Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information For:

In vitro studies of deferasirox derivatives as potential organelle-targeting traceable anti-cancer therapeutics

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Common abbreviations used:

Ac = acetyl
A549 = human lung adenocarcinoma cell line
CV = crystal violet
DCM = dichloromethane
DMEM = Dulbecco's Modified Eagle Medium (cell growth medium)
DMF = dimethylformamide
EDC*HCl = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
Et = ethyl
FBS = fetal bovine serum
logD = distribution coefficient (with octanol and pH buffered aqueous phase)
Me = methyl
Ms = mesyl
MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMR = nuclear magnetic resonance
NHS = N-hydroxysuccinimide
ORP = oxidation-reduction potential
P/S = penicillin/streptomycin
RPMI 1640 = cell growth medium
r.t. = room temperature
TEA = triethylamine
TFA = trifluoroacetic acid

1. Materials, Methods and Instruments

Chemicals. All materials were obtained from commercial sources at the highest purity available and used without further purification. Reactions were carried out under air unless otherwise specified. Flash column chromatography was performed on Sorbent silica gel (40-63 µm). Analytical thin layer chromatography (TLC) analyses were carried on glass-backed silica gel plates (200 µm, Sorbent Technologies).

NMR. The reported ^{1}H NMR and ^{13}C NMR spectra were measured on Varian Inova spectrometers at The University of Texas at Austin using $CDCl_3$ and DMSO-d_6 as the deuterated solvents. Chemical shifts are reported relative to the residual solvent proton signals. For the spin multiplicities the following abbreviations were used: s (singlet), d (doublet), t (triplet) q (quartet), and m (multiplet), as well as appropriate combinations of these. Coupling constants for protons (*J*) are given in Hertz (Hz). The NMR spectra were analyzed using the software *MestReNova v.10.0.2-15465* (Mestrelab Research S.L.). All deuterated solvents were purchased from Cambridge Isotope Laboratories.

HR-MS. High-resolution electrospray ionization (ESI) mass spectra were recorded on a VG ZAB2E instrument or VG AutoSpec apparatus.

HPLC. Purity and stability verifications using HPLC were performed on an Agilent Technologies 1200 series liquid chromatography system equipped with a diode-array (UV-vis) detector and an Agilent ZORBAX Eclipse Plus C18 narrow bore column (2.1 mm internal diameter, 50 mm length, 5 micron particle size; P.N. 959746-902). The organic solvent was methanol, and the aqueous solvent was water containing 0.1% formic acid. Elution was conducted using a gradient ramp from 5% organic up to 100% organic solvent.

pH determinations. pH measurements were performed at 20°C on a calibrated Accumet® AE-150 pH meter with a glass combination pH ORP titration electrode (Ag/AgCl in sat. KCl as the internal reference) and a temperature sensor.

UV-vis spectroscopy. UV-vis spectra were taken on a Varian Cary 5000 UV-Vis-NIR Spectrophotometer. A baseline correction was performed with the corresponding blank before each spectrum was taken.

Fluorescent spectroscopy. Fluorescence spectroscopic measurements were made using an Agilent Cary Eclipse fluorescence spectrofluorometer. The emission and

excitation slit widths were fixed at 10 nm. Where applicable, respective blanks were subtracted from the spectra.

Cell culture. A549, HCT116 and L929 cells were obtained from the American Type Culture Collection (ATCC). A549 and HCT116 cells were maintained in a RPMI-1640 Medium (Sigma, St. Louis, MO, USA) and L929 cells were maintained in DMEM (Sigma, St. Louis, MO, USA). Each Medium was supplemented with 10% fetal bovine serum (FBS, Sigma, St. Louis, MO, USA) and 2% Penicillin/Streptomycin (P/S, Sigma, St. Louis, MO, USA) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. Cells were split when they reached 90% confluency.

HeLa cells were maintained in a Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10 % fetal bovine serum (Gibco, Gland Island, NY, USA) in a humidified atmosphere of 5 % CO_2 and 95 % air at 37 °C.

Cell Viability Studies. Cells were harvested and plated on a 96-well plate in 100 µL growth medium at a density of 1500 cells per well (5000 cells per well for 24 h exposure time). The cells were left to grow in an incubator at 37°C under 5% CO_2 atmosphere for 24 h. The next day, appropriate concentrations of the compound under investigation were prepared via serial dilution in growth medium and 100 µL was added per well such that each row of wells contained the same concentration of compound. Unless otherwise indicated, the highest concentration of compound was 250 µM and 3-fold serial dilution was employed. Two rows of wells were treated with only 100 µL medium as reference.

For MTT assay:

After incubation with compound for 72 h (or 24 h, respectively), 50 µL of MTT solution (3 mg/mL MTT dye in medium without FBS- and P/S) was added to each well. After an additional incubation for 3 h, the supernatant was aspired off and 50 µL of DMSO was added to each well to solubilize the precipitated formazan dye. The optical density was measured for each well at 570 nm with an M5 microplate reader from Molecular Device, Sunnyvale, USA. From the mean absorbance of each row of well, a dose-response curve was produced which was then normalized to the wells containing untreated cells to allow for plate-to-plate comparison. The dose-response curves were subjected to non-linear regression analysis performed in Origin 2019b from Origin Labs, Inc to determine IC_{50} and HS values. Data is shown as the mean of 3 replicate experiments and error bars represent the standard deviation.

For CV assay:

After incubation with compound for 72 h, the media was aspired off and cells were carefully washed twice by slowly submerging the 96-well plate into a large, tap water filled dish followed by inverting the plate onto paper towel and gently tapping to remove the water without lifting of adhered cells. To each well was added 50 µL of crystal violet solution (0.5w% in methanol/distilled water (20:80V%)) and the plate was placed into the incubator for 10 min at 37°C. The crystal violet solution was removed through inverting the plate onto paper towel and the stained cells were again carefully washed twice through slow submergence into a large, tap water filled dish followed by inverting the plate, onto paper towel and gently tapping to remove the water without lifting of stained cells. To each well was added 100 µL ethanol to solubilize the dye. The optical density was measured for each well at 570 nm with an M5 microplate reader from Molecular Device, Sunnyvale, USA. From the mean absorbance of each row of well, a dose-response curve was produced which was then normalized to the wells containing untreated cells to allow for plate-to-plate comparison. The dose-response curves were subjected to non-linear regression analysis performed in Origin 2019b from Origin Labs, Inc to determine IC_{50} and HS values. Data is shown as the mean of 3 replicate experiments and error bars represent the standard deviation.

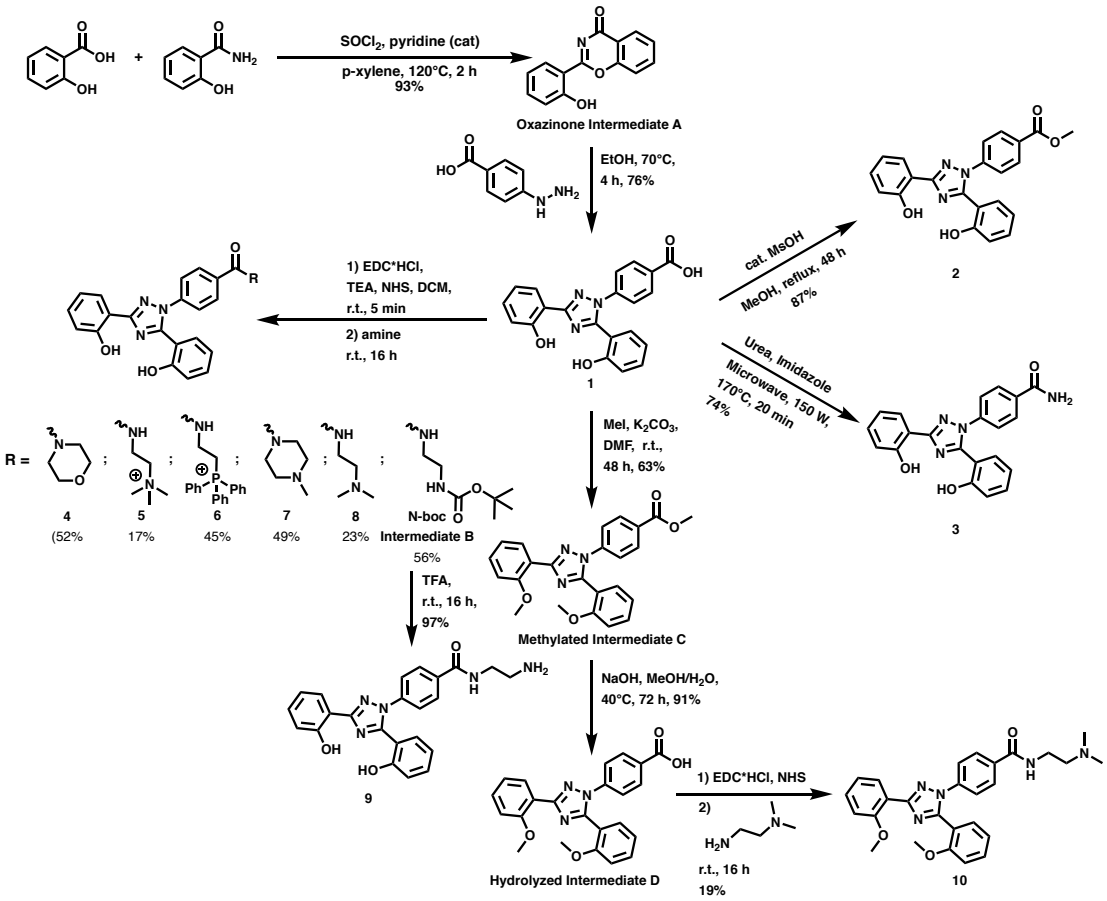
Co-localization Experiments with Lysotracker Red. Cells cultured in growth medium supplemented with 10 % FBS were added to a 24-well microplate. Cells were maintained in a humidified atmosphere of 5 % CO_2 and 95 % air at 37 °C overnight. Then, cells were incubated sequentially with **2** or **8** (20 µM), respectively, for 40 min and then Lysotracker® red (500 nM, Beyotime Biotechnology) for 30 min, and the cells on the microplate were rinsed with warm PBS and fixed with 4% paraformaldehyde for 20 min at room temperature. Immediately after sealing, the fluorescence was recorded and photographed with confocal laser scanning microscopy (Leica TCS SP8, Leica Microsystems, Wetzlar, Germany, red channel excitation at 405 nm, emission at 440−480 nm; Lyso tracker® channel excitation at 559 nm, emission at 580−620 nm).

Co-localization Experiments with Mitotracker Red. A549 cells were harvested and seeded at a density of 4 x 10^5 cells/dish in 35 mm dishes containing a poly-D lysine coated 10 mm glass diameter (Mat Tek P35GC-1.5- 10-C) overnight. Cells were then incubated with **5** (20 µM) for 40 min at 37°C. Post incubation, the media was removed, and cells were washed with PBS. To the cells was added a PBS solution containing 50 nM Mitotracker® Red FM (Invitrogen™) and cells were incubated for 30 min at 37°C. After incubation, the dye PBS solution was aspired off, the cells were washed with PBS. The cells were then imaged fluorescently on a Zeiss LSM 710 laser scanning confocal microscope using a Plan-Apo 63x/1.4 oil objective. The green channel was excited with a 405 nm laser, and the emission was detected spectrally from 482 - 555 nm. The red channel was excited with a 561 nm laser, and the emission was detected spectrally from 573 - 639 nm.

ROS visualization experiments. A549 cells were harvested and seeded at a density of 4 x 10^5 cells/dish in 35 mm dishes containing a poly-D lysine coated 10 mm glass diameter (Mat Tek P35GC-1.5- 10-C) overnight. Cells were then incubated at 37°C for 4 h with respective doses of compound **8** or the positive control ("Compound 1", a known redox active gold carbene complex from our lab[1]). Post incubation, the media was removed, and cells were washed with PBS. To the cells was added a PBS solution containing 100 nM Lysotracker® Red DND-99 (Invitrogen™) and cells were incubated for 60 min at 37°C. After incubation, the dye PBS solution was aspired off, the cells were washed with PBS and 2 µM H2DCFDA (Invitrogen™) ROS dye was added. Cells were incubated for 20 min, then the supernatant was aspired off and replaced with fresh PBS buffer. The cells were imaged fluorescently on a Zeiss LSM 710 laser scanning confocal microscope using a Plan-Apo 63x/1.4 oil objective. The green channel was excited with a 405 nm laser, and the emission was detected spectrally from 482 - 555 nm. The red channel was excited with a 561 nm laser, and the emission was detected spectrally from 573 - 639 nm.

2. Synthesis

2.1. Summary Scheme

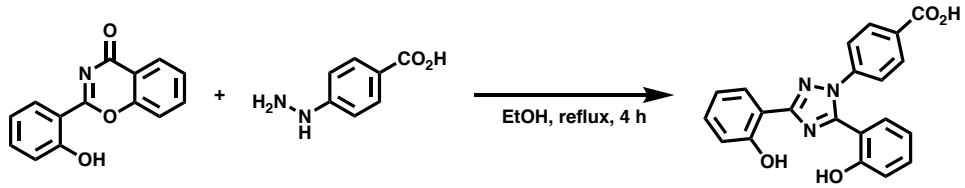


Scheme S1: Summary scheme for all syntheses relevant to the present study.

*2.2. Deferasirox **1***

4-(3,5-Bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid

This synthesis was adapted from the literature.[2]



In a 250 mL round bottom flask, 4-hydrazinobenzoic acid (10.5 g, 43.9 mmol) was heated at reflux in ethanol (100 mL) for 10 min. 2-(2-hydroxyphenyl)-4H-benzo[e][1,3]oxazin-4-one (7.40 g, 48.7 mmol, 1.1 equiv.) was added at once through a solid addition funnel and the reaction was stirred at reflux for 4 h. After cooling to room temperature, the reaction mixture was filtered off and the filter cake was washed once with cold ethanol (50 mL) and then twice with cold methanol (50 mL each). After drying under vacuum, the product was obtained as a pale brown powder (12.5 g. 33.5 mmol, 76%).

For biological studies, a portion of the product (5.0 g, 13 mmol) was recrystallized from ethanol (400 mL) to produce a white fluffy powder (2.9 g, 10 mmol, 58%).

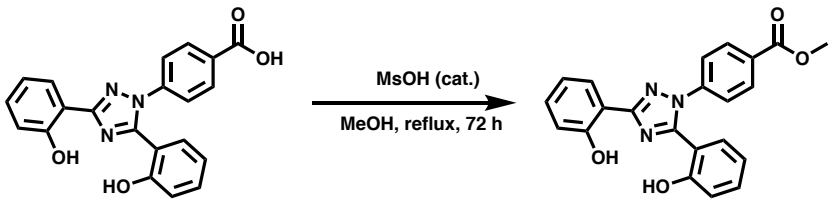
^{1}H-NMR (500 MHz, DMSO-d_6):
δ 13.09 (s, 1 H, COO-*H*), 10.81 (s, 1 H, O-*H*), 10.06 (s, 1 H, O-*H*), 8.06 (dd, *J* = 1.7, 7.8 Hz, 1 H, Ar*H*), 7.98-8.01 (m, 2 H, Ar*H*), 7.53 - 7.58 (m, 3 H, Ar*H*), 7.35 - 7.42 (m, 2 H, Ar*H*), 6.96 - 7.05 (m, 3 H, Ar*H*), 6.87 (dd, *J* = 1.0, 8.3 Hz, 1 H, Ar*H*)

^{13}C-NMR (125.75 MHz, DMSO-d_6):
δ 166.9, 160.4, 156.8, 155.6, 152.5, 141.7, 133.0, 131.9, 131.5, 131.0, 130.8, 127.2, 123.8, 120.2, 119.9, 117.5, 116.6, 114.9, 114.1

HR-MS (ESI): $C_{21}H_{15}N_3O_4$
Calculated ($[M+H]^+$): 374.1141
Found: 374.1143

2.3. Methyl Ester Derivative ***2***

Methyl 4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoate



To a 250 mL round bottom flask equipped with a reflux condenser was added 4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid (3.0 g, 8.04 mmol), methanol (200 mL) and methanesulfonic acid (1 mL). The reaction was stirred for 72 h under reflux while the reaction progress was monitored via TLC every 24 h (hexanes: ethyl acetate (75:25 V/V)). Once the reaction was deemed complete, the volatiles were removed under reduced pressure and the residue was taken up in DCM (100 mL). The organic layer was extracted twice with saturated aqueous sodium bicarbonate (100 mL per extraction), and once more with water (100 mL). The organic layer was concentrated and purified via column chromatography on silica gel (hexanes: ethyl acetate (75:25 V/V)). After drying under vacuum, the product was obtained as a white powder (2.7 g, 6.9 mmol, 87%).

^{1}H-NMR (500 MHz, DMSO-d_6):
δ 10.79 (s, 1 H, O-*H*), 10.04 (s, 1 H, O-*H*), 8.05 (d, *J* = 7.8 Hz, 1 H, Ar*H*), 8.01 (d, *J* = 8.5 Hz, 2 H, Ar*H*), 7.59 (d, *J* = 8.4 Hz, 2 H, Ar*H*), 7.56 (d, *J* = 8.1 Hz,1 H, Ar*H*), 7.35 - 7.43 (m, 2 H, Ar*H*), 6.96 - 7.05 (m, 3 H, Ar*H*), 6.86 (d, *J* = 8.3 Hz, 1 H, Ar*H*)

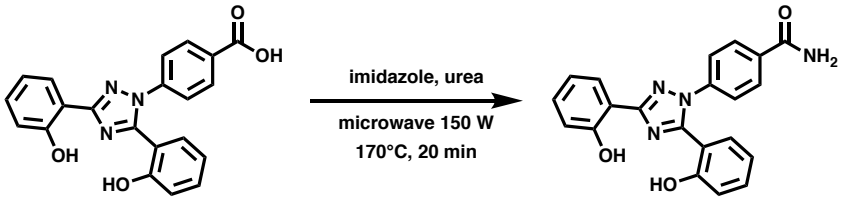
^{13}C-NMR (125.75 MHz, DMSO-d_6):
δ 165.4, 159.9, 156.3, 155.1, 152.1, 141.5, 132.6, 131.5, 131.1, 130.2, 129.3, 126.8, 123.4, 119.7, 119.5, 117.1, 116.1, 114.4, 113.6, 52.4

HR-MS (ESI): $C_{22}H_{17}N_3O_4$
Calculated ($[M+H]^+$): 388.1297
Found: 388.1299

*2.4. Primary Amide Derivative **3***

4-(3,5-Bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzamide

This synthesis was adapted from the literature.[3]



In a mortar, 4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid (300 mg, 0.8 mmol, 1.0 equiv .), imidazole (110 mg, 1.6 mmol, 2.0 equiv.) and urea (190 mg, 3.2 mmol, 4.0 equiv .) were ground to a fine powder. The mixture was transferred into a 10 mL microwave tube and allowed to react in a microwave reactor (CEM Discover S-Series, 150 W, 170°C, 20 min). The crude mixture obtained in this way was taken up in ethyl acetate (50 mL) and filtered. The filter cake was washed with ethyl acetate (3 times, 50 mL each). The combined filtrate was transferred into a separatory funnel, washed two times with saturated sodium bicarbonate (150 mL each) and once with brine (100 mL) and then dried over magnesium sulfate. After filtration and concentration under reduced pressure, the crude product was purified via flash column chromatography on silica gel (DCM: ethyl acetate (75:25 V/V)) to yield the product as a white powder (220 mg, 74%).

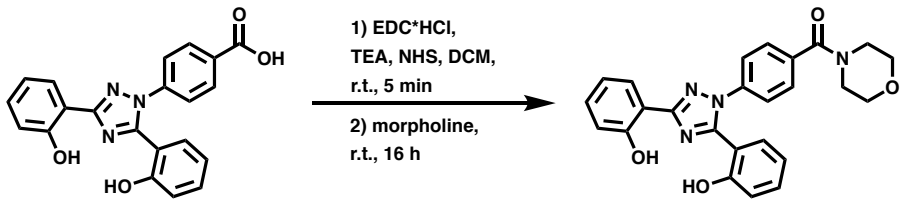
^{1}H-NMR (500 MHz, DMSO-d_6):
δ 10.81 (s, 1 H, O-*H*), 10.07 (s, 1 H, O-*H*), 8.01 – 8.09 (m, 2 H, 1 Ar*H*, 1 CON-*H*), 7.92 (d, *J* = 8.4 Hz, 2 H, Ar*H*), 7.45 - 7.55 (m, 4 H, 3 Ar*H*, 1 CON-*H*), 7.34 - 7.42 (m, 2 H, Ar*H*), 6.95 - 7.05 (m, 3 H, Ar*H*), 6.87 (d, *J* = 8.3 Hz, 1 H, Ar*H*)

^{13}C-NMR (125.75 MHz, DMSO-d_6):
δ 166.8, 159.8, 156.3, 155.3, 152.0, 139.9, 134.1, 132.5, 131.5, 131.1, 128.4, 126.8, 123.1, 119.7, 119.4, 117.1, 116.1, 114.5, 113.7

HR-MS (ESI): $C_{21}H_{16}N_4O_3$
Calculated ($[M+H]^+$): 373.1295
Found: 373.1306

2.5. Morpholine Derivative 4

(4-(3,5-Bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)phenyl)(morpholino)methanone



In a 100 mL round bottom flask, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDC•HCl, 770 mg, 4.0 mmol), 4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid (1.0 g, 2.7 mmol), triethylamine (0.6 mL, 4.0 mmol) and NHS (10 mg, catalyst) were dissolved in DCM (30 mL) and stirred at r.t. for 5 min. Morpholine (0.35 mL, 4.0 mmol) was added and the reaction was stirred at r.t. overnight for 16 h. The solvent was removed under reduced pressure, yielding a pale white-brown solid that was taken up in ethyl acetate (100 ml) and extracted three times with water (100 mL). The aqueous layers were combined and back-extracted once with ethyl acetate (100 mL). The combined organic layers were concentrated under reduced pressure and the crude product was purified via flash chromatography on silica gel (ethyl acetate: hexanes (50: 50 V/V)) to yield the product as a white powder (0.62 g, 1.4 mmol, 52%).

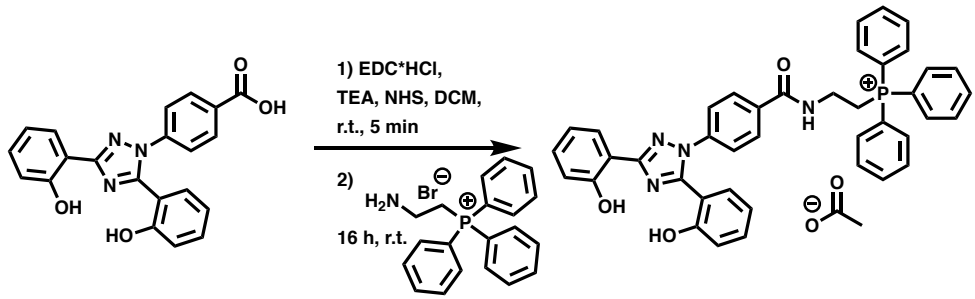
^{1}H-NMR (500 MHz, $CDCl_3$):
δ 11.39 (s, 1 H, O-*H*), 9.59 (s, 1 H, O-*H*), 8.14 (dd, *J* = 1.7, 7.8 Hz, 1 H, Ar*H*), 7.57 – 7.65 (m, 4 H, Ar*H*), 7.32 – 7.42 (m, 2 H, Ar*H*), 7.14 (dd, *J* = 1.2, 8.4 Hz, 1 H, Ar*H*), 7.02 – 7.10 (m, 2 H, Ar*H*), 6.99 (dd, *J* = 1.6, 8.0 Hz, 1 H, Ar*H*), 6.69 (ddd, *J* = 1.2, 7.3, 8.2 Hz, 1 H, Ar*H*), 3.36 – 4.00 (m, 8 H, -CH_2-)

^{13}C-NMR (125.75 MHz, $CDCl_3$, 65°C):
δ 169.0, 160.0, 158.4, 156.9, 152.6, 139.6, 137.5, 133.2, 132.0, 129.0, 127.9, 127.8, 126.6, 120.1, 119.3, 118.7, 117.4, 113.6, 110.4, 67.1, 45.5 (broad)*
*splits into a doublet at r.t. δ 45.5, *J* = 699 Hz, 2 C, -CH_2-

HR-MS (ESI): $C_{25}H_{22}N_4O_4$
Calculated ($[M+H]^+$): 443.1714
Found: 443.1714

2.6. Triphenylphosphonium Derivative ***5***

(2-(4-(3,5-Bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzamido)ethyl)triphenylphosphonium



In a 250 mL round bottom flask, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDC•HCl, 0.77 g, 4.0 mmol), 4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid (1.0 g, 2.7 mmol), triethylamine (TEA, 0.5 mL, 4.0 mmol) and N-hydroxysuccinimide (NHS, 10 mg, catalyst) were dissolved in DCM (20 mL) and stirred for 5 min at r.t. (2-Aminoethyl)triphenylphosphonium bromide (1.2 g, 3.2 mmol) was added and the reaction mixture was stirred for 16 h at r.t. Once the reaction was deemed complete, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The viscous residue was taken up in DCM (50 mL), loaded onto a silica gel column and purified via flash chromatography (gradient elution 95:05 DCM: methanol with 0.1% AcOH to 60:40 DCM: methanol with 1% AcOH) to yield the product as a brown powder (1.02 g, 45%).

^{1}H-NMR (500 MHz, $CDCl_3$):

δ 10.13 (s, 1 H, N-*H*), 8.12 (d, *J* = 8.1 Hz, 1 H, Ar*H*), 7.98 (d, *J* = 7.9 Hz, 2 H, Ar*H*), 7.68 - 7.78 (m, 9 H, Ar*H*), 7.57 - 7.63 (m, 6 H, Ar*H*), 7.43 (d, *J* = 8.0 Hz, 2 H, Ar*H*), 7.32 (t, *J* = 7.6 Hz, 1 H, Ar*H*), 7.26 (t, *J* = 7.6 Hz, 1 H, Ar*H*), 7.18 (d, *J* = 9.4 Hz, 1 H, Ar*H*), 7.04 (t, *J* = 8.2 Hz, 2 H, Ar*H*), 6.97 (t, *J* = 7.7 Hz, 1 H, Ar*H*), 6.76 (t, *J* = 7.7 Hz, 1 H, Ar*H*), 3.83 (s, 4 H, -CH_2-), 1.83 (s, 3 H, CH_3COO$^-$, counter anion)

^{13}C-NMR (125.75 MHz, $CDCl_3$):

δ 177.4, 167.1, 160,0, 157.4, 156.9, 152.5, 140.6, 135.2, 134.3, 133.7, 133.6, 132.7, 131.4, 130.6, 130.5, 129.3, 129.2, 127.4, 124.5, 119.7, 119.1, 118.4, 117.8, 117.7, 117.2, 113.9, 112.6, 34.11, 31.06, 23.74, 22.6, 22.2

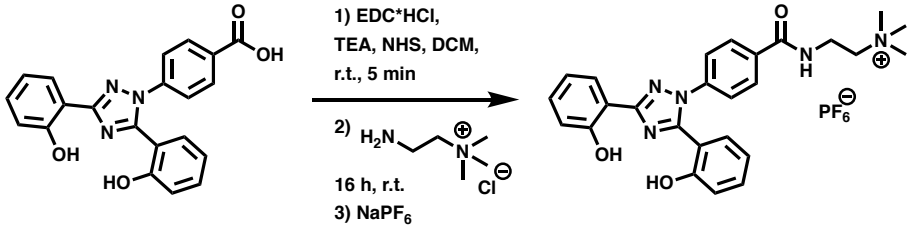
$C_{41}H_{34}N_4O_3P^+$

Calculated ([M]$^+$): 661.2363

Found: 661.2359

2.7. Quaternary Ammonium Derivative ***6***

2-(4-(3,5-Bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzamido)-*N*,*N*,*N*-trimethylethan-1-aminium hexafluorophosphate



In a 250 mL round bottom flask, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDC*HCl, 1.0 g, 5.4 mmol), 4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid (1.0 g, 2.7 mmol), triethylamine (TEA, 0.8 mL, 5.4 mmol) and N-hydroxysuccinimide (NHS, 10 mg, catalyst) were dissolved in DCM (40 mL) and stirred for 5 min at r.t. (2-Aminoethyl)trimethylammonium chloride hydrochloride (1.0 g, 5.7 mmol) was added and the reaction mixture was stirred for 16 h at r.t. Upon completion, the solvent was removed under reduced pressure and the residual pale white-brown solid was taken up in methanol (10 mL) due to limited solubility in other solvents. The crude product was purified via flash chromatography on silica gel (90:10 DCM: methanol to remove impurities, then 25:50:25 ethyl acetate: isopropyl alcohol: water to elute the product) to yield moderately pure product as an orange solid. This solid was taken up in the minimum amount of methanol needed to dissolve it fully (≈3 mL). Aqueous ammonium hexafluorophosphate (10 equiv. in 10 mL water) was added until the product precipitated as white powder (230 mg, 0.5 mmol 19%).

