# Mitoxantrone and Analogues Bind and Stabilise i-Motif Forming DNA Sequences

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**SUPPORTING INFORMATION for Scientific Reports**

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# 1. FRET MELTING EXPERIMENTS

The initial hits from the screen, were vetted to remove known cross-linking agents and were repeated again in both high (100 mM NaCl) and low (5 mM NaCl) salt buffer conditions to give 13 final hits which displayed a $\Delta T_m$ of at least 5°C (Fig. S1). Out of these, there were some compounds (eg. P2C5 and P9C11) which precipitated, rather than stabilised DNA so these were avoided. Out of the rest P9H8, **mitoxantrone**, offered the best stabilisation potential in both buffer conditions.

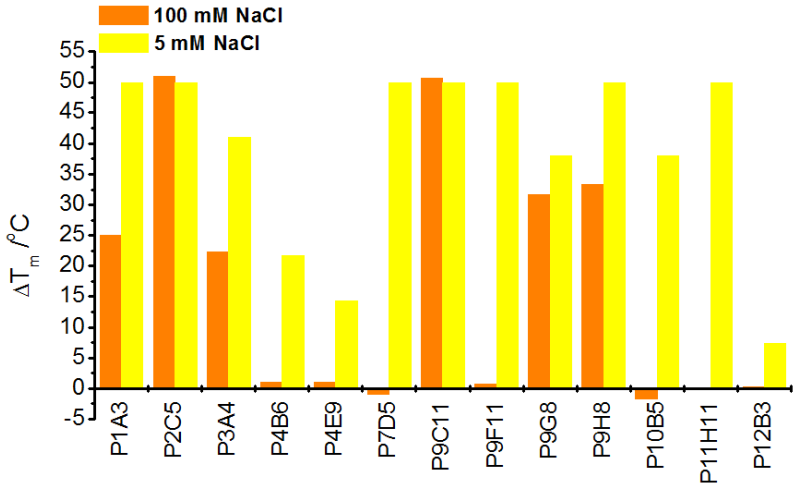



***Figure S1:*** **Comparison of the measured $\Delta T_m$ values of the 13 repeated compounds. DNA = 200 nM hTeloC$_{FRET}$, buffer = 10 mM sodium cacodylate and 100 mM or 5 mM NaCl at pH 5.5.**

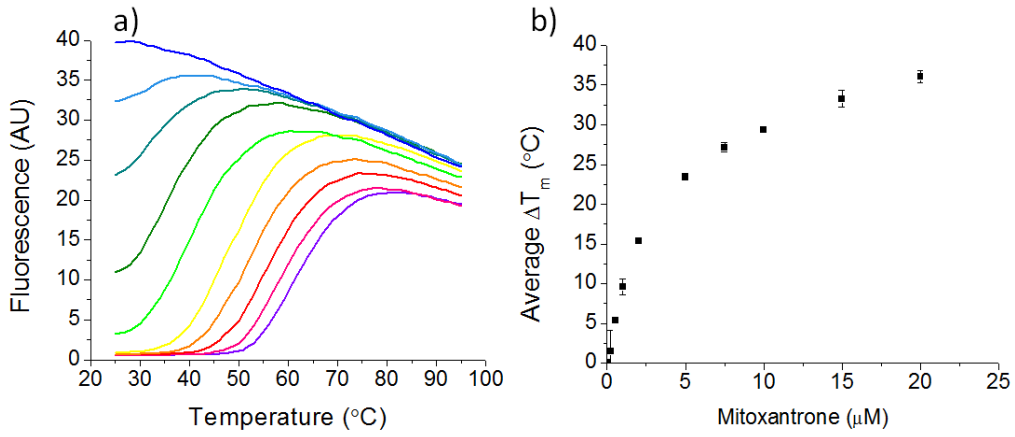



***Figure S2:*** **a) Example FRET melting curves for c-myc$_{FRET}$ (200 nM) with 0, 0.2, 0.5, 1, 2, 5, 7.5, 10 and 10 µM mitoxantrone in pH 7.4 10 mM sodium cacodylate and 100 mM NaCl. b) Plots of change in DNA melting temperature against concentration of mitoxantrone for c-myc in 10 mM sodium cacodylate at pH 7.4 with 100 mM, the error bars represent the standard deviation from three experiments.**

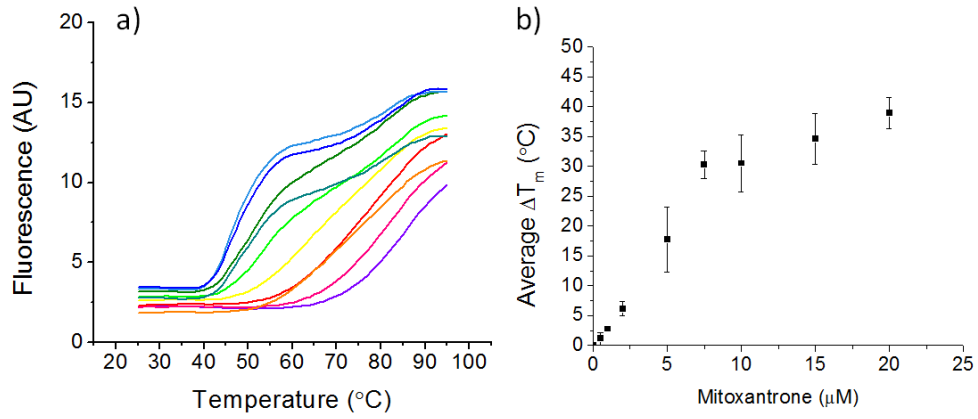



***Figure S3:*** **a) Example FRET melting curves for c-myc$_{FRET}$ (200 nM) with 0, 0.2, 0.5, 1, 2, 5, 7.5, 10 and 10 µM mitoxantrone in pH 5.5 10 mM sodium cacodylate and 100 mM NaCl. b) Plots of change in DNA melting temperature against concentration of mitoxantrone for c-myc in 10 mM sodium cacodylate at pH 5.5 with 100 mM, the error bars represent the standard deviation from three experiments.**

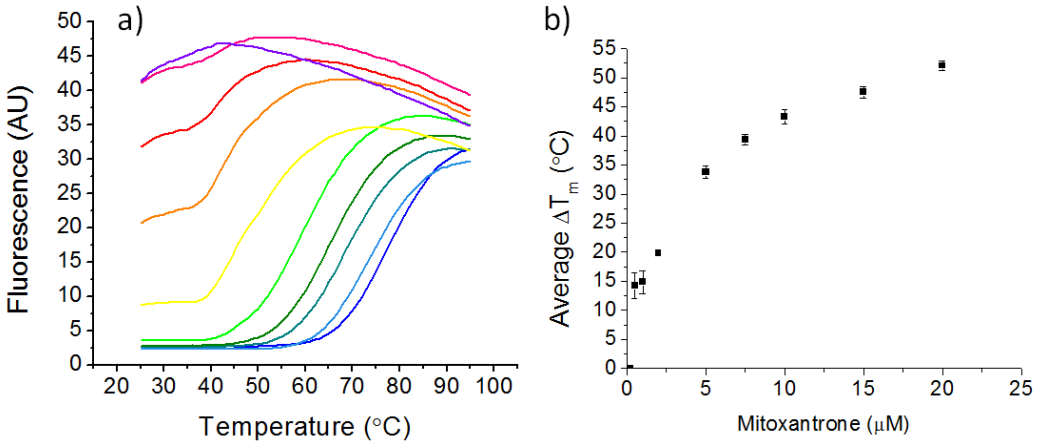



***Figure S4:*** **a) Example FRET melting curves for c-myc$_{FRET}$ (200 nM) with 0, 0.2, 0.5, 1, 2, 5, 7.5, 10 and 10 µM mitoxantrone in pH 6.6 10 mM sodium cacodylate and 100 mM NaCl. b) Plots of change in DNA melting temperature against concentration of mitoxantrone for c-myc in 10 mM sodium cacodylate at pH 6.6 with 100 mM, the error bars represent the standard deviation from three experiments.**

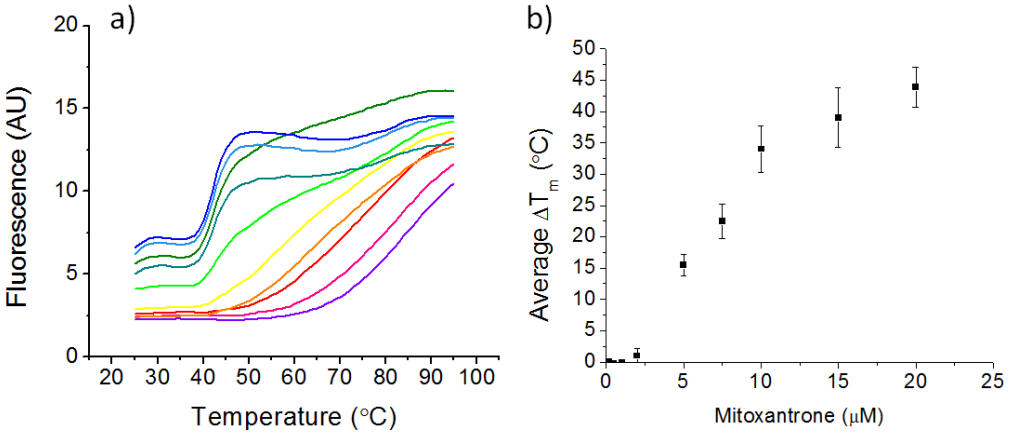



***Figure S5:*** **a) Example FRET melting curves for hTeloC$_{FRET}$ (200 nM) with 0, 0.2, 0.5, 1, 2, 5, 7.5, 10 and 10 µM mitoxantrone in pH 5.5 10 mM sodium cacodylate and 100 mM NaCl. b) Plots of change in DNA melting temperature against concentration of mitoxantrone for hTeloC in 10 mM sodium cacodylate at pH 5.5 with 100 mM, the error bars represent the standard deviation from three experiments.**

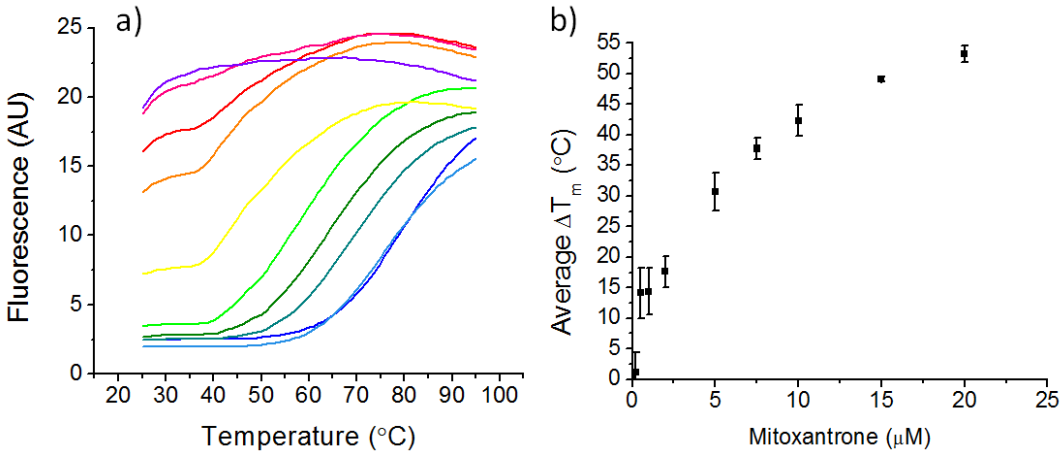



***Figure S6:*** **a) Example FRET melting curves for hTeloC$_{FRET}$ (200 nM) with 0, 0.2, 0.5, 1, 2, 5, 7.5, 10 and 10 µM mitoxantrone in pH 6.0 10 mM sodium cacodylate and 100 mM NaCl. b) Plots of change in DNA melting temperature against concentration of mitoxantrone for hTeloC in 10 mM sodium cacodylate at pH 6.0 with 100 mM, the error bars represent the standard deviation from three experiments.**

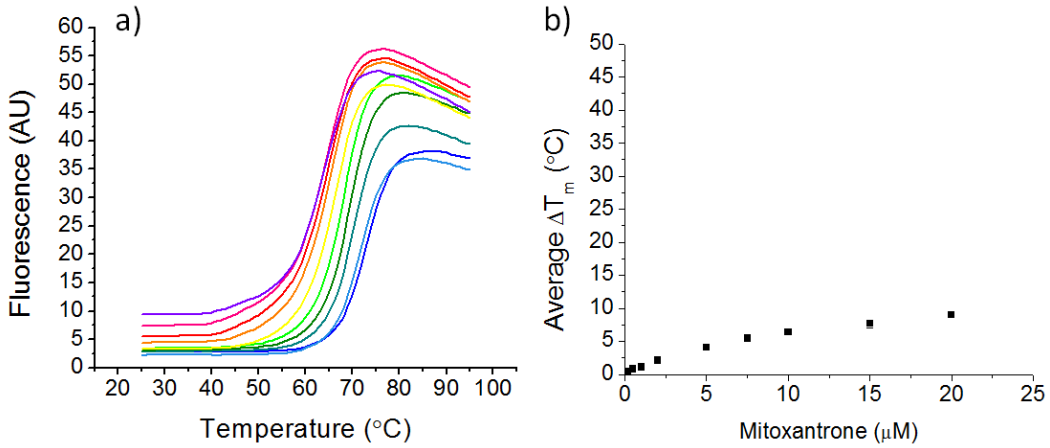



***Figure S7:*** **a) Example FRET melting curves for $DS_{FRET}$ (200 nM) with 0, 0.2, 0.5, 1, 2, 5, 7.5, 10 and 10 µM mitoxantrone in pH 7.4 10 mM sodium cacodylate and 100 mM NaCl. b) Plots of change in DNA melting temperature against concentration of mitoxantrone for DS in 10 mM sodium cacodylate at pH 7.4 with 100 mM, the error bars represent the standard deviation from three experiments.**

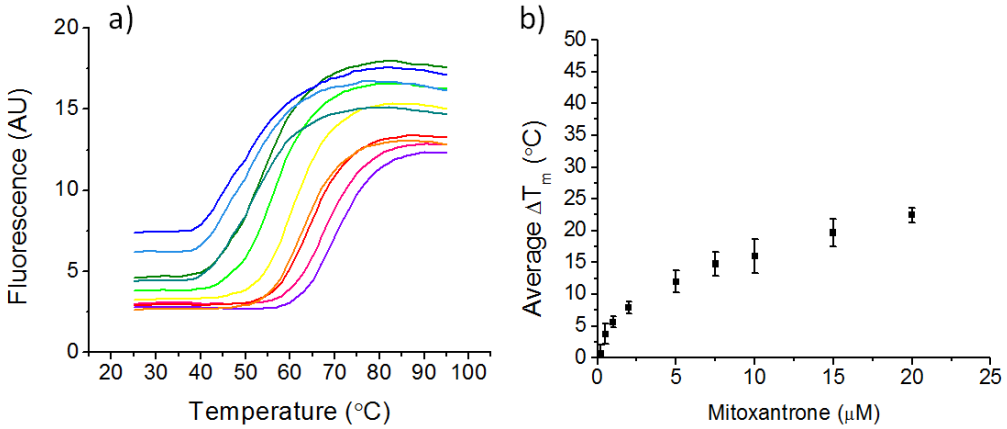



***Figure S8:*** **a) Example FRET melting curves for $hTeloG_{FRET}$ (200 nM) with 0, 0.2, 0.5, 1, 2, 5, 7.5, 10 and 10 µM mitoxantrone in pH 7.4 10 mM sodium cacodylate and 100 mM NaCl. b) Plots of change in DNA melting temperature against concentration of mitoxantrone for DS in 10 mM sodium cacodylate at pH 7.4 with 100 mM, the error bars represent the standard deviation from three experiments.**

