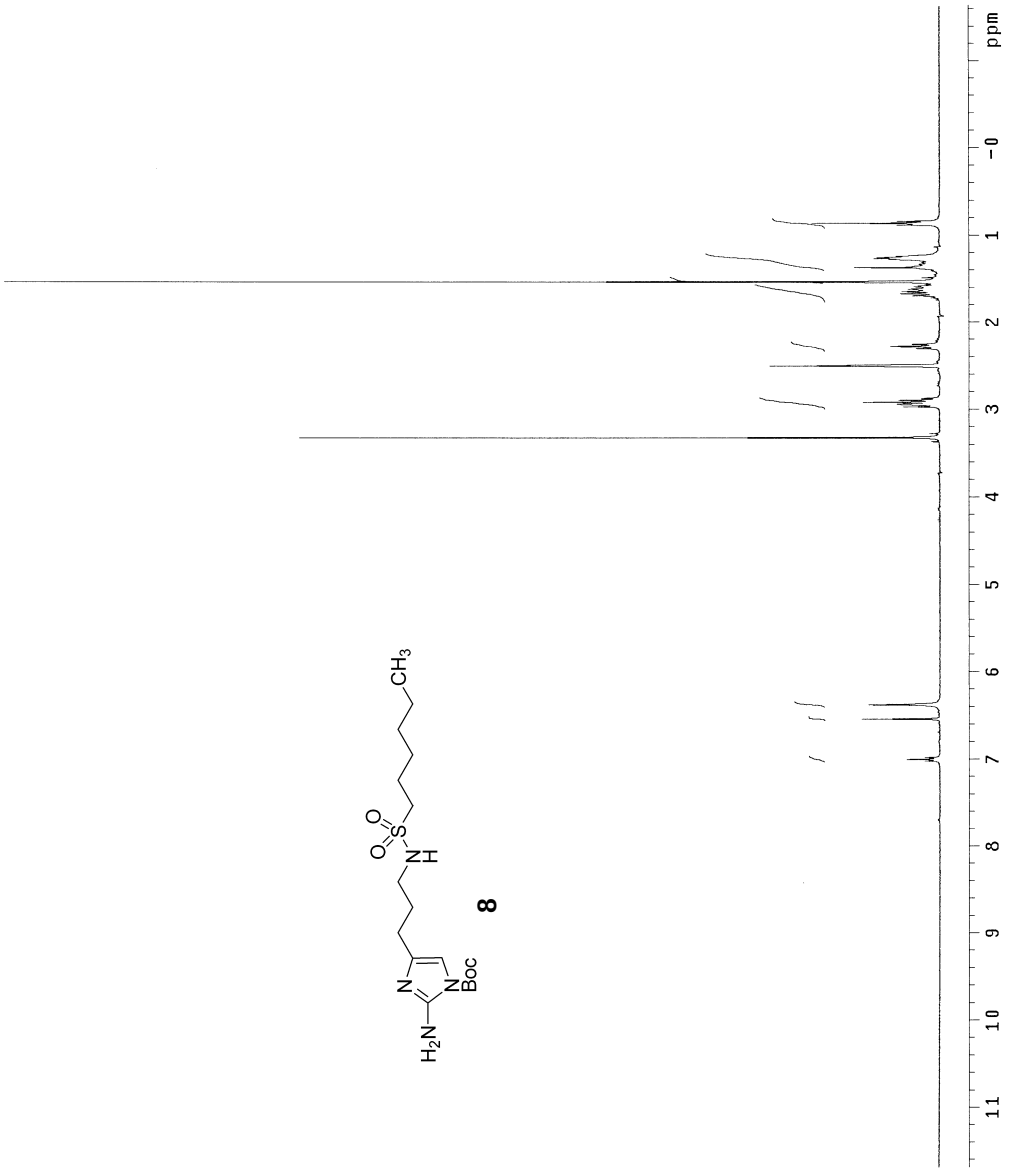
(6) Mukai, D.; Matsuda, N.; Yoshioka, Y.; Sato, M.; Yamasaki, T., Potential anthelmintics: polyphenols from the tea plant Camellia sinensis L. are lethally toxic to Caenorhabditis elegans. *J. Nat. Med.* **2008**, 62, (2), 155-159.