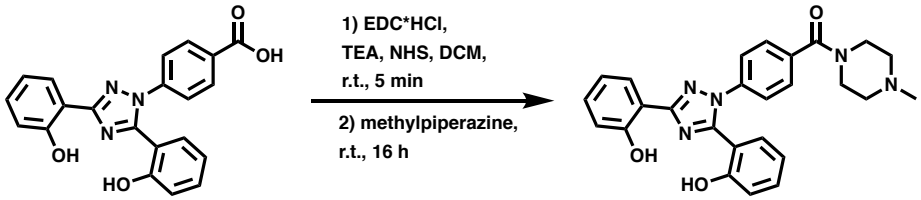
^{1}H-NMR (500 MHz, DMSO-d_6):
δ 10.81 (s, 1 H, O-*H*), 9.84 (s, 1 H, CON-*H*), 8.04 (dd, *J* = 1.5, 7.8 Hz, 1 H, Ar*H*), 7.90 (d, *J* = 8.3 Hz, 2 H, Ar*H*), 7.57 (d, *J* = 8.5 Hz, 2 H, Ar*H*), 7.51 (d, *J* = 7.8 Hz, 1 H, Ar*H*), 7.38 (td, *J* = 1.5, 7.0 Hz, 2 H, Ar*H*), 6.99 - 7.05 (m, 2 H, Ar*H*), 6.95 (d, *J* = 7.4 Hz, 1 H, Ar*H*), 6.86 (d, *J* = 7.6 Hz, 1 H, Ar*H*), 3.69 (d, *J* = 5.4 Hz, 2 H, CH_2), 3.69 (d, *J* = 6.5 Hz, 2 H, CH_2), 3.13 (s, 3 H, CH_3)

^{13}C-NMR (125.75 MHz, DMSO-d_6):
δ 165.7, 159.8, 156.3, 152.1, 140.1, 133.5, 132.4, 131.5, 131.0, 128.2, 126.7, 123.4, 119.7, 117.1, 116.2, 114.5, 113.7, 69.8, 63.8, 52.6, 33.8

HR-MS (ESI): $C_{26}H_{27}N_5O_3$
Calculated ([M+H]$^+$): 458.2187
Found: 458.2195

2.8. N-methylpiperazine Derivative ***7***

(4-(3,5-Bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)phenyl)(4-methylpiperazin-1-yl)methanone



In a 100 mL round bottom flask, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDC•HCl, 770 mg, 4.0 mmol), 4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid (1.0 g, 2.7 mmol), triethylamine (0.6 mL, 4.0 mmol) and NHS (10 mg, catalyst) were dissolved in DCM (30 mL) and stirred at r.t. for 5 min. N-methylpiperazine (0.45 mL, 4.0 mmol) was added and the reaction was stirred at r.t. overnight for 16 h. The volatiles were removed under reduced pressure, yielding a pale white-brown solid that was taken up in ethyl acetate (100 ml) and extracted three times with water (100 mL). The aqueous layers were combined and back-extracted once with ethyl acetate (100 mL). The combined organic layers were concentrated under reduced pressure and the crude product was purified via flash chromatography on silica gel (ethyl acetate: hexanes (50: 50 V/V)) to yield the product as a white powder (0.58 g, 1.3 mmol, 49%).

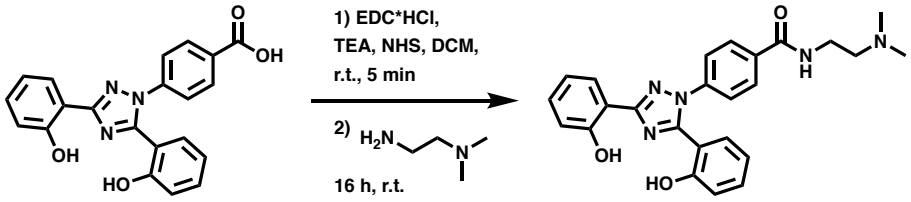
^{1}H-NMR (500 MHz, $CDCl_3$):
δ 11.30 (s, 1 H, O-*H*), 9.56 (s, 1 H, O-*H*), 8.06 (d, *J* = 10.4 Hz, 1 H, Ar*H*), 7.48 – 7.55 (m, 4 H, Ar*H*), 7.25 – 7.33 (m, 2 H, Ar*H*), 7.12 (d, *J* = 8.4 Hz, 1 H, Ar*H*), 6.98 – 7.10 (m, 3 H, Ar*H*), 6.67 – 6.71 (m, 1 H, Ar*H*), 3.85 (broad s, 2 H, -CH_2-), 3.50 (broad s, 2 H, -CH_2-), 2.53 (broad s, 2 H, -CH_2-), 2.41 (broad s, 2 H, -CH_2-), 2.35 (s, 3 H, -CH_3)

^{13}C-NMR (125.75 MHz, $CDCl_3$, r.t.):
δ 168.9, 159.6, 158.1, 156.6, 152.3, 139.2, 137.6, 133.2, 132.0, 128.9, 127.8, 126.4, 120.1, 119.3, 118.5, 117.3, 113.4, 110.1, 55.4*, 54.8*, 47.9*, 46.1, 42.4*
*the 4 -CH_2- protons from the piperazine ring produce 4 distinct peaks at r.t., similar to the -CH_2- protons in **4** (morpholine derivative) discussed above

HR-MS (ESI): $C_{26}H_{25}N_5O_3$
Calculated ($[M+H]^+$): 456.2030
Found: 456.2037

2.9. Dimethylethylenediamine Derivative ***8***

4-(3,5-Bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)-*N*-(2-(dimethylamino)ethyl)benzamide



In a 250 mL round bottom flask, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDC*HCl, 1.0 g, 5.4 mmol), 4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid (1.0 g, 2.7 mmol), triethylamine (TEA, 0.8 mL, 5.4 mmol) and N-hydroxysuccinimide (NHS, 10 mg, catalyst) were dissolved in DCM (40 mL) and stirred for 5 min at r.t. *N,N*-Dimethylethane-1,2-diamine (0.9 mL, 8.0 mmol) was added and the reaction mixture was stirred for 16 h at r.t. Once the reaction was deemed complete, the volatiles were removed under reduced pressure and the residual pale white-brown solid was taken up in ethyl acetate (100 ml) and extracted three times with water (100 mL each). The aqueous layers were combined and back-extracted once with ethyl acetate (100 mL). The combined organic layers were concentrated under reduced pressure and the crude product was purified via flash chromatography on silica gel (95:05 DCM: methanol) to yield the product as a white powder (0.24 g, 0.6 mmol, 23%).

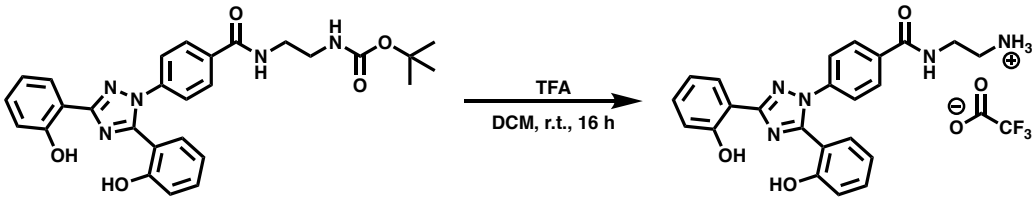
^{1}H-NMR (500 MHz, $CDCl_3$):
δ 8.12 (dd, *J* = 1.7, 7.8 Hz, 1 H, Ar*H*), 7.98 (d, *J* = 8.4 Hz, 2 H, Ar*H*), 7.57 (d, *J* = 8.5 Hz, 2 H, Ar*H*), 7.31 – 7.39 (m, 2 H, Ar*H*), 7.17 (broad s, 1 H, N-*H*), 7.10 (dd, *J* = 1.2, 8.4 Hz, 1 H, Ar*H*), 7.06 (dd, *J* = 1.1, 8.3 Hz, 1 H, Ar*H*), 7.02 (td, *J* = 1.1, 7.6 Hz, 1 H, Ar*H*), 6.97 (dd, *J* = 1.6, 8.0 Hz, 1 H, Ar*H*), 6.65 – 6.70 (m, 1 H, Ar*H*), 3.57 (q, *J* = 5.5 Hz, 2 H, -C*H*$_2$-), 2.60 (t, *J* = 5.9 Hz, 2 H, -C*H*$_2$-), 2.33 (s, 6 H, -C*H*$_3$)

^{13}C-NMR (125.75 MHz, $CDCl_3$):
δ 166.2, 159.7, 158.0, 156.7, 152.2, 140.5, 136.0, 133.1, 131.9, 128.9, 128.0, 127.7, 126.1, 120.0, 119.3, 118.4, 117.3, 113.4, 110.5, 57.8, 45.2, 37.3

HR-MS (ESI+): $C_{25}H_{25}N_5O_3$
Calculated ($[M+H]^+$): 444.2030
Found: 444.2035

2.10. Ethylenediamine Derivative ***9***

N-(2-Aminoethyl)-4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzamide



In a 250 mL round bottom flask, *tert*-butyl (2-(4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzamido)ethyl)carbamate (250 mg, 0.5 mmol), trifluoroacetic acid (TFA, 15 mL) and dichloromethane (20 mL) were stirred at r.t. overnight for 16 h. The volatiles were removed under reduced pressure to give a viscous, brown liquid that slowly solidified under vacuum to yield the TFA salt of the product as a crystalline pale-brown solid (250 mg, 97%).

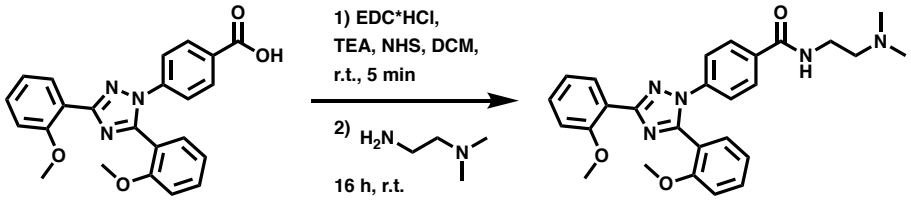
^{1}H-NMR (500 MHz, DMSO-d_6):
δ 8.70 (t, *J* = 5.3 Hz, CON-*H*) 8.05 (d, *J* = 7.8 Hz, 1 H, Ar*H*), 7.92 (d, *J* = 8.4 Hz, 2 H, Ar*H*), 7.84 (broad s, 3 H, -NH_3^+), 7.55 (d, *J* = 8.4 Hz, 2 H, Ar*H*), 7.51 (d, *J* = 7.4 Hz, 1 H, Ar*H*), 7.38 (q, *J* = 7.4 Hz, 2 H, ArH), 6.94 – 7.05 (m, 3 H, Ar*H*), 3.50 (q, *J* = 5.7 Hz, 2 H, -CH_2-), 2.99 (q, *J* = 5.7 Hz, 2 H, -CH_2-)

^{13}C-NMR (125.75 MHz, DMSO-d_6):
δ 165.9, 159.8, 158.5 (q, CF_3), 156.4, 155.4, 152.0, 140.0, 133.9, 132.5, 131.5, 131.1, 128.4, 126.8, 123.3, 119.7, 119.4, 117.1, 116.2, 114.5, 113.7, 38.7, 37.2

HR-MS (ESI): $C_{21}H_{15}N_3O_4$
Calculated ([M+H]$^+$): 416.1717
Found: 416.1728

*2.11. O-Methylated Dimethylethylenediamine Derivative **10***

4-(3,5-Bis(2-methoxyphenyl)-1*H*-1,2,4-triazol-1-yl)-*N*-(2-(dimethylamino)ethyl)benzamide



In a 250 mL round bottom flask, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDC*HCl, 400 mg, 2.1 mmol), 4-(3,5-bis(2-methoxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid (500 mg, 1.2 mmol), triethylamine (0.3 mL, 2.1 mmol) and NHS (10 mg, catalyst) were dissolved in DCM (20 mL) and stirred at r.t. for 5 min. *N,N*-Dimethylethyane-1,2-diamine (0.5 mL, 4.5 mmol) was added and the reaction was stirred at r.t. for 16 h. The volatiles were removed under reduced pressure, yielding a pale white-brown solid that was taken up in ethyl acetate (100 ml) and extracted three times with water (100 mL per extraction). The aqueous layers were combined and back-extracted once with ethyl acetate (100 mL). The combined organic layers were concentrated under reduced pressure and the crude product was purified via flash chromatography on silica gel (95:05 dichloromethane: methanol) to yield the product as a white powder (110 mg, 0.2 mmol, 19%).

^{1}H-NMR (500 MHz, $CDCl_3$):
δ 8.04 (d, *J* = 7.5, 1 H, Ar*H*), 7.92 (d, *J* = 8.4, 2 H, Ar*H*), 7.79 (s, 1 H, Ar*H*), 7.66 (d, *J* = 7.5, 1 H, Ar*H*), 7.38 - 7.48 (m, 4 H, Ar*H*), 7.02 - 7.10 (m, 3 H, Ar*H*), 6.81 (d, *J* = 8.4, 1 H, Ar*H*), 3.95 (s, 3 H, -CH_3), 3.69 (q, *J* = 5.4, 2 H, -CH_2-), 3.33 (s, 3 H, -CH_3), 2.88 (t, *J* = 5.6, 2 H, -CH_2-), 2.56 (s, 6 H, -CH_3)

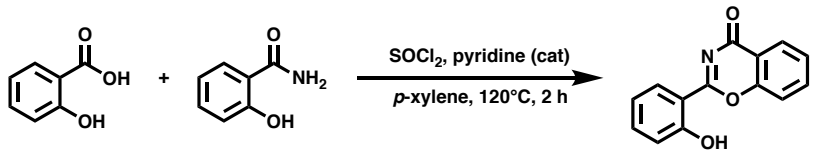
^{13}C-NMR (125.75 MHz, $CDCl_3$):
δ 166.7, 160.8, 157.8, 156.8, 152.2, 141.8, 132.8, 132.2, 131.7, 131.4, 130.7, 128.2, 123.1, 121.2, 120.7, 120.1, 118.0, 111.9, 111.5, 58.5, 56.2, 55.0, 44.7, 36.3, 29.4

HR-MS (ESI): $C_{27}H_{29}N_5O_3$
Calculated ($[M+H]^+$): 472.2343
Found: 472.2348

*2.12. Oxazinone Intermediate **A***

2-(2-Hydroxyphenyl)-4H-benzo[e][1,3]oxazin-4-one

This synthesis was adapted from the literature.[2]



In a 250 ml round bottom flask equipped with a reflux condenser, salicylic acid (11.1 g, 80.2 mmol, 1.1 eq), salicylamide (10.0 g, 72.9 mmol, 1.0 eq) and pyridine (0.5 ml, catalyst) were dissolved in *o*-xylene (50 ml) and the solution was heated to 80°C. $SOCl_2$ (5.0 ml, 69.3 mmol 0.9 eq) was added dropwise over the course of 5 min. The reaction mixture was heated further to 120°C, stirred for 1 h, and then another aliquot of $SOCl_2$ (5.0 ml, 69.3 mmol 0.9 eq) was added dropwise over the course of 5 min. The reaction was stirred for another 1 h at 120°C. After cooling, the volatiles were removed from the reaction mixture under reduced pressure and to the residue was added ethanol (50 mL) and acetic acid (1 mL). The resulting suspension was cooled to 4°C in a refrigerator for 10 min and the precipitate was filtered off, washed with cold ethanol (three times, 50 mL each) and dried under vacuum to yield the product as a yellow-green powder (17.9 g, 93%).

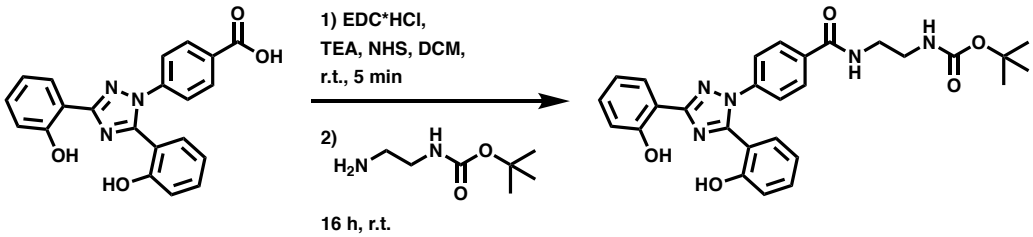
^{1}H-NMR (400 MHz, $CDCl_3$):
δ 12.73 (s., 1 H, O-*H*), 8.22 (dd, *J* = 1.7, 8.2 Hz, 1 H, Ar*H*), 8.12 (ddd , *J* = 0.4, 1.7, 8.1 Hz, 1 H, Ar*H*), 7.81 (ddd, *J* = 1.7, 7.4, 8.4 Hz, 1 H, Ar*H*), 7.51-7.57 (m, 3 H, Ar*H*), 7.10 (ddd, *J* = 0.4, 1.2, 8.5 Hz, 1 H, Ar*H*), 7.01 (ddd, *J* = 1.2, 7.1, 8.2 Hz, 1 H, Ar*H*)

^{13}C-NMR (100.6 MHz, $CDCl_3$):
δ 165.3, 164.0, 163.2, 154.2, 136.9, 135.8, 128.7, 128.1, 127.3, 119.5, 118.9, 118.4, 117.0, 111.3

HR-MS (ESI): $C_{14}H_9NO_3$
Calculated ($[M+H]^+$): 240.0661
Found: 240.0658

*2.13. N-boc-ethylenediamine Intermediate **B***

tert-Butyl (2-(4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzamido)ethyl)carbamate



In a 250 mL round bottom flask, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt (EDC•HCl, 2.03 g, 10.6 mmol, 1.1 equiv.) and 4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid (3.60 g, 9.6 mmol, 1.0 equiv.) were dissolved in DCM (50 mL) and stirred at r.t. for 5 min. *N*-Boc-ethylenediamine (1.5 g, 1.5 mL, 9.6 mmol, 1.0 equiv.) was added and the reaction was stirred at r.t. overnight for 16 h. The solvent was removed under reduced pressure, yielding a pale white-brown solid that was taken up in ethyl acetate (100 ml) and extracted three times with water (100 mL each). The aqueous layers were combined and back-extracted once with ethyl acetate (100 mL). The combined organic layers were concentrated under reduced pressure and the crude product was purified via flash chromatography on silica gel (hexanes: ethyl acetate (40:60 V/V to 80:20 V/V)) to yield the product as a white powder (2.80 g, 5.4 mmol, 56%).

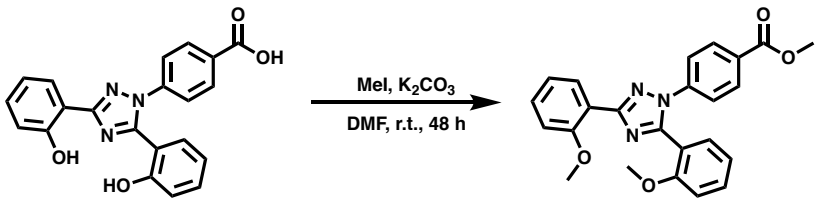
^{1}H-NMR (400 MHz, $CDCl_3$):
δ 8.12 (dd, *J* = 1.7, 7.9 Hz, 1 H, Ar*H*), 8.03 (d, *J* = 8.5 Hz, 2 H, Ar*H*), 7.63 (broad s, 1 H, CON-*H*), 7.57 (d, *J* = 8.5 Hz, 2 H, Ar*H*), 7.30 – 7.40 (m, 2 H, Ar*H*), 7.14 (d, *J* = 8.3 Hz, 1 H, Ar*H*), 7.07 (d, *J* = 8.4 Hz, 1 H, Ar*H*), 7.03 (t, *J* = 7.5 Hz, 1 H, Ar*H*), 6.90 (dd, *J* = 1.5, 8.0 Hz, 1 H, Ar*H*), 6.64 (dd, *J* = 7.0, 8.4 Hz, 1 H, Ar*H*), 5.02 (broad s, 1 H, CON-*H*), 3.56 – 3.62 (m, 2 H, -CH_2-), 3.41 – 3.47 (m, 2 H, -CH_2-), 1.42 (s, 1 H, -CH_3)

^{13}C-NMR (100.6 MHz, $CDCl_3$):
δ 161.2, 159.5, 158.2, 156.7, 152.2, 140.5, 135.9, 133.2, 132.0, 129.0, 127.8, 127.7, 126.4, 120.1, 119.2, 118.6, 117.3, 113.3, 110.0, 80.6, 43.2, 39.9, 28.5

HR-MS (ESI): $C_{21}H_{15}N_3O_4$
Calculated ($[M–H]^-$): 514.2096
Found: 514.2095

*2.14. Trimethylated Intermediate **C***

Methyl 4-(3,5-bis(2-methoxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoate



In a 100 mL round bottom flask, 4-(3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid (2.0 g, 5.4 mmol) and potassium carbonate (4.4 g, 32 mmol) were dissolved in DMF (20 mL) at r.t. Via a syringe, methyl iodide (4.0 mL, 64 mmol) was added and the reaction mixture was stirred for 48 h. Then, the solvent was removed under reduced pressure and the residue taken up in DCM (50 mL) and filtered. The filter cake was washed three times with DCM (50 mL per washing) and the filtrate concentrated under reduced pressure. The crude product was purified via column chromatography on silica gel (increasing gradient: hexanes: ethyl acetate (80:20 V/V to 50:50 V/V)). After drying under vacuum, the product was obtained as a pale-yellow powder (1.4 g, 63%).

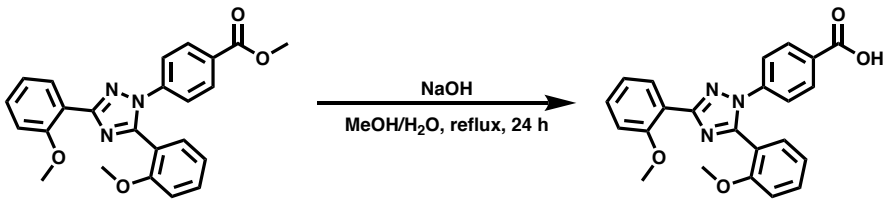
^{1}H-NMR (500 MHz, $CDCl_3$):
δ 8.03-8.07 (m, 1 H, Ar*H*), 7.98-8.02 (m, 2 H, Ar*H*),), 7.68-7.71 (m, 1 H, Ar*H*), 7.37 - 7.48 (m, 4 H, Ar*H*), 7.02-7.12 (m, 3 H, Ar*H*), 7.16 (dd, 1 H, *J* = 1.2, 8.4 Hz, Ar*H*), 6.80 (d, *J* = 8.3 Hz, 1 H, Ar*H*), 3.96 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.30 (s, 3 H, OCH_3)

^{13}C-NMR (125.75 MHz, $CDCl_3$):
δ 166.4, 160.9, 157.7, 156.6, 152.3, 142.8, 132.3, 131.7, 131.3, 130.8, 130.4, 129.1, 122.8, 121.2, 120.7, 119.9, 118.0, 111.8, 111.4, 56.2, 54.9, 52.4

HR-MS (ESI): $C_{24}H_{21}N_3O_4$
Calculated ($[M+H]^+$): 416.1605
Found: 416.1606

*2.15. Hydrolyzed Intermediate **D***

4-(3,5-Bis(2-methoxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoic acid



In a 250 mL round bottom flask, methyl 4-(3,5-bis(2-methoxyphenyl)-1*H*-1,2,4-triazol-1-yl)benzoate (2.0 g, 4.8 mmol), aqueous sodium hydroxide (1 N, 20 mL) and methanol (20 mL) were stirred for 72 h at 40°C. After the reaction was cooled to r.t., the volatiles were removed under reduced pressure and the aqueous liquor was acidified with aqueous hydrochloric acid (1 N) until a bulky white precipitate had formed. The suspension was filtered, and the filter cake washed three times with water (50 mL each washing). The filter cake was dried under vacuum to yield the product as a white powder (1.76 g, 91%).