## 2. CIRCULAR DICHROISM EXPERIMENTS

The CD spectrum of hTeloC in 10 mM sodium cacodylate and 100 mM NaCl at pH 5.5 has a negative signal at 255 nm and a positive signal at 288 nm, consistent with i-motif structure.[1] Titration of 10 µM **mitoxantrone** into the i-motif sample did not alter the signal, but further additions of ligand resulted in a decrease in the positive signal at 288 nm and a shift towards longer wavelengths (Fig. S9). The resulting signals are not consistent with any known DNA structure and are most likely to be the result of an induced CD signal, which indicates binding between the ligand and the DNA.[2]

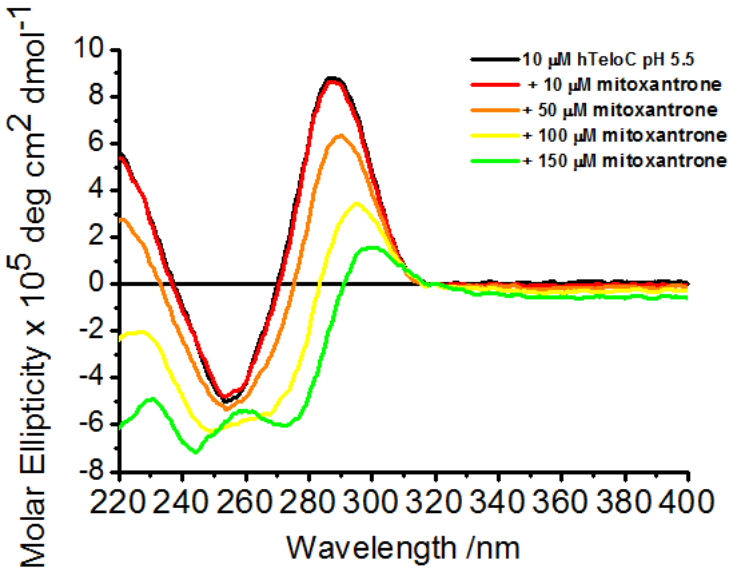



***Figure S9:*** **Example circular dichroism experiments of hTeloC (10 µM in 10 mM sodium cacodylate and 100 mM NaCl at pH 5.5) with 0-150 µM mitoxantrone.**

## 3. SURFACE PLASMON RESONANCE (SPR) EXPERIMENTS

**Table S1: Dissociation Constants ($K_d$, µM) Determined by SPR in pH 5.5 10 mM sodium cacodylate, 100 mM NaCl, 0.05% Tween-20 and 5% DMSO.**

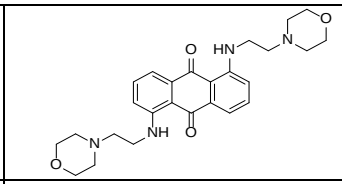
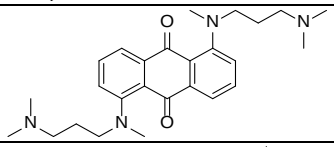
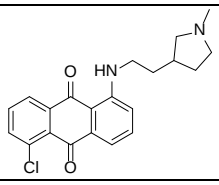
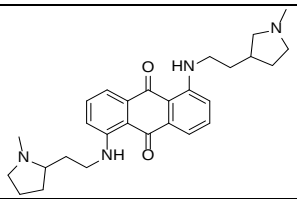
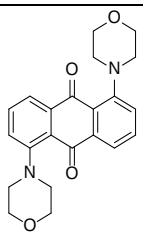
| Compound | Dissociation constant, $K_d$ (µM) | | |
|---|---|---|---|
| | hTeloC | c-myc | DS |
| **Mitoxantrone** | 12 ± 3 | 12 ± 3 | 71 ± 22 |
| **1** | 31 ± 5 | 34 ± 7 | 181 ± 90 |
| **2** | 99 ± 30 | 251 ± 70 | 15 ± 2 |
| **3** | NSB | NSB | NSB |
| **4** | 80 ± 15 | 92 ± 20 | 34 ± 12 |
| **5** | 36 ± 10 | 42 ± 12 | 33 ± 11 |
| **6** | NSB | NSB | 8.3 ± 1.4 |

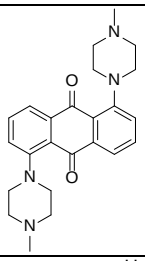
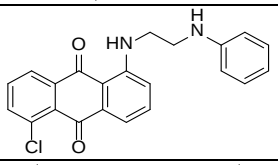
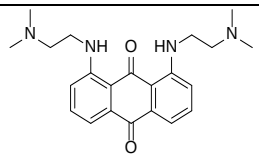
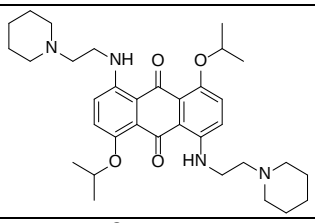
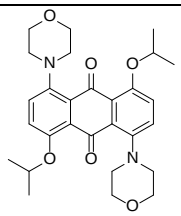
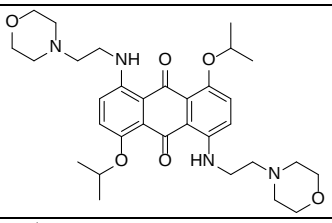
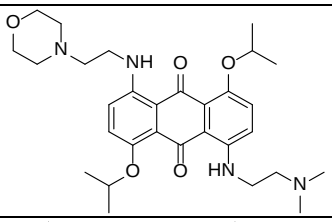
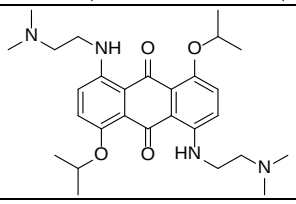
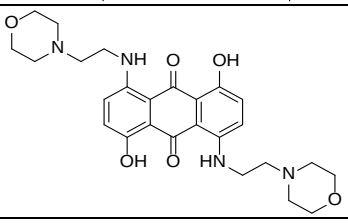
## 4.

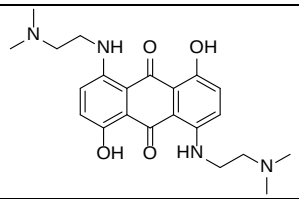
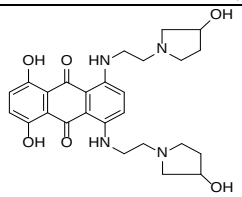
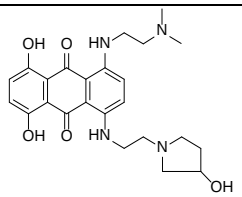
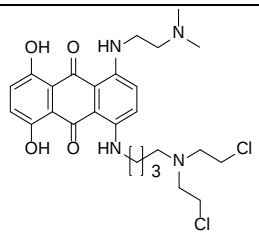
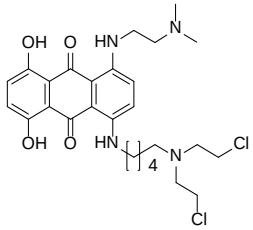
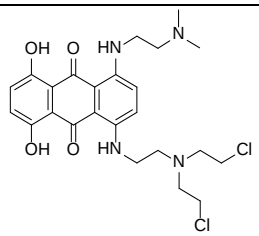
## 5. ANALOGUE LIBRARY FRET SCREEN

The screening experiments were performed once (Fig. S10). Key ligands which did not show remarkable interaction with ds DNA were repeated another two times and these are given in the main manuscript.

***Figure S10.*** **Numbering and structures of the anthraquinones, source or synthesis route, and stabilisation potentials ($\Delta T_m$) determined by FRET melting.**

| Compound | | Source/ Route | $\Delta T_m$ at 10 µM [ligand] with 0.2 µM [DNA] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | hTeloC pH 5.5 | hTeloC pH 6.0 | cMycC pH 5.5 | cMycC pH 6.6 | hTeloG pH 7.4 | DS pH 7.4 |
| **6** |  | Fig. S11 | 1.9 | 4.3 | 0.2 | -1 | 1 | -1 |
| **7** |  | Fig. S11 | -1.3 | 0 | -1.3 | -8 | 3.2 | -2.9 |
| **8** |  | Fig. S11 | 0.5 | 2.8 | 1 | 8.7 | 8.5 | 1.9 |
| **9** |  | Fig. S11 | 22.1 | 23.3 | 21.5 | 20.6 | 19 | 9.1 |
| **10** |  | Fig. S11 | 0.1 | -0.6 | -2 | -16.5 | 2 | -1.9 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **11** |  | Fig. S11 | -0.3 | 7.3 | 0.8 | -4 | 3.8 | -0.4 |
| **12** |  | Fig. S11 | -0.5 | 0 | -1.4 | -14.9 | 1.5 | -2.2 |
| **13** |  | Fig. S11 | 8.6 | 12.3 | 3 | 18.5 | 17.2 | 6.1 |
| **14** |  | Fig. S12 | -4.5 | 11 | -3.8 | 4 | -1 | -1.5 |
| **15** |  | Fig. S12 | -0.2 | -1.3 | 0.4 | -14 | 0.5 | -2.2 |
| **16** |  | Fig. S12 | -1.5 | -0.9 | -1.7 | -0.1 | 1.3 | -0.9 |
| **17** |  | Fig. S12 | -2 | -0.8 | -1.8 | -0.4 | 1 | -0.9 |
| **18** |  | Fig. S12 | 0.6 | -1.5 | -1.4 | -10 | 3.5 | -2.3 |
| **19** |  | Fig. S12 | 1.2 | -0.2 | 4.2 | 5.2 | 0.3 | -0.8 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **20** |  | Fig. S12 | 5.4 | 19 | 5.7 | 20 | 16 | 6.8 |
| **21** |  | Fig. S13 | 29 | 38.5 | 30.2 | 30.9 | 18.6 | 6.2 |
| **22** |  | Fig. S13 | 35.2 | 40.5 | 28.2 | 35.2 | 21.3 | 8.5 |
| **23** |  | Previously described[3] | >49.4 | >58 | >44.2 | >60 | >49 | >28.8 |
| **24** |  | Previously described[3] | >49.2 | >63.5 | >44.2 | >57.7 | >46.8 | >28.7 |
| **25** |  | Previously described[3] | >49.3 | >63.6 | >44.3 | >57.8 | >46.9 | >28.8 |

# 6. SYNTHETIC PROTOCOLS AND CHARACTERISATION OF MITOXANTRONE ANALOGUES

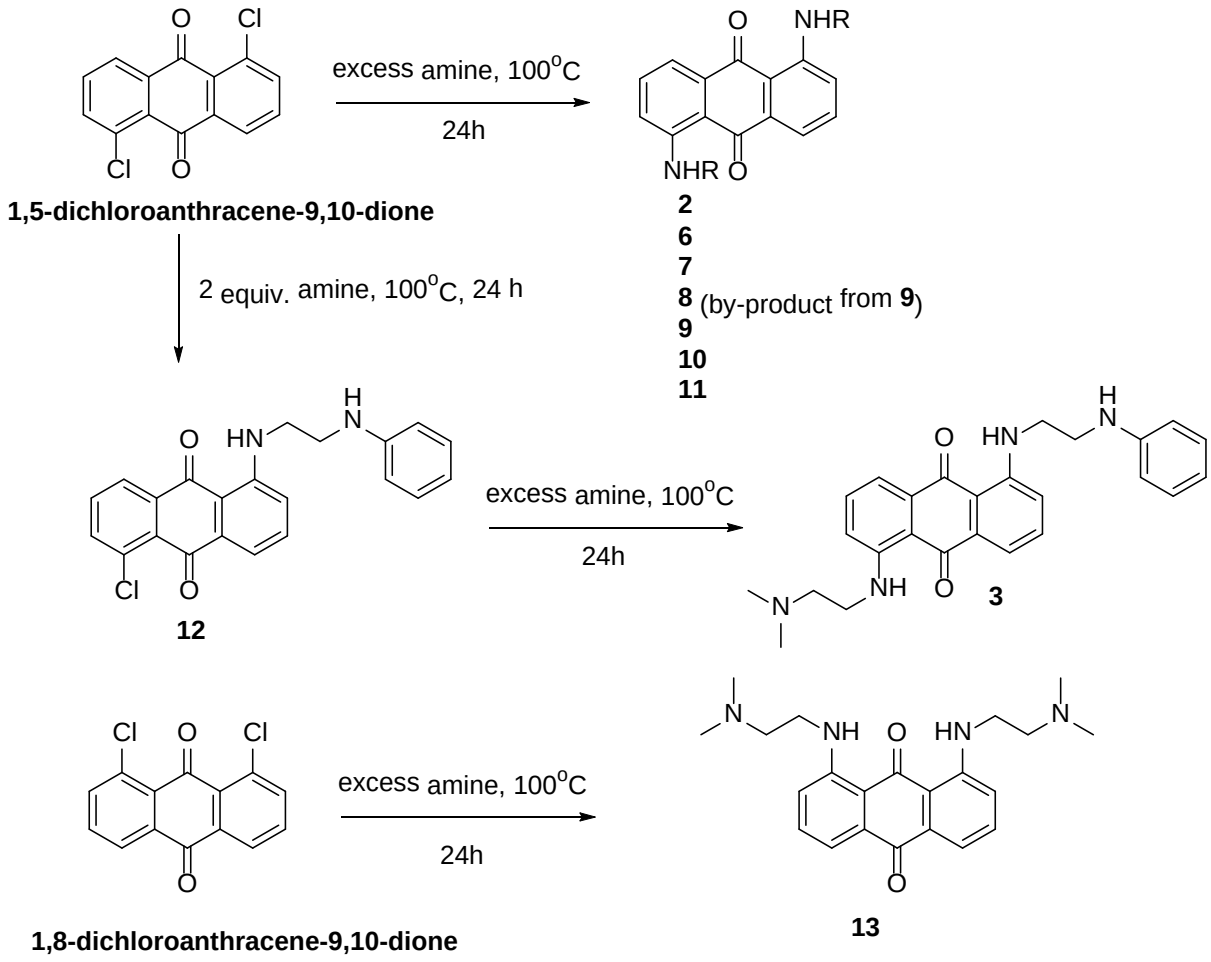
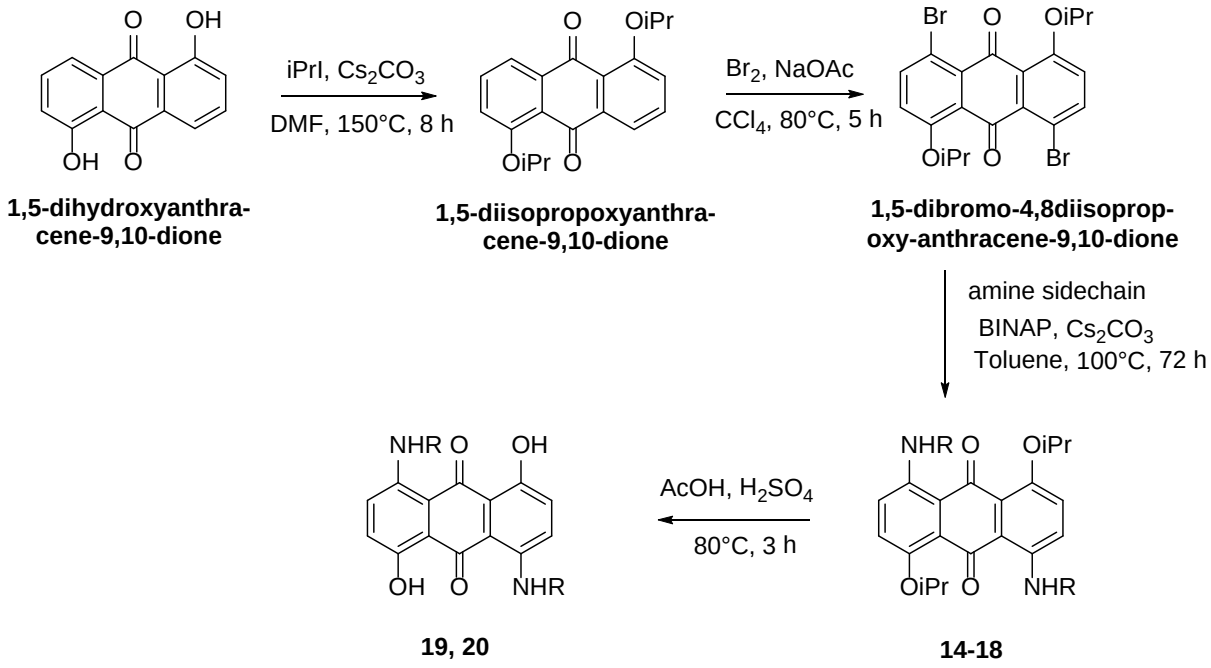