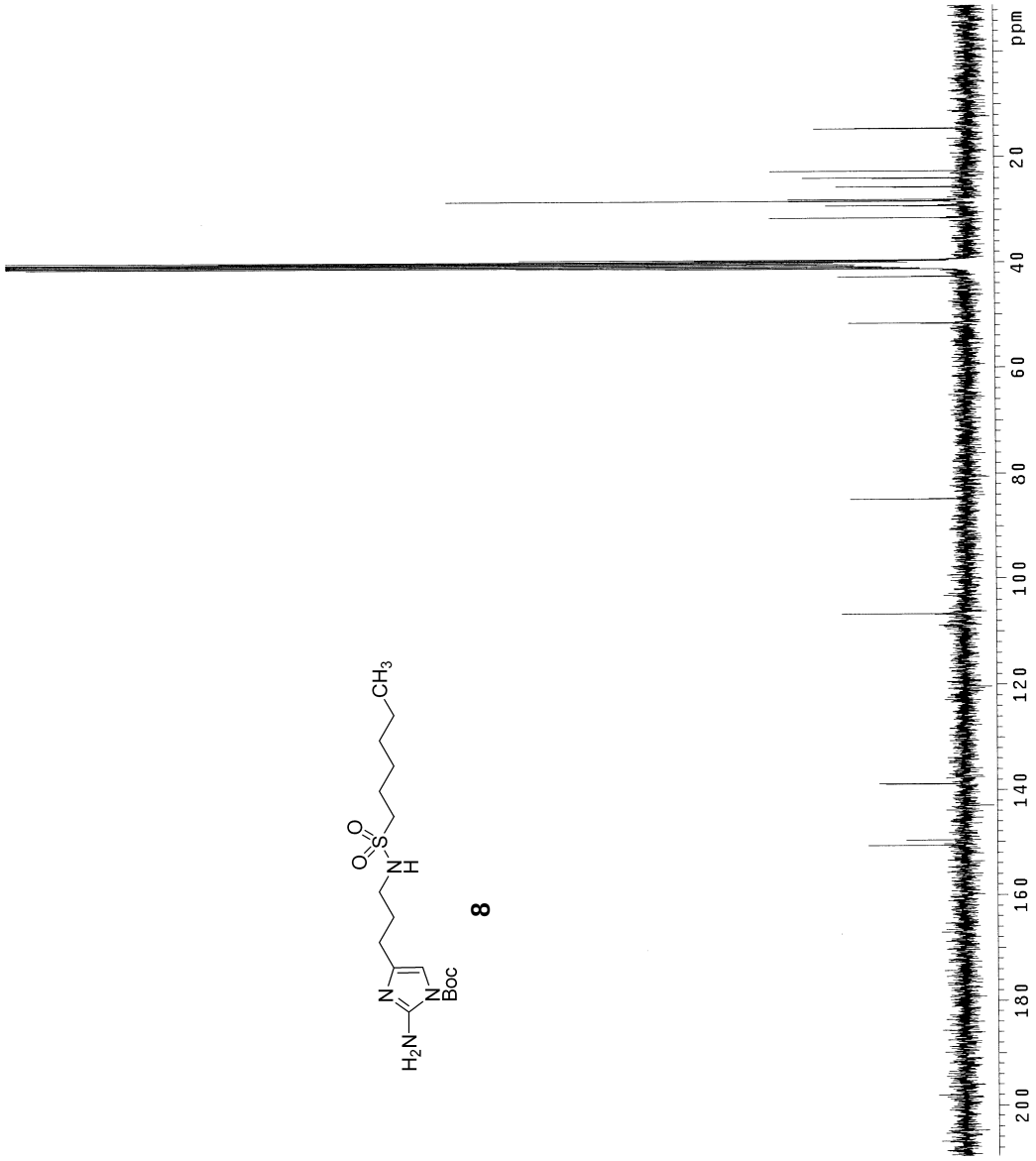
(7) Lewis, J. A.; Fleming, J. T., *Basic culture methods: Life cycle of Caenorhabditis elegans*. Academic Press: **1995**; Vol. 48, p 3-29.

(8) Steiernagle, T., edited by Hope, I., *Maintenance of C. elegans: A Practical Approach*. Oxford University Press: **1999**.

