Supplementary Material

1  Inhibition of ERCC1-XPF Endonuclease Activity

Supplementary Figure 1. ERCC1-XPF mediated cleavage of the stem-loop DNA substrate, in which the FAM signal is quenched, releases the fluorescently tagged octanucleotide. Triplicate measurements of the control (DMSO) and the effect of 10 μM of compounds 4 and 6 on the incision activity is shown.

2  PLA Control Experiments

Supplementary Figure 2. A549 cells were seeded in 6-well culture dishes (Thermo Fishes Scientific) at a density of 0.3 X 10^6 cells/well in a final volume of 3 ml. The cells were left to adhere for 24 h before adding 2 μM of compound 4 or compound 6. The chambers were incubated for 24 h and then washed with PBS and the cells were solubilized with RIPA buffer in the presence of protease inhibitor cocktail (Cell Signaling Technology) plus 2 mM EDTA. Proteins were dissolved in sample buffer and separated in a 4-20 % polyacrylamide gel and electrophoretically transferred to
nitrocellulose. Proteins were visualized using ERCC1 (A73368-100, 1:1000; EpiGentek), XPF (LS-C173159, 1:1000; LifeSpan BioSciences, Seattle, WA) and B-Actin (sc-47778, 1:1000; Santa Cruz) primary antibodies followed by secondary anti-mouse or anti-rabbit secondary HRP-conjugated secondary antibodies. ECL chemiluminescent substrate (Promega) was used to detect the proteins.

3 Cytotoxicity Analysis

Supplementary Figure 3. Cytotoxicity assay using colony forming assay

4 HCT-116 Derived XPF Knockout Cells

For CRISPR deletion of XPF from HCT-116 cells, the pSpCas9(BB)-2A-GFP (pX458) vector (Addgene plasmid # 48138) was used. The short guide sequence to target XPF exon one was: 5' - GGGCTAGTAGTGCGCCCG - 3' and target XPF exon eight was: 5' - CTATATCAGTCGCGCCG - 3'. All short guide sequences oligonucleotides were synthesized, annealed and cloned into pX458 vector and were confirmed by DNA sequencing at DNA laboratory, University of Calgary. The DNA plasmid (pX458 containing XPF short guide RNA sequence, 5 µg) was transfected into HCT-116 cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Forty-eight hours after transfection, cells were harvested and genomic DNA was isolated using the KAPA Express Extract Kit (Kapa Biosystems) according to the manufacturer’s instructions. Genomic DNA fragments of XPF around the short guide RNA site were amplified by PCR. The primers used to amplify the genomic region of XPF exon one were: Forward: 5' - CACTAGGAGTGGCTTCTG-3', Reverse: 5' - TCTCTGTGTCATCGCGTAGT-3' and exon eight were: Forward: 5' - TCGGGTGAAGGAATAAGGG-3', Reverse: 5' - AATTCTTTCCGGTGCTATTCTTC-3'. PCR products were used for Surveyor nuclease mutation detection assays using the SURVEYOR Mutation Detection Kit (IDT) according to manufacturer’s protocol. After transfection, GFP containing cells were sorted into 96-well plates by flow cytometry, at the Flow Cytometry Facility, University of Calgary. Single cells were then expanded for further analysis (i.e., Western blot and DNA sequencing). All CRISPR gene knockout clones were screened by Western blot using anti-XPF antibody (MA12060, ThermoFisher). The membrane was also probed with Mre11 antibody (Novus) as a loading control. For DNA sequencing confirmation of
XPF knockout clones, DNA fragments of XPF knockout clones around the short guide RNA site were amplified by PCR. PCR products were subcloned into pEGFP-C2 vector (Clontech) and plasmid DNA from individual clones were sent for Sanger DNA sequencing at DNA laboratory, University of Calgary to confirm all indels.

**Allele 1**

| Allele 1 | XPF-E1 | ATGGAGTCAGGGCAGCAGCCGCTCGACGGATCGAGTACGAGC
| Allele 1 | 1-2-1a | ATGGAGTCAGGGCAGCAGCCGCTCGACGGATCGAGTACGAGC
| Allele 1 | XPF-E1 | GACAGCTTGTCGGAAACTGCTCAGACACTGACGGGCTTAGTAGTGTACC
| Allele 1 | 1-2-1a | GACAGCTTGTCGGAAACTGCTCAGACACTGACGGGCTTAGTAGTGTACC
| Allele 1 | XPF-E1 | GACAGCTTGTCGGAAACTGCTCAGACACTGACGGGCTTAGTAGTGTACC
| Allele 1 | 1-2-1a | GACAGCTTGTCGGAAACTGCTCAGACACTGACGGGCTTAGTAGTGTACC
| Allele 1 | XPF-E1 | ATGGAGTCAGGGCAGCAGCCGCTCGACGGATCGAGTACGAGCGA
| Allele 1 | 1-2-1a | ATGGAGTCAGGGCAGCAGCCGCTCGACGGATCGAGTACGAGCGA

**Allele 2**

| Allele 2 | XPF-E1 | ATGGAGTCAGGGCAGCAGCCGCTCGACGGATCGAGTACGAGC
| Allele 2 | 1-2-1b | ATGGAGTCAGGGCAGCAGCCGCTCGACGGATCGAGTACGAGC
| Allele 2 | XPF-E1 | CAGCTTGTCGGAACTGCTCAGACACTGACGGGCTTAGTAGTGTACC
| Allele 2 | 1-2-1b | CAGCTTGTCGGAACTGCTCAGACACTGACGGGCTTAGTAGTGTACC
| Allele 2 | XPF-E1 | ATGGAGTCAGGGCAGCAGCCGCTCGACGGATCGAGTACGAGC
| Allele 2 | 1-2-1b | ATGGAGTCAGGGCAGCAGCCGCTCGACGGATCGAGTACGAGC

**Supplementary Figure 4.** Sequence analysis of the HCT116 XPF-/− cell clone (1-2-1a). 60 nucleotides were inserted and one nucleotide was mutated in Allele 1; one nucleotide was deleted in Allele 2. The targeted sequences in the WT exon 1 allele (XPF-E1) corresponding to the guide RNA is underlined.

