ecoAO: A simple system for the study of human aldehyde oxidase role in drug metabolism

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Supporting Information

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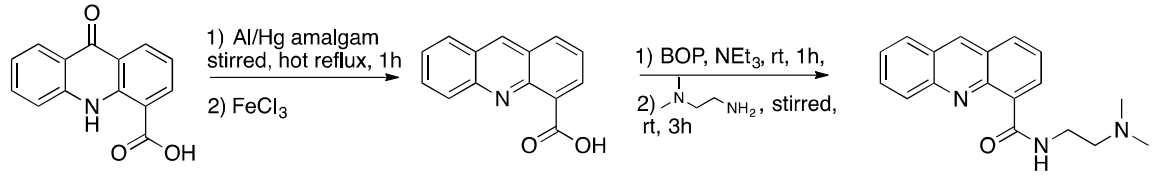
Synthesis of DACA (*N*-(2-(dimethylamino)ethyl)acridine-4-carboxamide)



Scheme S1. Synthesis of DACA.

5.0 grams of 4-carboxyacridone was suspended in 50% aqueous ethanol under reflux. Triethylamines was added dropwise just enough to dissolve the acridone. In a separate beaker, immersed Al foil (cut in small pieces) to a 200 mL of ethanol containing mercury (II) chloride for a minute. The soaked Al foil was added to reflux solution of acridone and allowed to react from 30 minutes to an hour as monitored by TLC. After the reaction is completed it is filtered and washed with 1 N KOH (in 50% aqueous ethanol). The filtrate was collected and strongly acidified by adding concentrated hydrochloric acid. It is then added with 10 g of ferric chloride and heated for 45 min to 1 h under reflux. The mixture is filtered and concentrated in vacuo just enough to remove most of the ethanol. It is partitioned between water and ethyl acetate. The organic layer is concentrated and the 4-carboxy-acridine (acridine R_f = 0.55, 9:1 dichloromethane - methanol), was recrystallized in acetone. The crystallized acridine was dissolved in anhydrous dichloromethane. The 4-carboxyacridine (500 mg) was resuspended to 20 mL anhydrous dichloromethane. Triethylamine was added dropwise until the acid becomes soluble. 1.2 grams (1.2 eq) of benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) was added. The reaction was stirred for 1 hr at room temperature. 0.25 mL of the amine (*N*,*N*-dimethylethylenediamine, 1.0 eq, d = 0.807 g/mL) was added slowly dropwise. The reaction was stirred at room temperature as monitored by TLC (DACA R_f = 0.15, 9:1 dichloromethane - methanol). After 3 hr, the reaction mixture was concentrated in vacuo and chromatographed over silica gel (solvent: 9:1:0.1, dichloromethane-MeOH-NH_4OH).

4-carboxy-acridine. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 9.04 (s, 1H), 8.94 (dd, *J* = 7.1, 1.5 Hz, 1H), 8.34 – 8.21 (m, 2H), 8.17 – 8.07 (m, 1H), 7.95 (ddd, *J* = 8.8, 6.7, 1.4 Hz, 1H), 7.75 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.75 – 7.63 (m, 1H).