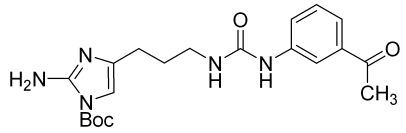
(9) Khoukhi, N.; Vaultier, M.; Carrie, R., Synthesis and reactivity of methyl gamma-azido butyrates and ethyl delta-azido valerates and of the corresponding acid chlorides as useful reagents for the aminoalkylation. *Tetrahedron* **1987**, 43, (8), 1811-1822.

***Representative ^{1}H NMR and ^{13}C NMR Spectra*:**

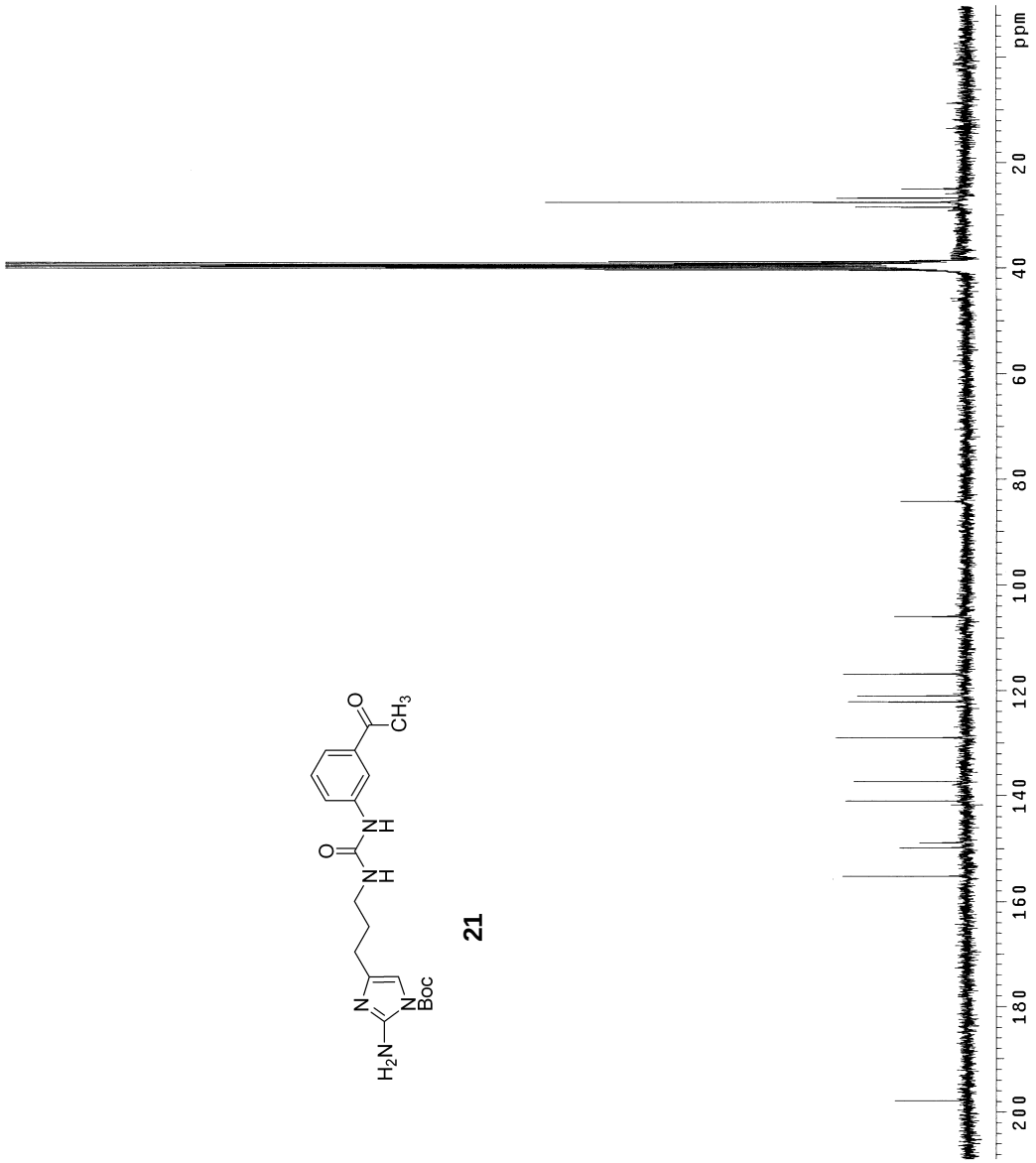




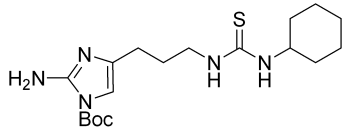
8



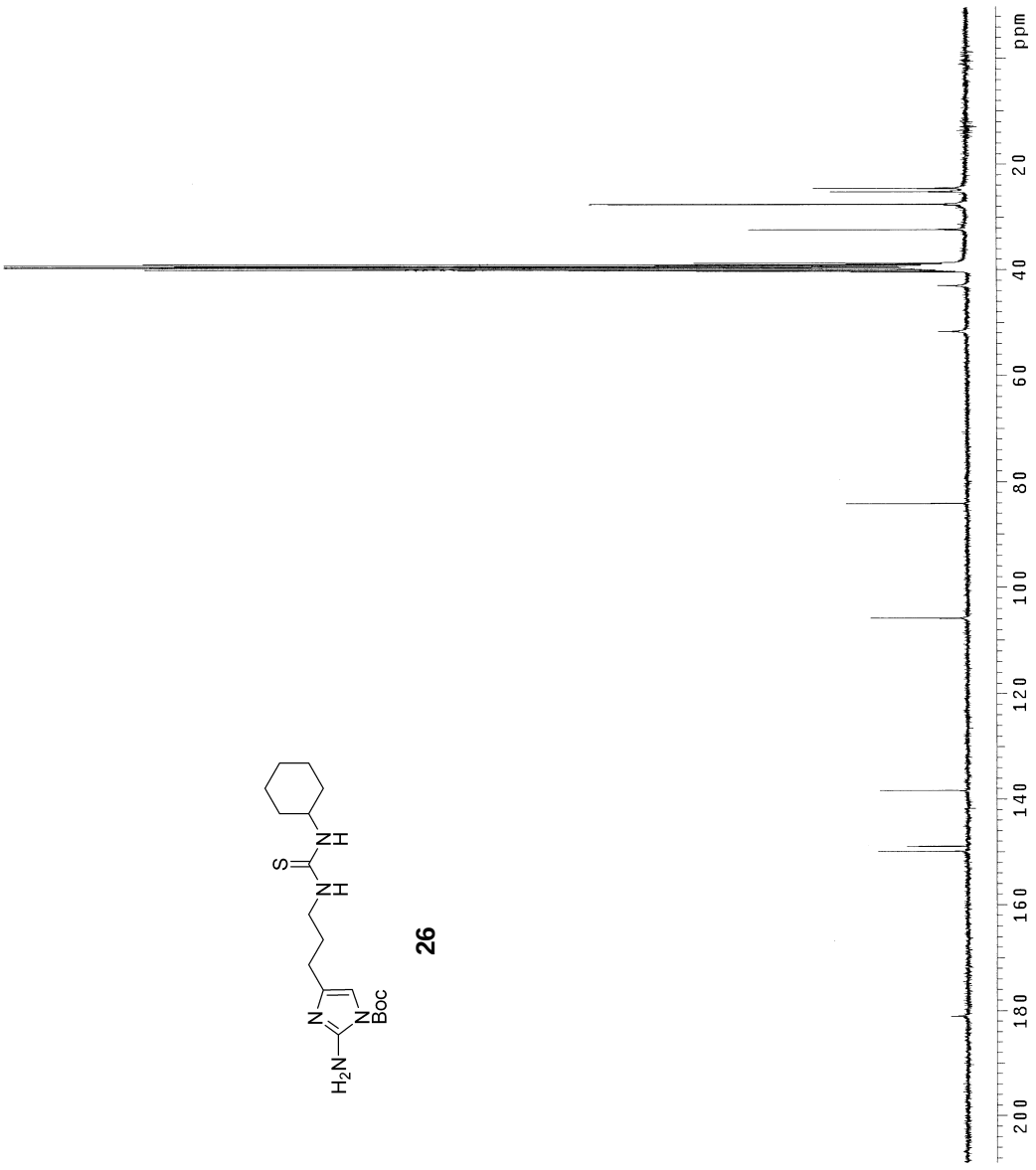