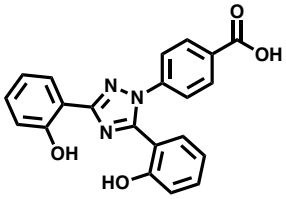
^{1}H-NMR (500 MHz, DMSO-d_6):
δ 7.98 (d, *J* = 8.3 Hz, 2 H, Ar*H*), 7.86 (d, *J* = 8.2 Hz, 1 H, Ar*H*), 7.64 (d, *J* = 8.2 Hz, 1 H, Ar*H*), 7.55 (t, *J* = 8.4 Hz,1 H, Ar*H*), 7.47 (t, *J* = 8.0 Hz,1 H, Ar*H*), 7.43 (d, *J* = 8.3 Hz, 2 H, Ar*H*), 7.19 (d, *J* = 8.4 Hz, 1 H, Ar*H*), 7.15 (t, *J* = 7.5 Hz,1 H, Ar*H*), 7.09 (t, *J* = 8.0 Hz,1 H, Ar*H*), 7.05 (d, *J* = 8.4 Hz, 1 H, Ar*H*), 3.86 (s, 3 H, OCH_3), 3.30 (s, 3 H, OCH_3)

^{13}C-NMR (125.75 MHz, $CDCl_3$):
δ 166.5, 159.9, 157.3, 156.1, 151.7, 141.7, 132.4, 131.2, 130.9, 130.6, 130.2, 130.1, 122.8, 121.0, 120.3, 119.6, 117.3, 112.3, 111.9, 55.7, 54.9

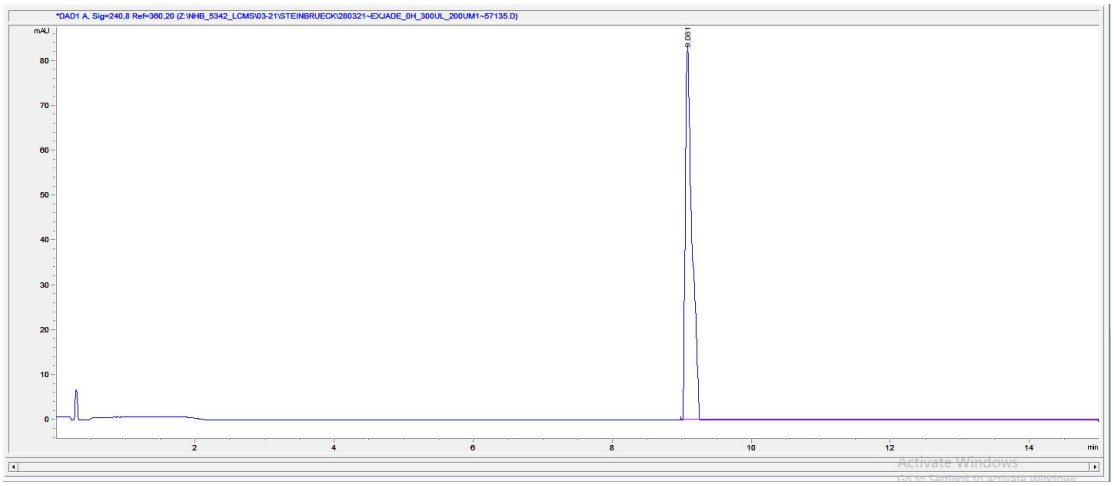
HR-MS (ESI): $C_{23}H_{19}N_3O_4$
Calculated ($[M+H]^+$): 402.1448
Found: 402.1450

3. ^{1}H-NMR, ^{13}C-NMR and HRMS Spectra; Liquid Chromatograms

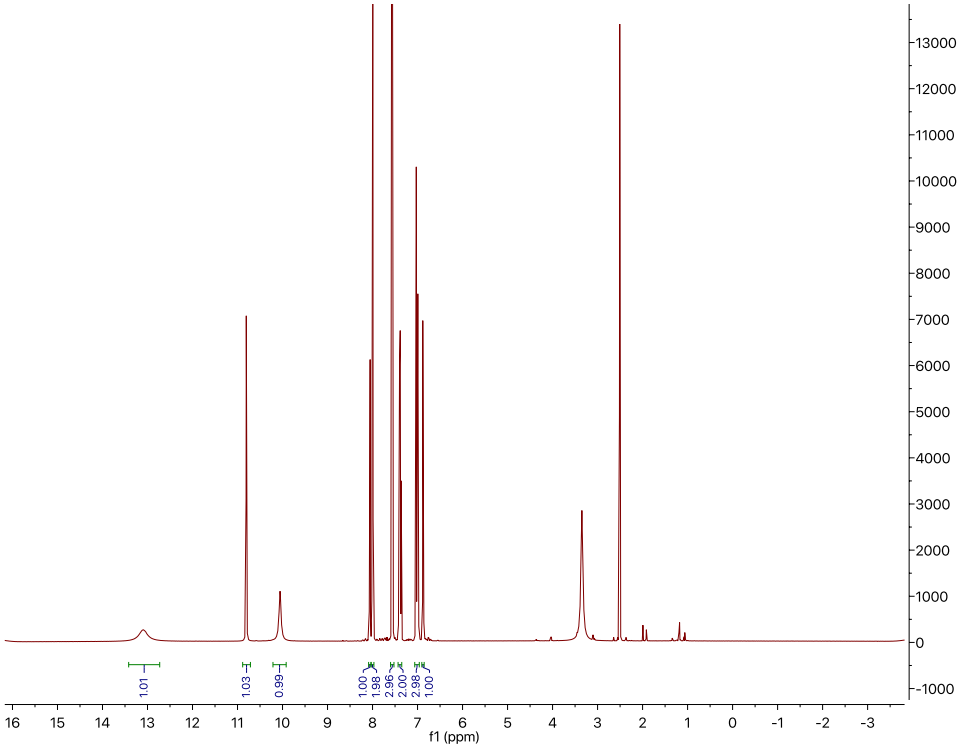
*3.1. Deferasirox **1***



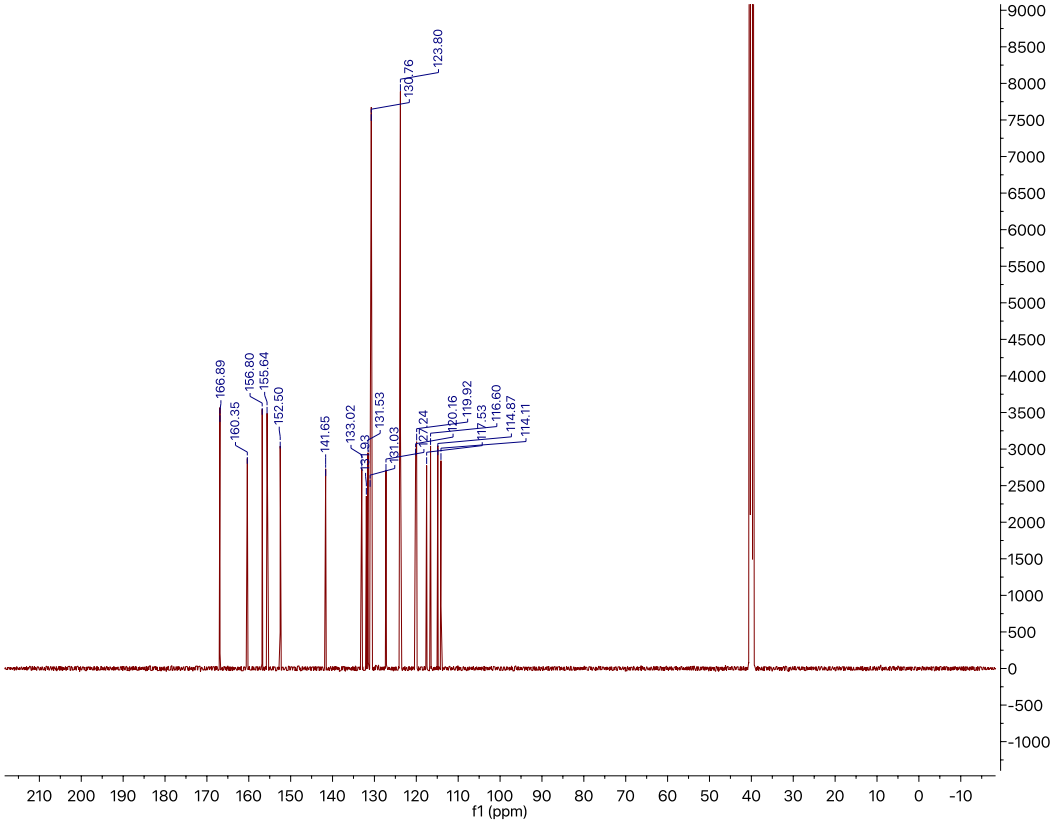
1



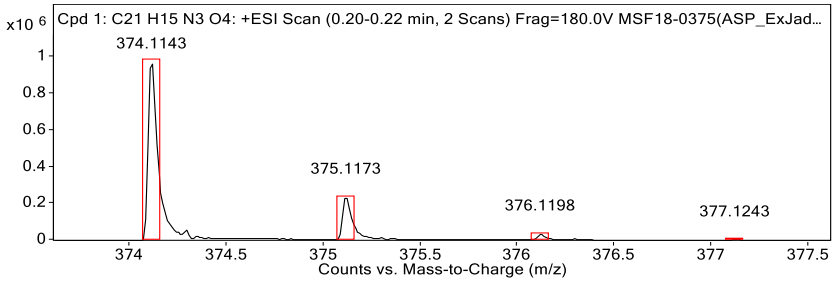
HPLC trace of **1** monitored at 240 nm



^{1}H-NMR spectrum of **1** (400 MHz, DMSO-d_6)



^{13}C-NMR spectrum of **1** (100.6 MHz, DMSO-d_6)



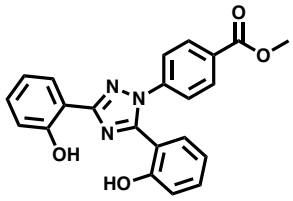
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
374.1143	374.1135	1	983483	C21H15N3O4	(M+H)+	-2.06
375.1173	375.1166	1	236734	C21H15N3O4	(M+H)+	-1.68
376.1198	376.1193	1	36037	C21H15N3O4	(M+H)+	-1.28
377.1243	377.1218	1	3565	C21H15N3O4	(M+H)+	-6.62
378.0973	378.1244	1	720	C21H15N3O4	(M+H)+	71.53

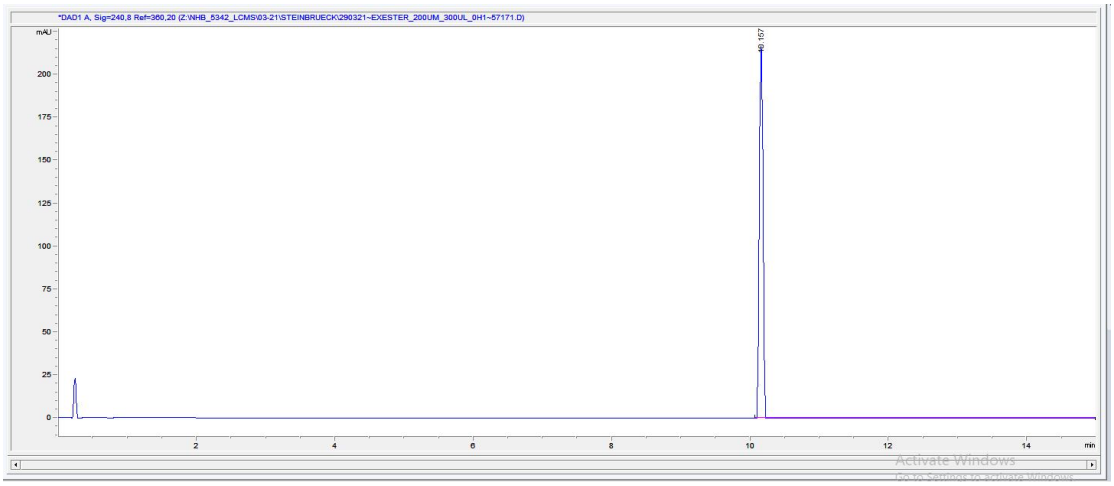
--- End Of Report ---

HRMS (ESI) analysis of **1**

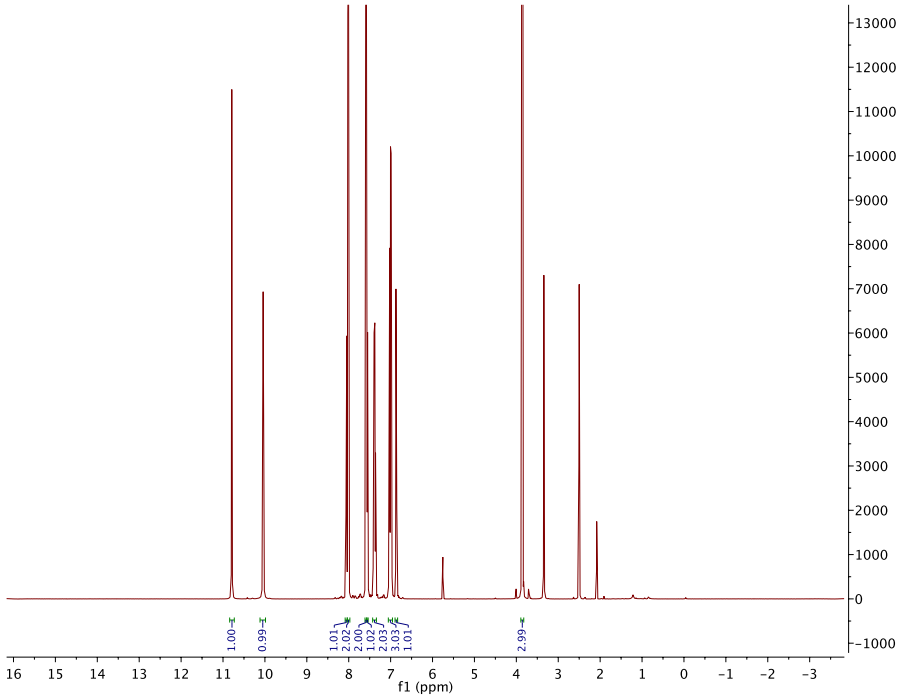
3.2. Methyl Ester Derivative ***2***



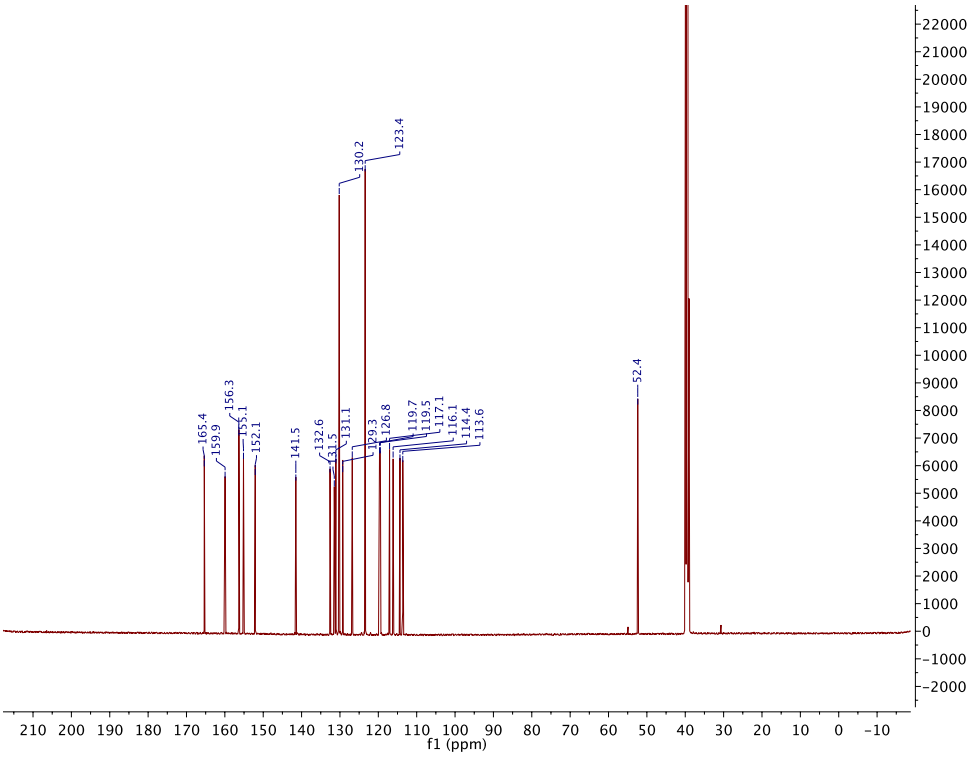
2



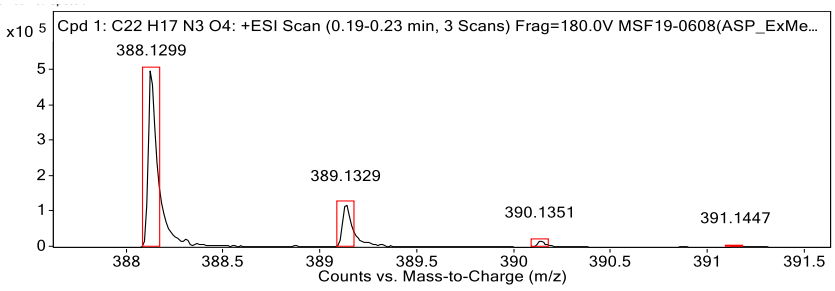
HPLC trace of **2** monitored at 240 nm



^{1}H-NMR spectrum of **2** (500 MHz, DMSO-d_6)



^{13}C-NMR spectrum of **2** (125.75 MHz, DMSO-d_6)



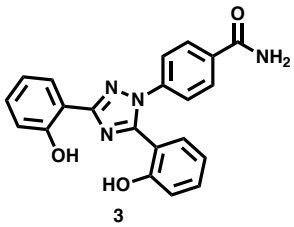
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
388.1299	388.1292	1	506091	C22H17N3O4	(M+H)+	-1.82
389.1329	389.1323	1	121523	C22H17N3O4	(M+H)+	-1.52
390.1351	390.1350	1	18362	C22H17N3O4	(M+H)+	-0.34
391.1447	391.1376	1	2392	C22H17N3O4	(M+H)+	-18.26

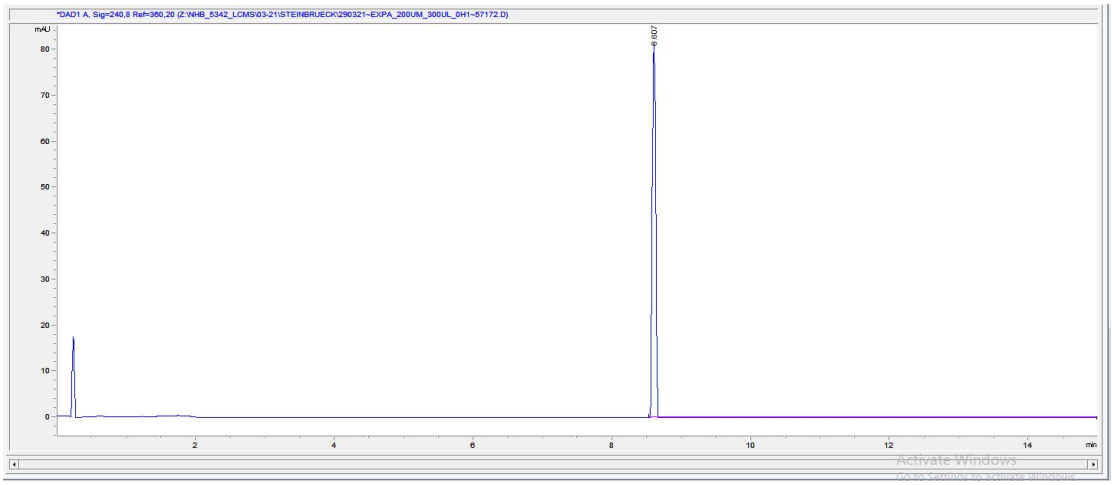
--- End Of Report ---

HRMS (ESI) analysis of **2**

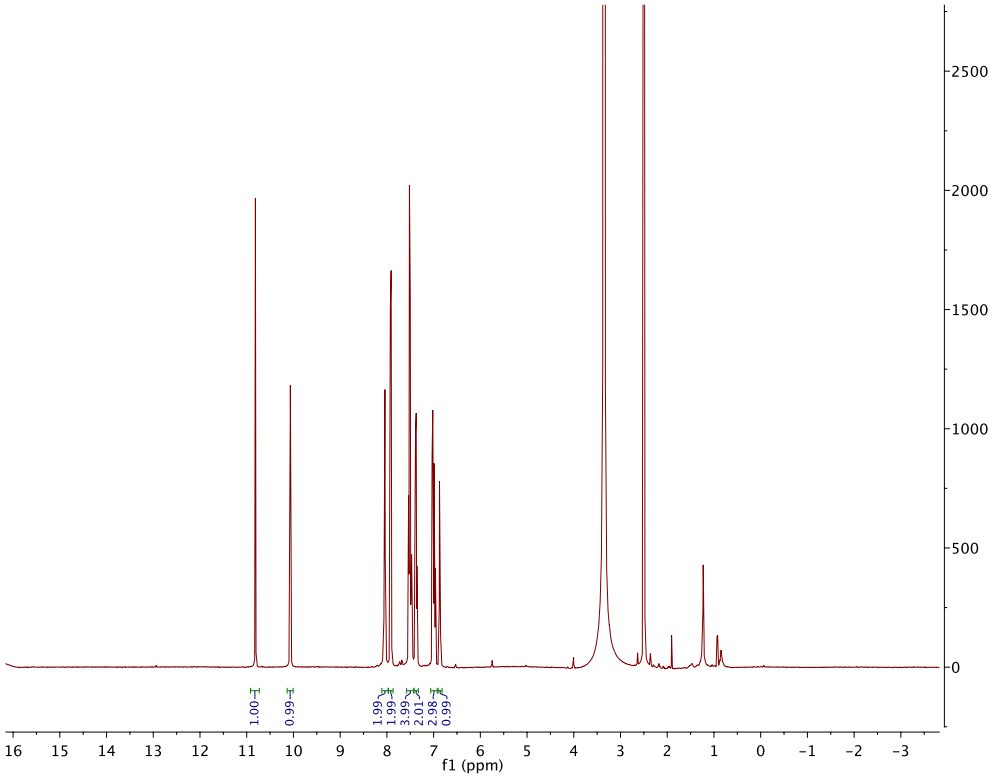
*3.3. Primary Amide Derivative **3***



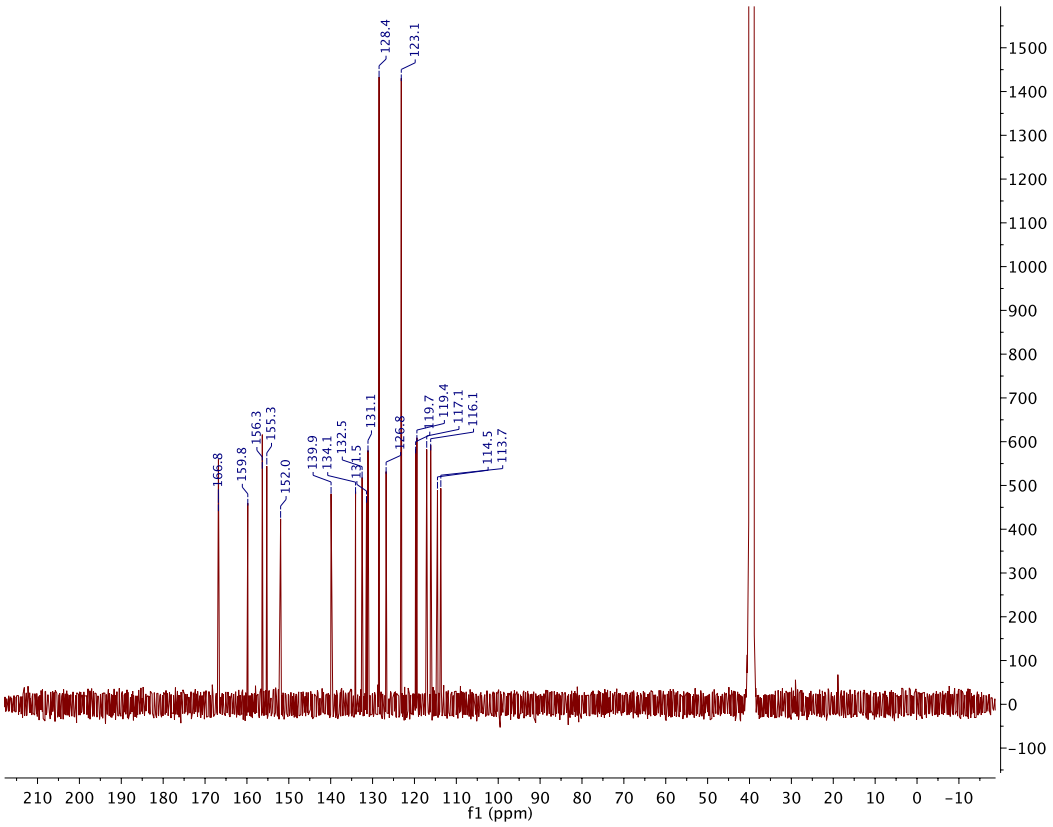
3



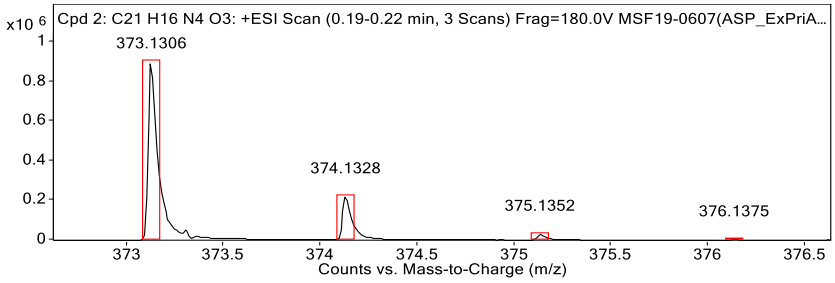
HPLC trace of **3** monitored at 240 nm



^{1}H-NMR spectrum of **3** (500 MHz, DMSO-d_6)



^{13}C-NMR spectrum of **3** (125.75 MHz, DMSO-d_6)



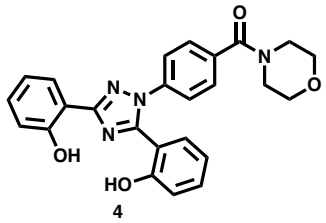
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
373.1306	373.1295	1	905134	C21H16N4O3	(M+H)+	-2.83
374.1328	374.1325	1	220676	C21H16N4O3	(M+H)+	-0.62
375.1352	375.1352	1	31259	C21H16N4O3	(M+H)+	-0.08
376.1375	376.1378	1	3194	C21H16N4O3	(M+H)+	0.79