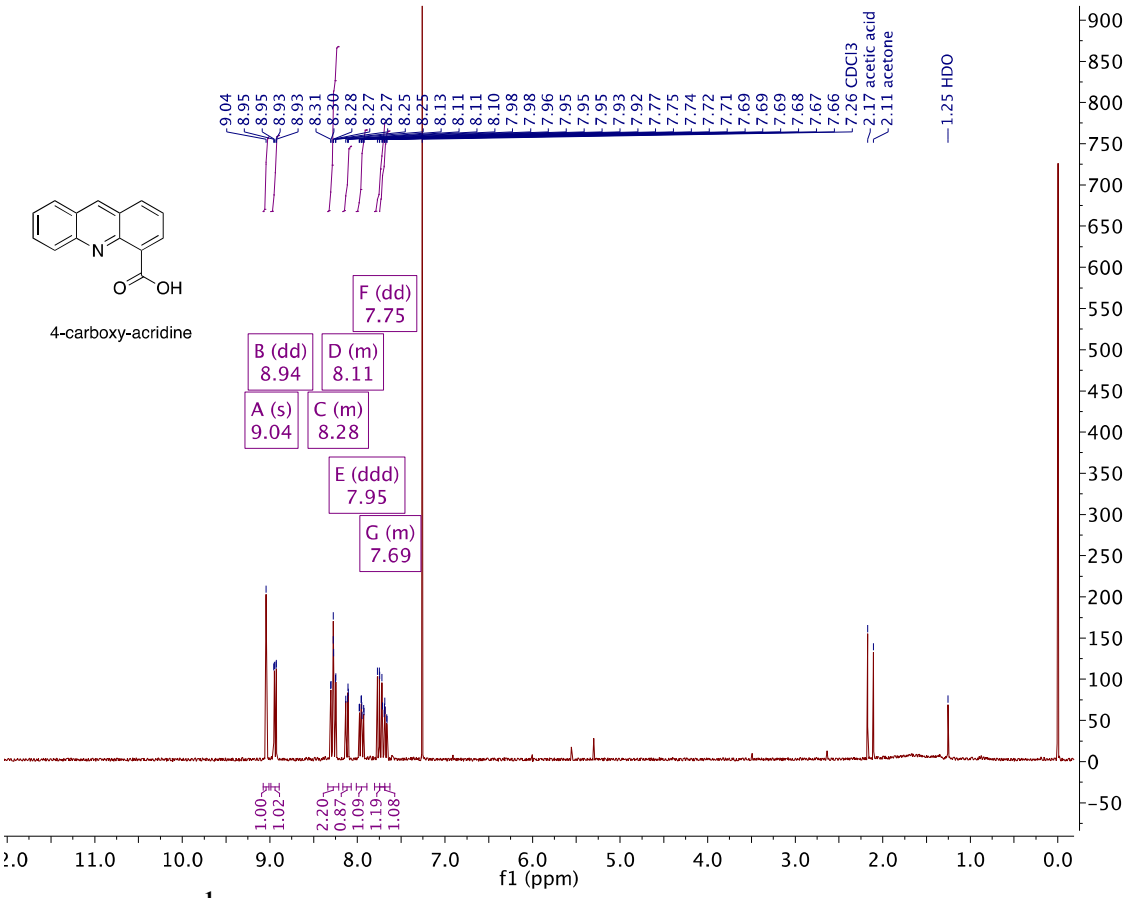


Figure S1. ^{1}H NMR of purified 4-carboxy-acridine (300 MHz, $CDCl_3$, 20 °C).

DACA (N-(2-(dimethylamino)ethyl)acridine-4-carboxamide). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.69 (t, J = 5.8 Hz, 1H), 9.38 (s, 1H), 8.77 (dd, J = 7.1, 1.6 Hz, 1H), 8.49 – 8.42 (m, 2H), 8.27 (d, J = 8.5 Hz, 1H), 8.04 – 7.98 (m, 1H), 7.83 – 7.71 (m, 2H), 3.95 (q, J = 5.9 Hz, 2H), 3.45 (s, 2H), 2.92 (s, 6H). LC-MS (ESI)$^+$: found m/z = 294.2 $[M+H]^+$, (calculated for $C_{17}H_{17}N_6O^+$: 294.16).

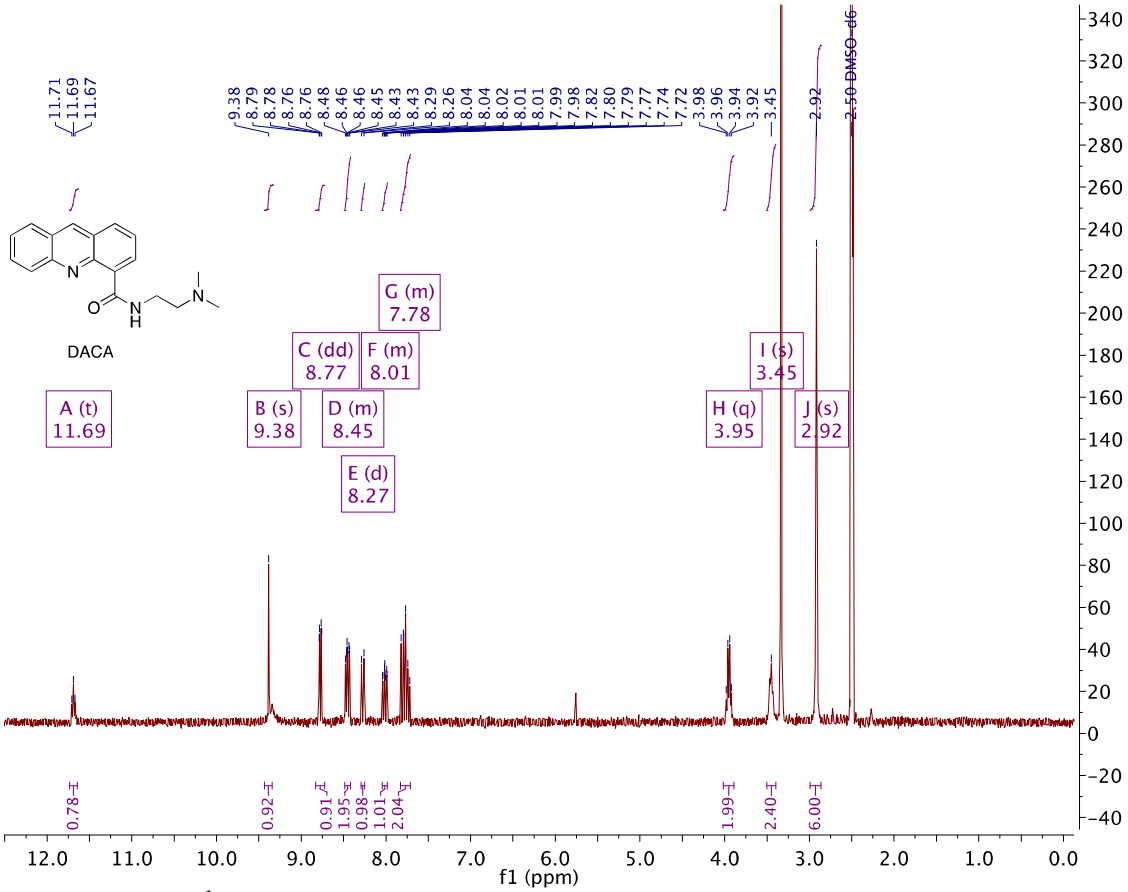
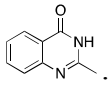