### 5 Compound Synthetic Procedures and Characterization

**General Information**
Reactions were carried out in flame or oven dried glassware under a positive nitrogen atmosphere unless otherwise stated. Transfer of anhydrous solvents and reagents was accomplished with oven-dried syringes or cannulae. Solvents and some reagents were distilled before use. Commercially available reagents were used without further purification. Thin layer chromatography was performed on glass plates precoated with 0.25 mm silica gel. Flash chromatography was performed on 230–400 mesh silica gel with the indicated eluents. Nuclear magnetic resonance (NMR) spectra were recorded in indicated deuterated solvents and are reported in ppm in the presence of TMS as internal standard and coupling constants (J) are reported in hertz (Hz). The spectra are referenced to residual solvent peaks: chloroform-d (7.26 ppm, 1H; 77.26 ppm, 13C), DMSO-d6 (2.50 ppm, 1H; 39.51 ppm, 13C), acetone-d6 (2.05 ppm, 1H; 206.68 and 29.92 ppm, 13C) and methanol-d4 (3.31 ppm, 1H; 49.00 ppm, 13C). Proton nuclear magnetic spectra (1H NMR) and carbon nuclear magnetic resonance spectra (13C NMR) were recorded at 500/400 and 125/100 MHz respectively. Mass spectra were recorded by using electrospray ionization (ESI).

4-((6-Chloro-2-methoxyacridin-9-yl)amino)-2-((4-(2-(diisopropylamino)ethyl)piperazin-1-yl)methyl)phenol (6)

Compound 20 (93 mg, 0.19 mmol) was dissolved in DCM (2.5 ml). Diisopropylamine (30 μl, 21 mg, 0.21 mmol) and acetic acid (3 μl, 3 mg, 0.06 mmol) were added and allowed to stir for 30 minutes before addition of NaBH(OAc)3 (48 mg, 0.23 mmol). The reaction mixture was allowed to stir at room temperature overnight. After the completion of the reaction, as indicated by TLC, the reaction was quenched with satd. NaHCO3 solution, then 1 M NaOH solution. The reaction mixture was then adjusted to pH = 8-9 by addition of 1M HCl. Extraction with DCM and drying of organic extracts with Na2SO4, filtration and removal of solvent under reduced pressure afforded the crude product. The compound was purified by column chromatography and recrystallization with MeCN produced a brown solid (84 mg, 76%). 1H NMR (400 MHz, Chloroform-d) δ 8.14 (d, J = 2.1 Hz, 1H), 8.04 (d, J = 9.4 Hz, 1H), 7.89 (d, J = 9.1 Hz, 1H), 7.41 (dd, J = 9.5, 2.7 Hz, 1H), 7.06 (d, J = 2.7 Hz, 1H), 6.89 – 6.81 (m, 1H), 6.76 (d, J = 8.5 Hz, 1H), 6.54 (s, 1H), 6.44 (s, 1H), 3.74 (s, 3H), 3.57 (s, 2H), 2.98 (p, J = 6.5 Hz, 2H), 2.54 (dd, J = 10.0, 6.0 Hz, 2H), 2.39 (dd, J = 9.8, 6.2 Hz, 2H), 1.55 (broad s, 8H), 0.99 (d, J = 6.5 Hz, 12H); 13C NMR (175 MHz, Chloroform-d) δ 156.3, 153.5, 148.4, 147.5, 143.0, 136.5, 134.9, 131.7, 128.5, 125.5, 125.3, 124.4, 122.0, 120.1, 119.8, 117.9, 116.9, 99.6, 61.3, 60.6, 55.4, 53.5, 53.4, 52.5, 49.3, 43.0, 29.7, 20.8, 1.0. HRMS (ESI) m/z calculated for C33H31ClN5O2 [M-H] -574.2954; found 574.2945.

4-((6-Chloro-2-methoxyacridin-9-yl)amino)-2-((4-(3-((2,3-dihydro-1H-inden-2-yl)(methyl)amino)propyl)piperazin-1-yl)methyl)phenol (8)
Compound 25 (142 mg, 0.28 mmol) was dissolved in DCM (5 ml). N-methyl-2,3-dihydro-1H-inden-2-amine (46 mg, 0.31 mmol) and acetic acid (5 μl, 5 mg, 0.08 mmol) were added and allowed to stir for 30 minutes before addition of NaBH(OAc)₃ (72 mg, 0.34 mmol). The reaction mixture was allowed to stir at room temperature overnight. After the completion of the reaction, as indicated by TLC, the reaction was quenched with satd. NaHCO₃ solution, then 1 M NaOH solution. The reaction mixture was then adjusted to pH = 8-9 by addition of 1M HCl. Extraction with DCM and drying of organic extracts with Na₂SO₄, filtration and removal of solvent under reduced pressure afforded the crude product. The compound was purified by column chromatography and recrystallization with MeCN produced a brown oil (23 mg, 16%). HRMS (ESI) m/z calculated for C₃₈H₄₃ClN₅O₂ [M+H]⁺ 636.3100; found 636.3096

2-((4-(2-(Benzylationo)ethyl)piperazin-1-yI)methyl)-4-((6-chloro-2-methoxyacridin-9-yl)amino)phenol (10)