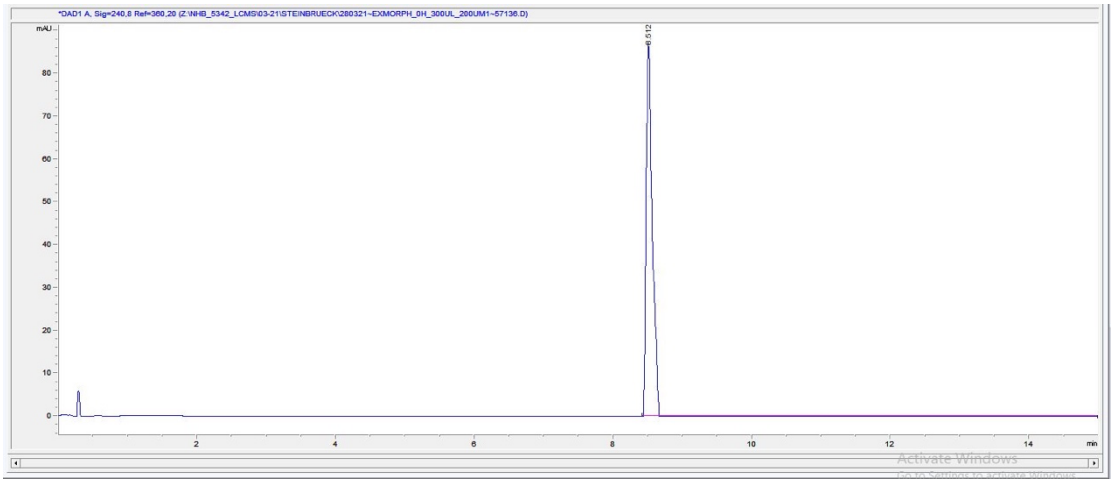
--- End Of Report ---

HRMS (ESI) analysis of **3**

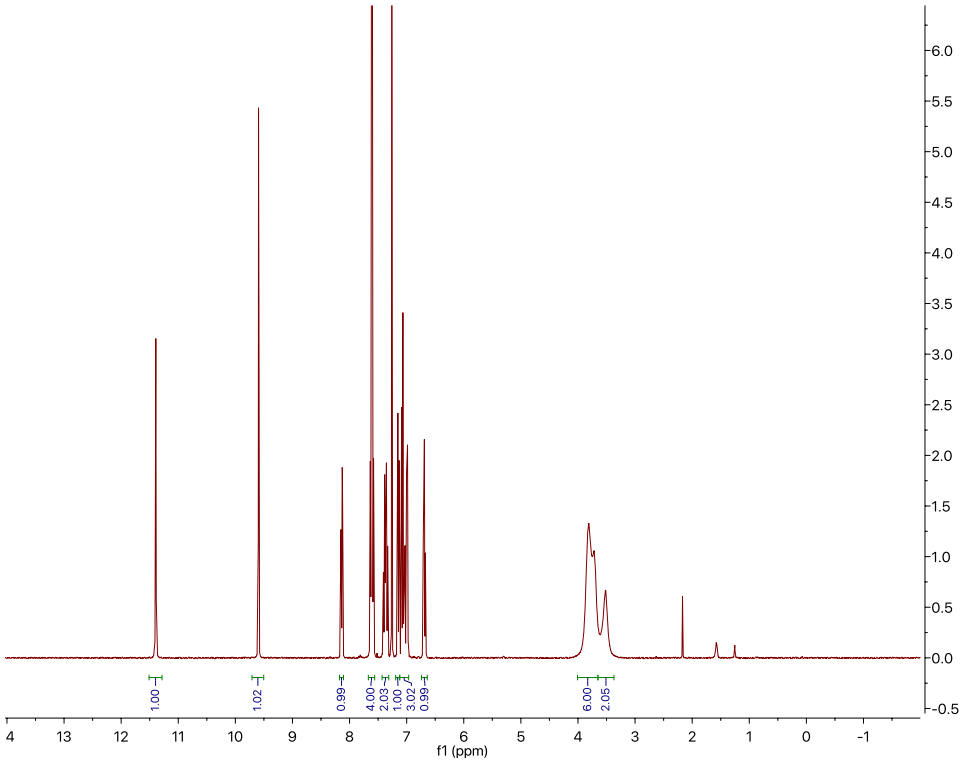
3.4. Morpholine Derivative 4



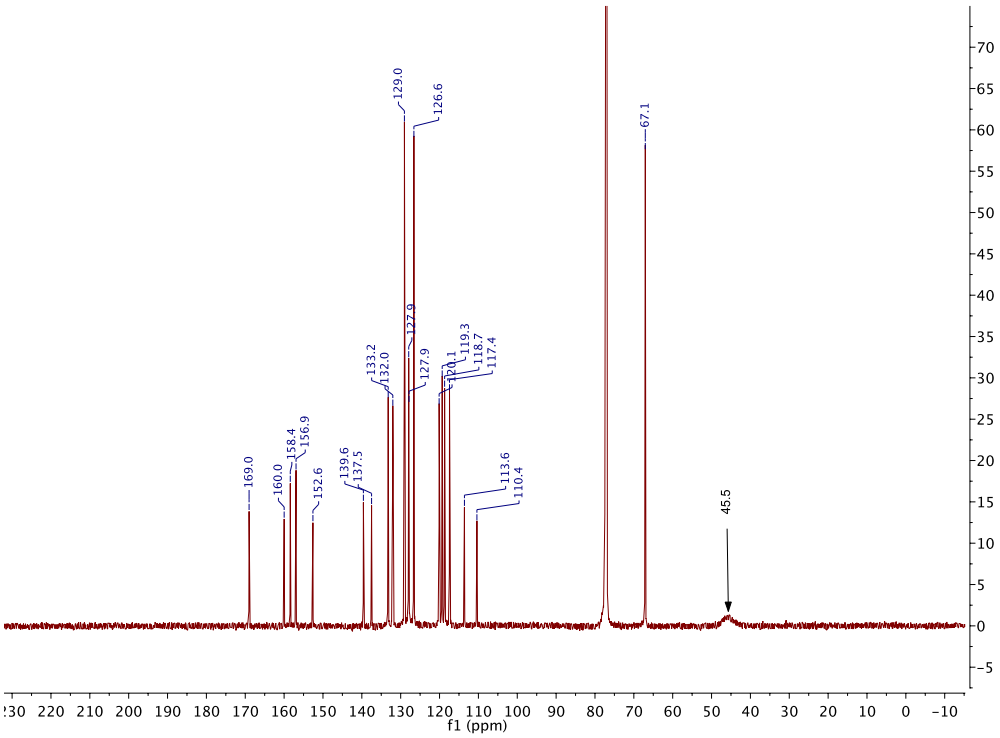
4



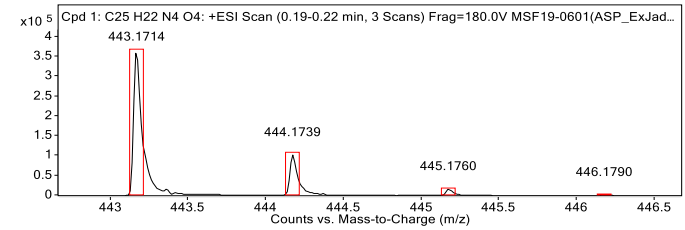
HPLC trace of **4** monitored at 240 nm



^{1}H-NMR spectrum of **4** (500 MHz, $CDCl_3$)



^{13}C-NMR spectrum of **4** (125.75 MHz, $CDCl_3$)



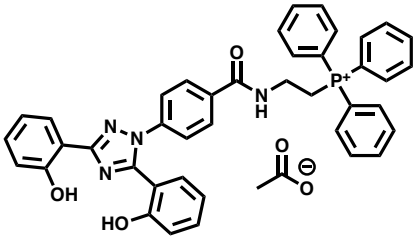
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
443.1714	443.1714	1	368624	C25H23N4O4	(M+H)+	-0.01
444.1739	444.1744	1	102880	C25H23N4O4	(M+H)+	1.16
445.1760	445.1772	1	17206	C25H23N4O4	(M+H)+	2.75
446.1790	446.1798	1	2307	C25H23N4O4	(M+H)+	1.89
447.1652	447.1824	1	475	C25H23N4O4	(M+H)+	38.44

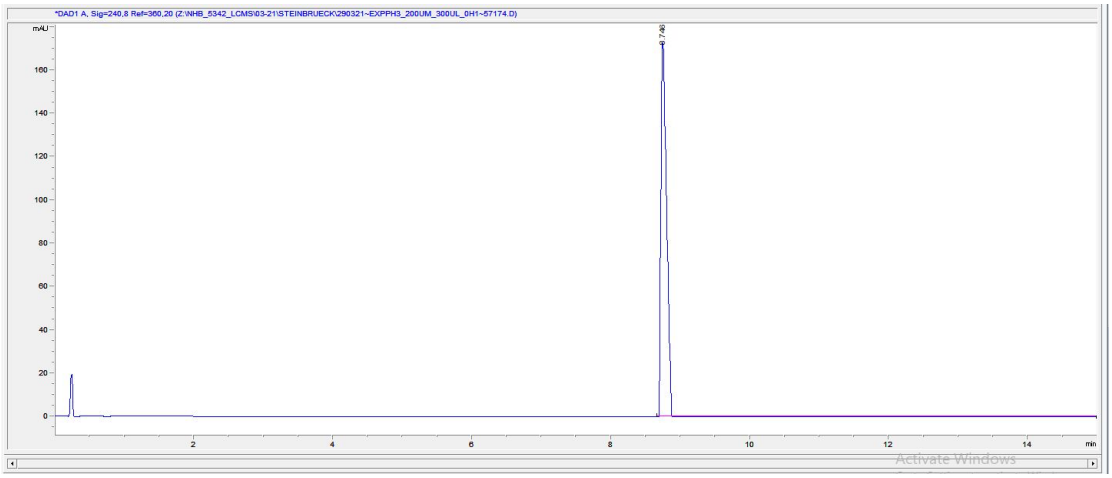
--- End Of Report ---

HRMS (ESI) analysis of **4**

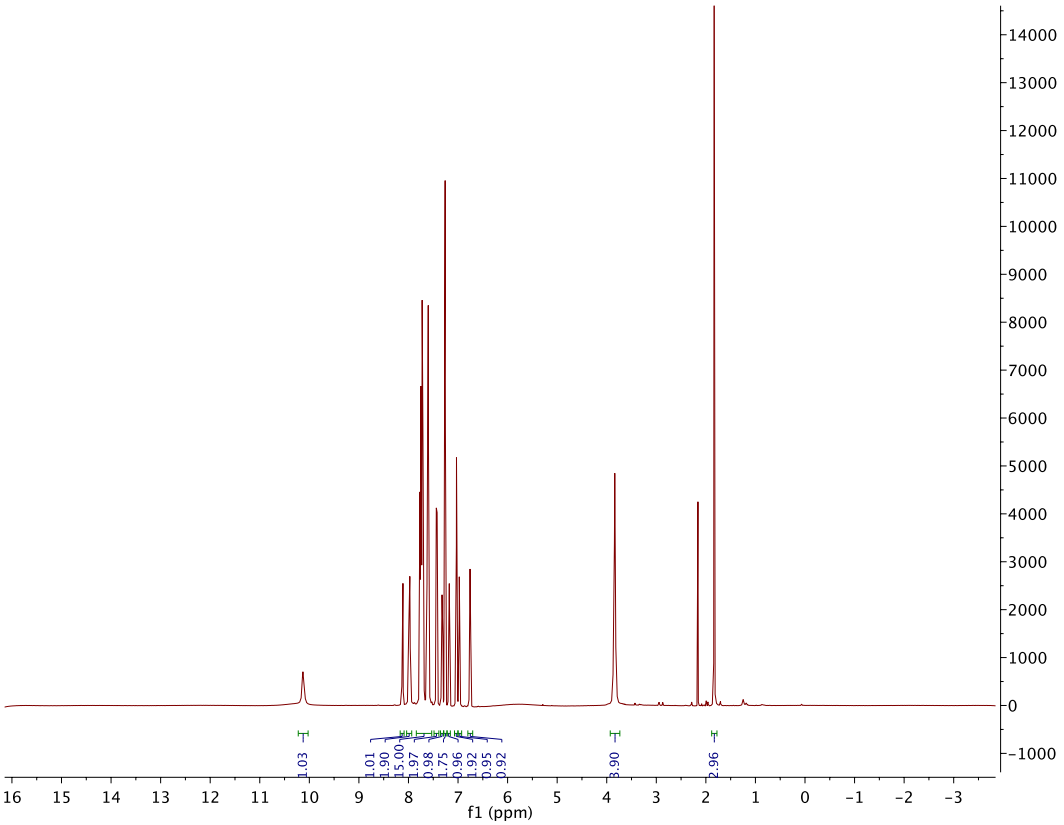
*3.5. Triphenylphosphonium Derivative **5***



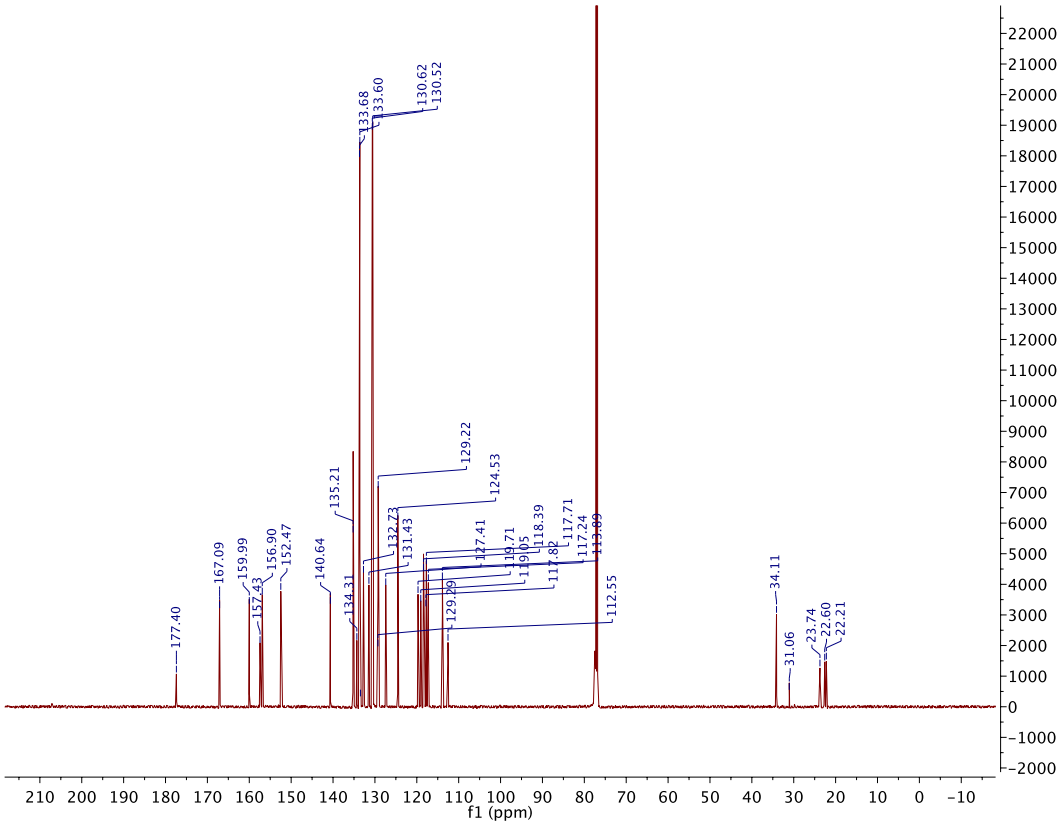
5



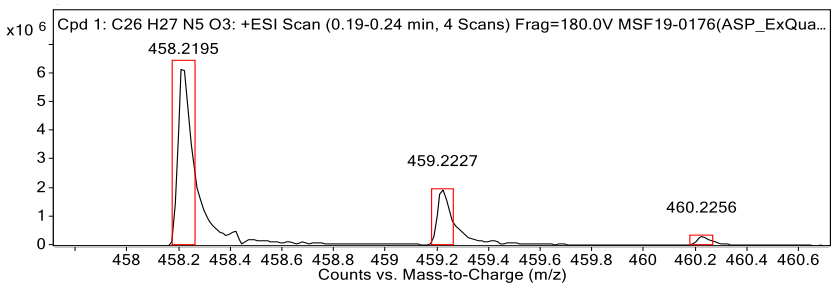
HPLC trace of **5** monitored at 240 nm



^{1}H-NMR spectrum of **5** (500 MHz, $CDCl_3$)



^{13}C-NMR spectrum of **5** (125.75 MHz, $CDCl_3$)



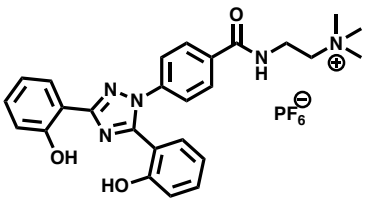
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
458.2195	458.2187	1	6397503	C26H27N5O3	(M+H)+	-1.84
459.2227	459.2217	1	1967543	C26H27N5O3	(M+H)+	-2.23
460.2256	460.2245	1	343212	C26H27N5O3	(M+H)+	-2.41
461.2270	461.2271	1	35026	C26H27N5O3	(M+H)+	0.31
462.2307	462.2297	1	3280	C26H27N5O3	(M+H)+	-2.07

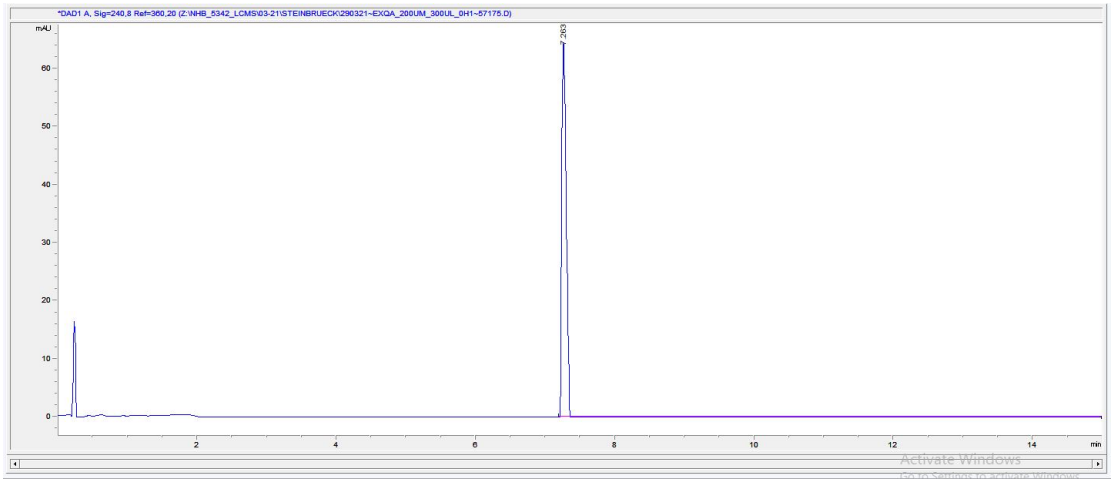
--- End Of Report ---

HRMS (ESI) analysis of **5**

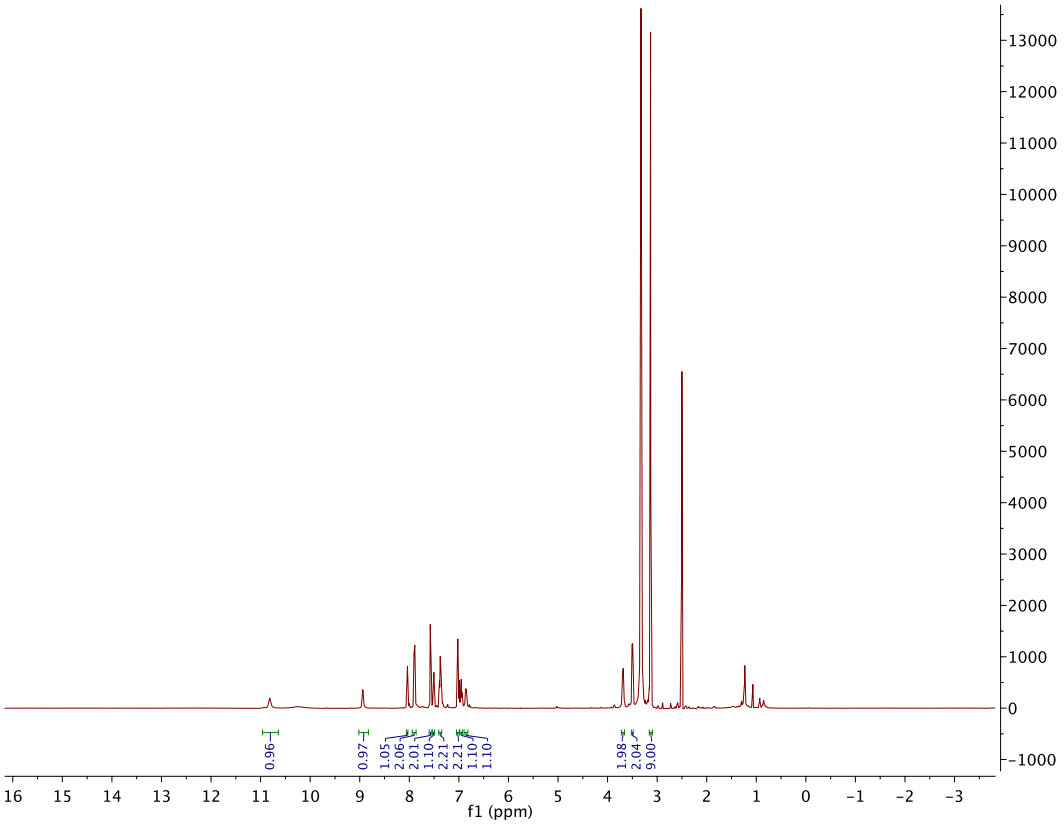
*3.6. Quaternary Ammonium Derivative **6***



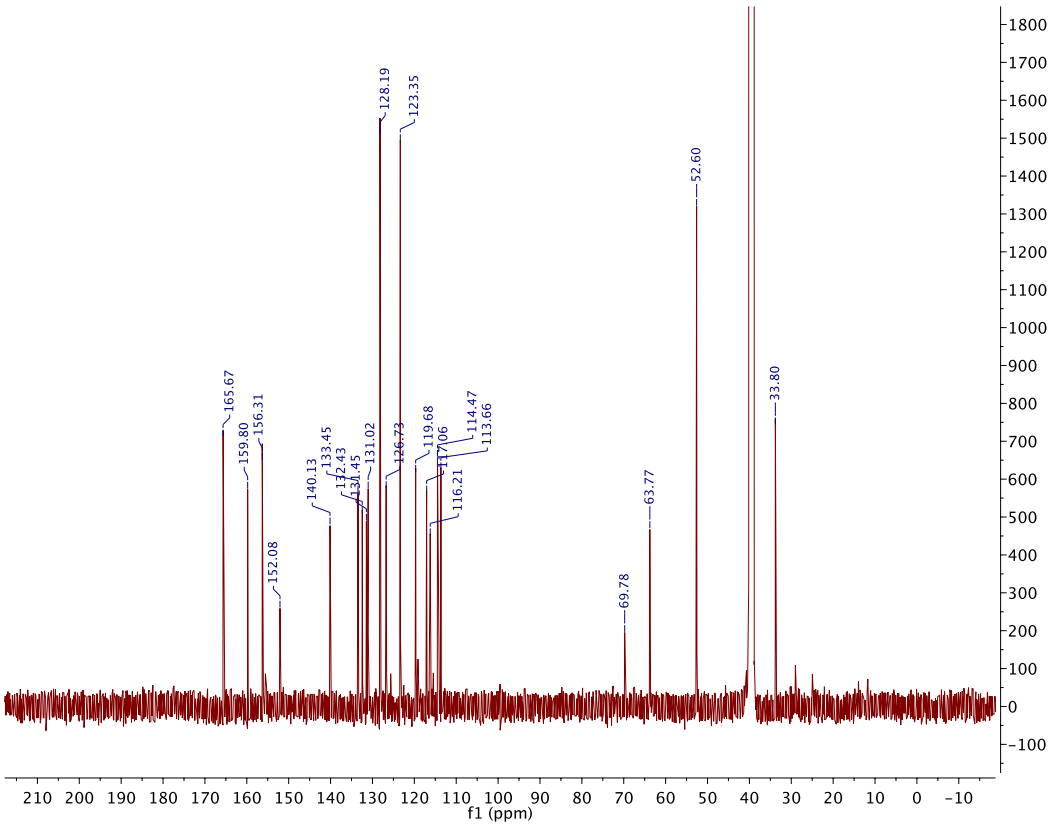
6



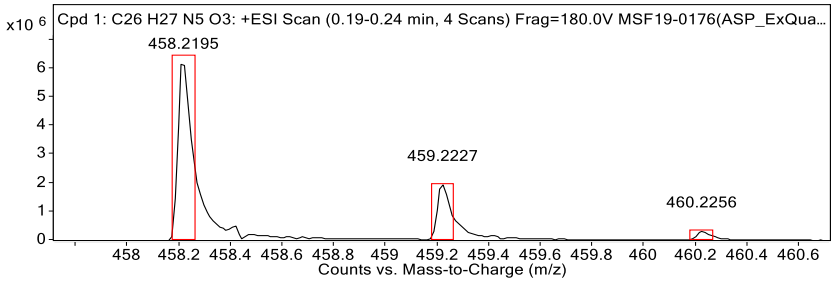
HPLC trace of **6** monitored at 240 nm



^{1}H-NMR spectrum of **6** (500 MHz, DMSO-d_6)



^{13}C-NMR spectrum of **6** (125.75 MHz, DMSO-d_6)



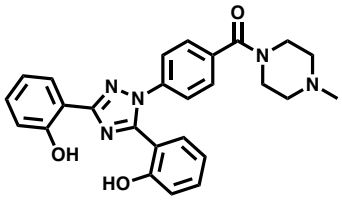
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
458.2195	458.2187	1	6397503	C26H27N5O3	(M+H)+	-1.84
459.2227	459.2217	1	1967543	C26H27N5O3	(M+H)+	-2.23
460.2256	460.2245	1	343212	C26H27N5O3	(M+H)+	-2.41
461.2270	461.2271	1	35026	C26H27N5O3	(M+H)+	0.31
462.2307	462.2297	1	3280	C26H27N5O3	(M+H)+	-2.07

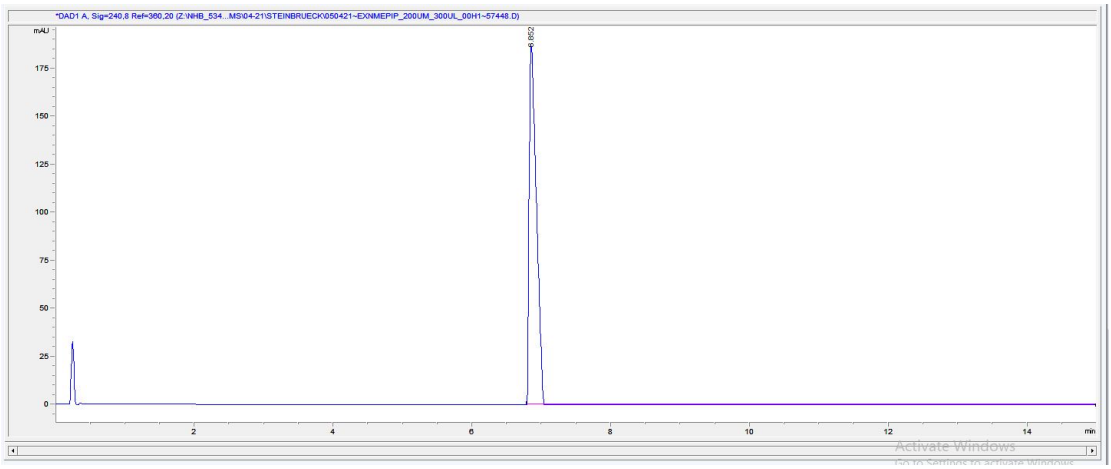
--- End Of Report ---

HRMS (ESI) analysis of **6**

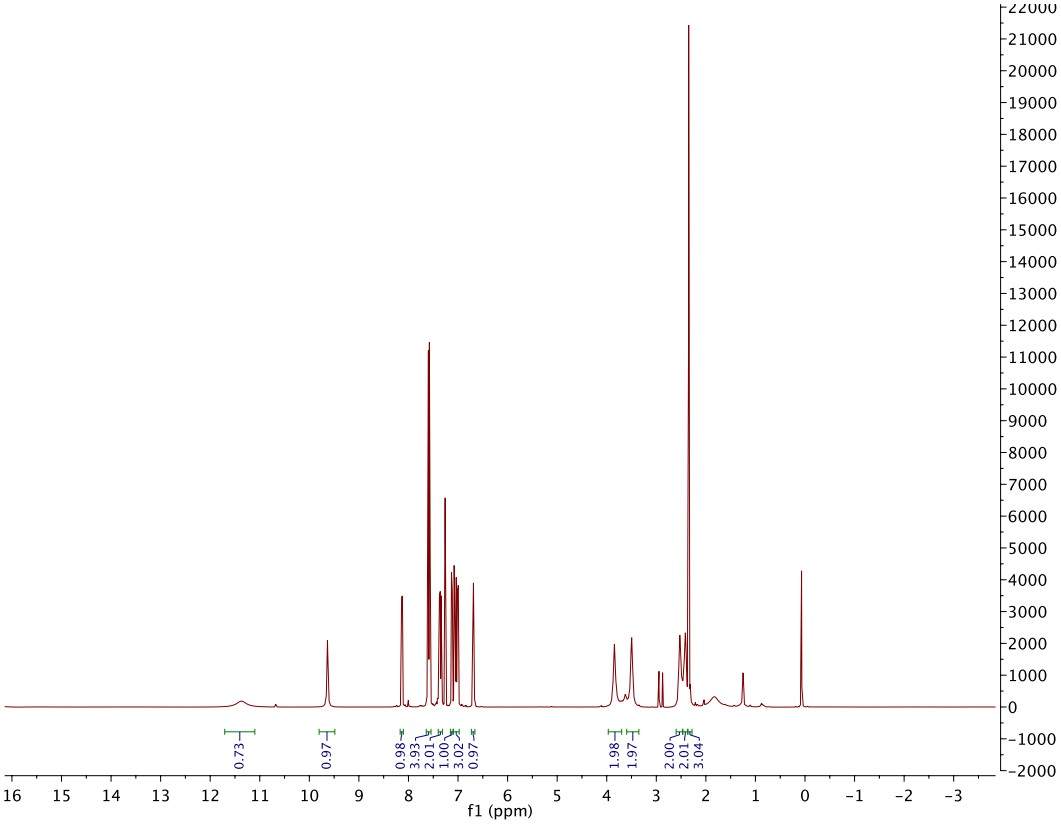
3.7. N-methylpiperazine Derivative ***7***