***Figure S11:*** **General routes to 1-mono and 1,5-disubstituted aminoanthracene-9,10-diones**




**1,5-dihydroxyanthracene-9,10-dione**

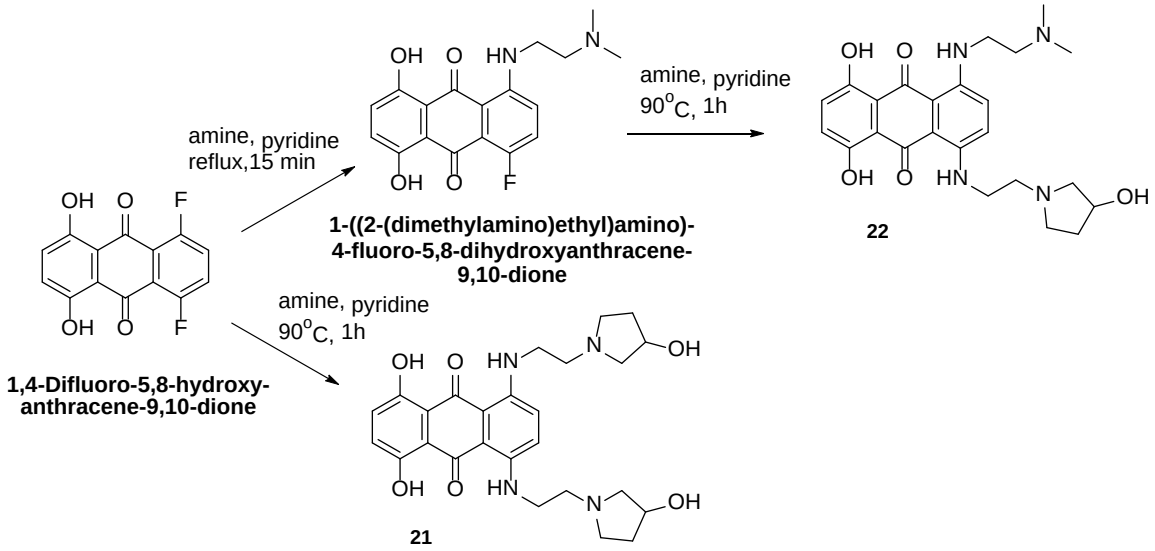
**1,5-diisopropoxyanthracene-9,10-dione**

**1,5-dibromo-4,8diisopropoxy-anthracene-9,10-dione**

**19, 20**

**14-18**

***Figure S12:*** **General route to 1,5-dihydroxylated and isopropyl-protected aminoanthracene-9,10-diones**




**1,4-Difluoro-5,8-hydroxy-anthracene-9,10-dione**

**1-((2-(dimethylamino)ethyl)amino)-4-fluoro-5,8-dihydroxyanthracene-9,10-dione**

**22**

**21**

***Figure S13:*** **General route to 1,4-disubstituted aminoanthracene-9,10-diones**

**1,5-bis((2-morpholinoethyl)amino)anthracene-9,10-dione (6)**

The method follows that of **2** using 1,5-dichloroanthracene-9,10-dione (50 mg, 0.181 mmol) and 2-morpholinoethylamine (1 mL). The product **6** was afforded as a red solid (67.9 mg, 81%).

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.84 (s, 2H, Ar-*NH*), 7.60 (d, *J* = 7.2 Hz, 2H, *ArH*), 7.54 (d, *J* = 8.4 Hz, 2H, *ArH*), 6.98 (d, *J* = 8.4 Hz, 2H, *ArH*), 3.81 (m, 8H, $CH_2OCH_2$), 3.45 (dd, *J* = 6.4 Hz, 4H, $NHCH_2CH_2$) 2.76 (t, *J* = 6.4 Hz, 4H, $NHCH_2CH_2$), 2.58 (m, 8H, $NCH_2CH_2O$); $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 186.37 (2C, *C*=O), 151.37 (2C, C1 & C6), 136.35 & 113.27, 135.17, 116.36 & 114.91, 67.06, 56.96, 53.57, 39.93; m/z 465 ([M+H]$^+$, 100%); HRMS (*m/z*): [M + H]$^+$ calcd for $C_{26}H_{32}N_4O_4$, 465.2496; found, 465.2487.

**1,5-bis((3-(dimethylamino)propyl)(methyl)amino)anthracene-9,10-dione (7)**

The method follows that of **2** using 1,5-dichloroanthracene-9,10-dione (50 mg, 0.181 mmol) and $N^1,N^1,N^2$-trimethylethane-1,2-diamine (1 mL). The title compound **7** was afforded as a red powder (48.9 mg, 62%).

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.61 (d, *J* = 7.2 Hz, 2H, *ArH*), 7.43 (m, 2H, *ArH*), 7.19 (d, *J* = 8.2 Hz, 2H, *ArH*), 3.32 (t, 4H, *J* = 7.2 Hz, $ArNCH_2CH_2CH_2$), 2.82 (s, 6H, $ArNCH_3$), 2.24 (t, 4H, *J* = 7.2 Hz, $ArNCH_2CH_2CH_2$), 2.15 (12H, s, $ArNCH_3$), 1.80 (4H, dd, *J* = 7.2 Hz, $ArNCH_2CH_2CH_2$); $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 182.28, 151.67, 138.21, 120.20, 133.14, 121.35, 117.11, 56.91, 52.78, 45.47, 42.13, 25.44; m/z 437 ([M+H]$^+$, 64%); HRMS (*m/z*): [M+H]$^+$ calcd for $C_{26}H_{36}N_4O_2$, 437.2911; found, 437.2901.

**1-chloro-5-((2-(1-methylpyrrolidin-3-yl)ethyl)amino)anthracene-9,10-dione (8)**

The method follows that of **2** using 1,5-dichloroanthracene-9,10-dione (50 mg, 0.181 mmol) and 2-(1-methylpyrrolidin-3-yl)ethylamine (1 mL). The title compound, a by-product from the synthesis of **9**, was afforded as a red powder (12.7 mg, 19%).

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.56 (s, 1H, Ar-*NH*), 8.21 (d, *J* = 7.6 Hz, 1H, *ArH*), 7.55 (m, 4H, *ArH*), 6.97 (dd, *J* = 7.2 and 4.0 Hz, 1H, *ArH*), 3.33 (m, 2H, NH*CH*$_2$CH$_2$), 3.10 (t, *J* = 8.0 Hz, 1H), 2.34 (s, 3H, N*CH*$_3$), 1.5-2.27 (m, 8H); $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 183.38, 182.59, 151.29, 137.58, 135.76, 134.46, 129.36 & 112.27, 136.43, 135.17, 133.41, 126.23, 117.21 & 115.99, 64.40, 57.10, 40.52, 40.35, 32.67, 30.62, 22.03; m/z 369 ([M+H]$^+$, 100%); HRMS (*m/z*): [M+H]$^+$ calcd for $C_{21}H_{21}ClN_2O_2$, 369.1364; found, 369.1366.

**1-((2-(1-methylpyrrolidin-2-yl)ethyl)amino)-5-((2-(1-methylpyrrolidin-3-yl)ethyl)amino)-anthracene-9,10-dione (9)**

The method follows that of **2** using 1,5-dichloroanthracene-9,10-dione (50 mg, 0.181 mmol) and 2-(1-methylpyrrolidin-3-yl)ethylamine (1 mL). The title compound **9** was afforded as a red powder (50.7 mg, 61%).

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.64 (t, *J* = 4.8 Hz, 1H, Ar-*NH*), 7.46 (m, 4H, *ArH*), 6.90 (d, *J* = 8.0 Hz, 2H, *ArH*), 3.33 (m, 4H, NH*CH*$_2$CH$_2$), 3.10 (t, *J* = 8.6 Hz, 2H), 2.31 (s, 6H, N*CH*$_3$), 2.25-1.5 (m, 16H). $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 185.45, 151.34, 136.29, 112.95, 135.24, 116.34, 114.78, 64.31, 57.16, 40.54, 40.35, 32.91, 30.68, 22.00; m/z 361 ([M+H]$^+$, 30%); HRMS (*m/z*): [M+H]$^+$ calcd for $C_{28}H_{36}N_4O_2$, 461.2911; found, 461.2908.

**1,5-dimorpholinoanthracene-9,10-dione (10)**

The method follows that of **2** using 1,5-dichloroanthracene-9,10-dione (50 mg, 0.181 mmol) and morpholine (1 mL). The product **10** was afforded as a red powder (47.8 mg, 70 %).

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.84 (d, *J* = 7.6 Hz, 2H, *ArH*), 7.57 (t, *J* = 8.0 Hz, 2H, *ArH*), 7.22 (d, *J* = 8.4 Hz, 2H, *ArH*), 3.94 (m, 8H, NCH$_2$*CH*$_2$O), 3.12 (m, 8H, N*CH*$_2$CH$_2$O); $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 181.51, 151.50, 137.63, 121.14, 133.47, 121.95, 119.95, 66.01, 51.81; m/z 379 ([M+H]+, 100%); HRMS (*m/z*): [M + H]$^+$ calcd for $C_{22}H_{22}N_2O_4$, 379.1652; found, 379.1653.

**1,5-bis(4-methylpiperazin-1-yl)anthracene-9,10-dione (11)**

The method follows that of **2** using 1,5-dichloroanthracene-9,10-dione (50 mg, 0.181 mmol) and *N*-methylpiperazine (1 mL). The title compound **11** was afforded as a red powder (48.9 mg, 67%). $^{1}$H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.82 (d, *J* = 8.8 Hz, 2H, *ArH*), 7.53 (dd, *J* = 8.0 Hz, 2H, *ArH*), 7.23 (d, *J* = 8.4 Hz, 2H, *ArH*), 3.16 (m, 8H, ArN*CH*$_2$CH$_2$), 2.68 (m, 8H, ArNCH$_2$*CH*$_2$), 2.35 (s, 6H, N*CH*$_3$); $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 182.58, 152.52, 138.63, 122.05, 134.32, 123.12, 120.60, 55.12, 52.35, 46.12; m/z 405 ([M+H]$^+$, 20%); HRMS (*m/z*): [M+H]$^+$ calcd for $C_{24}H_{28}N_4O_2$, 405.2285; found, 405.2277.

**1-chloro-5-((2-(phenylamino)ethyl)amino)anthracene-9,10-dione (12)**

The method follows that of **2** using 1,5-dichloroanthracene-9,10-dione (50 mg, 0.181 mmol), *N*$^1$-phenylethane-1,2-diamine (49.6 µL, 0.379 mmol) and pyridine (1 mL). The title compound **12** was afforded as a red powder (35.4 mg, 52%).

$^{1}$H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.68 (t, *J* = 4.8 Hz, 1H, Ar*NH*), 8.21 (d, *J* = 7.6 Hz, 1H, *ArH*), 7.65 (d, *J* = 7.2 Hz, 1H, *ArH*), 7.53 (t, *J* = 7.6 Hz, 1H, *ArH*), 7.49 (m, 2H, *ArH*), 7.14 (t, *J* = 7.6 Hz, 2H, *ArH*), 6.99 (d, *J* = 7.2 Hz, 1H, *ArH*), 6.68 (t, *J* = 7.6 Hz, 1H, *ArH*), 6.62 (d, *J* = 8.0 Hz, 2H, *ArH*), 3.88 (br, s, 1H, CH$_2$*NH*Ph), 3.56 (q, *J* = 6.0 Hz, 2H, NH*CH*$_2$CH$_2$NHPh), 3.47 (t, *J* = 6.0 Hz, 2H, NHCH$_2$*CH*$_2$NHPh); $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 183.85, 182.51, 151.41, 147.35, 137.29, 136.56, 135.85, 134.36, 133.49, 129.42, 126.30, 120.84, 119.47, 118.05, 117.19, 116.37, 113.06, 113.05, 43.11, 42.20; m/z 377 ([M+H]$^+$, 100%); HRMS (*m/z*): [M+H]$^+$ calcd for $C_{22}H_{17}ClN_2O_2$, 377.1051; found, 377.1054.

**1,8-bis((2-(dimethylamino)ethyl)amino)anthracene-9,10-dione (13)**

The method follows that of **2** using 1,8-dichloroanthracene-9,10-dione (50 mg, 0.181 mmol) and *N*,*N*-dimethylethane-1,2-diamine (1 mL). The title compound **13** was afforded as a red powder (35 mg, 51%).

$^{1}$H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.65 (s, 2H, Ar-*NH*), 7.60 (d, *J* = 7.2 Hz, 2H, *ArH*), 7.44 (m, 4H, *ArH*), 6.94 (d, *J* = 8.4 Hz, 2H, *ArH*), 3.34 (q, *J* = 6.4 Hz, 4H, NH*CH*$_2$CH$_2$) 2.61 (t, *J* = 6.4 Hz, 4H, NHCH$_2$*CH*$_2$), 2.29 (s, 12H, N-*CH*$_3$); $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 184.92, 150.98, 134.38, 134.11,

117.60, 114.86, 114.63, 58.10, 45.68, 41.19; m/z 381 ($[M+H]^+$, 100%); HRMS (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{28}N_4O_2$, 381.2285; found, 381.2287.

**1,5-diisopropoxyanthracene-9,10-dione**

To a stirred solution of 1,5-dihydroxyanthracene-9,10-dione (2.0 g, 8.326 mmol, 1 eq.) in anhydrous DMF (17 mL) was added cesium carbonate (40.69 g, 124.88 mmol, 15 eq.). The resulting mixture was stirred and heated at 150°C under $N_2$. 2-iodopropane (25 mL, 249.77 mmol, 30 eq.) was added in portions of 3 mL over 8 hours. The purple colored reaction mixture was left to stir overnight at 150°C. After cooling to room temperature, the mixture was filtered and washed with $CH_2Cl_2$ through a short pad of silica to remove the cesium carbonate. The resulting orange/brown solution was concentrated *in vacuo* to afford a brown residue, which was purified by flash column chromatography using EtOAc:PE (1:9) to yield the title compound (2.36 g, 87%) as an orange/brown solid.

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.87 (2H, dd, *J* = 7.0, 1.5 Hz, *ArH*), 7.64 (2H, t, *J* = 7.9 Hz, *ArH*), 7.25 (2H, d, *J* = 8.1 Hz, *ArH*), 4.71 (2H, m, C*H*-$(CH_3)_2$), 1.48 (12H, d, *J* = 6.0 Hz, CH-(C$H_3$)$_2$); $^{13}$C NMR (100 MHz, $CDCl_3$): δ (ppm) 182.5.1, 158.4, 137.8, 134.6, 122.1, 120.0, 119.7, 72.3, 22.1; m/z 325 ($[M+H]^+$, 100%).