Compound 20 (77 mg, 0.16 mmol) was dissolved in DCM (2 ml). Benzylamine (16 μl, 16 mg, 0.21 mmol) and acetic acid (3 μl, 3 mg, 0.06 mmol) were added and allowed to stir for 30 minutes before addition of NaBH(OAc)₃ (40 mg, 0.19 mmol). The reaction mixture was allowed to stir at room temperature for 2 days. After the completion of the reaction, as indicated by TLC, the reaction was quenched with satd. NaHCO₃ solution, then 1 M NaOH solution. The reaction mixture was then adjusted to pH = 8-9 by addition of 1M HCl. Extraction with DCM and drying of organic extracts with Na₂SO₄, filtration and removal of solvent under reduced pressure afforded the crude product. The compound was purified by column chromatography and recrystallization with MeCN produced an orange solid (41 mg, 49%). ¹H NMR (500 MHz, Chloroform-d) δ 8.15 (s, 1H), 8.05 (d, J = 9.3
Hz, 1H), 7.91 (d, J = 9.3 Hz, 1H), 7.42 (d, J = 9.3 Hz, 1H), 7.19 (dd, J = 8.6, 7.4 Hz, 2H), 7.07 (s, 1H), 6.85 (dd, J = 8.5, 2.7 Hz, 1H), 6.78 (d, J = 8.6 Hz, 1H), 6.75 – 6.69 (m, 1H), 6.63 (dd, J = 8.6, 1.1 Hz, 2H), 6.55 (d, J = 2.7 Hz, 1H), 6.45 (s, 1H), 4.23 (s, 1H), 3.75 (s, 3H), 3.60 (s, 2H), 3.50 (s, 2H), 3.17 (t, J = 5.9 Hz, 2H), 2.98 – 2.32 (m, 10H).

$^{13}$C NMR (125 MHz, Chloroform-d) δ 156.4, 153.5, 148.5, 136.8, 135.1, 129.4, 125.3, 124.7, 122.1, 120.3, 119.9, 118.0, 117.6, 117.0, 113.0, 99.9, 61.4, 56.6, 55.5, 53.6, 52.8, 52.6, 40.4, 30.1, 29.8; HRMS (ESI) m/z calculated for C$_{33}$H$_{35}$ClN$_5$O$_2$ [M+H]$^+$ 568.2474; found 568.2471.

4-((6-Chloro-2-methoxyacridin-9-yl)amino)-2-((4-((thiophen-2-ylmethyl)amino)ethyl)piperazin-1-yl)methyl)phenol (11)

Compound 20 (93 mg, 0.19 mmol) was dissolved in DCM (2.5 ml). 2-Thiophenemethylamine (21 μl, 24 mg, 0.38 mmol) and acetic acid (3 μl, 3 mg, 0.06 mmol) were added and allowed to stir for 30 minutes before addition of NaBH(OAc)$_3$ (48 mg, 0.23 mmol). The reaction mixture was allowed to stir at room temperature for 3 days. After the completion of the reaction, as indicated by TLC, the reaction was quenched with satd. NaHCO$_3$ solution, then 1 M NaOH solution. The reaction mixture was then adjusted to pH = 8-9 by addition of 1M HCl. Extraction with DCM and drying of organic extracts with Na$_2$SO$_4$, filtration and removal of solvent under reduced pressure afforded the crude product. The compound was purified by column chromatography and recrystallization with MeCN produced a yellow solid (75 mg, 67%). $^1$H NMR (500 MHz, Chloroform-d) δ 1H NMR (500 MHz, Chloroform-d) δ 8.15 (s, 1H), 8.05 (d, J = 9.2 Hz, 1H), 7.91 (d, J = 9.2 Hz, 1H), 7.42 (d, J = 9.4 Hz, 1H), 7.21 (dd, J = 5.0, 1.2 Hz, 1H), 7.07 (s, 1H), 6.95 (dd, J = 5.0, 3.4 Hz, 1H), 6.93 – 6.91 (m, 1H), 6.85 (dd, J = 8.4, 2.1 Hz, 1H), 6.78 (d, J = 8.5 Hz, 1H), 6.55 (d, J = 2.7 Hz, 1H), 6.45 (s, 1H), 4.01 (s, 2H), 3.75 (s, 3H), 3.58 (s, 2H), 3.50 (s, 2H), 2.78 – 2.33 (m, 12H); HRMS (ESI) m/z calculated for C$_{32}$H$_{35}$ClN$_5$O$_2$S [M+H]$^+$ 588.2195; found 588.2191.

3-(4-((5-((6-Chloro-2-methoxyacridin-9-yl)amino)-2-hydroxybenzyl)piperazin-1-yl)-N,2,2-trimethylpropanamide (12)
6,9-Dichloro-2-methoxyacridine (68 mg, 0.21 mmol) and 32 (59 mg, 0.21 mmol) were dissolved in EtOH (4.5 ml), 5 drops concentrated HCl were added, and the mixture was heated at reflux overnight. The reaction was quenched with saturated NaHCO₃ solution and extracted with DCM. The combined organic extracts were dried with MgSO₄, filtered, and the solvent evaporated under reduced pressure to afford the crude product as a brown semisolid. The compound was purified by column chromatography, recrystallization from hexanes/DCM, and recrystallization from MeCN (29 mg, 24%). ¹H NMR (400 MHz, Chloroform-d) δ 8.06 (s, 1H), 7.97 (d, J = 9.4 Hz, 1H), 7.86 (d, J = 9.3 Hz, 1H), 7.36 (dd, J = 9.5, 2.7 Hz, 1H), 7.22 – 7.15 (m, 2H), 7.08 (s, 1H), 6.87 (dd, J = 8.6, 2.7 Hz, 1H), 6.77 (d, J = 8.6 Hz, 1H), 6.53 (s, 1H), 3.72 (s, 3H), 2.75 (d, J = 4.8 Hz, 3H), 2.52 (d, J = 81.2 Hz, 10H), 1.13 (s, 6H); ¹³C NMR (125 MHz, Chloroform-d) δ 177.9, 156.3, 153.4, 135.1, 125.3, 124.6, 121.9, 120.3, 119.9, 119.8, 117.0, 66.5, 61.2, 55.4, 54.9, 53.4, 52.9, 42.5, 30.9, 29.7, 26.1, 24.6, 14.1; HRMS (ESI) m/z calculated for C₃₁H₃₇ClN₅O₃ [M+H]+ 562.2579; found 562.2571.