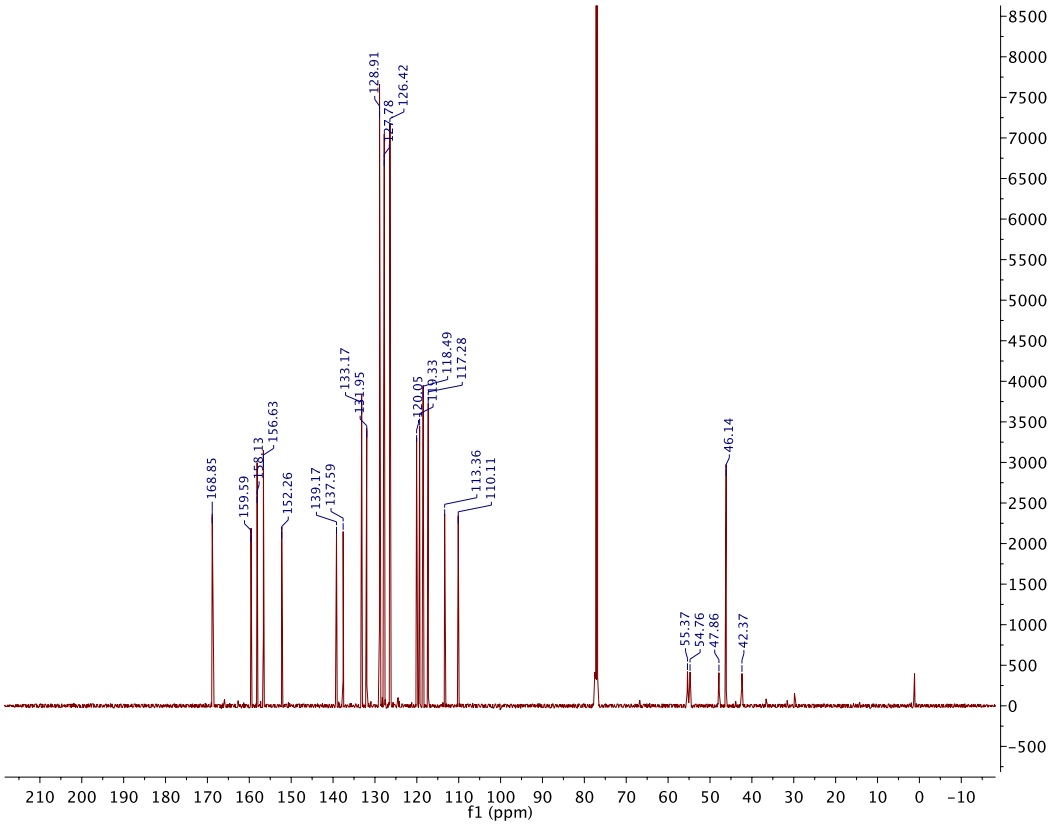
7



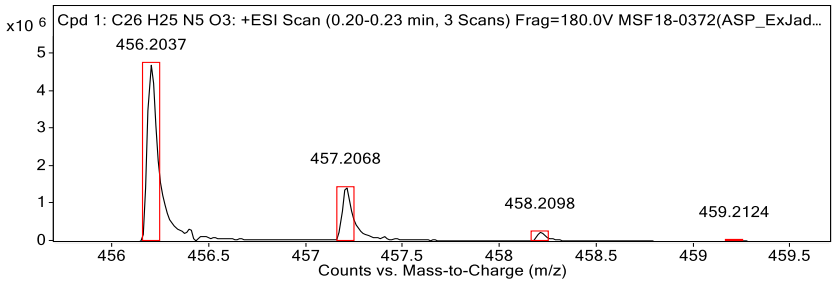
HPLC trace of **7** monitored at 240 nm



^{1}H-NMR spectrum of **7** (500 MHz, $CDCl_3$)



^{13}C-NMR spectrum of **7** (125.75 MHz, $CDCl_3$)



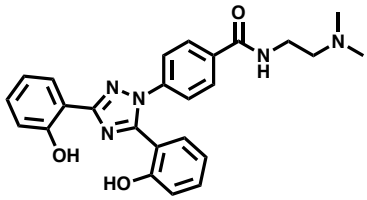
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
456.2037	456.2030	1	4740963	C26H25N5O3	(M+H)+	-1.53
457.2068	457.2060	1	1456381	C26H25N5O3	(M+H)+	-1.7
458.2098	458.2088	1	256610	C26H25N5O3	(M+H)+	-2.24
459.2124	459.2115	1	30908	C26H25N5O3	(M+H)+	-1.96
460.2212	460.2141	1	4184	C26H25N5O3	(M+H)+	-15.5

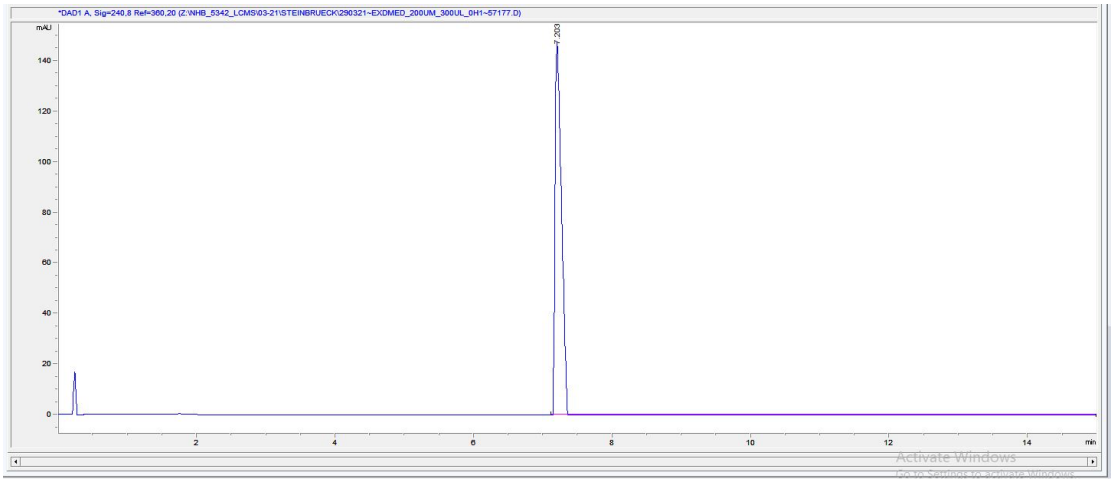
--- End Of Report ---

HRMS (ESI) analysis of **7**

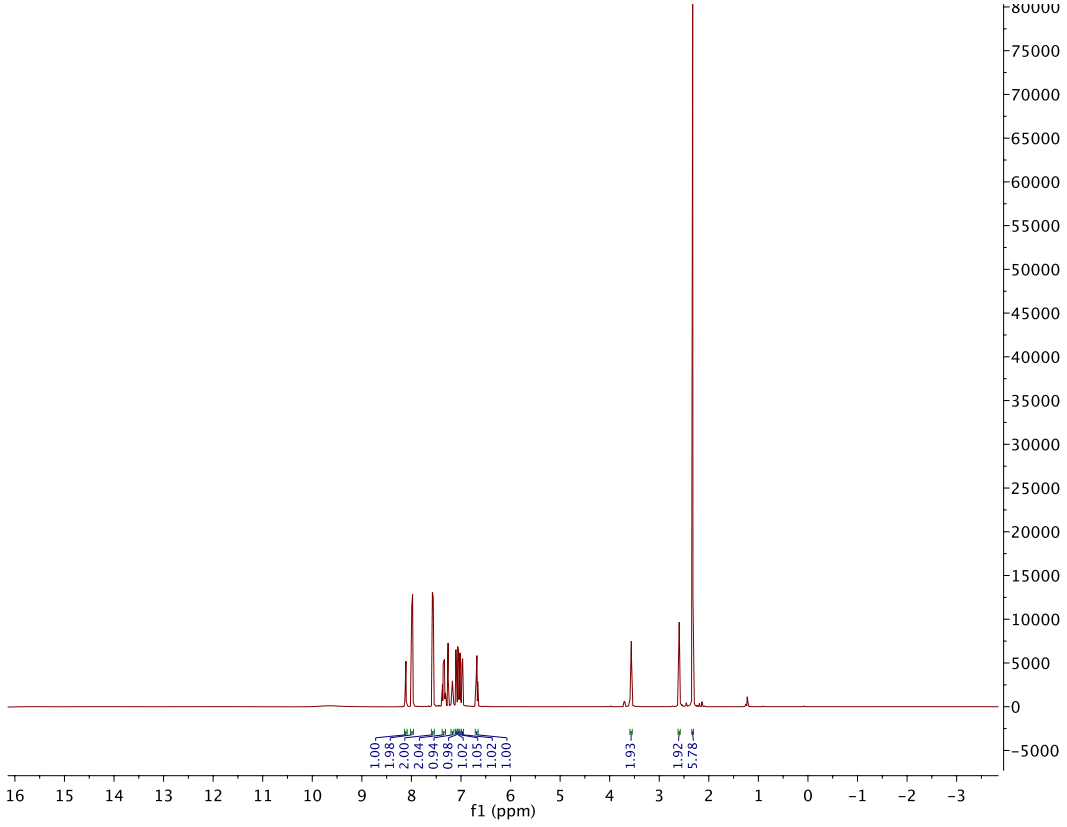
*3.8. Dimethylethylenediamine Derivative **8***



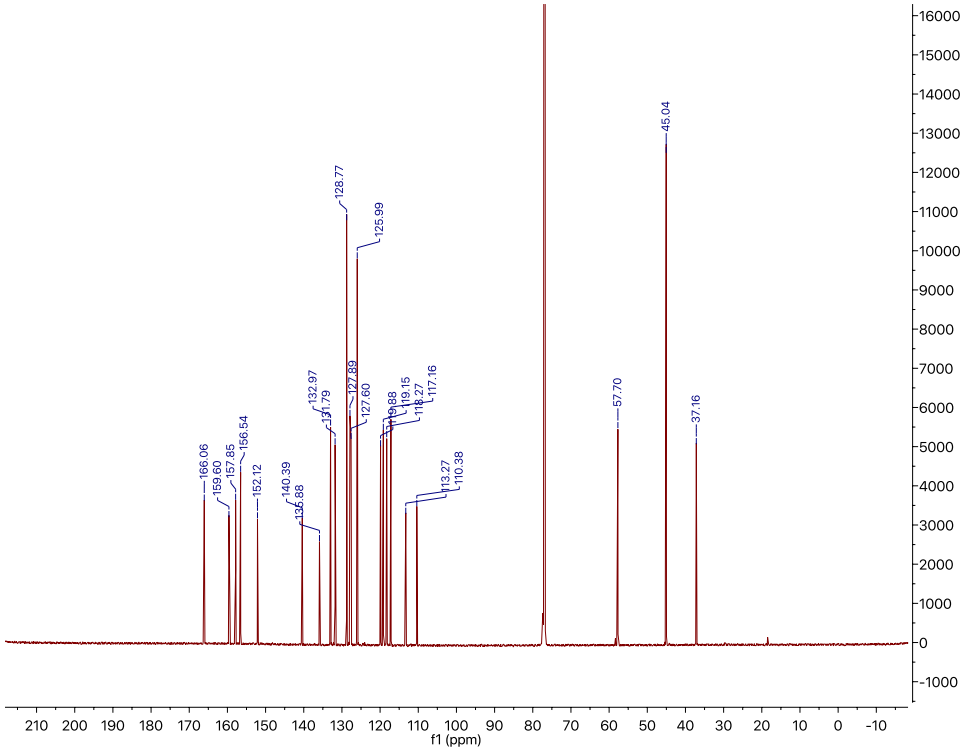
8



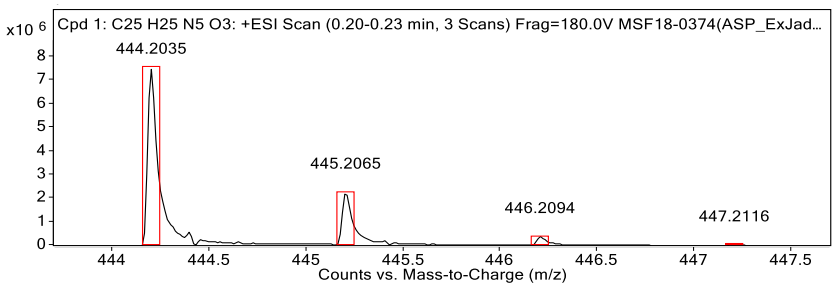
HPLC trace of **8** monitored at 240 nm



^{1}H-NMR spectrum of **8** (500 MHz, $CDCl_3$)



^{13}C-NMR spectrum of **8** (125.75 MHz, $CDCl_3$)



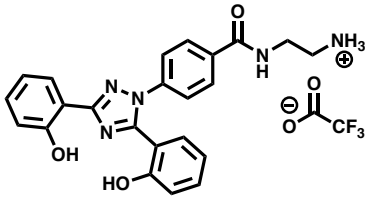
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
444.2035	444.2030	1	7473284	C25H25N5O3	(M+H)+	-0.99
445.2065	445.2060	1	2277215	C25H25N5O3	(M+H)+	-1.06
446.2094	446.2088	1	392031	C25H25N5O3	(M+H)+	-1.44
447.2116	447.2114	1	40285	C25H25N5O3	(M+H)+	-0.41

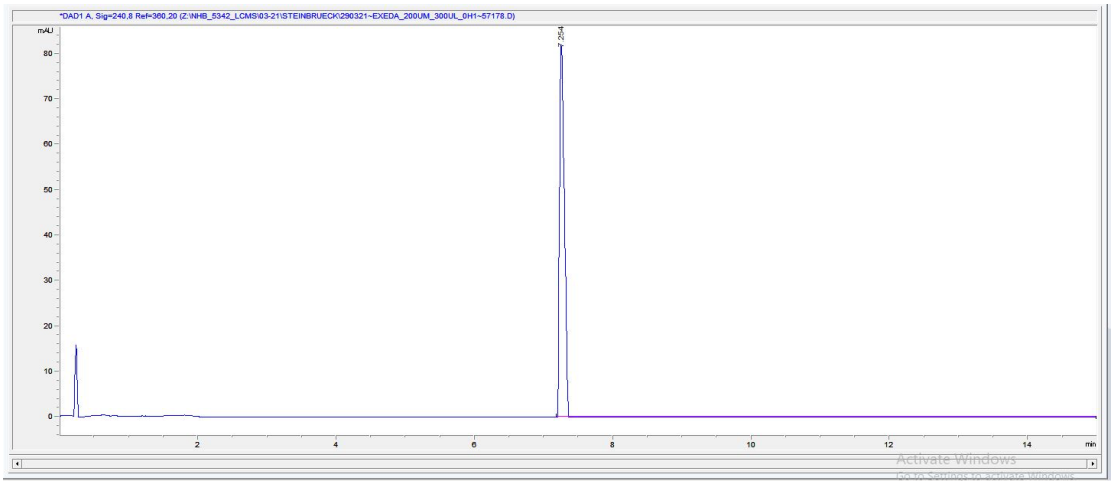
--- End Of Report ---

HRMS (ESI) analysis of **8**

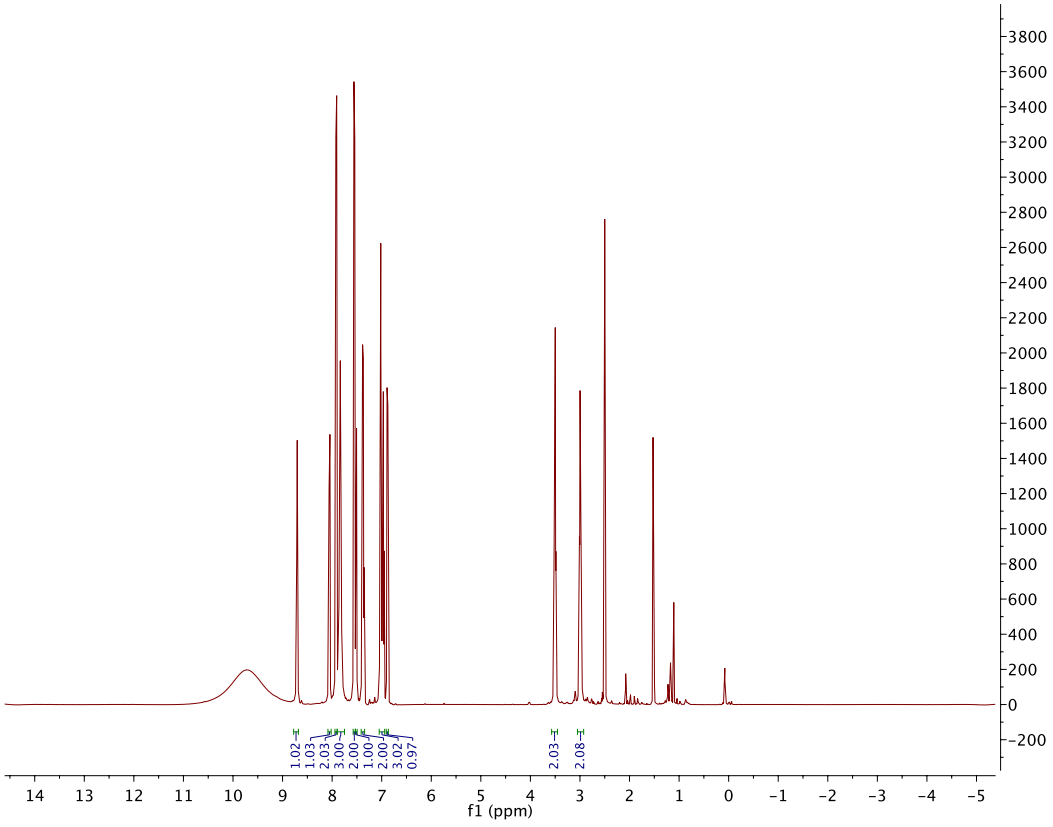
*3.9. Ethylenediamine Derivative **9***



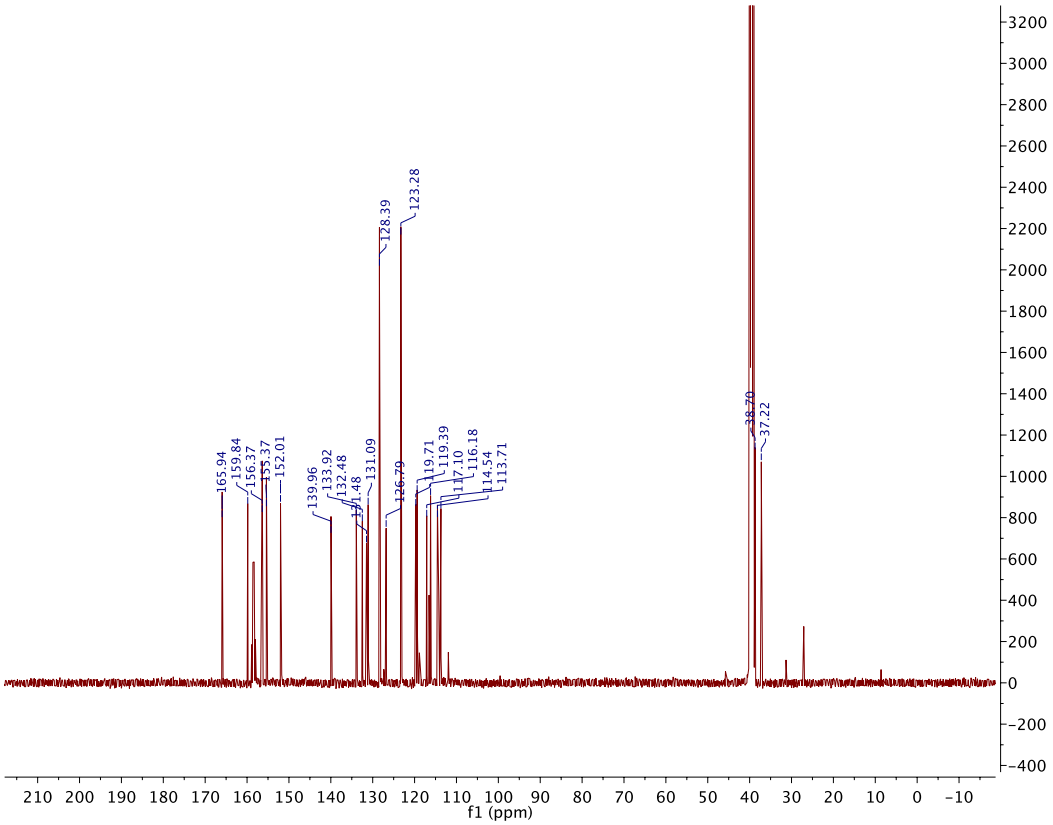
9



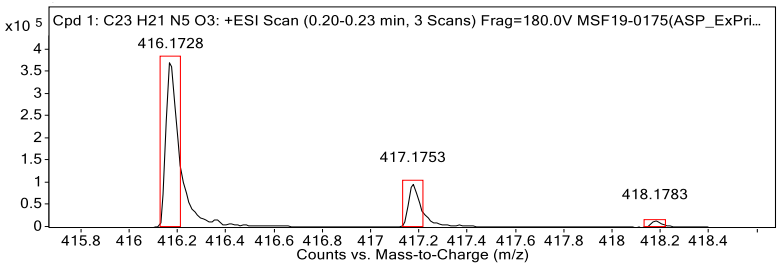
HPLC trace of **9** monitored at 240 nm



^{1}H-NMR spectrum of **9** (500 MHz, DMSO-d_6)



^{13}C-NMR spectrum of **9** (125.75 MHz, DMSO-d_6)



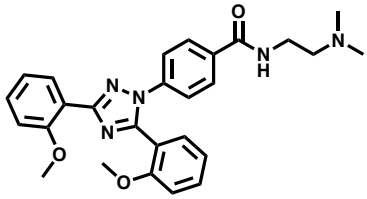
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
416.1728	416.1717	1	384495	C23H21N5O3	(M+H)+	-2.6
417.1753	417.1747	1	98604	C23H21N5O3	(M+H)+	-1.56
418.1783	418.1774	1	14843	C23H21N5O3	(M+H)+	-2.24
419.1857	419.1800	1	2215	C23H21N5O3	(M+H)+	-13.8
420.1409	420.1825	1	450	C23H21N5O3	(M+H)+	99.07

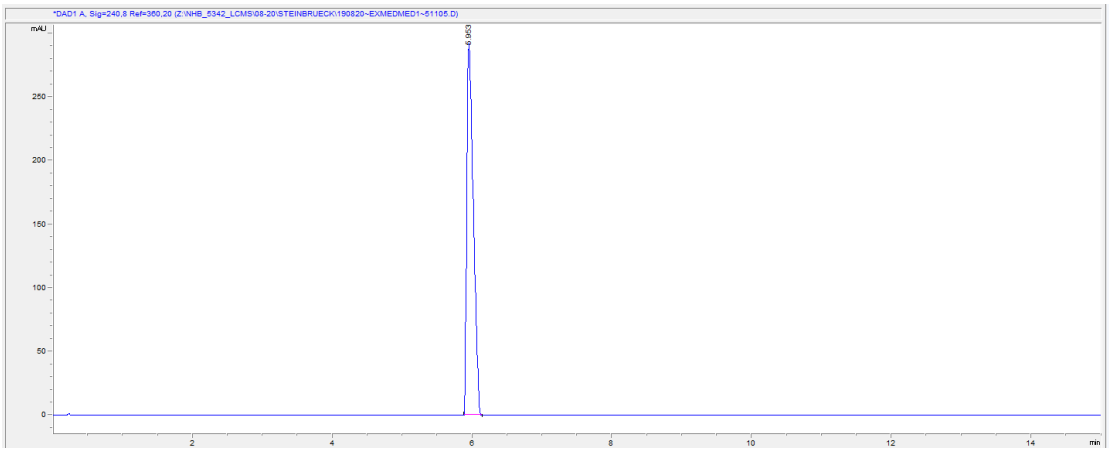
--- End Of Report ---

HRMS (ESI) analysis of **9**

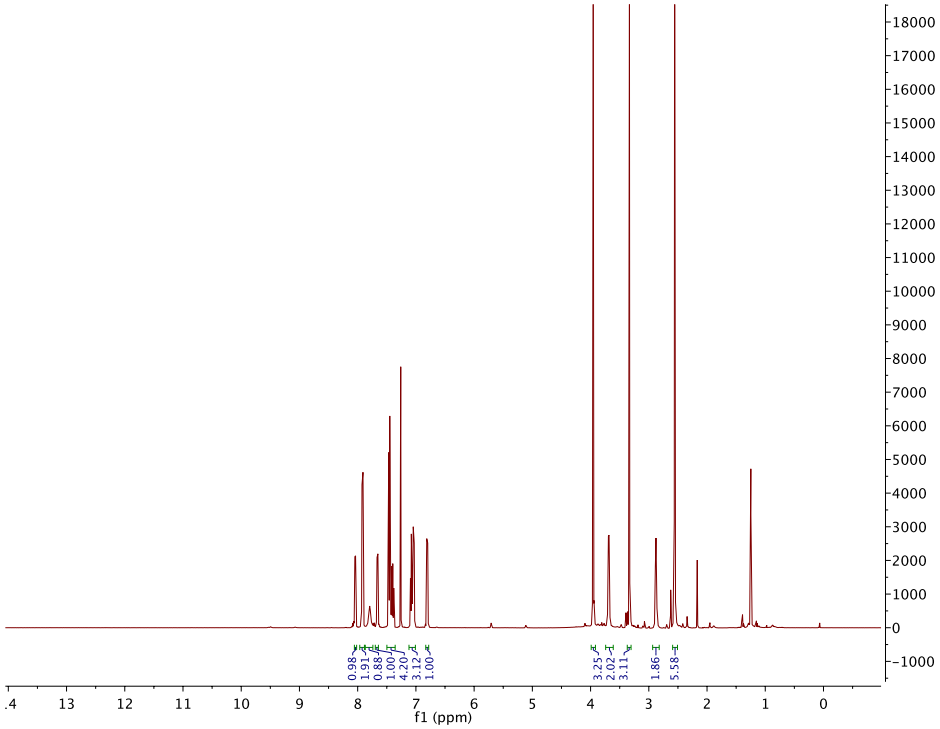
*3.10. O-Methylated Dimethylethylenediamine Derivative **10***