**1,5-dibromo-4,8-diisopropoxyanthracene-9,10-dione**

To a stirred solution of **1,5-diisopropoxylanthracene-9,10-dione** (1.0 g, 3.08 mmol, 1 eq.) in $CCl_4$ (25 mL) was added sodium acetate (1.64 g, 20.04 mmol, 6.5 eq.). The resulting solution was heated at 80°C and bromine (2.53 mL, 49.34 mmol, 16 eq.) in $CCl_4$ (6.5 mL) was added dropwise within 30 minutes. The resulting solution was heated at 80°C for 5 hours before it was concentrated *in vacuo* to give an orange residue. The resulting solid was purified by flash column chromatography using EtOAc:PE (1:9→2:8) to yield the title compound (0.99 g, 67%) as an orange solid.

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.71 (2H, d, *J* = 9.0, *ArH*), 7.02 (2H, d, *J* = 9.0 Hz, *ArH*), 4.58 (2H, m, C*H*-$(CH_3)_2$), 1.41 (12H, d, *J* = 6.1 Hz, CH-(C*H*$_3$)$_2$); $^{13}$C NMR (100 MHz, $CDCl_3$): δ (ppm) 182.2, 156.2, 139.5, 135.9, 126.8, 120.9, 110.1, 73.5, 21.9; m/z 481/483/485 ($[M+H]^+$, 50%).

**1,5-diisopropoxy-4,8-bis((2-(piperidin-1-yl)ethyl)amino)anthracene-9,10-dione (14)**

Toluene (4 mL) was added to palladium acetate (4.4 mg, 0.019 mmol) and BINAP ((±)-2,2′-Bis(diphenylphosphino)-1,1′-binaphthalene) (15.2 mg, 0.025 mmol) under argon. The resulting solution was degassed by bubbling argon through the solution for 30 minutes. 1,5-dibromo-4,8-diisopropoxyanthracene-9,10-dione (49 mg, 0.099 mmol), cesium carbonate (128.6 mg, 0.395 mmol) and 2-(piperidin-1-yl)ethylmine (1 mL) were then added to the solution, which was stirred and heated at 100°C for 72 hours under argon. The reaction mixture was then cooled to room temperature before it was filtered through a short pad of celite to remove the palladium and the cesium carbonate. The remaining solution was concentrated *in vacuo* and the resulting residue was purified by flash column chromatography using $CH_2Cl_2$:$CH_3OH$ (95:5→90:10) to yield the title compound **14** (41.1 mg, 72%) was afforded as a dark purple solid.

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.50 (t, *J* = 4.72 Hz, 2 H, Ar-*NH*), 7.11 (d, *J* = 9.2 Hz, 2H, *ArH*), 6.83 (d, *J* = 9.2 Hz, 2H, *ArH*), 4.23 (hept, *J* = 6.0 Hz, 2H, isopropyl-*CH*), 3.31 (m, 4 H, ArNH*CH*$_2$$CH_2$), 2.60 (t, *J* = 6.8 Hz, 4H, ArNHCH$_2$*CH*$_2$), 2.42 (m, 8H, N*CH*$_2$$CH_2CH_2$), 1.57 (m, 8H, $NCH_2$*CH*$_2$$CH_2$), 1.42 (m, 4H, $NCH_2CH_2$*CH*$_2$), 1.29 (d, *J* = 6.0 Hz, 12H, isopropyl-*CH*$_3$); $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 186.11, 147.52, 146.60, 130.58, 117.16, 126.31, 114.52, 75.26, 57.64, 54.68, 40.51, 26.03, 24.42, 22.41; m/z 577 ($[M+H]^+$, 40%); HRMS (*m/z*): $[M+H]^+$ calcd for $C_{34}H_{48}N_4O_4$, 577.3748; found, 577.3738.

**1,5-diisopropoxy-4,8-dimorpholinoanthracene-9,10-dione (15)**

The method follows that of **14** using palladium acetate (6.2 mg, 0.027 mmol), BINAP ((±)-2,2′-Bis(diphenylphosphino)-1,1′-binaphthalene) (21.3 mg, 0.035 mmol), 1,5-dibromo-4,8-diisopropoxyanthracene-9,10-dione (69 mg, 0.138 mmol), cesium carbonate (180 mg, 0.553 mmol)

and morpholinoethylamine (1 mL). The title compound **15** (46.5 mg, 66%) was afforded as a dark purple solid.

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.03 (m, 4H, *ArH*), 4.42 (hept, *J* = 6.0 Hz, 2H, isopropyl-*CH*), 3.83 (m, 8H, $NCH_2CH_2O$), 3.00 (m, 8 H, $NCH_2CH_2O$), 1.29 (d, *J* = 6.0 Hz, 12H, isopropyl-$CH_3$); $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 185.73, 150.58, 144.64, 129.11, 128.53, 123.72, 122.26, 73.30, 67.11, 53.20, 22.16; m/z 413 ([M+H]$^+$, 100%); HRMS (*m/z*): [M+H]$^+$ calcd for $C_{28}H_{34}N_2O_6$, 495.2490; found, 495.2481.

**1,5-diisopropoxy-4,8-bis((2-morpholinoethyl)amino)anthracene-9,10-dione (16)**

The method follows that of **14** using palladium acetate (9.3 mg, 0.041 mmol), BINAP ((±)-2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene) (32 mg, 0.052 mmol), 1,5-dibromo-4,8-diisopropoxyanthracene-9,10-dione (103 mg, 0.207 mmol), cesium carbonate (270 mg, 0.829 mmol) and morpholino (1 mL). The title compound **16** (61 mg, 61%) was afforded as a dark purple solid.

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.65 (t, *J* = 4.4 Hz, 2H, Ar-*NH*), 7.21 (d, *J* = 9.2 Hz, 2H, *ArH*), 6.91 (d, *J* = 9.2 Hz, 2H, *ArH*), 4.33 (hept, *J* = 6.0 Hz, 2H, isopropyl-*CH*), 3.81 (m, 8H, $CH_2OCH_2$), 3.40 (q, *J* = 6.8 Hz, 4H, $NHCH_2CH_2$) 2.74 (t, *J* = 6.8 Hz, 4H, $NHCH_2CH_2$), 2.58 (m, 8H, $CH_2CH_2OCH_2CH_2$), 1.38 (d, *J* = 6.0 Hz, 12H, isopropyl-$CH_3$). $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 185.48, 147.00, 145.78, 129.81, 116.54, 125.62, 114.01, 74.55, 66.42, 56.43, 52.91, 39.27, 21.83; m/z 581 ([M+H]$^+$, 52%); HRMS (*m/z*): [M+H]$^+$ calcd for $C_{32}H_{44}N_4O_6$, 581.3334; found, 581.3328.

**1-((2-(dimethylamino)ethyl)amino)-4,8-diisopropoxy-5-((2-morpholinoethyl)amino)anthracene-9,10-dione (17)**

The method is described under **16** and is a by-product, which was afforded as a dark purple solid (15.6 mg, 14 %).

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.56 (t, *J* = 4.8 Hz, 1H, Ar*NH*), 9.49 (t, *J* = 4.8 Hz, 1H, Ar*NH*), 7.13 (d, *J* = 9.6 Hz, 1H, *ArH*), 7.12 (d, *J* = 9.6 Hz, 1H, *ArH*), 6.84 (t, *J* = 9.6 Hz, 1H, *ArH*), 6.82 (d, *J* = 9.6 Hz, 1H, *ArH*), 4.33 (m, 2H, isopropyl-*CH*), 3.72 (m, 8H, $CH_2OCH_2$), 3.31 (m, 4H, $NHCH_2CH_2$), 2.65 (t, *J* = 6.8

Hz, 2H, $NHCH_2CH_2$), 2.59 (t, *J* = 6.8 Hz, 2H, $NHCH_2CH_2$), 2.49 (m, 4H, $CH_2CH_2OCH_2CH_2$), 2.28 (s, 6H, $NCH_3$), 1.30 (d, *J* = 6.0 Hz, 6H, isopropyl-$CH_3$), 1.28 (d, *J* = 6.0 Hz, 6H, isopropyl-$CH_3$). $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 186.20, 186.09, 147.00, 146.53, 146.45, 130.61, 130.44, 117.18, 117.11, 126.36, 126.17, 114.62, 114.58, 75.49, 75.14, 67.06, 58.10, 57.06, 53.54, 45.56, 41.11, 39.90, 22.45, 22.35; m/z 539 ($[M+H]^+$, 30%); HRMS (*m/z*): $[M+H]^+$ calcd for $C_{30}H_{42}N_4O_5$, 539.3228; found, 539.3218.

**1,5-bis((2-(dimethylamino)ethyl)amino)-4,8-diisopropoxyanthracene-9,10-dione (18)**

The method follows that of **14** using palladium acetate (3.72 mg, 0.016 mmol), BINAP ((±)-2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene) (12.8 mg, 0.021 mmol), 1,5-dibromo-4,8-diisopropoxyanthracene-9,10-dione (41.4 mg, 0.083 mmol), cesium carbonate (108 mg, 0.332 mmol) and *N*,*N*-dimethylethane-1,2-diamine (1 mL). The title compound **19** (32.6 mg, 79%) was afforded as a dark purple solid.

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.48 (t, *J* = 4.8 Hz, 2 H, *Ar-NH*), 7.12 (d, *J* = 9.2Hz, 2H, *ArH*), 6.83 (d, *J* = 9.2 Hz, 2H, *ArH*), 4.21 (hept, *J* = 6.0 Hz, 2H, isopropyl-*CH*), 3.31 (q, *J* = 6.8 Hz, 4 H, $NHCH_2CH_2$), 2.58 (t, *J* = 6.8 Hz, 4H, $NHCH_2CH_2$), 2.28 (s, 12H, $NCH_3$), 1.28 (d, *J* = 6.0 Hz, 12H, isopropyl-$CH_3$); $^{13}$C NMR (101 MHz, $CDCl_3$): δ (ppm) 186.19, 147.69, 146.56, 126.27, 114.58, 130.57, 117.13, 75.44, 58.11, 45.57, 41.12, 22.35; m/z 497 ($[M+H]^+$, 40%); HRMS (*m/z*): $[M+H]^+$ calcd for $C_{28}H_{40}N_4O_4$, 497.3122; found, 497.3119.

**1,5-dihydroxy-4,8-bis((2-morpholinoethyl)amino)anthracene-9,10-dione (19)**:

To a stirred solution of **14** (75 mg, 0.129 mmol) in acetic acid (25 mL) was dropwise added sulphuric acid (0.5 mL) over a period of 10 min. The resulting mixture was stirred at 80°C for 3 hours before it was allowed to cool to room temperature and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel using $CH_2Cl_2$:$CH_3OH$ (95:5→90:10 + 0.1% conc. $NH_3$ ammonia) to yield the title compound **19** (48 mg, 75%) as a dark blue solid.

$^1$H NMR (400 MHz, $CDCl_3$): δ (ppm) 14.01 (s, 2H, Ar-*OH*), 9.88 (t, *J* = 4.6 Hz, 2H, Ar*NH*), 7.15 (d, *J* = 9.6 Hz, 2H, *ArH*), 6.99 (d, *J* = 9.6 Hz, 2H, *ArH*), 3.71 (m, 8H, $CH_2OCH_2$), 3.39 (q, *J* = 6.4 Hz, 4H, $NHCH_2CH_2$)

2.67 (t, *J* = 6.4 Hz, 4H, $NHCH_2\mathit{CH_2}$), 2.49 (m, 8H, $\mathit{CH_2}CH_2OCH_2\mathit{CH_2}$). $^{13}C$ NMR (101 MHz, $CDCl_3$): δ (ppm) 186.69, 155.19, 146.87, 128.76, 115.21, 120.99, 109.66, 67.09, 56.83, 53.46, 39.94; m/z 497 ($[M+H]^+$, 100%); HRMS (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{32}N_4O_6$, 497.2395; found, 497.2388.

**1,5-bis((2-(dimethylamino)ethyl)amino)-4,8-dihydroxyanthracene-9,10-dione (20)**

The method follows that of **14** using **18** (25 mg, 0.050 mmol), acetic acid (20 mL) and sulphuric acid (0.5 mL). The product **20** was afforded as a dark blue powder (11.3 mg, 55 %).

$^1H$ NMR (400 MHz, $CDCl_3$): δ (ppm) 13.95 (s, 2H, Ar*OH*), 9.74 (t, *J* = 4.4 Hz, 2H, Ar*NH*), 7.14 (d, *J* = 9.2 Hz, 2H, *ArH*), 6.98 (d, *J* = 9.2 Hz, 2H, *ArH*), 3.37 (q, *J* = 6.4 Hz, 4H, $NH\mathit{CH_2}CH_2$), 2.59 (t, *J* = 6.4 Hz, 4H, $NHCH_2\mathit{CH_2}$), 2.27 (s, 12H, $N\mathit{CH_3}$); $^{13}C$ NMR (101 MHz, $CDCl_3$): δ (ppm) 186.64 (2C, *C*=O), 155.11 (2C, C4 & C9), 147.00 (2C, C1 & C6), 128.76 & 120.82 (4C, C2, C3, C7 & C8), 115.18 & 109.57 (4C, C11, C12, C13 & C14), 58.21 (2C, $NH\mathit{CH_2}CH_2$), 45.64 (4C, $N\mathit{CH_3}$), 41.16 (2C, $NHCH_2\mathit{CH_2}$); m/z 413 ($[M+H]^+$, 100%); HRMS (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{28}N_4O_4$, 413.2183; found, 413.2183.

**1,4-Bis-([2-(3-hydroxypyrrolidine)ethyl]amino)-5,8-dihydroxy-anthracene-9,10-dione (21)**

1,4-Difluoro-5,8-hydroxyanthracene-9,10-dione (75 mg, 0.272 mmol) and 1-(2-aminoethyl)-pyrrolidin-3-ol (1 g, 7.7 mmol) were stirred in pyridine (2 mL) at 90 °C for 1 h. The reaction mixture was added to ice-cold brine and set aside at 4°C overnight. The precipitated solid was isolated by filtration and lyophilised. The desired product was purified by flash chromatography, initially eluting with $CH_2Cl_2/CH_3OH$ (95:5) to remove non-polar impurities, followed by a gradual increase of $CH_2Cl_2$ to $CH_2Cl_2/CH_3OH$ (85:15). The chromatographed product was then crystallised from $CHCl_3$ affording the title compound **21** was afforded as a dark blue powder (59.2 mg, 44%) as a dark blue powder (0.11 g, 34%).