4-((6-Chloro-2-methoxyacridin-9-yl)amino)-2-((4-(2-methoxyethyl)piperazin-1-yl)methyl)phenol (15)

6,9-Dichloro-2-methoxyacridine (89 mg, 0.32 mmol) and 35 (85 mg, 0.32 mmol) were dissolved in EtOH (7 ml), 3 drops concentrated HCl were added, and the mixture was heated at reflux overnight. The reaction was quenched with saturated NaHCO₃ solution and extracted with DCM. The combined organic extracts were dried with MgSO₄, filtered, and the solvent evaporated under reduced pressure to afford the crude product as an orange semisolid. The compound was purified by column chromatography and recrystallization from MeCN (86 mg, 53%). ¹H NMR (500 MHz, Chloroform-d) δ 8.13 (s, 1H), 8.03 (s, 1H), 7.90 (d, J = 9.3 Hz, 1H), 7.40 (d, J = 9.4 Hz, 1H), 7.08 (s, 1H), 6.84
(dd, \( J = 8.5, 2.7 \) Hz, 1H), 6.77 (d, \( J = 8.6 \) Hz, 1H), 6.56 (d, \( J = 2.7 \) Hz, 1H), 6.47 (s, 1H), 3.74 (s, 3H), 3.59 (s, 2H), 3.51 (t, \( J = 5.5 \) Hz, 2H), 3.36 (s, 3H), 2.60 (t, \( J = 5.5 \) Hz, 10H); HRMS (ESI) \( m/z \) calculated for \( \text{C}_{28}\text{H}_{32}\text{ClN}_{4}\text{O}_{3} \ [\text{M+H}^+ \) 207.2157; found 207.2156.

1-(2,2-Dimethoxyethyl)piperazine (16)

![Image of compound 16]

Piperizine (2.50 g, 29.0 mmol), 2-bromoacetaldehyde dimethyl acetal (1.7 ml, 2.44 g, 14.5 mmol), and triethylamine (2.0 ml, 1.47 g, 14.5 mmol) were dissolved in EtOH (17 ml) and heated at reflux overnight. After completion of the reaction, as indicated by TLC, the reaction mixture was allowed to cool to room temperature, filtered and the solvent evaporated under reduced pressure. The crude mixture was purified by column chromatography to afford the product (1.66 g, 66%). \(^1\)H NMR (500 MHz, Chloroform-\( d \)) \( \delta \) 4.53 (t, \( J = 5.2 \) Hz, 1H), 3.37 (s, 6H), 2.90 (t, \( J = 4.9 \) Hz, 4H), 2.54 – 2.47 (m, 6H), 1.83 (s, 1H); \(^{13}\)C NMR (100 MHz, Chloroform-\( d \)) \( \delta \) 102.6, 60.1, 53.8, 53.4; HRMS (ESI) \( m/z \) calculated for \( \text{C}_{8}\text{H}_{19}\text{N}_{2}\text{O}_{2} \ [\text{M+H}^+ \) 175.1440; found 175.1440.

2-((4-(2,2-Dimethoxyethyl)piperazin-1-yl)methyl)-4-nitrophenol (17)

![Image of compound 17]

2-chloromethyl-4-nitrophenol hydrochloride (1.42 g, 6.35 mmol) and 16 (1.44 g, 8.25 mmol) were dissolved in DCM (52 ml). Triethylamine (1.1 ml, 835 mg, 0.73 mmol) was added in 3 portions and the reaction mixture was allowed to stir at room temperature overnight. After completion of the reaction, as indicated by TLC, the solvent was removed under reduced pressure and the crude mixture was purified by column chromatography to afford the product (2.04 g, 97%). \(^1\)H NMR (500 MHz, Chloroform-\( d \)) \( \delta \) 8.10 (dd, \( J = 9.0, 2.8 \) Hz, 1H), 7.96 (d, \( J = 2.8 \) Hz, 1H), 6.86 (d, \( J = 9.0 \) Hz, 1H), 4.52 (t, \( J = 5.2 \) Hz, 1H), 3.80 (s, 2H), 3.38 (s, 6H), 2.81 – 2.50 (m, 10H); \(^{13}\)C NMR (175 MHz, Chloroform-\( d \)) \( \delta \) 164.4, 140.1, 125.4, 124.8, 121.0, 116.5, 102.5, 60.7, 59.4, 53.5, 53.3, 52.3.; HRMS (ESI) \( m/z \) calculated for \( \text{C}_{15}\text{H}_{24}\text{N}_{3}\text{O}_{5} \ [\text{M+H}^+ \) 326.1710; found 326.1707.

4-Amino-2-((4-(2,2-dimethoxyethyl)piperazin-1-yl)methyl)phenol (18)
MeOH (40 ml) was added to a 3-neck round bottomed flask containing 10% Pd/C (50 mg), before addition of a solution of 17 (1.91 g, 5.87 mmol) in MeOH (50 ml) was added. The reaction vessel was evacuated and backfilled with nitrogen 3 times, then evacuated and backfilled with H₂ 3 times before being allowed to stir at room temperature for 7 days. After the completion of the reaction, as indicated by TLC, the reaction mixture was filtered over celite and the solvent removed under reduced pressure. The crude product was purified by column chromatography (1.62 g, 93%). ¹H NMR (500 MHz, Chloroform-d) δ 6.66 (d, J = 8.4 Hz, 1H), 6.56 (dd, J = 8.4, 2.8 Hz, 1H), 6.39 (d, J = 2.8 Hz, 1H), 4.52 (t, J = 5.2 Hz, 1H), 3.61 (s, 2H), 3.37 (s, 6H), 2.79–2.50 (m, 10H); ¹³C NMR (100 MHz, Chloroform-d) δ 150.4, 138.5, 121.8, 116.6, 116.0, 102.4, 61.4, 59.7, 53.7, 53.5, 52.4; HRMS (ESI) m/z calculated for C₁₅H₂₆N₃O₃ [M+H]⁺ 296.1970; found 296.1960.