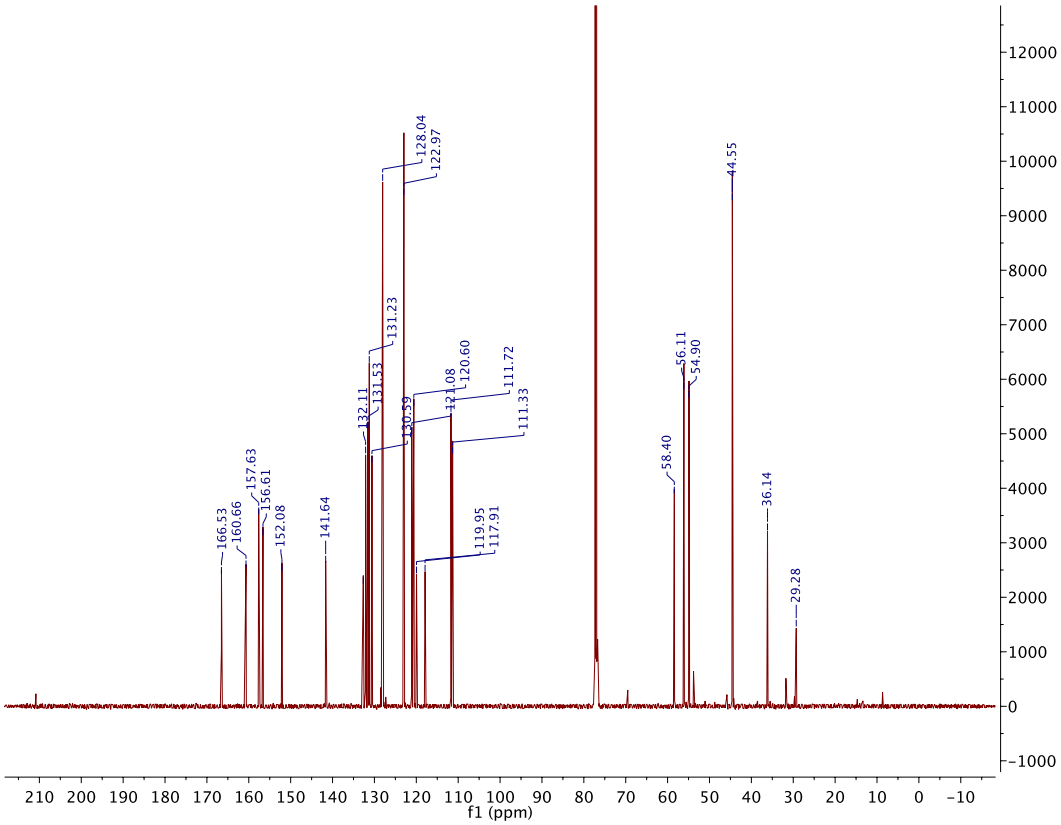
10



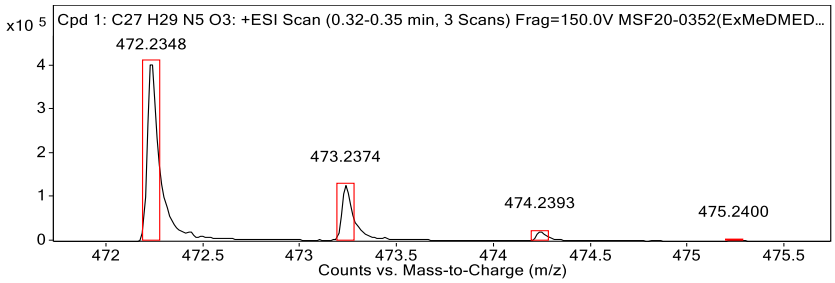
HPLC trace of **10** monitored at 240 nm



^{1}H-NMR spectrum of **10** (500 MHz, $CDCl_3$)



^{13}C-NMR spectrum of **10** (125.75 MHz, $CDCl_3$)



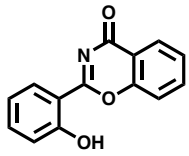
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
236.6213			506046			
472.2348	472.2343	1	412045	C27H29N5O3	(M+H)+	-0.97
473.2374	473.2373	1	128379	C27H29N5O3	(M+H)+	-0.04
474.2393	474.2401	1	22506	C27H29N5O3	(M+H)+	1.8
475.2400	475.2428	1	2657	C27H29N5O3	(M+H)+	5.95
476.2758	476.2455	1	429	C27H29N5O3	(M+H)+	-63.75

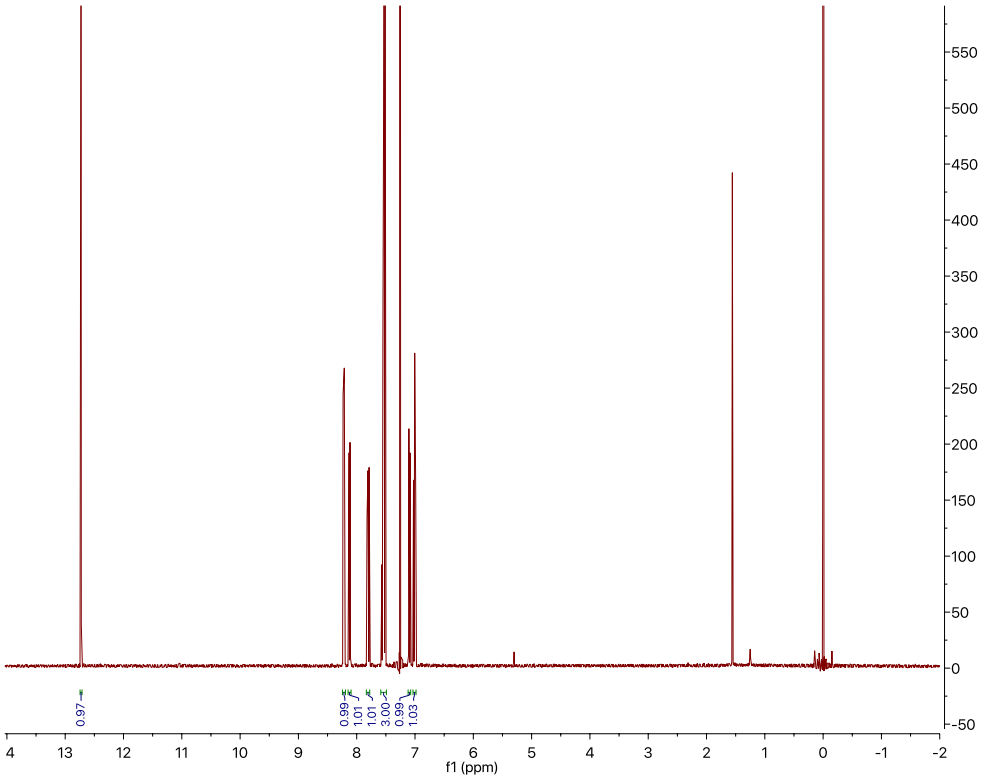
--- End Of Report ---

HRMS (ESI) analysis of **10**

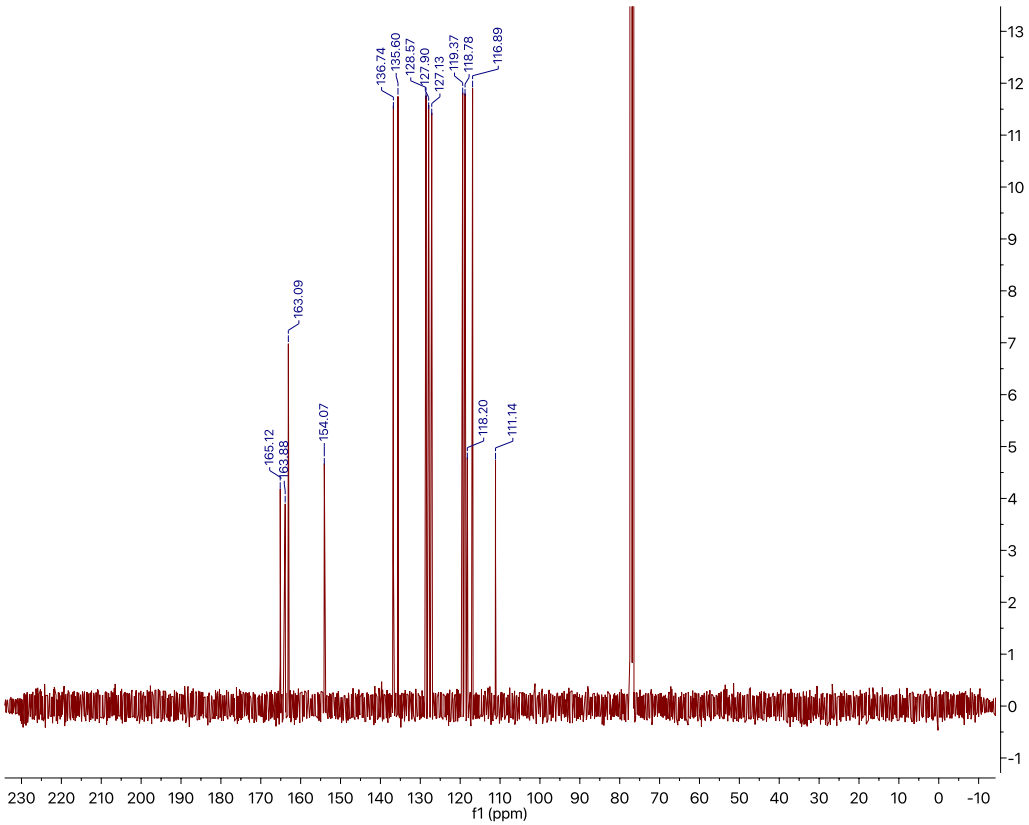
*3.11. Oxazinone Intermediate **A***



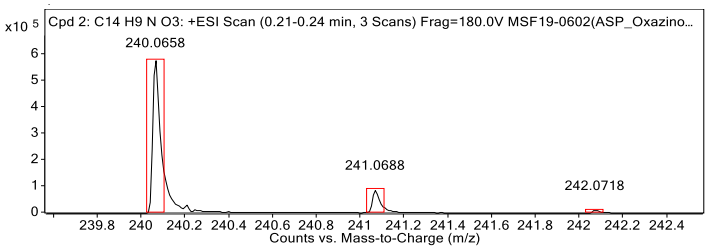
Oxazinone Intermediate A



^{1}H-NMR spectrum of Intermediate **A** (400 MHz, $CDCl_3$)



^{13}C-NMR spectrum of Intermediate **A** (100.6 MHz, $CDCl_3$)



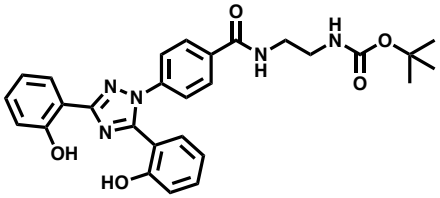
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
240.0658	240.0655	1	581677	C14H9NO3	(M+H)+	-1.24
241.0688	241.0688	1	88067	C14H9NO3	(M+H)+	-0.37
242.0718	242.0712	1	11116	C14H9NO3	(M+H)+	-2.31
243.0168	243.0738	1	502	C14H9NO3	(M+H)+	234.28

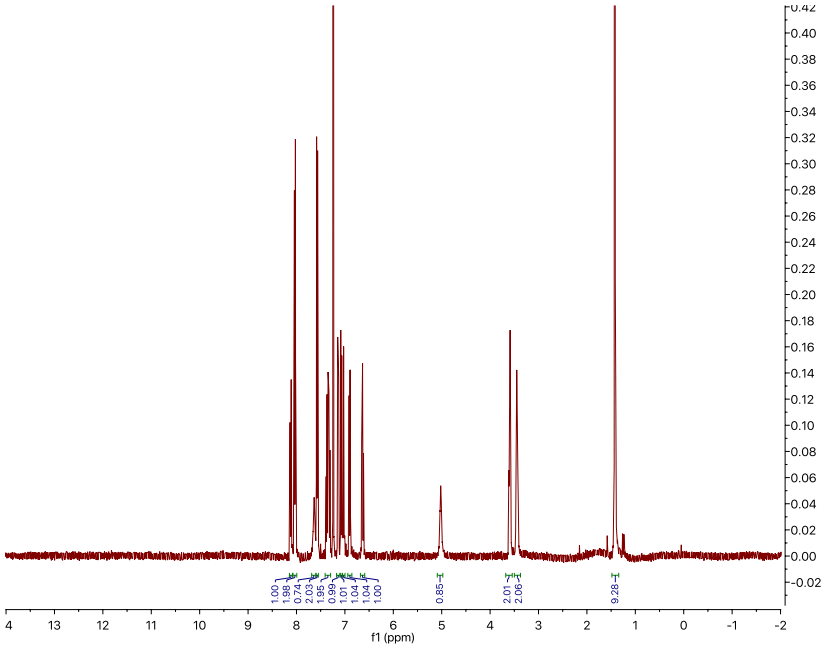
--- End Of Report ---

HRMS (ESI) analysis of Intermediate **A**

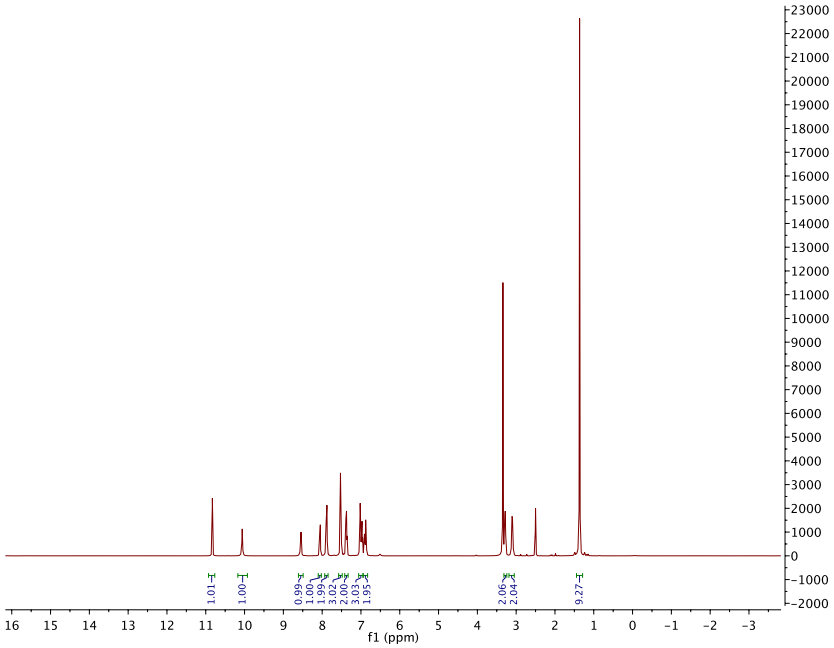
*3.12. N-boc-ethylenediamine Intermediate **B***



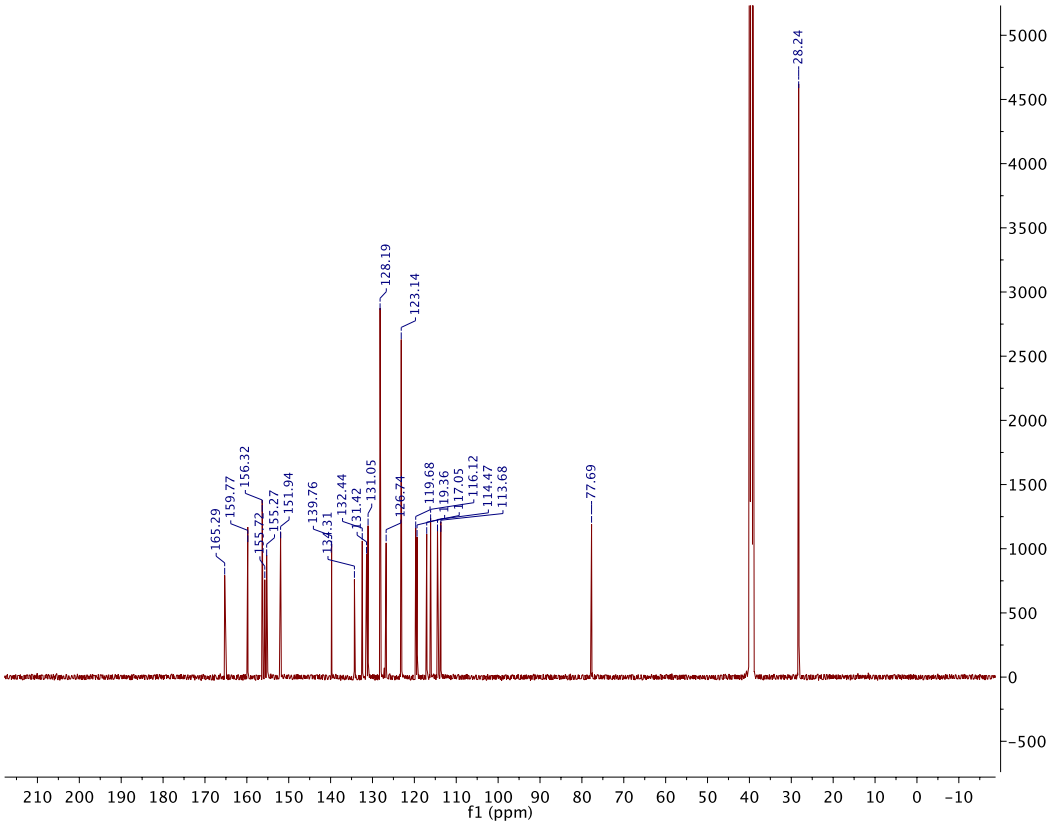
N-boc-ethylenediamine Intermediate B



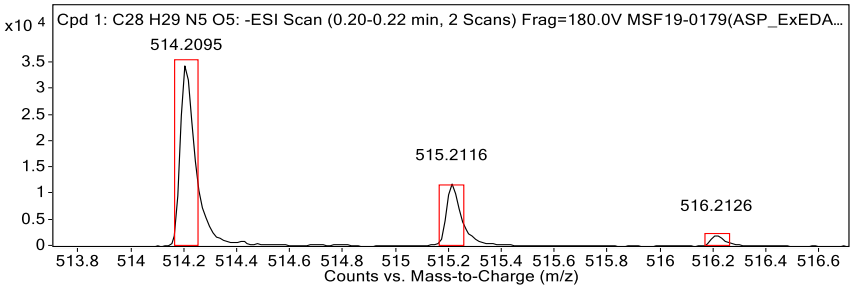
^{1}H-NMR spectrum of Intermediate **B** (400 MHz, $CDCl_3$)



^{1}H-NMR spectrum of Intermediate **B** (500 MHz, DMSO-d_6)



^{13}C-NMR spectrum of Intermediate **B** (125.75 MHz, DMSO-d_6)



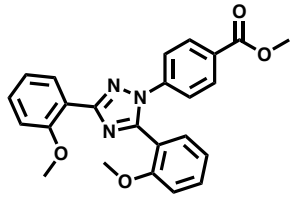
MS Spectrum Peak List

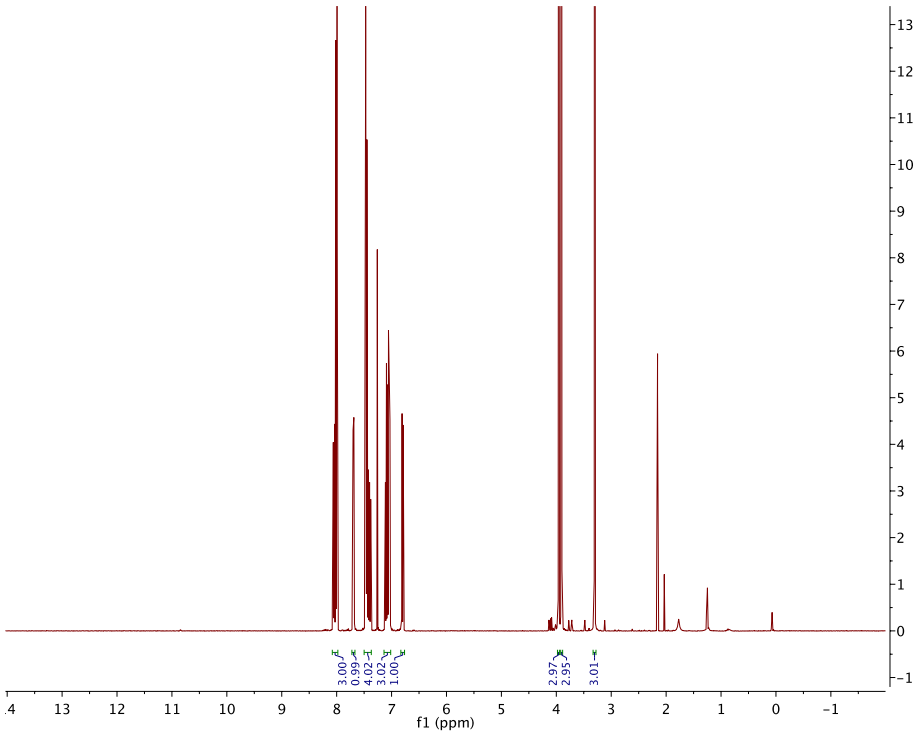
Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
514.2095	514.2096	1	34897	C28H29N5O5	(M-H)-	0.17
515.2116	515.2126	1	11925	C28H29N5O5	(M-H)-	1.99
516.2126	516.2153	1	2237	C28H29N5O5	(M-H)-	5.23
517.2195	517.2180	1	383	C28H29N5O5	(M-H)-	-3
518.2590	518.2205	1	33	C28H29N5O5	(M-H)-	-74.18

--- End Of Report ---

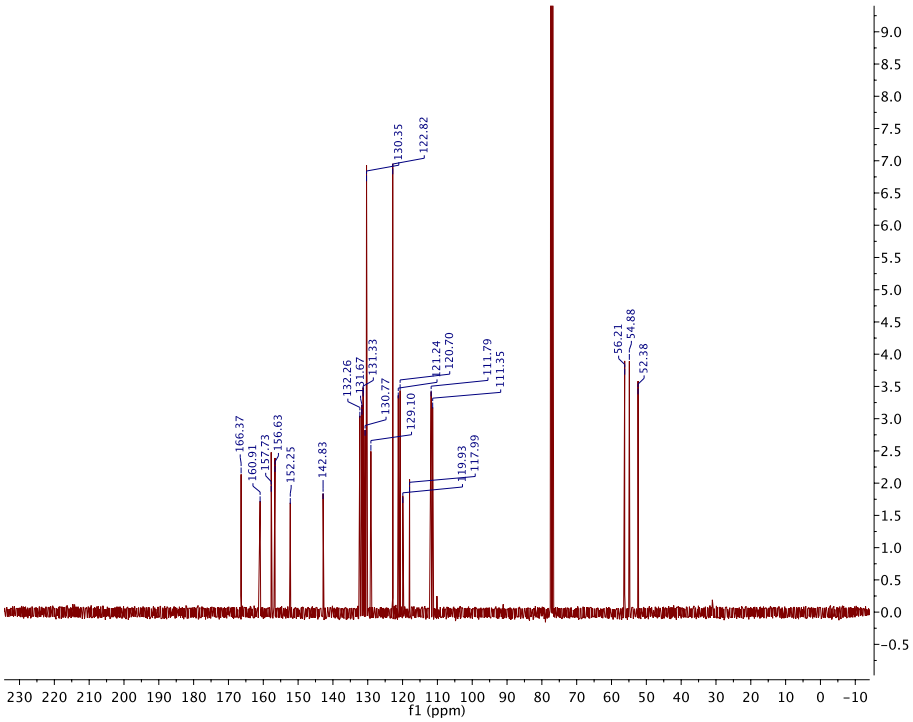
HRMS (ESI) analysis of Intermediate **B**

*3.13. Trimethylated Intermediate **C***

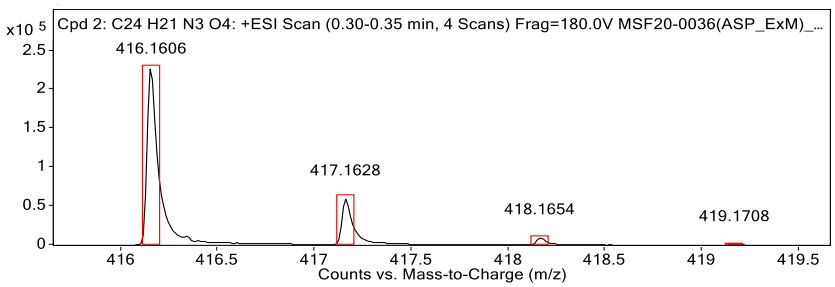




^{1}H-NMR spectrum of Intermediate **C** (500 MHz, $CDCl_3$)



^{13}C-NMR spectrum of Intermediate **C** (125.75 MHz, $CDCl_3$)



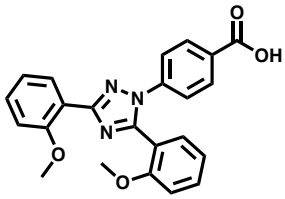
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
416.1606	416.1605	1	230535	C24H21N3O4	(M+H)+	-0.18
417.1628	417.1636	1	60114	C24H21N3O4	(M+H)+	1.93
418.1654	418.1664	1	9998	C24H21N3O4	(M+H)+	2.37
419.1708	419.1690	1	1186	C24H21N3O4	(M+H)+	-4.22
420.1646	420.1716	1	209	C24H21N3O4	(M+H)+	16.65