$^1H$ NMR (270 MHz, DMSO/$CDCl_3$(1:1): δ (ppm) 13.55 (s, 2H, ArO*H*), 10.55 (t, *J* = 4.4 Hz, 2H, ArN*H*), 7.3 (m, 2H, Ar*H*), 7.05 (s, 2H, Ar*H*), 4.10 (m, 2H, $CH_2C\mathit{H}OH$), 3.60 (q, *J* = 7.0 Hz 4H, $HNC\mathit{H_2}CH_2N$), 2.80 (t, q, *J* = 7.0 Hz 4H, $HNCH_2C\mathit{H_2}N$), 2.75 (m, 10H, ring-*H* and O*H*), 2.05 (m, 2H, ring-*H*), 1.60 (m, 2H, ring-*H*); $^{13}C$ NMR (62.9 MHz, DMSO/$CDCl_3$(1:1): δ (ppm) 183.21, 154.38, 146.43, 124.90, 123.79,

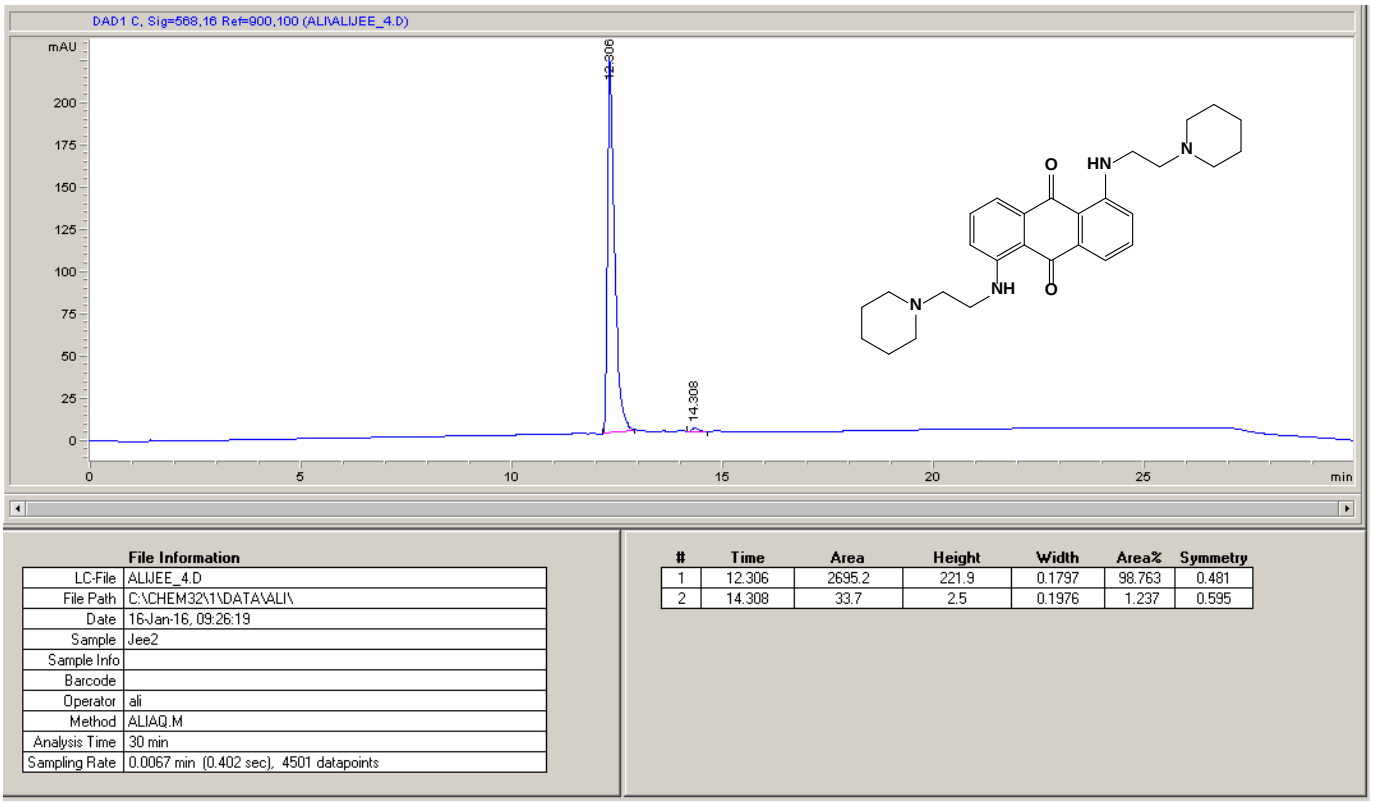
115.04, 107.01, 69.26, 62.33, 54.42, 52.18, 41.22, 34.73, 28.01; m/z 497 ([M+H]$^+$, 100%); Anal. Calcd for $C_{26}H_{32}N_4O_6$: C, 62.88; H, 6.51; N, 11.28. Found: C, 62.50; H, 6.54; N, 11.00.

**1-([(2-Dimethylamino)ethyl]amino)-4-([2-(3-hydroxypyrrolidine)ethyl]amino)-5,8-dihydroxy-anthracene-9,10-dione (22)**

The method follows that of **21** using 1-(2-Dimethylamino)ethylamino-4-fluoro-5,8-dihydroxy-anthracene-9,10-dione (45 mg, 0.13 mmol), 1-(2-aminoethyl)-pyrrolidin-3-ol (880 mg, 6.77 mmol), pyridine (1 mL). The product **22** was afforded as a dark blue powder (24 mg, 41 %)

$^1$H NMR (270 MHz, DMSO/$CDCl_3$(1:1): δ (ppm) 13.5 (s, 2H, ArO*H*) 10.5 (t, *J* = 4.4 Hz, 2H, ArN*H*), 7.4 (m, 2H, Ar*H*), 7.1 (s, 2H, Ar*H*), 4.25 (m, 1H, $CH_2CHOH$), 3.53 (q, *J* = 6.8 Hz, 4H, $HNCH_2CH_2N$), 2.75 (m, 2H, ring-*H* and O*H*), 2.55 (t, *J* = 6.8 Hz, 4H, HNCH2C$H_2$N), 2.38 (m, 1H, ring-*H*), 2.3 (s, 6H, $NCH_3$), 2.00 (m, 2H, ring-*H*), 1.62 (m, 1H, ring-*H*); $^{13}$C NMR (62.9 MHz, DMSO/$CDCl_3$(1:1): δ (ppm) 183.16, 154.38, 146.90, 124.90, 123.79, 114.78, 107.18, 69.26, 62.33, 54.42, 52.18, 41.22, 34.19; m/z 455 ([M+H]$^+$, 100%).

## 7. HPLC TRACES




**File Information**

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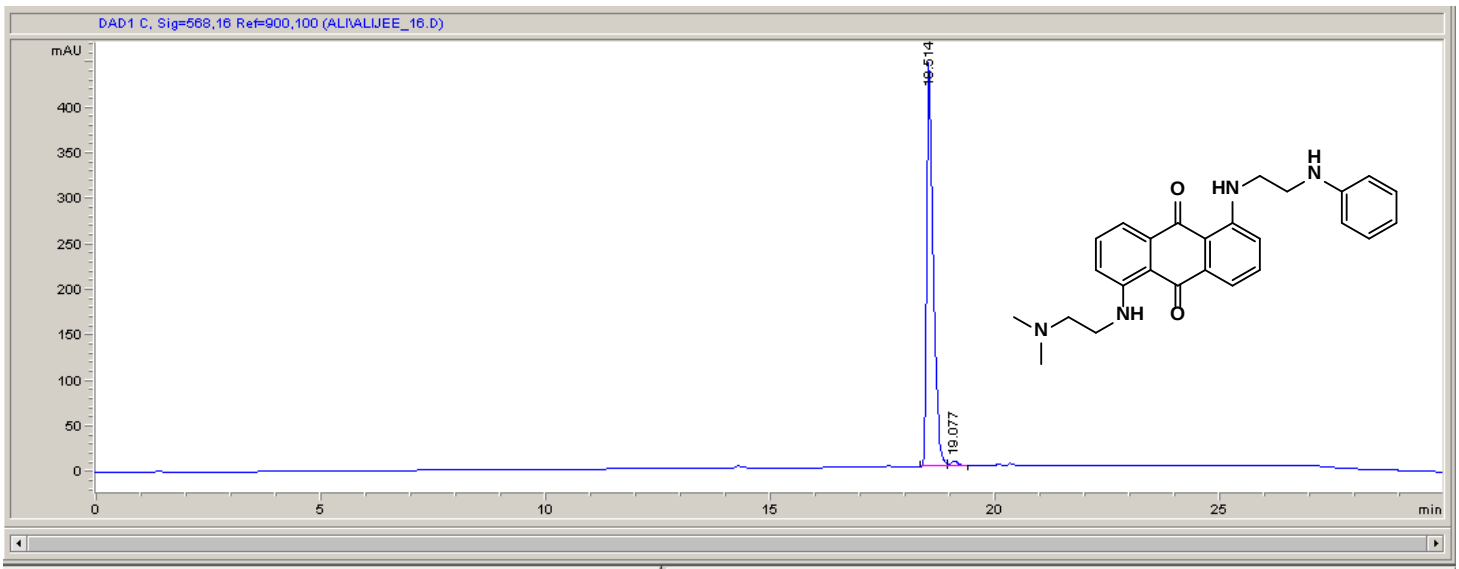
| Sample | Jee2 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 12.306 | 2695.2 | 221.9 | 0.1797 | 98.763 | 0.481 |
| 2 | 14.308 | 33.7 | 2.5 | 0.1976 | 1.237 | 0.595 |

**Compound 2**

## Compound 3




**File Information**

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|---|---|
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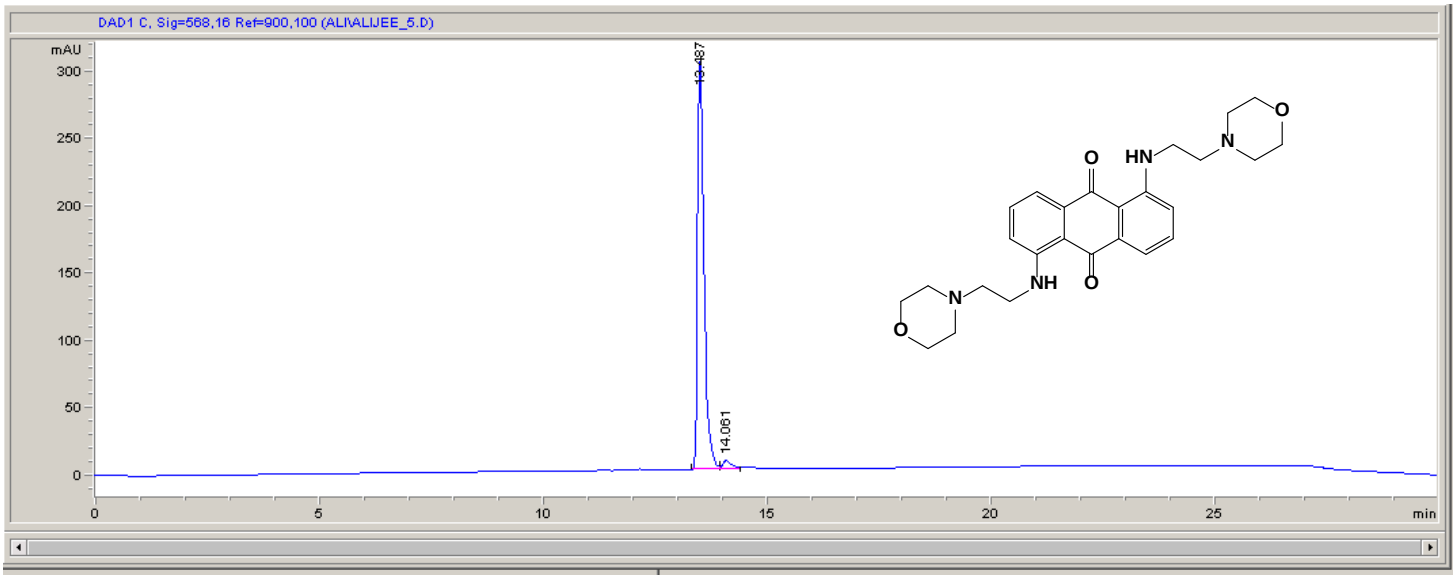
| Sample | Jee25 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 18.514 | 4656.8 | 444.1 | 0.1603 | 98.559 | 0.52 |
| 2 | 19.077 | 68.1 | 6.1 | 0.156 | 1.441 | 0.675 |

## Compound 6




**File Information**

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|---|---|
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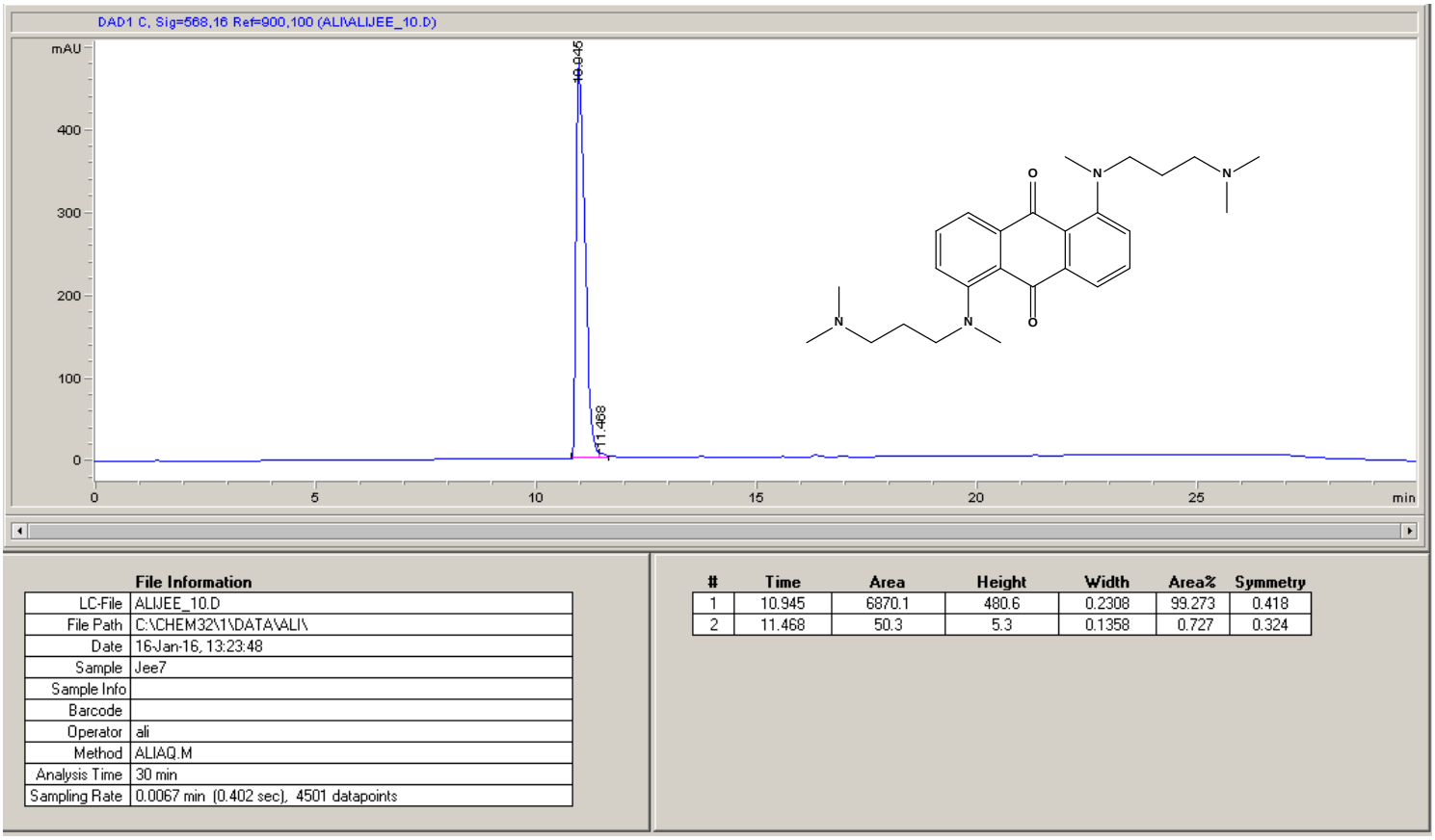
| Sample | Jee3 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 13.487 | 3010.7 | 302.8 | 0.148 | 97.115 | 0.621 |
| 2 | 14.061 | 89.4 | 6.9 | 0.1843 | 2.885 | 0.538 |