4-((6-Chloro-2-methoxyacridin-9-yl)amino)-2-((4-(2,2-dimethoxyethyl)piperazin-1-yl)methyl)phenol (19)

6,9-Dichloro-2-methoxyacridine (540 mg, 1.94 mmol) and 18 (573 mg, 1.94 mmol) were dissolved in MeOH (40 ml), 6 drops concentrated HCl were added, and the mixture was heated at reflux overnight. The reaction was quenched with saturated NaHCO₃ solution and extracted with DCM. The combined organic extracts were dried with MgSO₄, filtered, and the solvent evaporated under reduced pressure to afford the crude product as an orange solid. The compound was purified by column chromatography and recrystallization from MeCN (889 mg, 85%). ¹H NMR (500 MHz, Chloroform-d) δ 8.13 (s, 1H), 8.03 (s, 1H), 7.89 (d, J = 9.3 Hz, 1H), 7.40 (d, J = 9.4 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.07 (s, 1H), 6.84 (dd, J = 8.6, 2.7 Hz, 1H), 6.78 (d, J = 8.5 Hz, 1H), 6.54 (d, J = 2.7 Hz, 1H), 6.51 (s, 1H), 4.52 (t, J = 5.2 Hz, 1H), 3.73 (s, 3H), 3.58 (s, 2H), 3.38 (s, 6H), 2.77–2.41 (m, 10H); ¹³C NMR (125 MHz, Chloroform-d) δ 156.3, 153.4, 143.1, 136.5, 134.9, 131.7, 128.4, 125.4, 125.2, 124.5, 122.0, 120.1, 119.8, 117.9, 116.9, 116.0, 102.5, 99.6, 61.3, 59.6, 55.3, 53.6, 53.4, 52.4; HRMS (ESI) m/z calculated for C₂₉H₃₄ClN₄O₄ [M+H]⁺ 537.2263; found 537.2257.

2-(4-((6-Chloro-2-methoxyacridin-9-yl)amino)-2-hydroxybenzyl)piperazin-1-yl)acetaldehyde (20)
Compound 19 (321 mg, 0.60 mmol) was dissolved in DCM (25 ml) and cooled to 0 °C. BBr₃ (1.32 ml, 1.32 mmol) was added dropwise and allowed to stir at 0 °C for 15 minutes. After completion of the reaction, as indicated by TLC, the reaction was carefully quenched by addition of H₂O and the pH adjusted to 8 with a saturated NaHCO₃ solution. The reaction mixture was extracted with DCM and the combined organic extracts dried with Na₂SO₄, filtered, and evaporated. The crude product was purified by column chromatography to afford the product as an orange solid (222 mg, 75%). 

**1H NMR (400 MHz, Chloroform-d)** δ 9.68 (s, 1H), 8.09 (s, 1H), 7.99 (d, J = 9.4 Hz, 1H), 7.87 (d, J = 9.2 Hz, 1H), 7.41 – 7.34 (m, 1H), 7.22 (d, J = 9.2 Hz, 1H), 7.08 (s, 1H), 6.86 (dd, J = 8.6, 2.7 Hz, 1H), 6.77 (d, J = 8.6 Hz, 1H), 6.57 (s, 1H), 3.73 (s, 2H), 3.60 (s, 1H), 3.22 (d, J = 1.4 Hz, 2H), 2.62 (s, 5H); HRMS (ESI) m/z calculated for C₂₈H₃₆ClN₄O₃ [M+H]⁺ 505.2001; found 505.2000.

**1-(3,3-Diethoxypropyl)piperazine (21)**

Piperizine (2.57 g, 29.9 mmol), 3-chloropropionaldehyde diethyl acetal (1.8 ml, 2.49 g, 15.0 mmol), and triethylamine (2.1 ml, 1.52 g, 15.0 mmol) were dissolved in EtOH (17 ml) and heated at reflux overnight. After completion of the reaction, as indicated by TLC, the reaction mixture was allowed to cool to room temperature, filtered and the solvent evaporated under reduced pressure. The crude mixture was purified by column chromatography to afford the product (1.51 g, 47%). 

**1H NMR (400 MHz, Chloroform-d)** δ 4.56 (t, J = 5.7 Hz, 1H), 3.64 (dq, J = 9.4, 7.1 Hz, 2H), 3.49 (dq, J = 9.4, 7.0 Hz, 2H), 2.90 (t, J = 4.9 Hz, 4H), 2.51 – 2.33 (m, 6H), 1.86 – 1.75 (m, 2H), 1.19 (t, J = 7.1 Hz, 6H); 

**13C NMR (100 MHz, Chloroform-d)** δ 101.7, 61.2, 54.6, 54.4, 46.0, 30.9, 15.4; HRMS (ESI) m/z calculated for C₁₁H₂₅N₂O₂ [M+H]⁺ 217.1911; found 217.1909.

**3-((4-(3,3-Diethoxypropyl)piperazin-1-yl)methyl)-4-hydroxybenzoic acid (22)**
2-chloromethyl-4-nitrophenol hydrochloride (652 mg, 2.93 mmol) and 21 (822 mg, 3.80 mmol) were dissolved in DCM (15 ml). Triethylamine (0.5 ml, 385 mg, 3.80 mmol) was added in 3 portions and the reaction mixture was allowed to stir at room temperature overnight. After completion of the reaction, as indicated by TLC, the solvent was removed under reduced pressure and the crude mixture was purified by column chromatography to afford the product (1.04 g, 97%).

**1H NMR** (500 MHz, Chloroform-d) δ 8.07 (dd, \(J = 9.0, 2.8\) Hz, 1H), 7.93 (d, \(J = 2.7\) Hz, 1H), 6.83 (d, \(J = 9.0\) Hz, 1H), 4.56 (t, \(J = 5.6\) Hz, 1H), 3.78 (s, 2H), 3.63 (dq, \(J = 9.4, 7.0\) Hz, 2H), 3.53 – 3.44 (m, 2H), 2.91 – 2.27 (m, 10H), 1.85 – 1.75 (m, 2H), 1.19 (t, \(J = 7.1\) Hz, 6H); **13C NMR** (125 MHz, Chloroform-d) δ 164.5, 140.1, 125.4, 124.8, 121.1, 116.5, 101.3, 61.2, 60.7, 53.6, 52.7, 52.5, 31.1, 15.3; HRMS (ESI) \(m/z\) calculated for C\(_{18}\)H\(_{30}\)N\(_3\)O\(_5\) [M+H]\(^+\) 368.2180; found 368.2180.