21



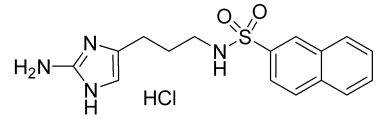
21



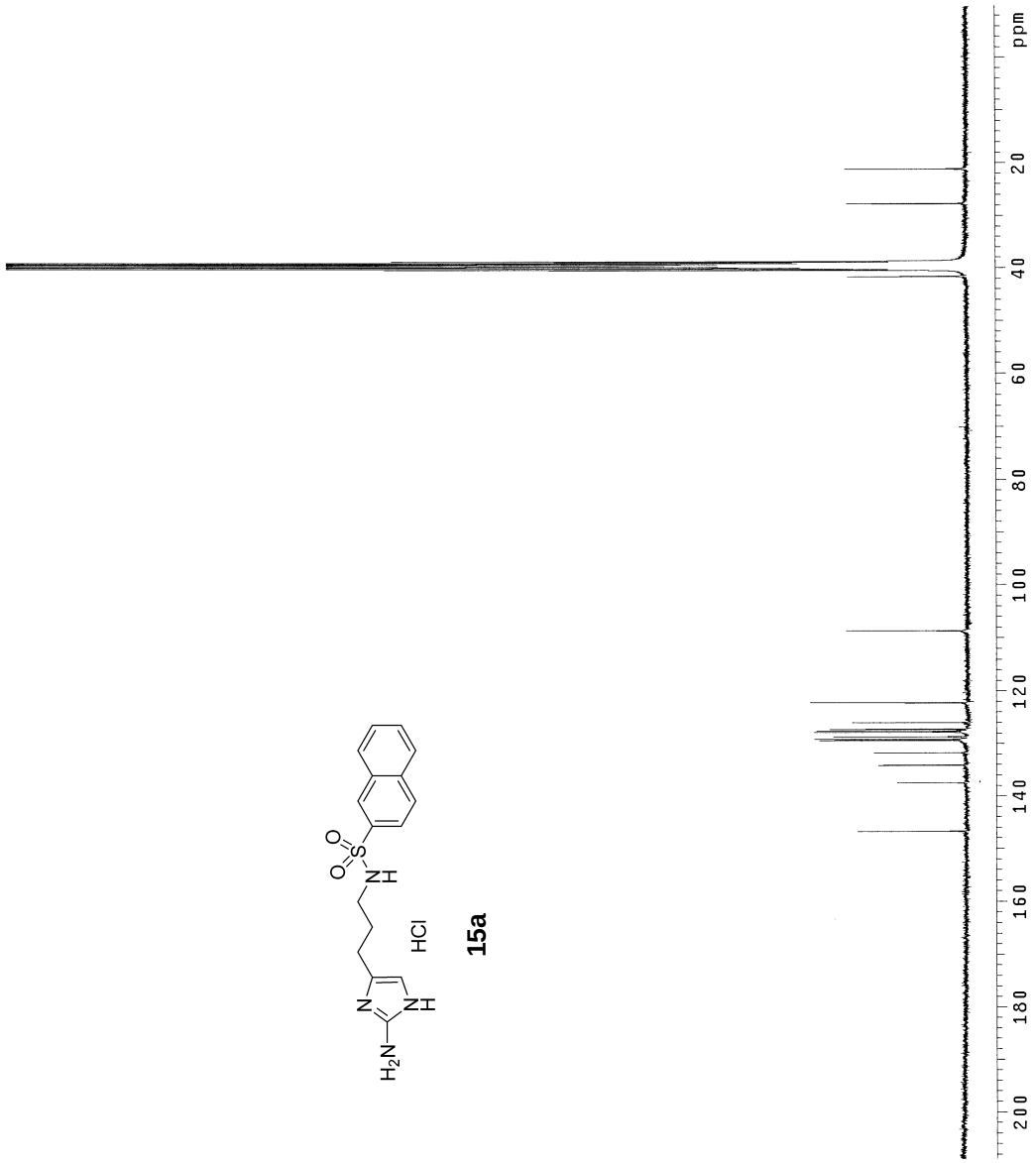
26



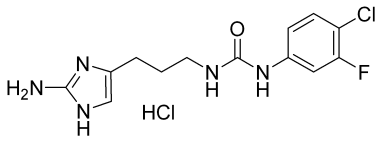
26