## Compound 7




| File Information | |
|---|---|
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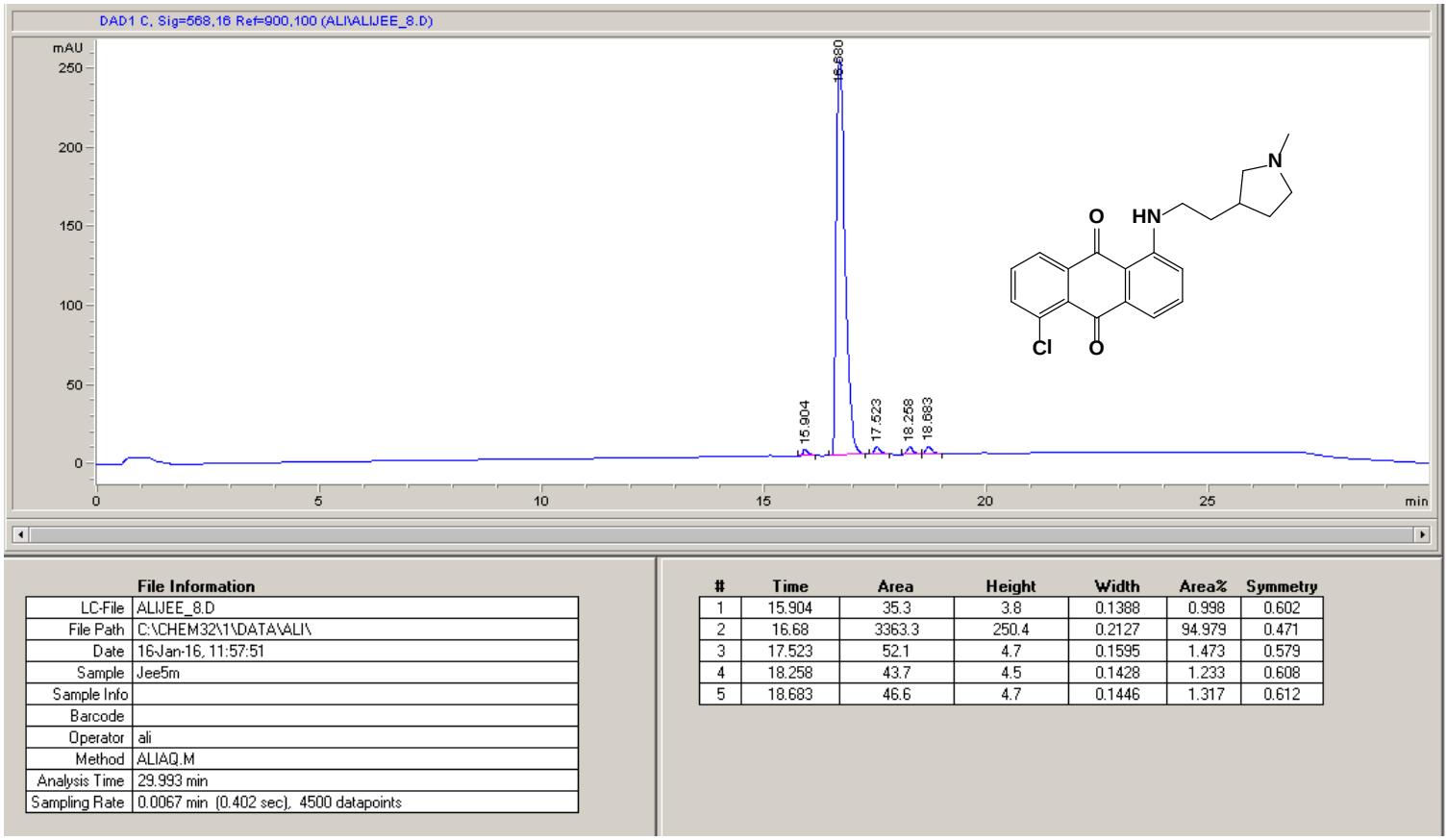
| Date | 16-Jan-16, 13:23:48 |
| Sample | Jee7 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 10.945 | 6870.1 | 480.6 | 0.2308 | 99.273 | 0.418 |
| 2 | 11.468 | 50.3 | 5.3 | 0.1358 | 0.727 | 0.324 |

## Compound 8




| File Information | |
|---|---|
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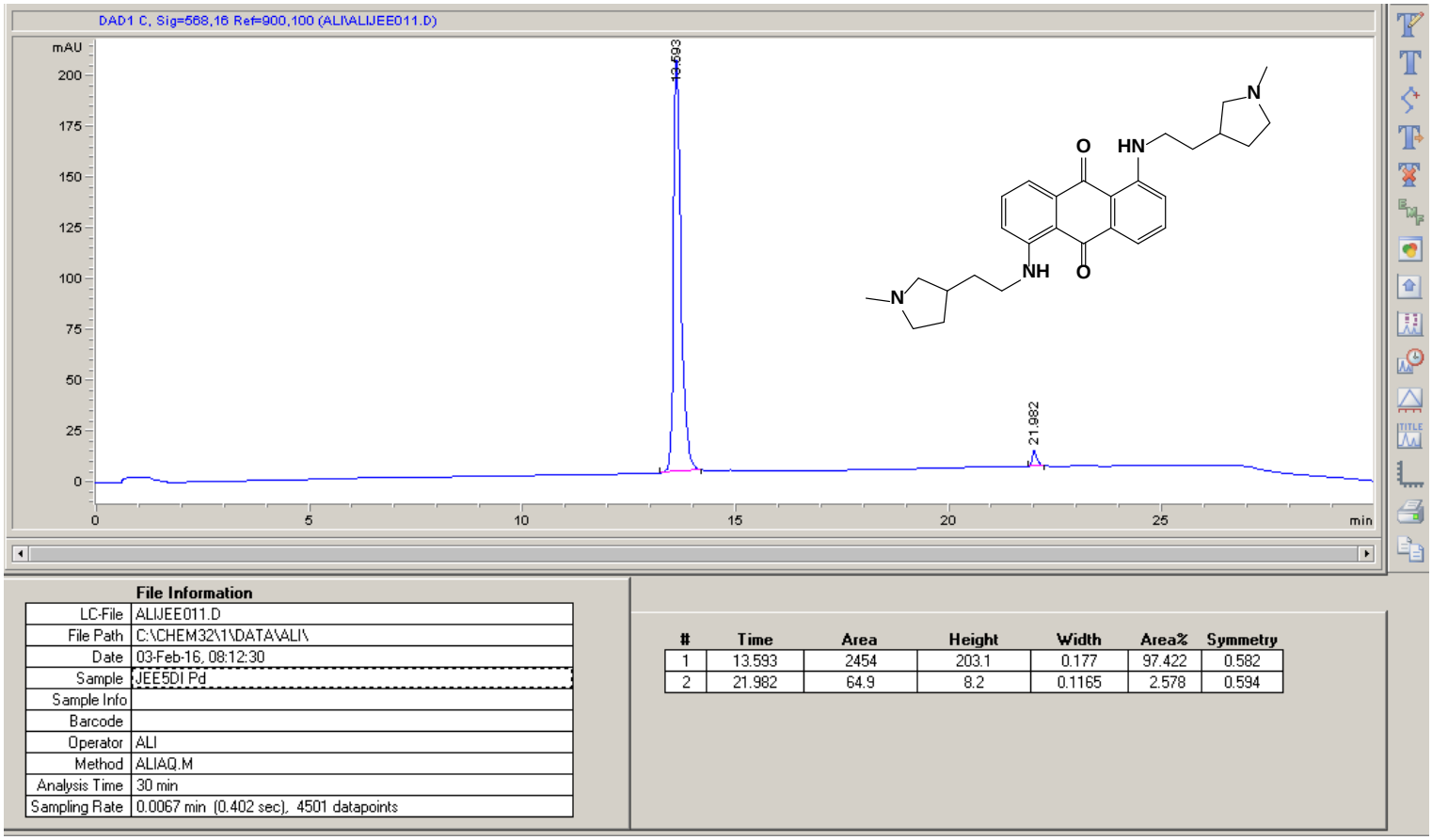
| Date | 16-Jan-16, 11:57:51 |
| Sample | Jee5m |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 29.993 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4500 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 15.904 | 35.3 | 3.8 | 0.1388 | 0.998 | 0.602 |
| 2 | 16.68 | 3363.3 | 250.4 | 0.2127 | 94.979 | 0.471 |
| 3 | 17.523 | 52.1 | 4.7 | 0.1595 | 1.473 | 0.579 |
| 4 | 18.258 | 43.7 | 4.5 | 0.1428 | 1.233 | 0.608 |
| 5 | 18.683 | 46.6 | 4.7 | 0.1446 | 1.317 | 0.612 |

## Compound 9




**File Information**

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|---|---|
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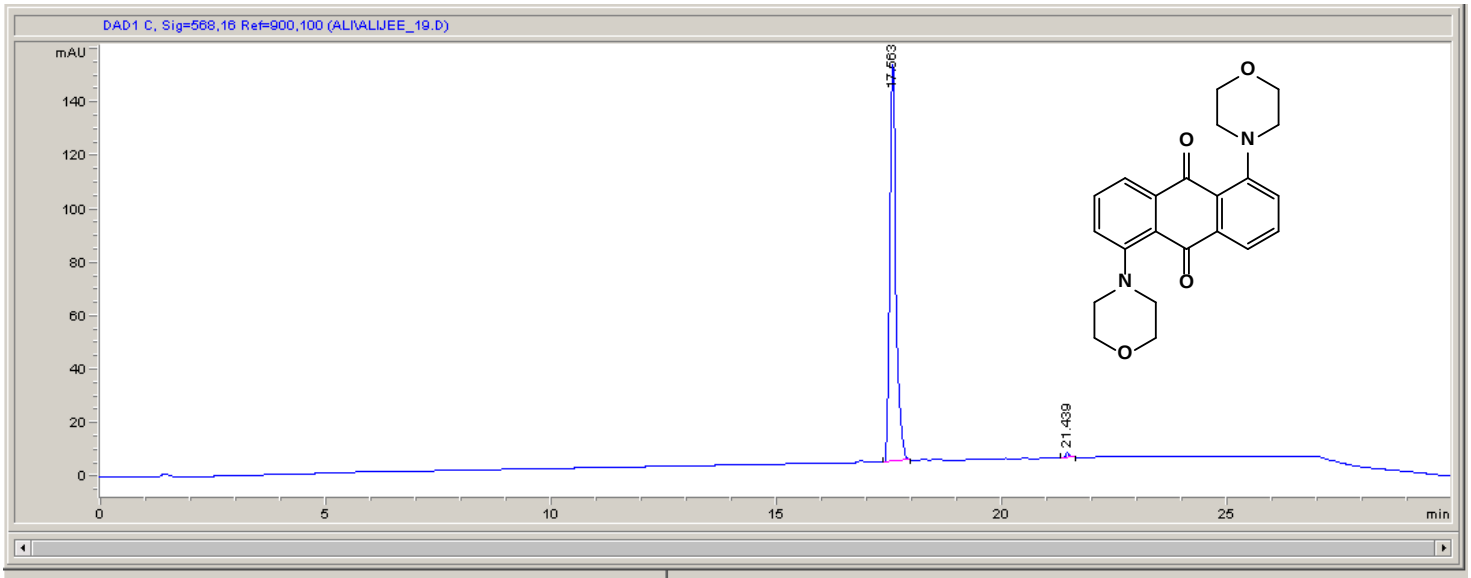
| Sample | JEE5DI Pd |
| Sample Info | |
| Barcode | |
| Operator | ALI |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 13.593 | 2454 | 203.1 | 0.177 | 97.422 | 0.582 |
| 2 | 21.982 | 64.9 | 8.2 | 0.1165 | 2.578 | 0.594 |

## Compound 10




**File Information**

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|---|---|
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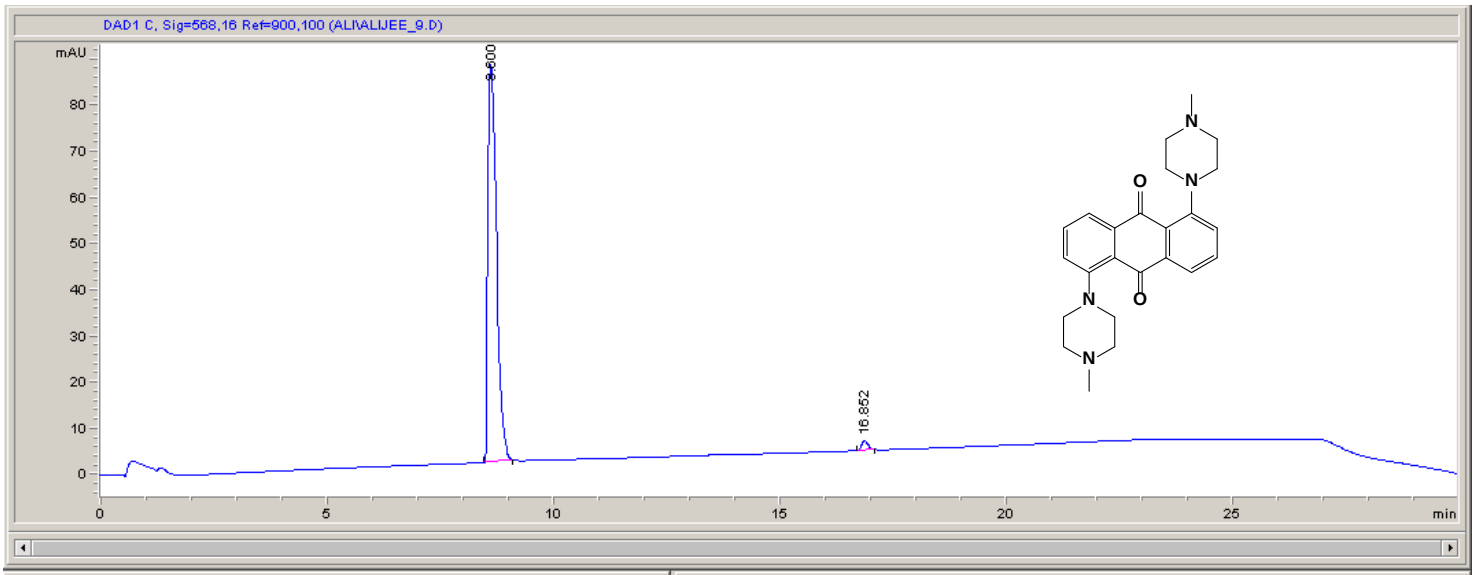
| Date | 17-Jan-16, 11:27:33 |
| Sample | Jee9 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 17.563 | 1405.3 | 148.6 | 0.1403 | 99.069 | 0.679 |
| 2 | 21.439 | 13.2 | 1.8 | 0.1074 | 0.931 | 0.601 |