**4-Amino-2-((4-(3,3-dioethoxypropyl)piperazin-1-yl)methyl)phenol (23)**

MeOH (10 ml) was added to a 3-neck round bottomed flask containing 10% Pd/C (35 mg), before addition of a solution of 22 (438 mg, 1.19 mmol) in MeOH (10 ml) was added. The reaction vessel was evacuated and backfilled with nitrogen 3 times, then evacuated and backfilled with H\(_2\) 3 times before being allowed to stir at room temperature for 7 days. After the completion of the reaction, as indicated by TLC, the reaction mixture was filtered over celite and the solvent removed under reduced pressure. The crude product was purified by column chromatography (385 mg, 97%). **1H NMR** (400 MHz, Chloroform-d) δ 6.65 (d, \(J = 8.4\) Hz, 1H), 6.55 (dd, \(J = 8.5, 2.8\) Hz, 1H), 6.38 (d, \(J = 2.8\) Hz, 1H), 4.56 (t, \(J = 5.6\) Hz, 1H), 3.69 – 3.56 (m, 4H), 3.49 (dq, \(J = 9.5, 7.0\) Hz, 2H), 2.83 – 2.26 (m, 10H), 1.81 (q, \(J = 6.3\) Hz, 2H), 1.19 (t, \(J = 7.0\) Hz, 6H); **13C NMR** (100 MHz, Chloroform-d) δ 150.4, 138.5, 121.8, 116.6, 116.2, 116.0, 101.5, 61.5, 61.2, 53.8, 53.1, 52.5, 31.0, 15.4; HRMS (ESI) \(m/z\) calculated for C\(_{18}\)H\(_{32}\)N\(_3\)O\(_3\) [M+H]\(^+\) 338.2440; found 338.2430.

**4-((6-Chloro-2-methoxyacridin-9-yl)amino)-2-((4-(3,3-dimethoxypropyl)piperazin-1-yl)methyl)phenol (24)**
6,9-Dichloro-2-methoxyacridine (308 mg, 1.11 mmol) and 23 (373 mg, 1.11 mmol) were dissolved in MeOH (23 ml), 3 drops concentrated HCl were added, and the mixture was heated at reflux overnight. The reaction was quenched with saturated NaHCO₃ solution and extracted with DCM. The combined organic extracts were dried with MgSO₄, filtered, and the solvent evaporated under reduced pressure to afford the crude product as red solid. The compound was purified by column chromatography and recrystallization from MeCN (338 mg, 56%). ¹H NMR (500 MHz, Chloroform-d) δ 8.17 (s, 1H), 8.07 (d, J = 9.4 Hz, 1H), 7.92 (d, J = 9.3 Hz, 1H), 7.44 (d, J = 9.5 Hz, 1H), 7.08 (d, J = 6.4 Hz, 1H), 6.87 (d, J = 8.6 Hz, 1H), 6.80 (d, J = 8.5 Hz, 1H), 6.57 (s, 1H), 6.46 (s, 1H), 4.46 (t, J = 5.7 Hz, 1H), 3.77 (s, 2H), 3.34 (s, 6H), 2.92 – 2.34 (m, 10H), 2.20 (s, 3H), 1.81 (q, J = 6.8 Hz, 2H); HRMS (ESI) m/z calculated for C₃₃H₃₆ClN₄O₄ [M+H]⁺ 551.2420; found 551.2418.

3-(4-(5-((6-Chloro-2-methoxyacridin-9-yl)amino)-2-hydroxybenzyl)piperazin-1-yl)propanal (25)

Compound 24 (177 mg, 0.31 mmol) was dissolved in DCM (10 ml) and cooled to 0 °C. 1M BBr₃ in DCM (0.7 ml) was added dropwise and allowed to stir at 0 °C for 15 minutes. After completion of the reaction, as indicated by TLC, the reaction was carefully quenched by addition of H₂O and the pH adjusted to 8 with a saturated NaHCO₃ solution. The reaction mixture was extracted with DCM and the combined organic extracts dried with Na₂SO₄, filtered, and evaporated. The crude product was purified by column chromatography to afford the product as an orange solid (153 mg, 97%). The impure mixture obtained was used without further purification.
Methyl 2,2-dimethyl-3-oxopropanoate (27)

\[
\begin{align*}
&\text{O} \\
&\text{O} \\
&\text{Me}
\end{align*}
\]

A solution of oxalyl chloride (0.39 ml, 552 mg, 4.35 mmol) in DCM (9ml) was cooled to -78°C. A mixture of DMSO (0.4 ml, 443 mg, 5.67 mmol) and DCM (0.5 ml) was added dropwise and allowed to stir for 5 minutes before addition of a solution of methyl 3-hydroxy-2,2-dimethylpropanoate (500 mg, 3.78 mmol) in DCM (2 ml) was added dropwise and allowed to stir for a further 5 minutes before the dropwise addition of triethylamine (2.62 ml, 1.91 g, 18.9 mmol). The reaction mixture was slowly allowed to warm to room temperature. After completion of the reaction, as indicated by TLC, Et₂O and H₂O were added and the combined organic extracts were dried with MgSO₄, filtered, and evaporated. The product was purified by column chromatography (169 mg, 34%). ¹H NMR (500 MHz, Chloroform-d) δ 9.67 (s, 1H), 3.77 (s, 3H), 1.37 (s, 6H); ¹³C NMR (125 MHz, Chloroform-d) δ 199.0, 173.2, 53.9, 52.6, 19.7; HRMS (EI) m/z calculated for C₆H₁₁O₃ [M+H]⁺ 131.0708; found 131.0710.