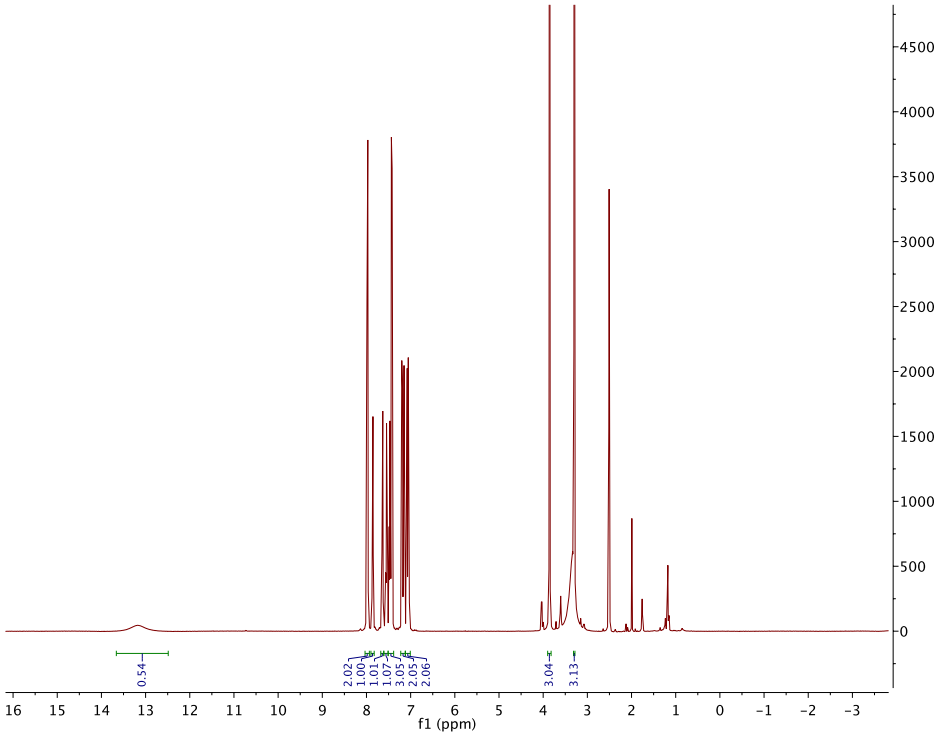
--- End Of Report ---

HRMS (ESI) analysis of Intermediate **C**

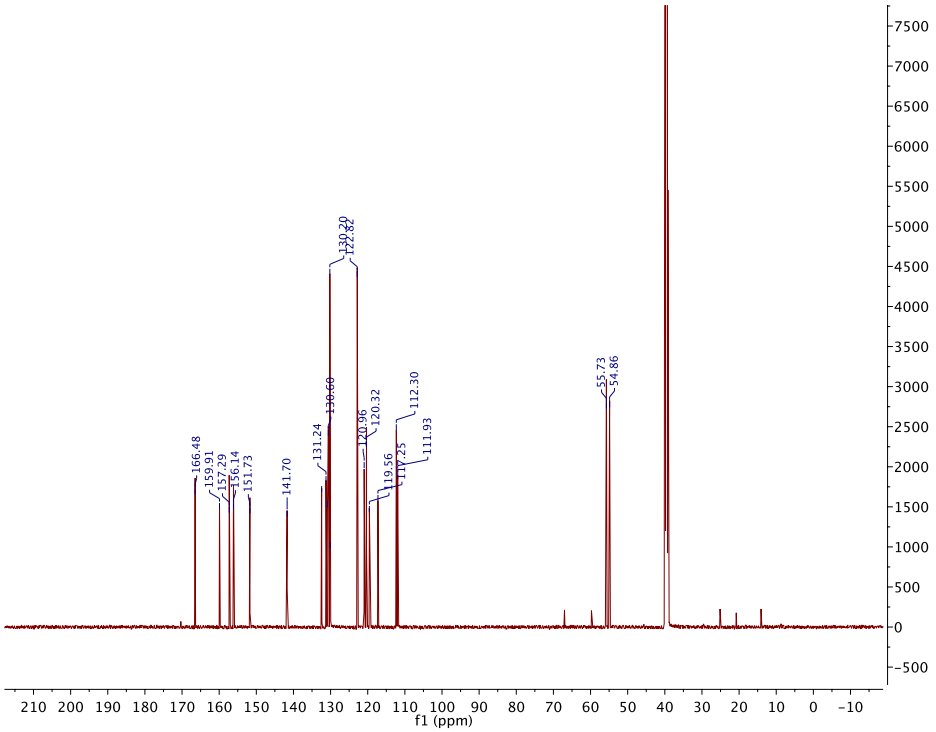
*3.14. Hydrolyzed Intermediate **D***



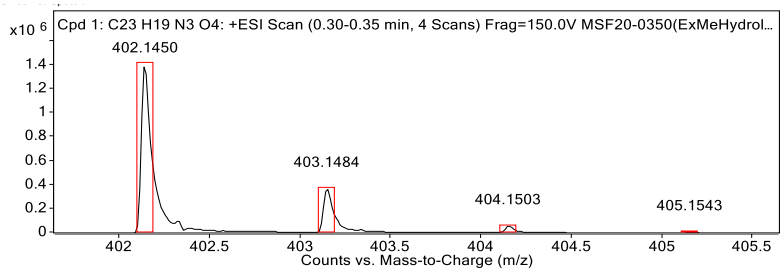
Hydrolyzed Intermediate D



^{1}H-NMR spectrum of Intermediate **D** (500 MHz, DMSO-d_6)



^{13}C-NMR spectrum of Intermediate **D** (125.75 MHz, DMSO-d_6)



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
402.1450	402.1448	1	1416644	C23H19N3O4	(M+H)+	-0.43
403.1484	403.1480	1	368951	C23H19N3O4	(M+H)+	-1.22
404.1503	404.1507	1	57094	C23H19N3O4	(M+H)+	1.03
405.1543	405.1533	1	6626	C23H19N3O4	(M+H)+	-2.58
406.1635	406.1559	1	1226	C23H19N3O4	(M+H)+	-18.83

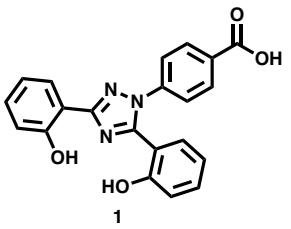
--- End Of Report ---

HRMS (ESI) analysis of Intermediate **D**

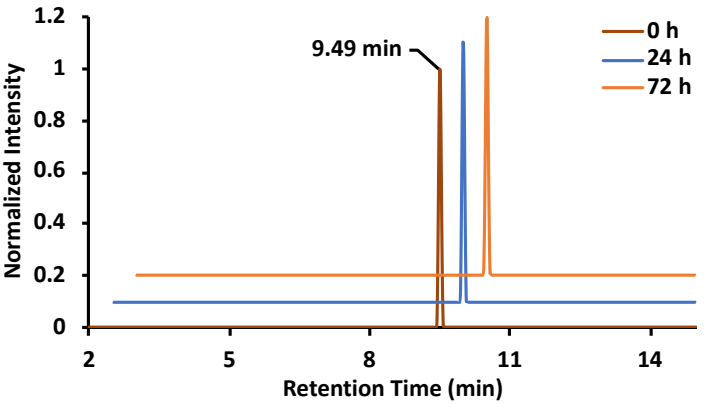
4. Compound Stability Test and HPLC Retention Times

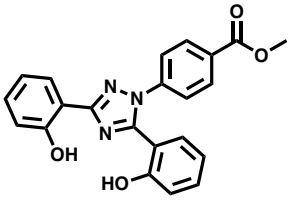
Procedure:

10 mM stock solutions in DMSO were prepared for compounds **1**-**9**. Into a glass vial was added 1 mL of a PBS buffer solution and 20 µL of compound from the appropriate tock solution resulting in a final concentration of 200 µM for each compound, respectively. Vials were kept in a warm water bath at 37°C for 72 h. Aliquots of each compound were taken after 0 h, 24 h and 72 h, respectively, and analyzed via HPLC with UV detection at 240 nm. Chromatograms are shown with an off-set of 0.5 min along the abscissa and 0.1 along the ordinate to facilitate intercomparisons of the 3 evaluated time points.

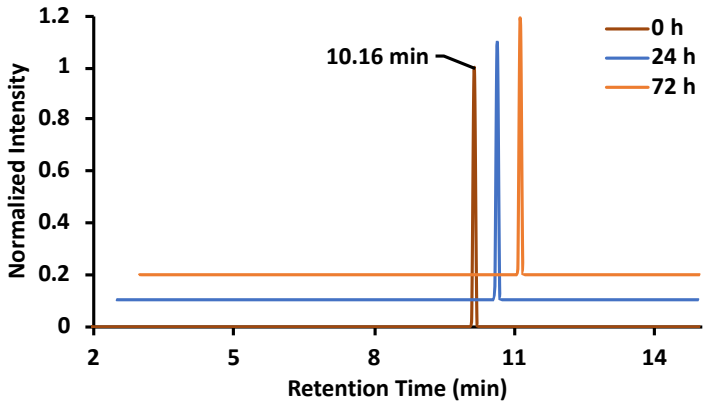


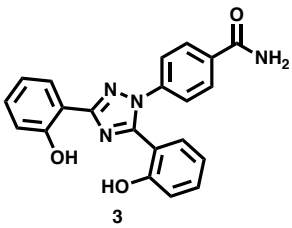
1



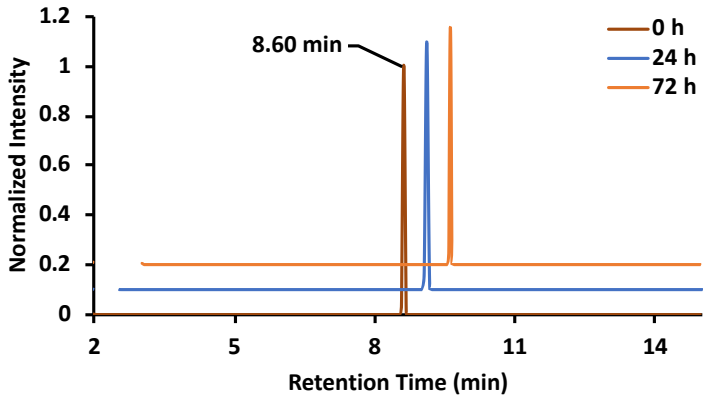


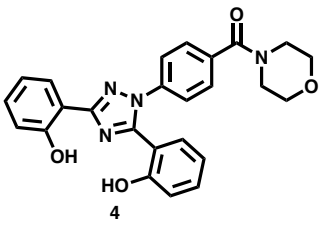
2



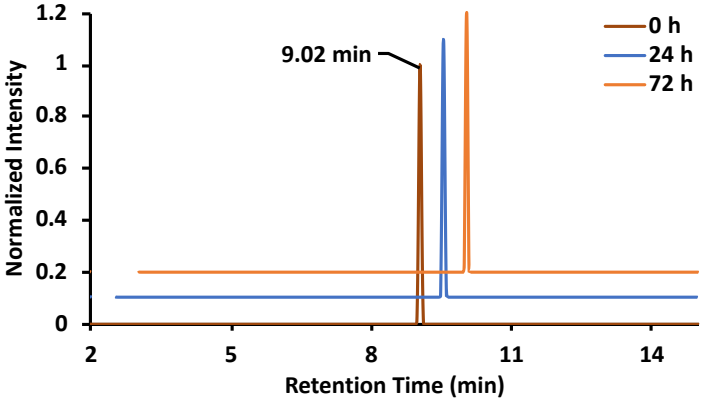


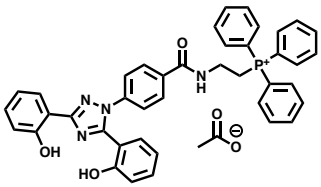
3



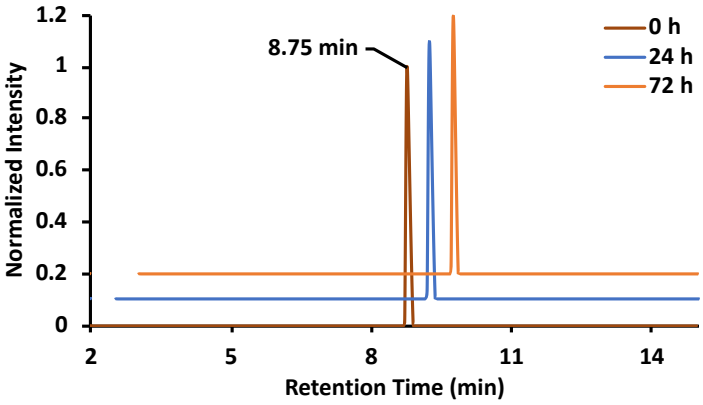

O
N
O
N
N
OH
HO

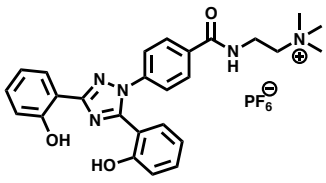
4


1.2
1
0.8
0.6
0.4
0.2
0
Normalized Intensity
9.02 min
0 h
24 h
72 h
2
5
8
11
14
Retention Time (min)

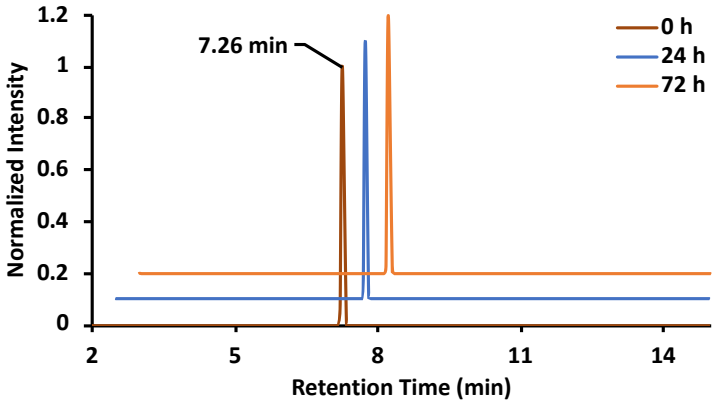

O
N
H
P+
O
O
N
N
OH
HO

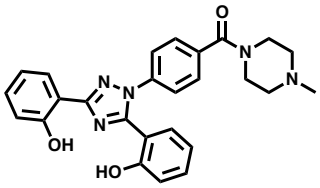
5


1.2
1
0.8
0.6
0.4
0.2
0
Normalized Intensity
8.75 min
0 h
24 h
72 h
2
5
8
11
14
Retention Time (min)

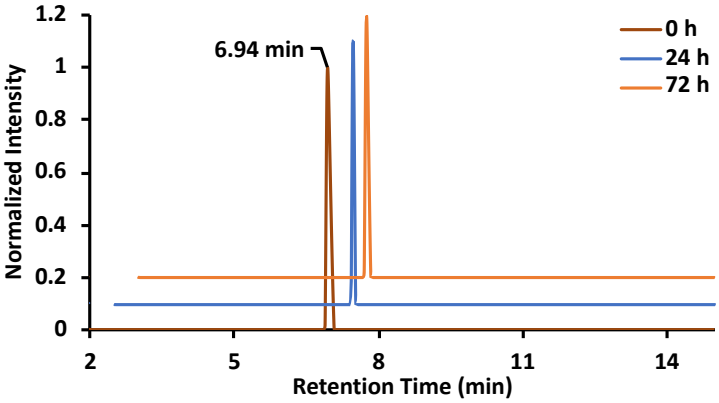

O
N
H
N
PF6
OH
HO

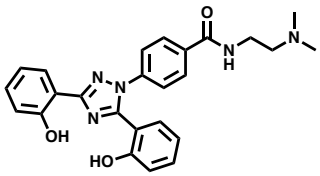
6


1.2
1
0.8
0.6
0.4
0.2
0
Normalized Intensity
7.26 min
0 h
24 h
72 h
2
5
8
11
14
Retention Time (min)

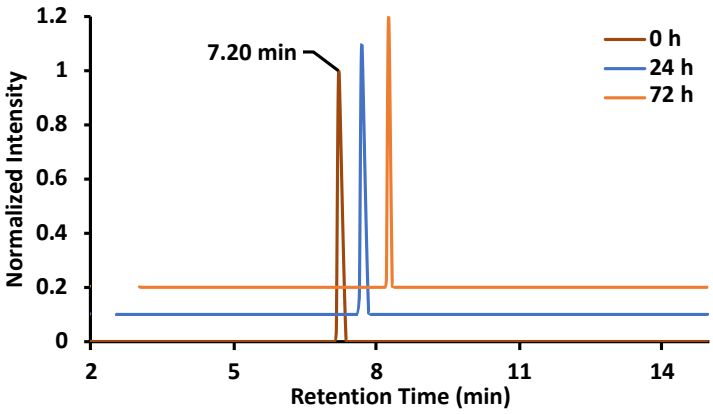

O
N
N
OH
HO

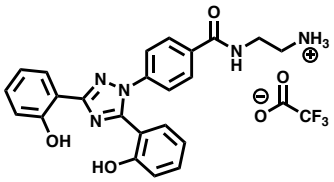
7


1.2
1
0.8
0.6
0.4
0.2
0
Normalized Intensity
6.94 min
0 h
24 h
72 h
2
5
8
11
14
Retention Time (min)

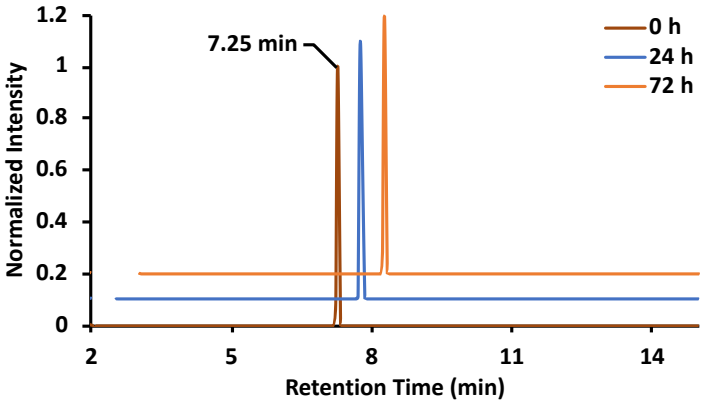

O
N
H
N
N
N
OH
HO

8


1.2
1
0.8
0.6
0.4
0.2
0
Normalized Intensity
7.20 min
0 h
24 h
72 h
2
5
8
11
14
Retention Time (min)


O
N
H
NH3
N
N
N
OH
HO
O
O
CF3

9


1.2
1
0.8
0.6
0.4
0.2
0
Normalized Intensity
7.25 min
0 h
24 h
72 h
2
5
8
11
14
Retention Time (min)

5. LogD$_{7.2}$ and logD$_{4.5}$ Determination

500 mL aliquots of purified octanol and aqueous phase were thoroughly mixed in a 2 L separatory funnel to saturate the two phases. For compounds **1**-**9**, 20 µM standard solutions were prepared in aqueous phase-saturated octanol, respectively. 5 mL standard solutions containing the compound in question were then pipetted into 15 mL centrifuge tubes, and a 5 mL aliquot of the octanol-saturated aqueous phase was added. The resulting samples were shaken for 10 min and allowed to sit overnight for 18 hours. The samples were then centrifuged for 30 min (3000 g, 20° C) and aliquots of the octanol phase were withdrawn and analyzed via UV-vis spectroscopy. Experiments were conducted in triplicate. The concentrations of the deferasirox derivatives in the octanol phase were quantified using to their absorbance at 300 nm. logD was defined as:

$$logD = log\left(\frac{A_0}{A_0 - A_S}\right)$$

where A_0= absorbance of 20 µM compound in aqueous phase- saturated octanol
A_S= absorbance of compound in octanol phase after shaking with aqueous phase

Aqueous phases:
pH 7.2 (logD$_{7.2}$): PBS buffer solution
pH 4.5 (logD$_{4.5}$): 0.1 M AcOH/AcONa buffer

LogD values >3 could not be determined via this method due to the sensitivity limit of the UV-vis instrument that did not allow to distinguish absorbance differences below 0.001.

Compound	Log D$_{7.2}$	Log D$_{4.5}$	HPLC Retention time
1	1.16 ± 0.02	>3	9.49 min
2	>3	>3	10.16 min
3	>3	n.d.	8.60 min
4	>3	n.d.	9.02 min
5	2.56 ± 0.33	n.d.	8.75 min
6	1.02 ± 0.03	n.d.	7.26 min
7	2.07 ± 0.07	1.41 ± 0.01	6.94 min
8	2.15 ± 0.07	1.35 ± 0.03	7.20 min
9	1.18 ± 0.02	1.24 ± 0.03	7.25 min

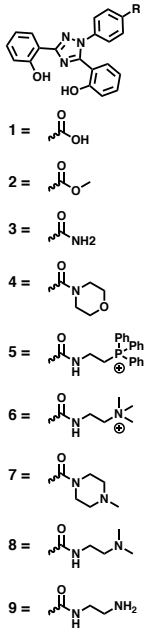


Table S1: logD values for **1**-**9** at pH = 7.2 and pH = 4.5, respectively. For comparison, HPLC retention times on a C18 reverse phase column using methanol and acidified water as solvent system are included. n.d. = not determined.

6. Cell Viability Studies

MTT and CV assay in HCT-116, L929, A549 cell lines:
The highest concentration of the compound: 250 µM; a 3-fold serial dilution was employed.

MTT assays in A549 after pre-incubation of compounds with HSA:
The highest concentration of compound and HSA, respectively: 100 µM (1:1); a 2-fold serial dilution was employed.

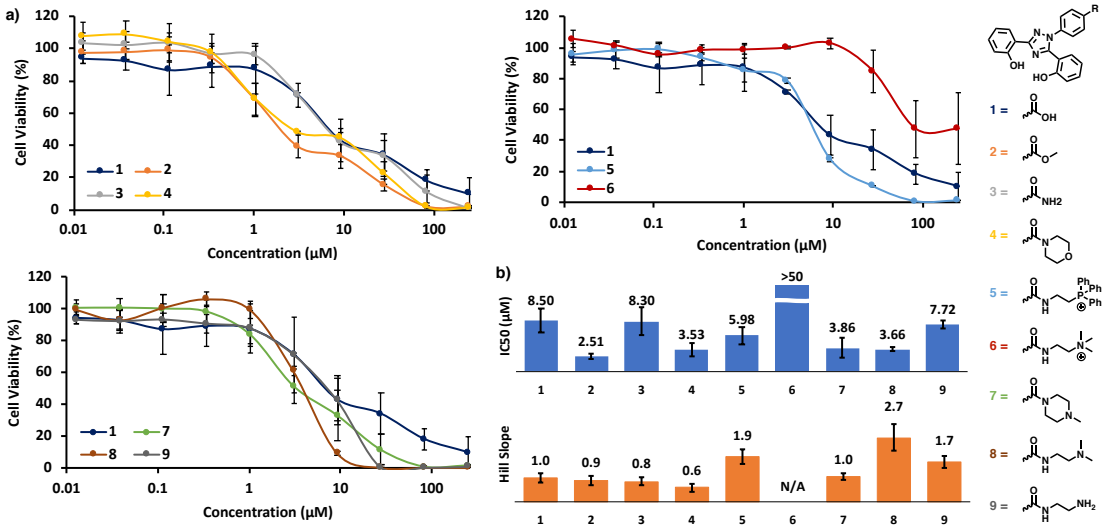


Figure S1: a) Proliferation profiles in A549 cells after 72 h of exposure to varying concentrations of **1**-**9** with the highest concentration = 250 µM. b) Corresponding IC_{50} and HS values derived from the proliferation profiles.

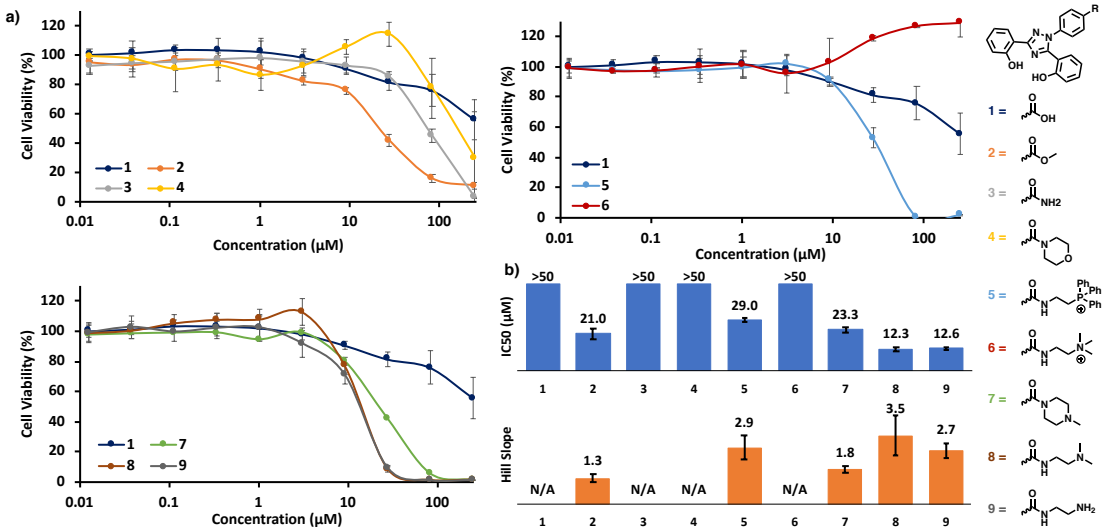


Figure S2: a) Proliferation profiles in A549 cells after 24 h of exposure to varying concentrations of **1**-**9** with the highest concentration = 250 µM. b) Corresponding IC_{50} and HS values derived from the proliferation profiles

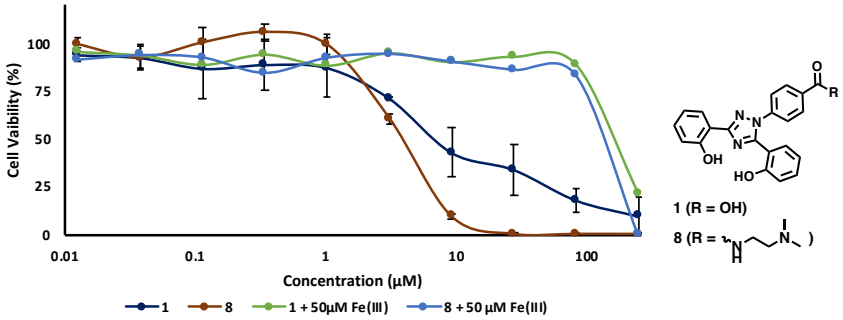


Figure S3: Proliferation profiles of A549 cells exposed to varying concentrations of **1** and **8** in the presence and absence of 50 µM supplemental $FeCl_3$, respectively. exposure time was 72 h and highest concentration = 250 µM.

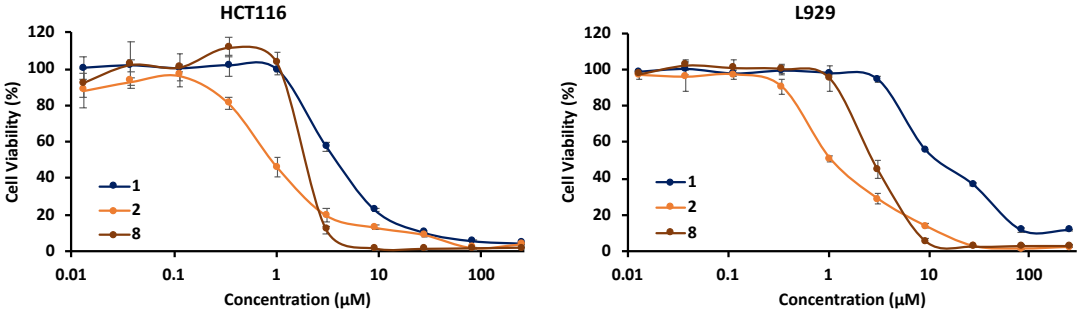


Figure S4: Proliferation profiles of HCT116 colon cancer cells (left) and L929 murine fibroblast cells (right) exposed to varying concentrations of **1**, **2** and **8,** respectively. Exposure time was 72 h and highest concentration = 250 µM.