## Compound 11




| File Information | |
|---|---|
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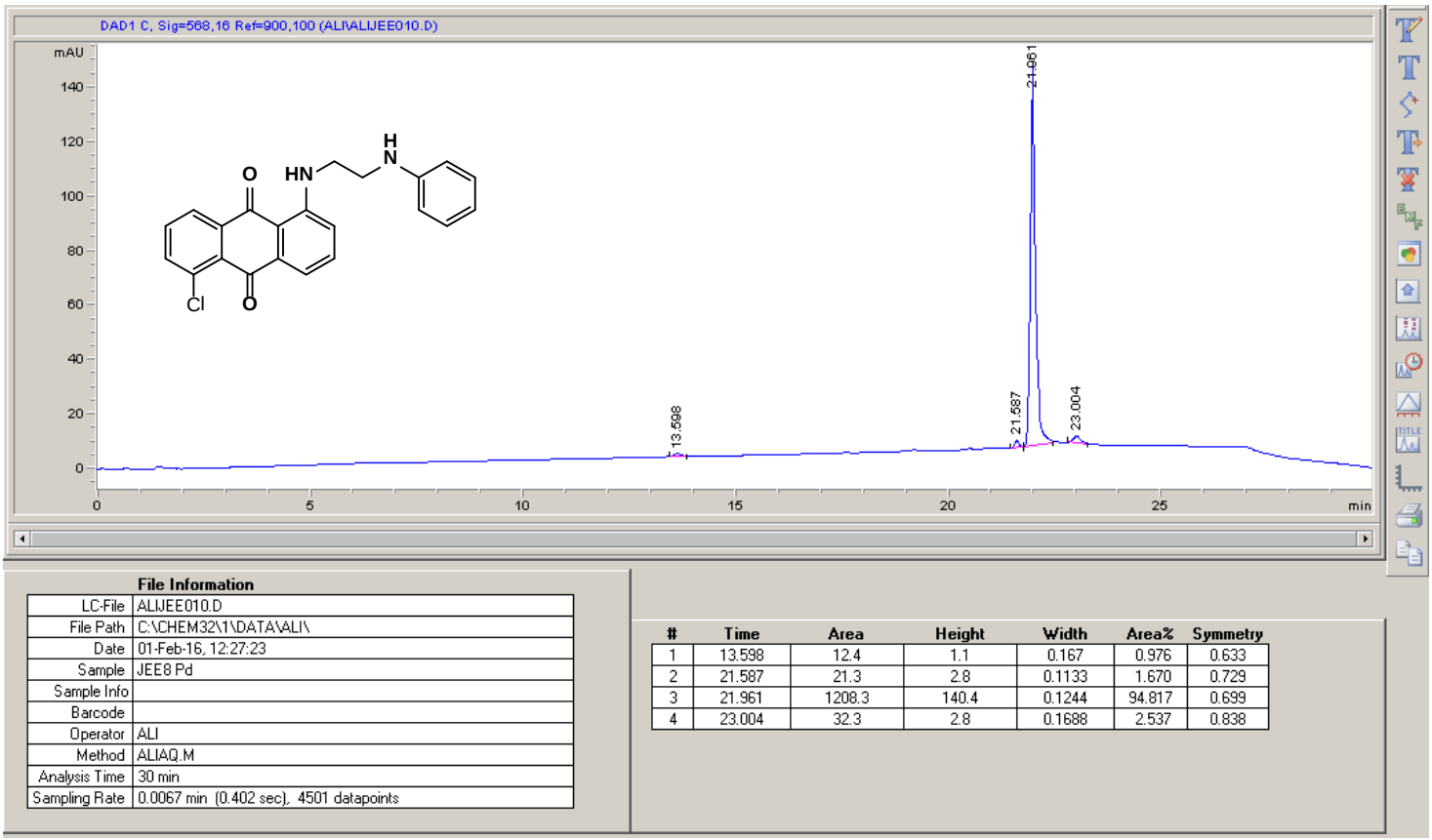
| File Path | C:\CHEM32\1\DATA\ALI\ |
| Date | 16-Jan-16, 12:37:45 |
| Sample | Jee6 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 8.6 | 1109 | 86.1 | 0.2042 | 98.138 | 0.451 |
| 2 | 16.852 | 21 | 2.1 | 0.1444 | 1.862 | 0.604 |

## Compound 12

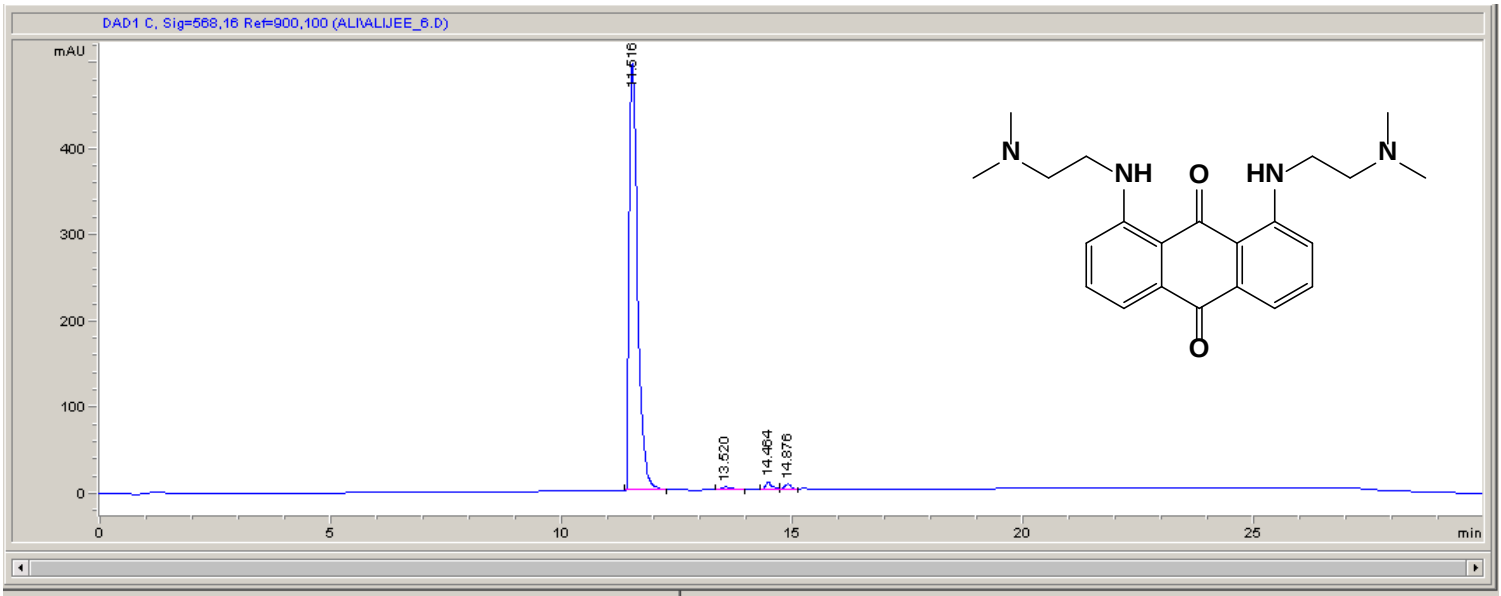



**File Information**

| | |
|---|---|
| LC-File | ALIJEE010.D |
| File Path | C:\CHEM32\1\DATA\ALI\ |
| Date | 01-Feb-16, 12:27:23 |
| Sample | JEE8 Pd |
| Sample Info | |
| Barcode | |
| Operator | ALI |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 13.598 | 12.4 | 1.1 | 0.167 | 0.976 | 0.633 |
| 2 | 21.587 | 21.3 | 2.8 | 0.1133 | 1.670 | 0.729 |
| 3 | 21.961 | 1208.3 | 140.4 | 0.1244 | 94.817 | 0.699 |
| 4 | 23.004 | 32.3 | 2.8 | 0.1688 | 2.537 | 0.838 |

## Compound 13




**File Information**

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|---|---|
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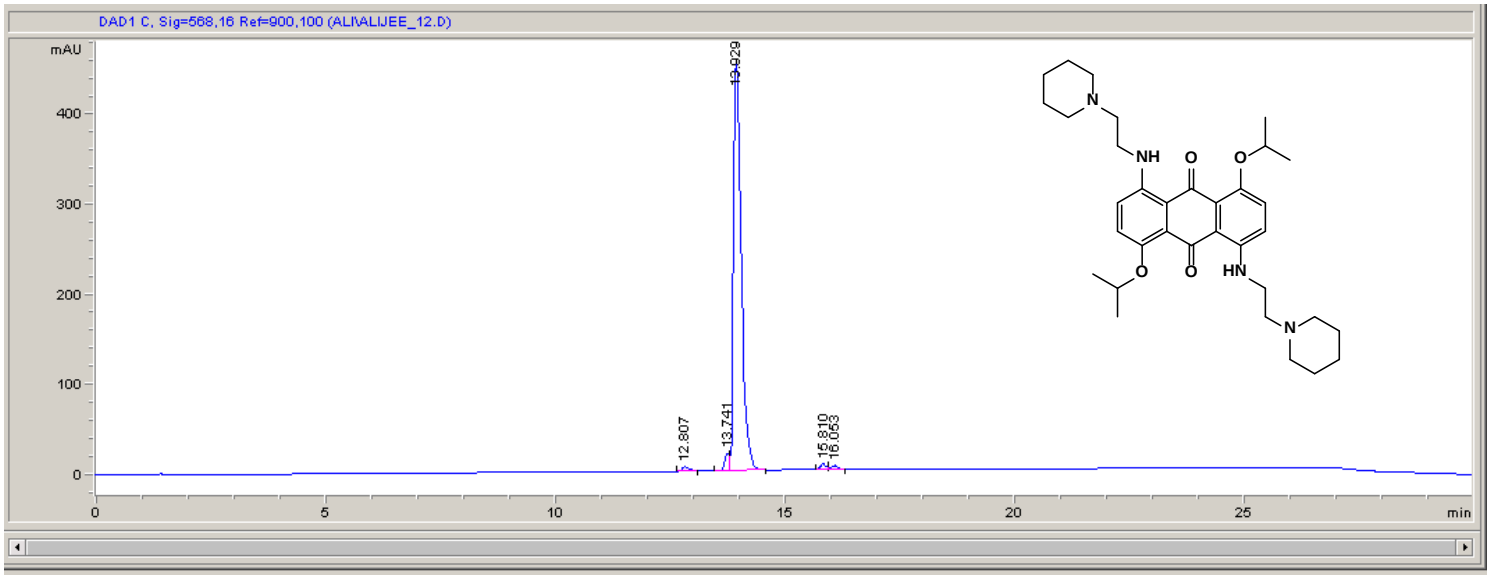
| Date | 16-Jan-16, 10:43:03 |
| Sample | Jee4 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 11.516 | 6141.3 | 495.2 | 0.1907 | 96.489 | 0.472 |
| 2 | 13.52 | 60.2 | 3.5 | 0.2308 | 0.946 | 0.433 |
| 3 | 14.464 | 93.5 | 9.2 | 0.1489 | 1.469 | 0.583 |
| 4 | 14.876 | 69.8 | 6.7 | 0.1541 | 1.096 | 0.753 |

## Compound 14




**File Information**

| | |
|---|---|
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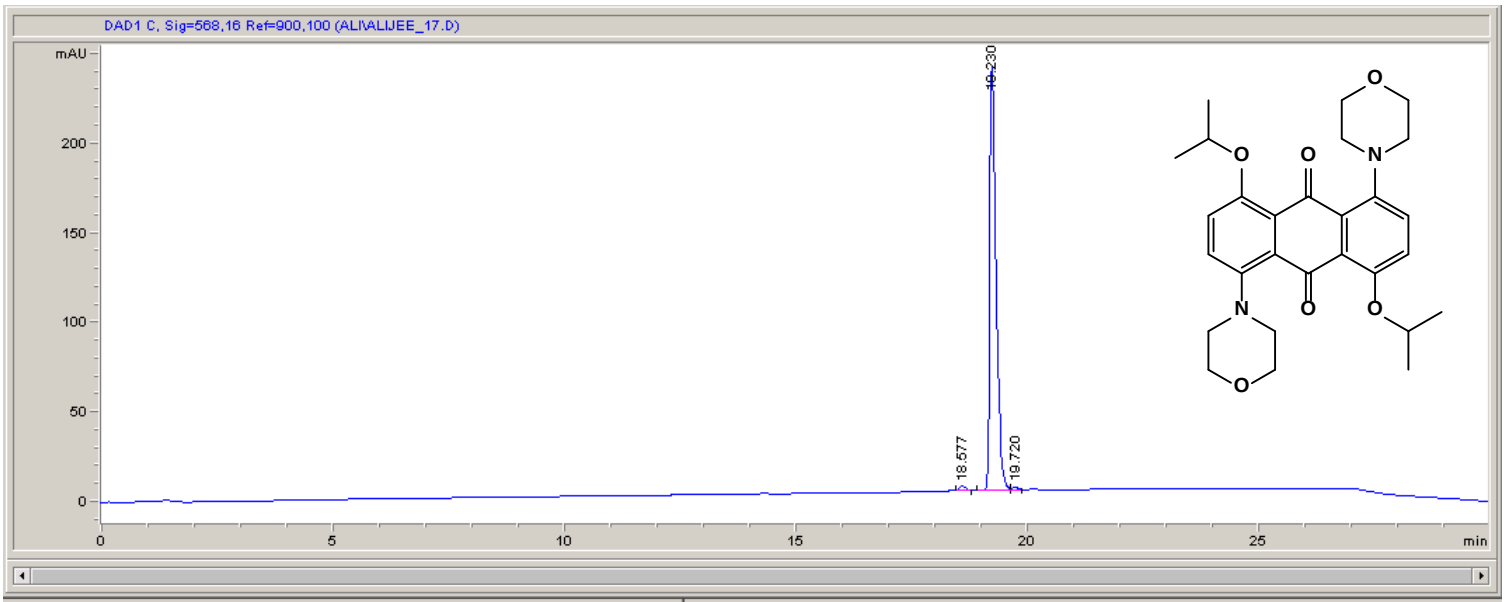
| Date | 16-Jan-16, 15:02:00 |
| Sample | Jee16 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 12.807 | 47.6 | 4.7 | 0.1509 | 0.882 | 0.579 |
| 2 | 13.741 | 128.4 | 19.5 | 0.1007 | 2.381 | 1.77 |
| 3 | 13.929 | 5099 | 454.2 | 0.167 | 94.576 | 0.517 |
| 4 | 15.81 | 61.6 | 6.7 | 0.1346 | 1.143 | 0.75 |
| 5 | 16.053 | 54.9 | 4.7 | 0.1671 | 1.018 | 0.726 |

## Compound 15




**File Information**

| | |
|---|---|
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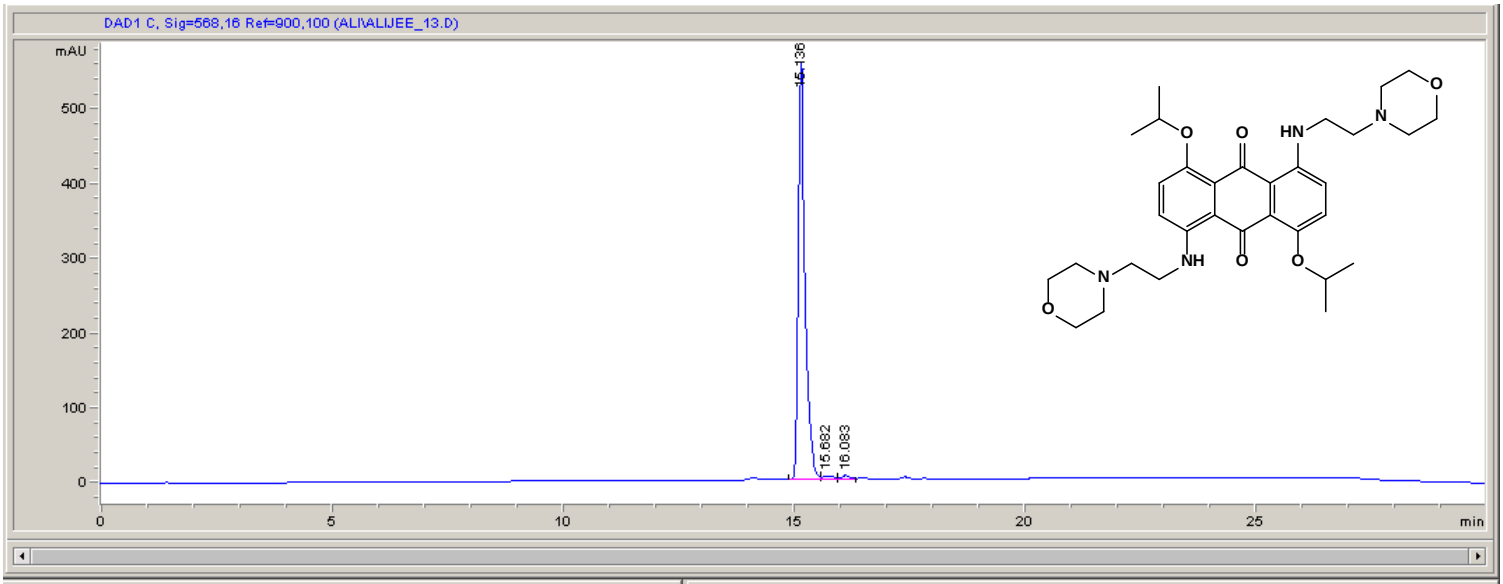
| File Path | C:\CHEM32\1\DATA\ALI\ |
| Date | 17-Jan-16, 10:13:19 |
| Sample | Jee12 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 18.577 | 27.3 | 2.8 | 0.1423 | 1.208 | 0.659 |
| 2 | 19.23 | 2218.5 | 236.8 | 0.1393 | 98.030 | 0.601 |
| 3 | 19.72 | 17.2 | 2.2 | 0.1108 | 0.761 | 0.697 |