4-Nitro-2-(piperazin-1-ylmethyl)phenol (28)

\[
\begin{align*}
&\text{O} \\
&\text{N} \\
&\text{H} \\
&\text{N} \\
&\text{H}
\end{align*}
\]

2-Hydroxy-5-nitrobenzaldehyde (1.0 g, 6.0 mmol) was dissolved in DCM (50 ml). Boc-piperazine (1.2 g, 6.6 mmol) and acetic acid (0.10 ml, 0.11 g, 1.8 mmol) were added and allowed to stir for 30 minutes before addition of NaBH(OAc)₃ (1.5 g, 7.2 mmol). The reaction mixture was allowed to stir at room temperature overnight. After the completion of the reaction, as indicated by TLC, the reaction was quenched with satd. NaHCO₃ solution, then 1 M NaOH solution. The reaction mixture was then adjusted to pH = 8-9 by addition of 1M HCl. Extraction with DCM and drying of organic extracts with Na₂SO₄, filtration and removal of solvent under reduced pressure afforded the crude boc-protected product, which was purified by column chromatography (1.74 g, 86%). The boc-protected product was dissolved in DCM (20 ml) and TFA (4 ml) and allowed to stir at room temperature for 2 days. After the completion of the reaction, as indicated by TLC, the reaction mixture was quenched with a saturated solution of NaHCO₃ and extracted with DCM. The combined organic extracts were dried with MgSO₄, filtered, and the solvent removed under reduced pressure. The crude product was purified by column chromatography (quantitative yield). ¹H NMR (700 MHz, Chloroform-d) δ 8.09 (dd, J = 9.0, 2.8 Hz, 1H), 7.94 (dd, J = 2.8, 0.9 Hz, 1H), 6.84 (d, J = 8.9 Hz, 1H), 3.78 (s, 2H), 3.01 – 2.43 (m, 9H); ¹³C NMR (175 MHz, Chloroform-d) δ 164.4, 125.4, 124.8;

---

¹Ng SS, Ho CY, Jamison TF. Nickel-catalyzed coupling of alkenes, aldehydes, and silyl triflates. *J Am Chem Soc* (2006) 128:11513–11528. doi:10.1021/ja062866w
121.0, 116.5, 61.3, 53.5, 53.4, 45.7; HRMS (ESI) m/z calculated for C₁₁H₁₆N₃O₃ [M+H]+ 238.1186; found 238.1184.

Methyl 3-(4-(2-hydroxy-5-nitrobenzyl)piperazin-1-yl)-2,2-dimethylpropanoate (29)

![Structure of 29]

Compound 27 (470 mg, 3.61 mmol) was dissolved in DCM (25 ml). Compound 28 (777 mg, 3.28 mmol) and acetic acid (0.10 ml, 0.11 g, 1.8 mmol) were added and allowed to stir for 30 minutes before addition of NaBH(OAc)₃ (56μl, 59 mg, 1.0 mmol). The reaction mixture was allowed to stir at room temperature overnight. After the completion of the reaction, as indicated by TLC, the reaction was quenched with satd. NaHCO₃ solution, then 1 M NaOH solution. The reaction mixture was then adjusted to pH = 8-9 by addition of 1M HCl. Extraction with DCM and drying of organic extracts with Na₂SO₄, filtration and removal of solvent under reduced pressure afforded the crude product, which was purified by column chromatography (990 mg, 86%). ¹H NMR (400 MHz, Chloroform-d) δ 8.08 (dd, J = 9.0, 2.8 Hz, 1H), 7.92 (d, J = 2.7 Hz, 1H), 6.83 (d, J = 9.0 Hz, 1H), 3.76 (s, 2H), 3.65 (s, 3H), 2.68 – 2.46 (m, 8H), 1.16 (s, 6H).

3-(4-(2-Hydroxy-5-nitrobenzyl)piperazin-1-yl)-2,2-dimethylpropanoic acid (30)

![Structure of 30]

Compound 29 (515 mg, 1.50 mmol) was dissolved in THF (4 ml) and H₂O (4 ml). LiOH.H₂O (123 mg, 2.93 mmol) was added and the reaction was heated at reflux for 6 hours. The reaction mixture was allowed to cool to room temperature and the THF was evaporated under reduced pressure. Extractions of the aqueous layer with DCM at pH = 14, pH = 1, and pH = 7 were carried out and the combined organic extracts were dried with MgSO₄, filtered, and evaporated under reduced pressure to afford the desired product (280 mg, 57%). ¹H NMR (700 MHz, Chloroform-d) δ 8.11 (dd, J = 9.0, 2.7 Hz, 1H), 7.96 (d, J = 2.7 Hz, 1H), 6.87 (d, J = 9.0 Hz, 1H), 3.83 (s, 2H), 2.96 – 2.55 (m, 8H), 1.23 (s, 6H); ¹³C NMR (175 MHz, Chloroform-d) δ 177.6, 163.7, 140.5, 125.6, 124.9, 120.5, 116.6, 65.2, 60.5, 54.4, 52.2, 41.5, 25.2.

3-(4-(2-Hydroxy-5-nitrobenzyl)piperazin-1-yl)-N,2,2-trimethylpropanamide (31)
Compound 30 (236 mg, 0.78 mmol) was dissolved in DCM (7 ml). DMF (7 drops) then thionyl chloride (0.17 ml, 279 mg, 2.34 mmol) was added and the reaction mixture allowed to stir at room temperature overnight. After the completion of the reaction, as indicated by TLC, the solvent was evaporated under reduced pressure to afford the crude acid chloride as a colourless solid, which was dissolved in DCM (20 ml). Methylamine (2M in THF, 2.0 ml) was added and the reaction mixture was allowed to stir at room temperature overnight. After the completion of the reaction, as indicated by TLC, the reaction was quenched with H$_2$O and extracted with DCM. The combined organic extracts were washed with brine, dried with MgSO$_4$, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography to afford the desired product (256 mg, 84%).