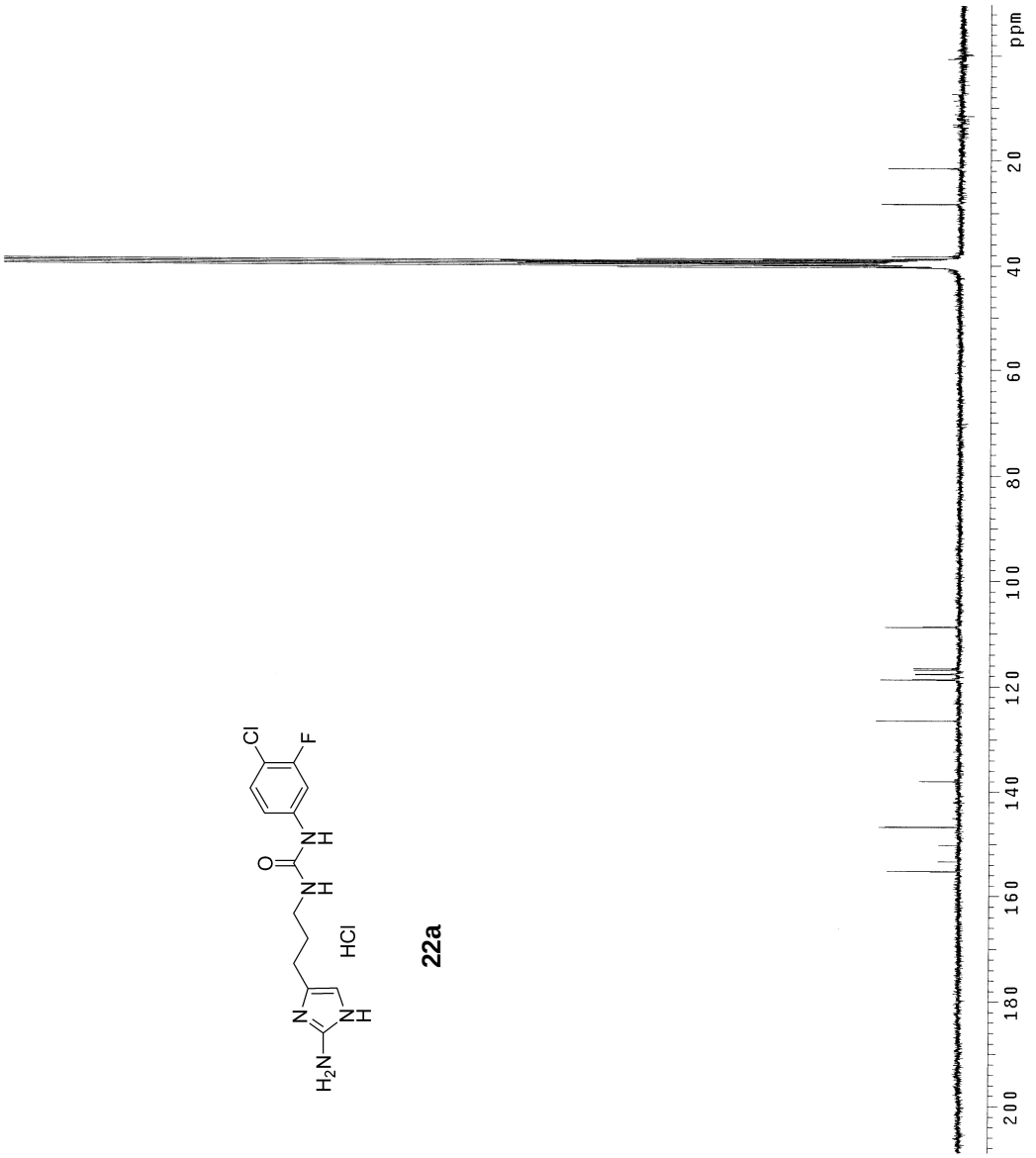
15a



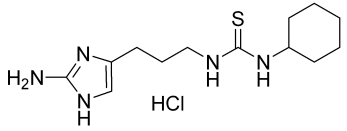
15a



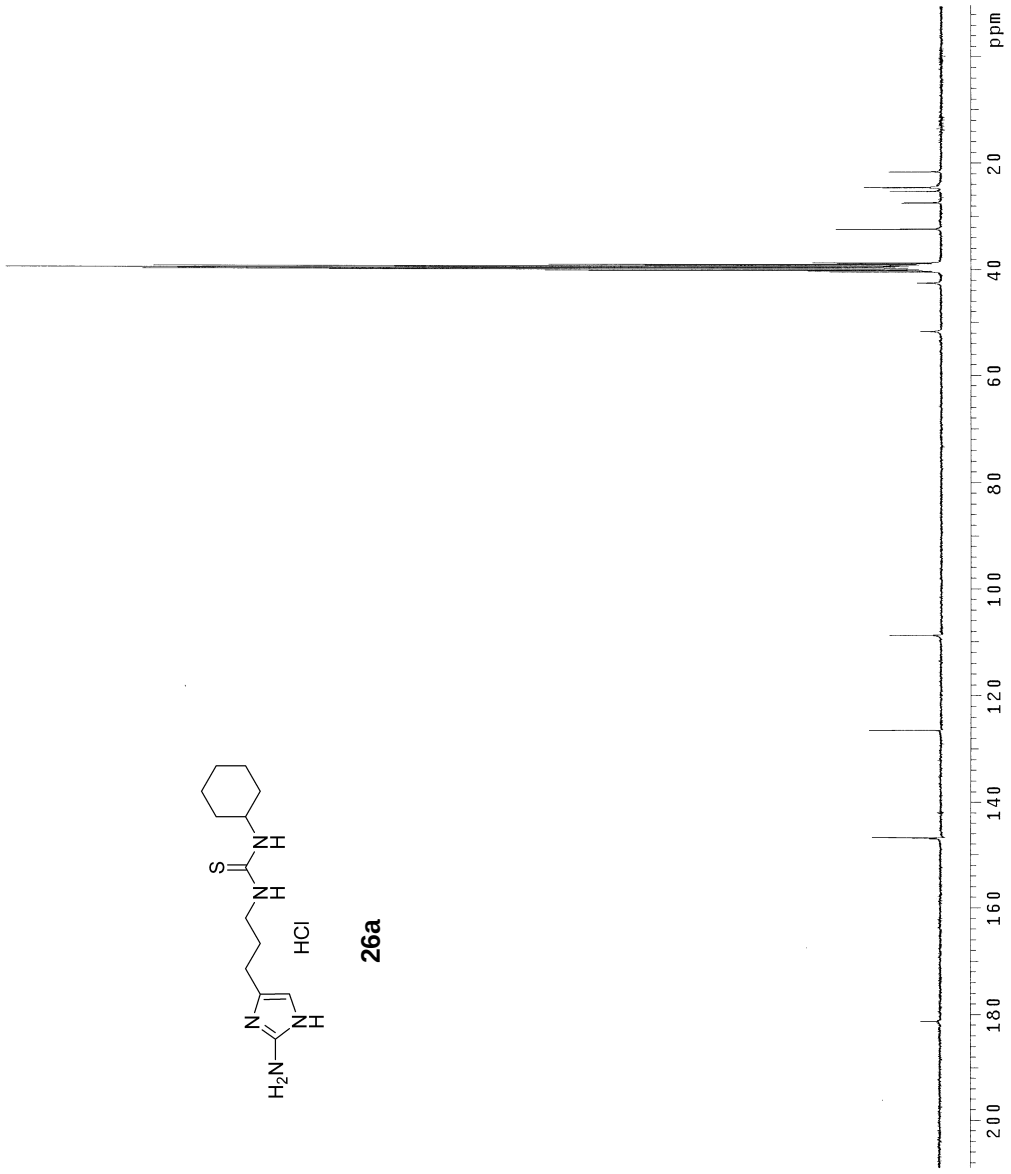
22a



22a



26a



HCl

26a