## Compound 16




| File Information | |
|---|---|
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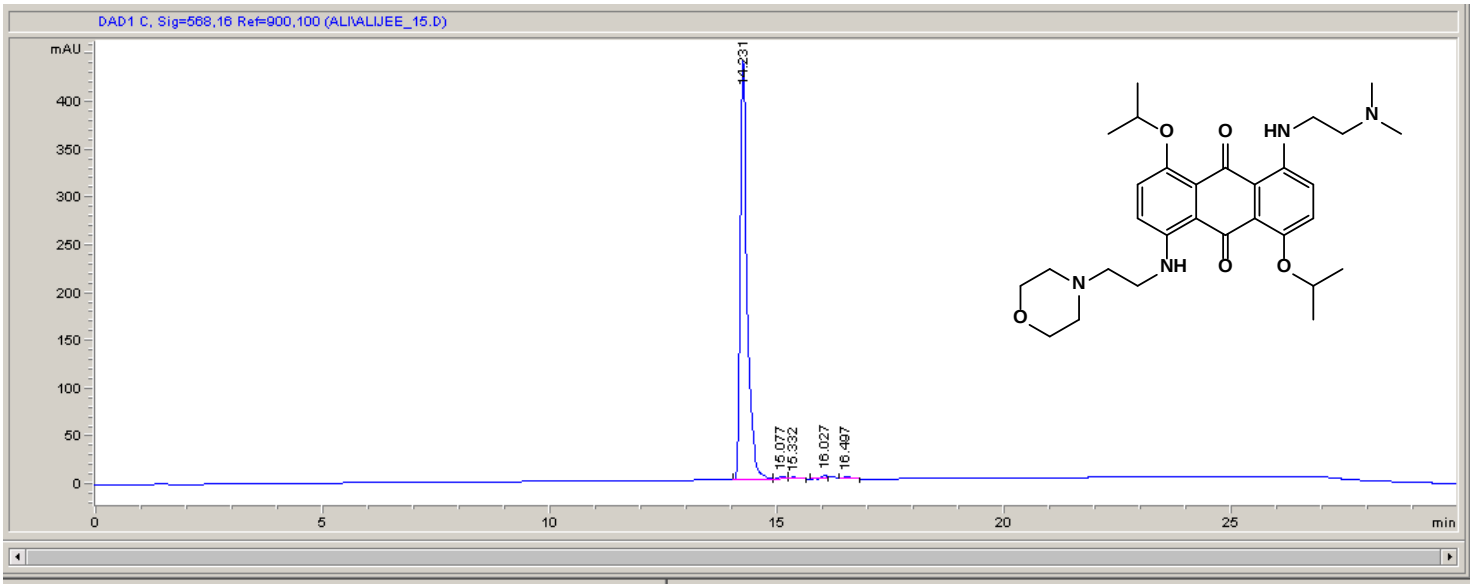
| Date | 16-Jan-16, 15:42:22 |
| Sample | Jee18 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 15.136 | 5787.1 | 556.5 | 0.1553 | 97.587 | 0.569 |
| 2 | 15.682 | 80 | 4.6 | 0.2286 | 1.348 | 0.388 |
| 3 | 16.083 | 63.1 | 5.3 | 0.1701 | 1.065 | 0.615 |

## Compound 17




**File Information**

| | |
|---|---|
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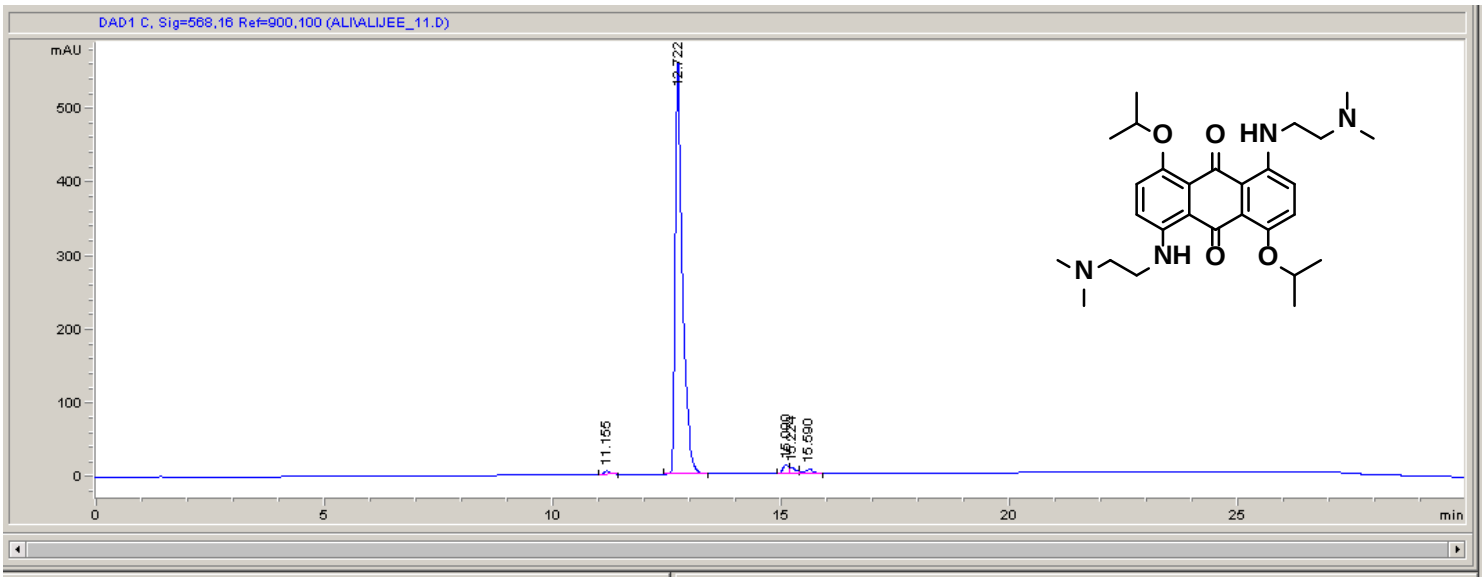
| Date | 17-Jan-16, 08:56:04 |
| Sample | Jee22 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 14.231 | 4639.7 | 437.5 | 0.1577 | 96.797 | 0.527 |
| 2 | 15.077 | 40.5 | 3.5 | 0.1686 | 0.845 | 0.826 |
| 3 | 15.332 | 34.8 | 2.7 | 0.18 | 0.726 | 0.565 |
| 4 | 16.027 | 40.5 | 4.1 | 0.1423 | 0.845 | 1.234 |
| 5 | 16.497 | 37.7 | 3.2 | 0.1659 | 0.787 | 0.537 |

**Compound 18**




| File Information | |
|---|---|
| LC-File | ALIJEE_11.D |
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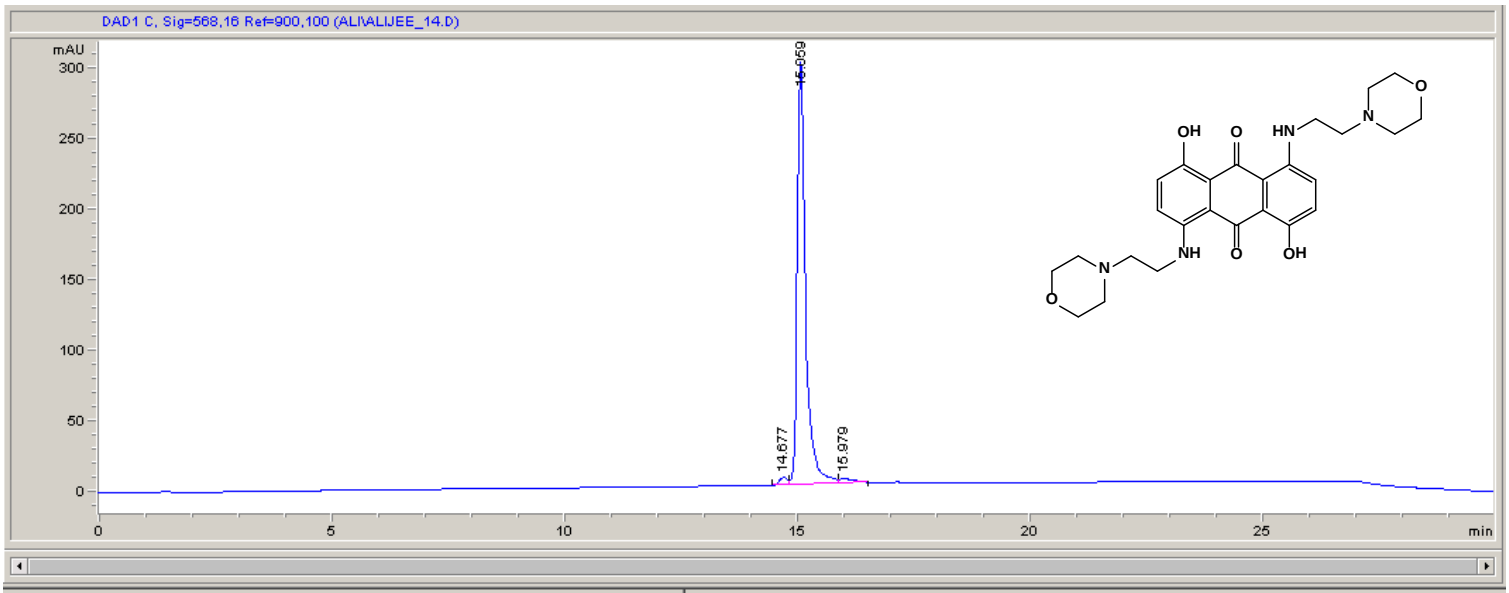
| Sample | Jee11 |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 29.993 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4500 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 11.155 | 51 | 5 | 0.1492 | 0.771 | 0.519 |
| 2 | 12.722 | 6307.6 | 558.8 | 0.1697 | 95.378 | 0.491 |
| 3 | 15.09 | 104.1 | 11.8 | 0.1311 | 1.575 | 0.933 |
| 4 | 15.224 | 70.8 | 8 | 0.1276 | 1.070 | 0.331 |
| 5 | 15.59 | 79.7 | 6.3 | 0.178 | 1.205 | 1.066 |

## Compound 19




**File Information**

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|---|---|
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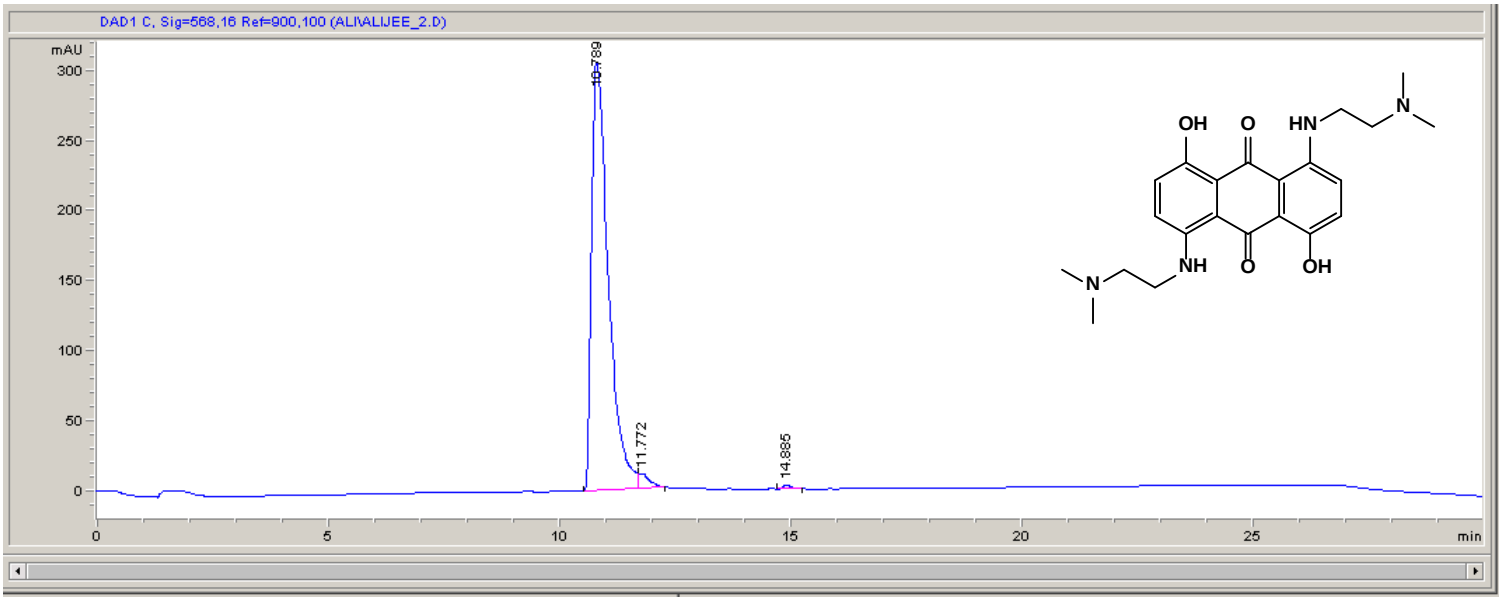
| Sample | Jee18DP |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 14.677 | 62 | 5.1 | 0.1847 | 1.627 | 0.761 |
| 2 | 15.059 | 3673.1 | 299.1 | 0.1733 | 96.330 | 0.634 |
| 3 | 15.979 | 77.9 | 3.8 | 0.2644 | 2.043 | 0.334 |

## Compound 20




**File Information**

| | |
|---|---|
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| File Path | C:\CHEM32\1\DATA\ALI\ |
| Date | 15-Jan-16, 12:27:23 |
| Sample | Jee11DP |
| Sample Info | |
| Barcode | |
| Operator | ali |
| Method | ALIAQ.M |
| Analysis Time | 30 min |
| Sampling Rate | 0.0067 min (0.402 sec), 4501 datapoints |

| # | Time | Area | Height | Width | Area% | Symmetry |
|---|---|---|---|---|---|---|
| 1 | 10.789 | 7812.2 | 305.3 | 0.3885 | 97.367 | 0.429 |
| 2 | 11.772 | 174.7 | 10.9 | 0.2245 | 2.177 | 0.295 |
| 3 | 14.885 | 36.5 | 2.9 | 0.1811 | 0.455 | 0.474 |

## 8. SUPPORTING INFORMATION REFERENCES


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