$^1$H NMR (500 MHz, Chloroform-d) $\delta$ 8.07 (dd, $J$ = 9.0, 2.8 Hz, 1H), 7.92 (d, $J$ = 2.8 Hz, 1H), 6.94 (s, 1H), 6.83 (d, $J$ = 9.0 Hz, 1H), 3.77 (s, 2H), 2.76 (d, $J$ = 4.8 Hz, 3H), 2.72 – 2.40 (m, 9H), 1.13 (s, 6H); $^{13}$C NMR (125 MHz, Chloroform-d) $\delta$ 177.8, 164.3, 140.2, 125.4, 124.8, 121.0, 116.6, 66.4, 60.6, 54.6, 52.8, 42.6, 26.2, 24.4; HRMS (ESI) $m/z$ calculated for C$_{17}$H$_{27}$N$_4$O$_4$ [M+H]$^+$ 351.2027; found 351.2028.

3-(4-(5-Amino-2-hydroxybenzyl)piperazin-1-yl)-N,2,2-trimethylpropanamide (32)

MeOH (5 ml) was added to a 3-neck round bottomed flask containing 10% Pd/C (20 mg), before addition of a solution of 31 (148 mg, 0.42 mmol) in MeOH (5 ml) was added. The reaction vessel was evacuated and backfilled with nitrogen 3 times, then evacuated and backfilled with H$_2$ 3 times before being allowed to stir at room temperature for 7 days. After the completion of the reaction, as indicated by TLC, the reaction mixture was filtered over celite and the solvent removed under reduced pressure. The crude product was purified by column chromatography (77 mg, 57%). $^1$H NMR (700 MHz, Chloroform-d) $\delta$ 6.65 (d, $J$ = 8.4 Hz, 1H), 6.55 (dd, $J$ = 8.4, 2.8 Hz, 1H), 6.36 (d, $J$ = 2.8 Hz, 1H), 3.59 (s, 2H), 2.76 (d, $J$ = 4.8 Hz, 3H), 2.70 – 2.38 (m, 9H), 1.12 (s, 6H); HRMS (ESI) $m/z$ calculated for C$_{17}$H$_{29}$N$_4$O$_2$ [M+H]$^+$ 321.2285; found 321.2284.

Tert-butyl 4-(2-methoxyethyl)piperazine-1-carboxylate (33)
1-(2-Hydroxyethyl)piperazine (1.0 g, 7.7 mmol) was dissolved in MeOH (22 ml). Boc₂O (1.8 ml, 1.7 g, 7.7 mmol) was added dropwise and the reaction mixture was allowed to stir at room temperature overnight. The solvent was evaporated to afford crude tert-butyl 4-(2-hydroxyethyl)piperazine-1-carboxylate which was used without further purification. NaH (40 %, 88 mg, 2.2 mmol) was dissolved in THF (10 ml) and cooled to 0 °C, tert-butyl 4-(2-hydroxyethyl)piperazine-1-carboxylate (421 mg, 1.8 mmol) in THF (10 ml) was added and the reaction mixture was allowed to stir at 0 °C for 1 hour. Methyl iodide (137 μl, 312 mg, 2.2 mmol) was added and the reaction mixture was allowed to warm to room temperature and stir overnight. After the completion of the reaction, as indicated by TLC, the reaction was quenched with H₂O and extracted with EtOAc. The combined organic extracts were dried with Na₂SO₄, filtered, the solvent removed under reduced pressure to afford the product (422 mg, 95%).

\[ ^1H \text{ NMR (400 MHz, Chloroform-}d) \delta 3.51 (t, J = 5.6 \text{ Hz, 2H}), 3.45 (t, J = 5.0 \text{ Hz, 4H}), 3.35 (s, 3H), 2.58 (t, J = 5.6 \text{ Hz, 2H}), 2.43 (t, J = 5.1 \text{ Hz, 4H}), 1.45 (s, 9H); \]

\[ ^13C \text{ NMR (100 MHz, Chloroform-}d) \delta 154.8, 79.6, 70.1, 59.0, 58.1, 53.4, 28.5, -9.1. \]

1-(2-Methoxyethyl)piperazine (TFA salt) (34)

Compound 33 (412 mg, 1.69 mmol) was dissolved in DCM (7.0 ml) and TFA (1.4 ml) and allowed to stir at room temperature overnight. After the completion of the reaction, as indicated by TLC, DCM was evaporated and residual TFA was co-evaporated with toluene to afford the product as a beige solid that was used without further purification (quantitative yield).

4-Amino-2-((4-(2-methoxyethyl)piperazin-1-yl)methyl)phenol (35)

Compound 34 (630 mg, 2.4 mmol), acetaminophen (250 mg, 1.6 mmol), and formaldehyde (37% aq. Soln, 0.8 ml) were dissolved in isopropanol (12 ml). The reaction mixture was heated at reflux overnight. After the completion of the reaction, as indicated by TLC, the solvent was evaporated under reduced pressure and the crude mixture purified by column chromatography to afford N-(4-
hydroxy-3-((4-(2-methoxyethyl)piperazin-1-yl)methyl)phenylacetamide (192 mg, 56%), which was used without further purification. N-(4-hydroxy-3-((4-(2-methoxyethyl)piperazin-1-yl)methyl)phenyl)acetamide (166 mg, 0.54 mmol) was dissolved in 6M aqueous HCl (1.5 ml). The reaction was heated at reflux for 2 hours. After the completion of the reaction, as indicated by TLC, the reaction mixture was allowed to cool to room temperature, neutralized and extracted with DCM. The combined organic extracts were dried with MgSO₄, filtered, and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography (98 mg, 68%). ¹H NMR (400 MHz, Chloroform-d) δ 6.65 (d, J = 8.4 Hz, 1H), 6.55 (dd, J = 8.4, 2.8 Hz, 1H), 6.38 (d, J = 2.8 Hz, 1H), 3.61 (s, 2H), 3.51 (t, J = 5.5 Hz, 2H), 3.35 (s, 3H), 2.84 – 2.39 (m, 10H); HRMS (ESI) m/z calculated for C₁₄H₂₄N₃O₂ [M+H]+ 266.1863; found 266.1864.

NMR Spectra