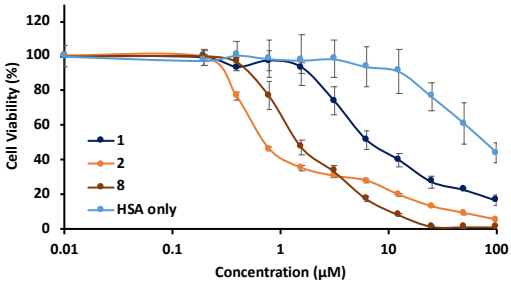
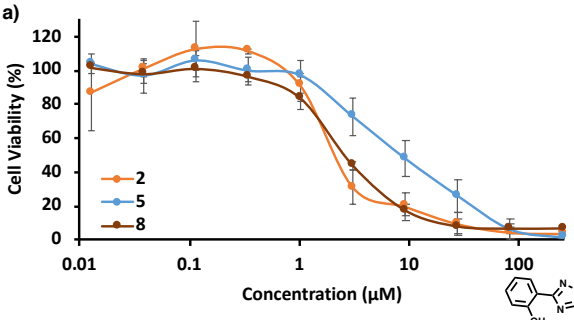


Figure S5: Proliferation profiles of A549 cells exposed to varying concentrations of **1**, **2** and **8**, respectively, in the presence of 1 equivalent of HSA. Exposure time was 72 h and highest concentration = 100 µM.

Comp.	A549	HCT 116	L929	A549 + HSA
1	8.5 ± 2.0 µM	3.5 ± 0.3 µM	11.6 ± 1.9 µM	7.4 ± 0.6 µM
2	2.5 ± 0.7 µM	1.0 ± 0.1 µM	1.3 ± 0.2 µM	0.9 ± 0.1 µM
8	3.7 ± 0.3 µM	2.7 ± 0.3 µM	2.8 ± 0.3 µM	1.7 ± 0.2 µM

*Table S2: IC_{50} values of **1, 2** and **8** in A549, HCT116 and L929 cells after 72 h incubation as determined via MTT assay. Pre-incubation with HSA resulted in lower IC_{50} values (greater potency) for **2** and **8** in A549 cells under otherwise identical conditions.*

a)



b)

Compound	CV assay A549 IC_{50} (µM)	MTT assay A549 IC_{50} (µM)
2	2.2 ± 0.3	2.5 ± 0.7
5	8.6 ± 1.4	6.0 ± 0.6
8	2.4 ± 0.1	3.7 ± 0.3

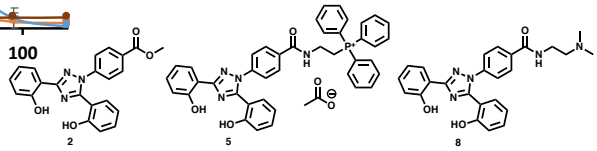


Figure S6: a) Proliferation profiles of A549 cells exposed to varying concentrations of **2**, **5** and **8**, respectively, as determined by crystal violet (CV) staining. Exposure time was 72 h and the highest concentration was 250 µM. b) IC_{50} values using CV staining. The corresponding values derived from MTT staining are shown for convenience.

7. Binding of 1, 2 and 8 to HSA as Determined via Stern-Volmer Plot

To a solution of 5 µM HSA in deionized water was added incremental amounts of each respective chelator from a 1 mM stock solution in DMSO. Emission profiles were collected with excitation at 290 nm; excitation and emission slit width was 5 nm, respectively. From the fluorescence intensity at 360 nm, Stern-Volmer plots were constructed in accord with the Stern-Volmer equation:

$$\frac{F_0}{F_x} - 1 = K_{SV}[Q] = k_q \tau_0 [Q]$$

With F_0, F_x = fluorescence intensity at 360 nm in the absence (F_0) and presence (F_x) of quencher Q,
K_{SV}= Stern–Volmer quenching constant,
k_q= the bimolecular rate constant of the quenching reaction, and
τ_0= the average fluorescent lifetime ($\tau_0 = 5 * 10^{-9} s$ = fluorescent lifetime of the relevant tryptophan moieties in HSA)[4–6]

Modification of the Stern–Volmer equation allowed us to estimate n, the number of equivalents of quencher that bind to HSA, and K_a, the binding constant between the quencher and HSA,[4–6] as follows:

$$\log\left(\frac{F_0}{F_x} - 1\right) = n * \log[Q] + \log K_a$$

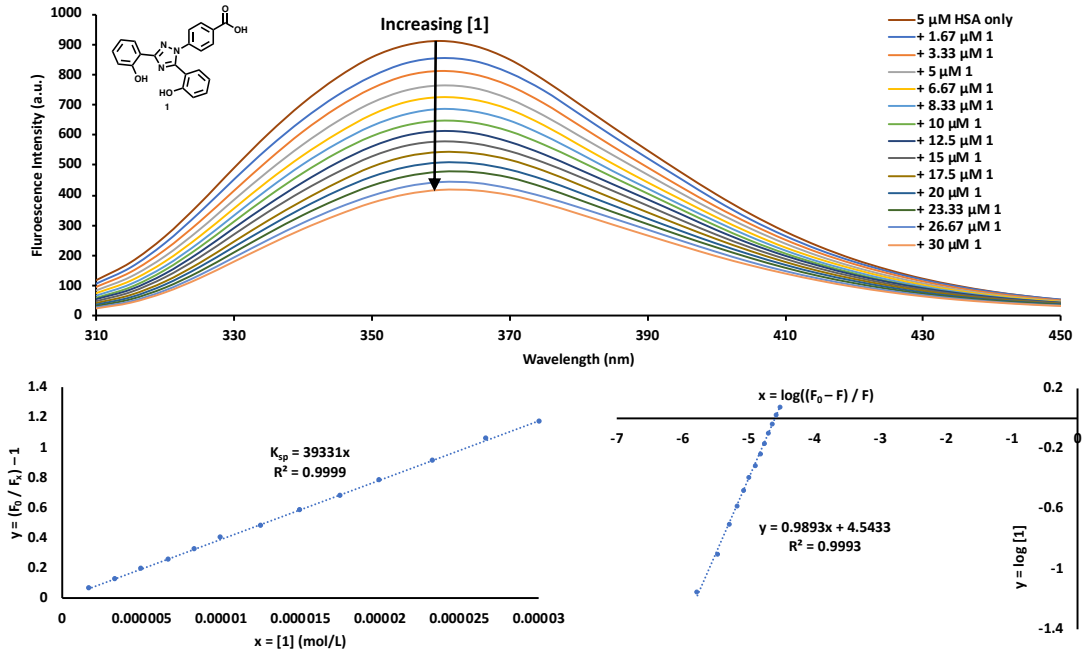


Figure S7: a) Emission profiles of 5 µM HSA titrated with **1**, b) Stern-Volmer plot: F_0, F_x = fluorescence intensity at 360 nm (emission of HSA) in the absence (F_0) and presence (F_x) of quencher **1**, c) double logarithm plot

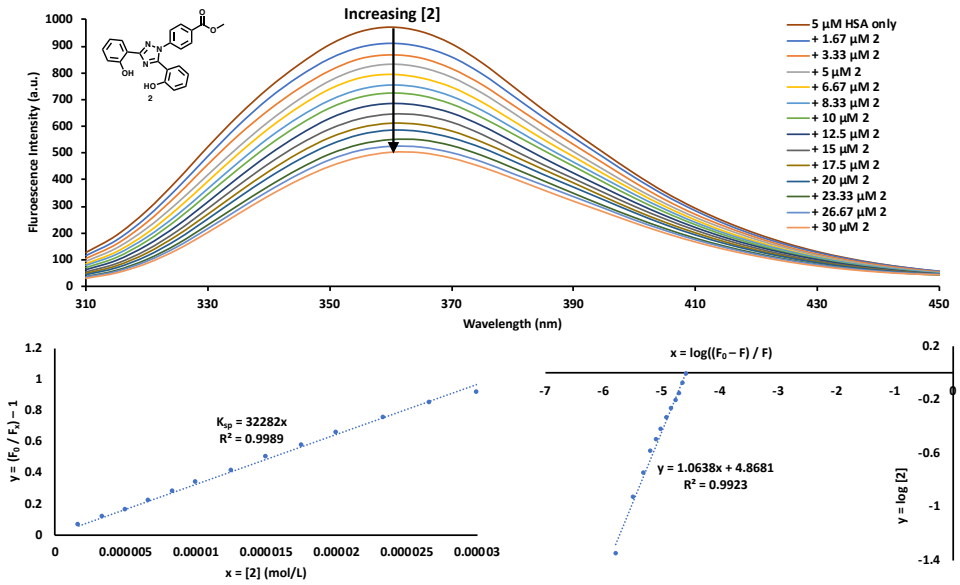


Figure S8: a) Emission profiles of 5 µM HSA titrated with **2**, b) Stern-Volmer plot: F_0, F_x = fluorescence intensity at 360 nm (emission of HSA) in the absence (F_0) and presence (F_x) of quencher **2**, c) double logarithm plot

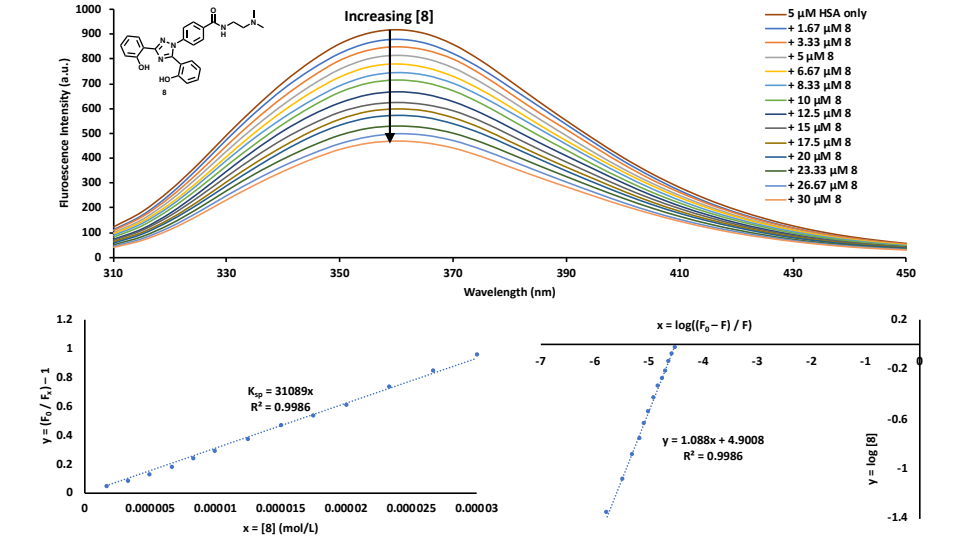


Figure S9: a) Emission profiles of 5 µM HSA titrated with **8**, b) Stern-Volmer plot: F_0, F_x = fluorescence intensity at 360 nm (emission of HSA) in the absence (F_0) and presence (F_x) of quencher **8**, c) double logarithm plot

Compound	K_{SV} (M^{-1})	k_q (M^{-1} s^{-1})	*n*	K_a (M^{-1})
1	3.93 * 10^4	7.86 * 10^{12}	0.99	3.49 * 10^4
2	3.23 * 10^4	6.46 * 10^{12}	1.06	7.38 * 10^4
8	3.11 * 10^4	6.22 * 10^{12}	1.09	7.94 * 10^4

Table S3: Summary of Stern-Volmer and double logarithm plot results. k_q for all evaluated compounds exceeded the diffusion limit of ≈6 * 10^9 M^{-1} s^{-1} and thus involvement of static quenching through binding to HSA is invoked.[4,7] For each compound, a 1:1 binding stoichiometry with HSA was inferred from the slope *n* of the double logarithm plot, which was used to generate the corresponding association constant, K_a, from the intercept of the plot.

8. UV-vis and Fluorescence Spectroscopy

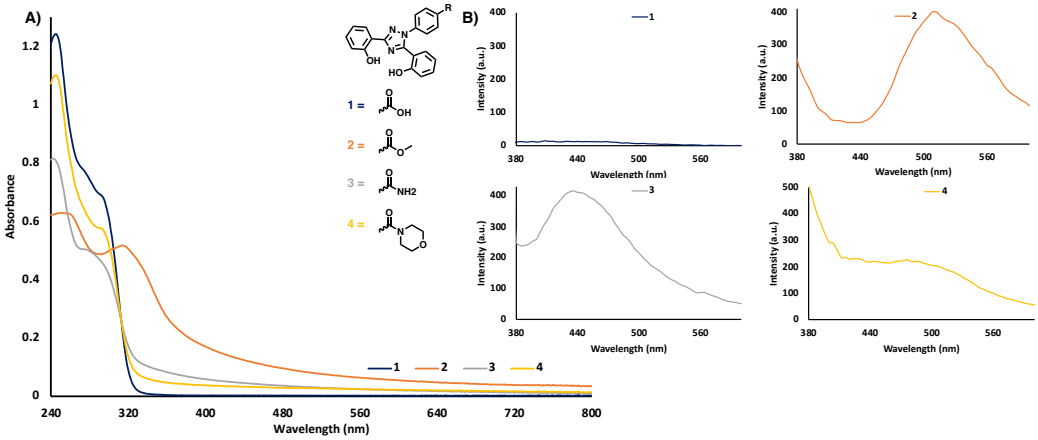


Figure S10: Spectra of **1**, **2**, **3** and **4** at 50 µM in PBS (pH = 7.2) containing <0.5% DMSO: A) UV-vis absorbance spectra B) Fluorescence emission spectra with excitation at 360 nm and a slit width of 10 nm (5 nm for **2**)

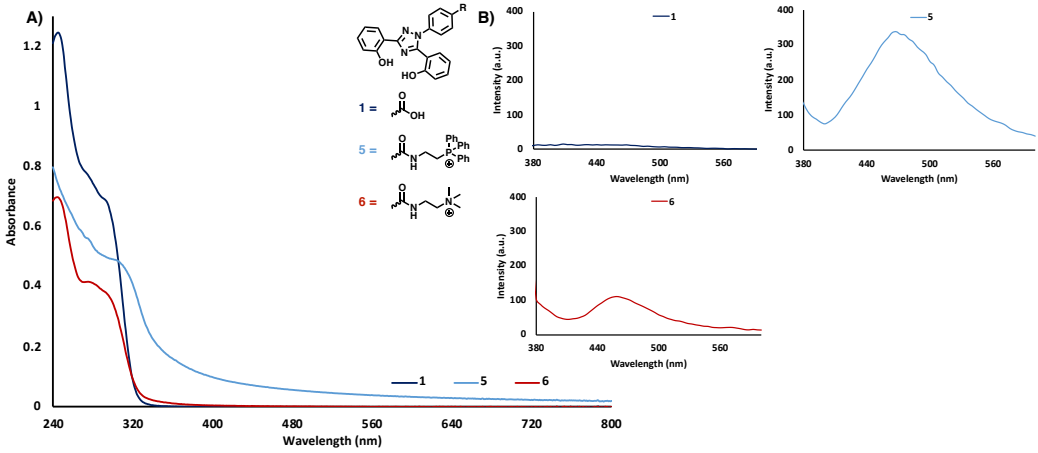


Figure S11: Spectra of **1**, **5** and **6** at 50 µM in PBS (pH = 7.2) containing <0.5% DMSO: A) UV-vis absorbance spectra B) Fluorescence emission spectra with excitation at 360 nm and a slit width of 10 nm (5 nm for **5**)

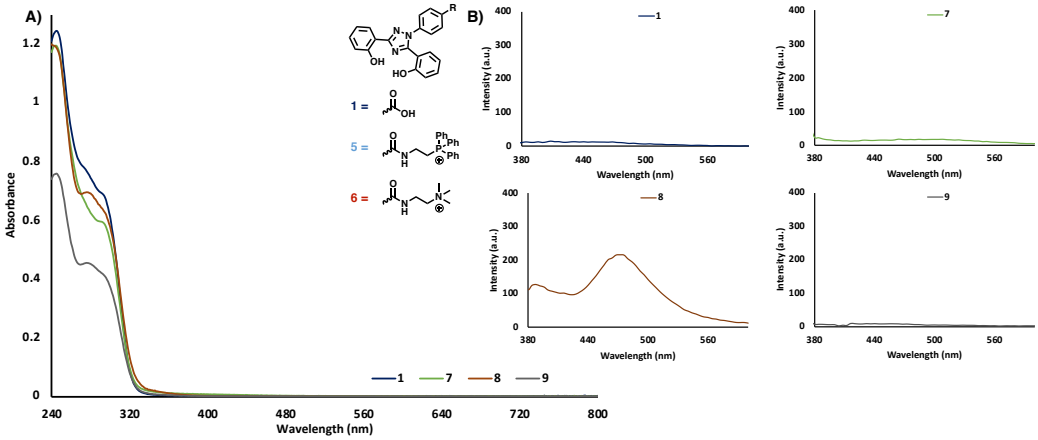


Figure S12: Spectra of **1**, **7**, **8** and **9** at 50 µM in PBS (pH = 7.2) containing <0.5% DMSO: A) UV-vis absorbance spectra B) Fluorescence emission spectra with excitation at 360 nm and a slit width of 10 nm

9. Cell Imaging

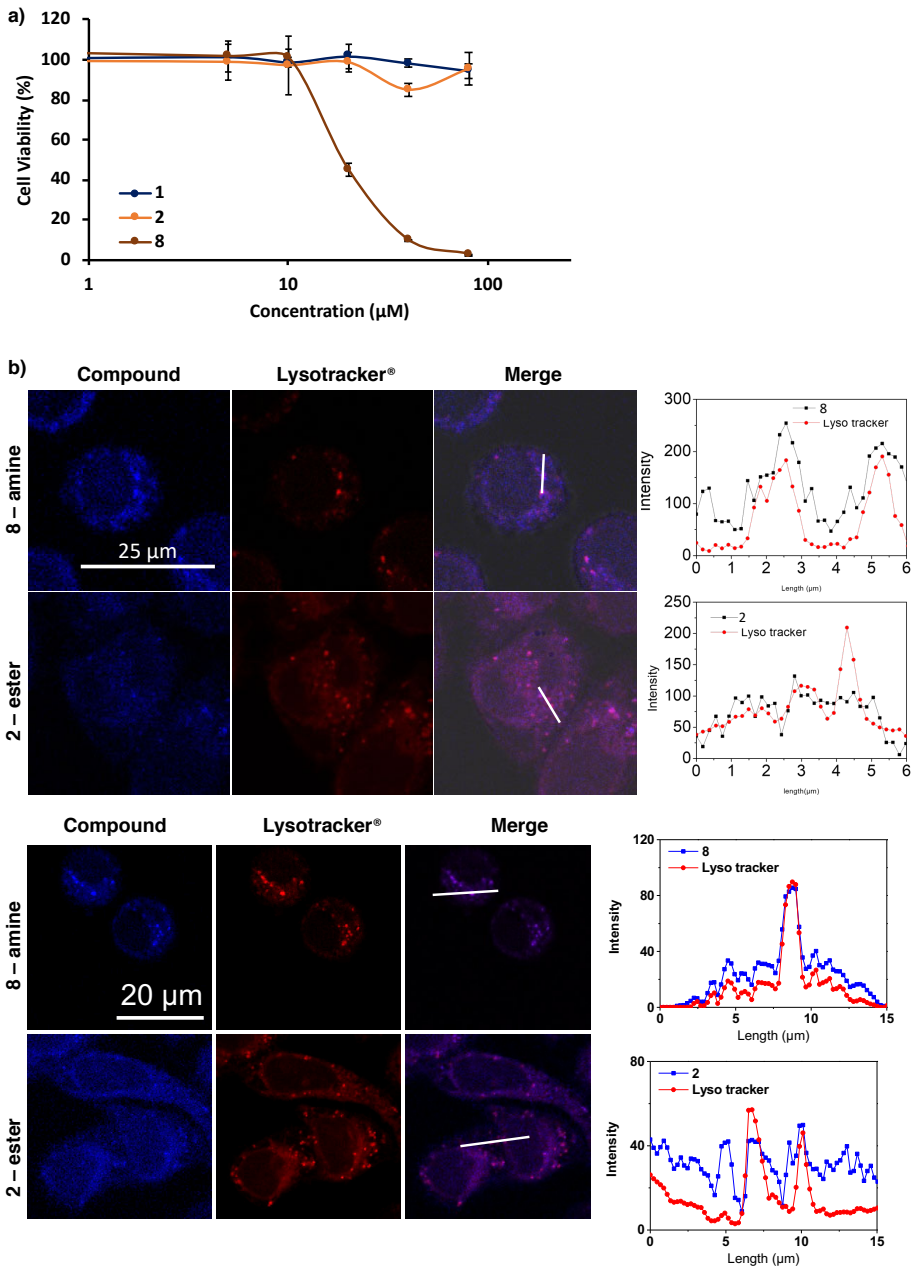


Figure S13: a) Proliferation profiles of HeLa cells 24 h after exposure to varying concentrations of **1**, **2** and **8**, respectively, as determined by MTT assay. Highest concentration = 80 µM; a 2-fold serial dilution was employed. b) Confocal microscopy imaging of **2** and **8** (20 µM) in HeLa cells. Colocalization was observed between **8** and Lysotracker® red. Blue channel: Ex/Em = 405/ 440–480 nm. Red channel: Ex/Em = 559/ 580–620 nm.

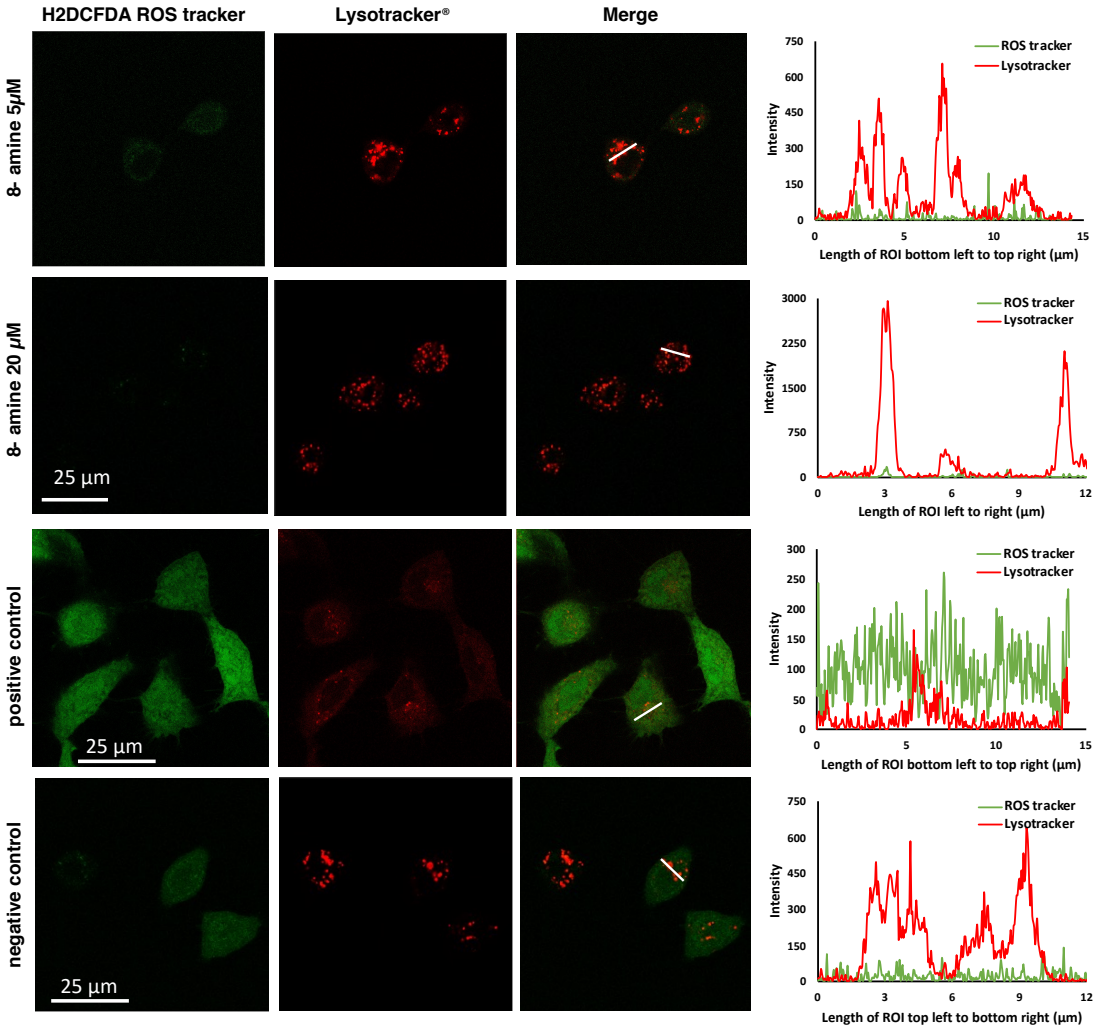


Figure S14: Confocal microscopy imaging of A549 cells stained with Lysotracker® red (100 nM) and H2DCFDA dye (2 μM) after treatment with **8** (5 μM or 20 μM, respectively), "Compound 1" developed in our lab, an established ROS inducer[7] (2.5 μM, positive control), or no prior treatment (negative control). ROS production was not observed for **8**. Green channel: Ex/Em = 488/500-550 nm. Red channel: Ex/Em = 561/573-639 nm.

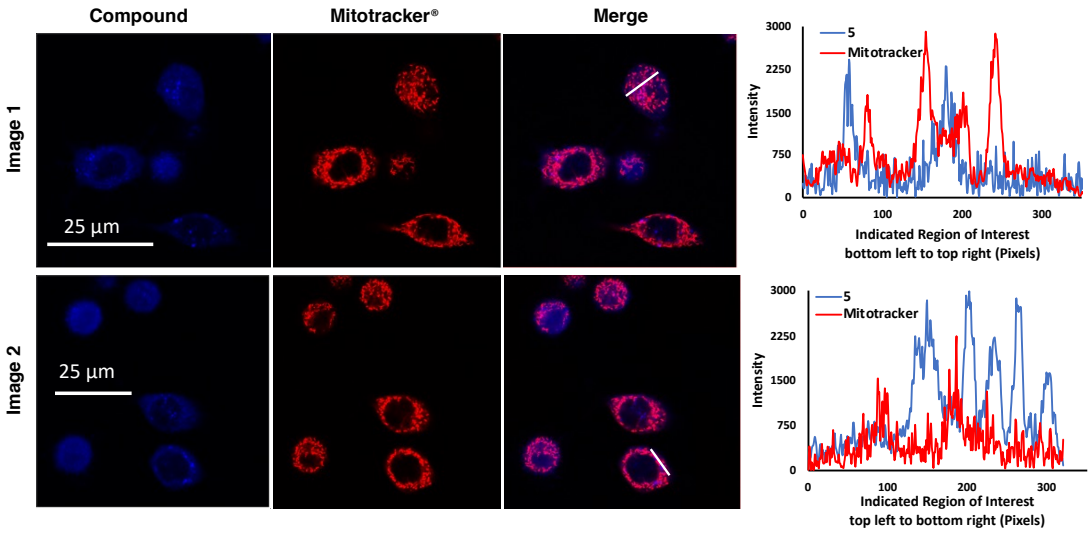


Figure S15: Confocal microscopy imaging of **5** (20 μM) in A549 cells. Colocalization was not observed between **5** and Mitotracker® red. Blue channel: Ex/Em = 405/ 500-550 nm. Red channel: Ex/Em = 561/ 573–639 nm.

10. References

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