Figure S2. ^{1}H NMR of purified DACA (*N-(2-(dimethylamino)ethyl)acridine-4-carboxamide)* (300 MHz, DMSO-d_6, 20 °C).

LC-MS/MS Chromatogram

The internal standard used is 2-methyl-4(*3H*)-quinazolinone,  .

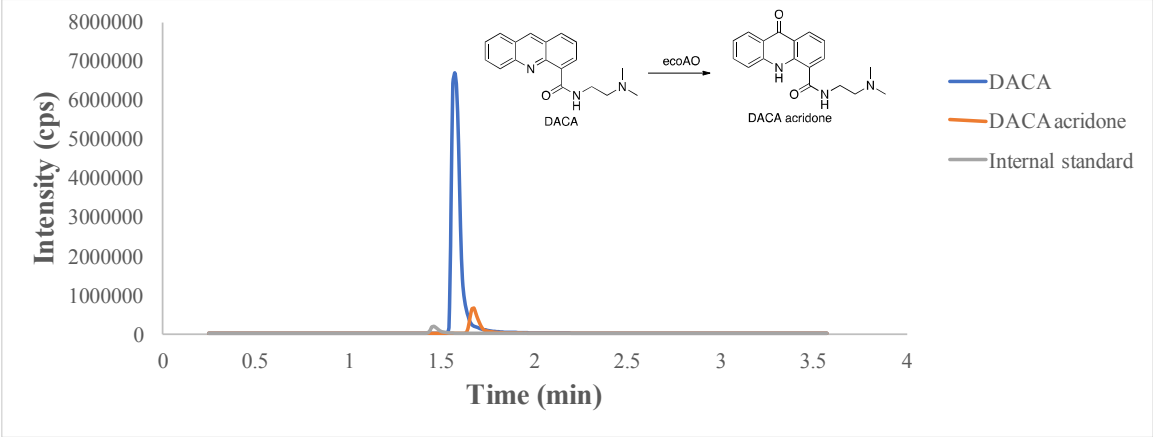


Figure S3. LC-MS/MS chromatogram of DACA and DACA acridone.

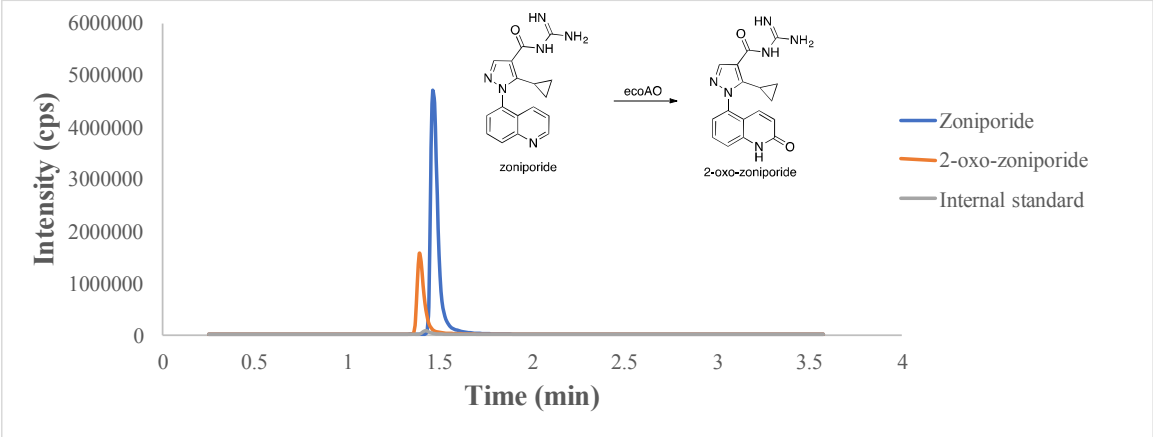


Figure S4. LC-MS/MS chromatogram of zoniporide and 2-oxo-zoniporide.

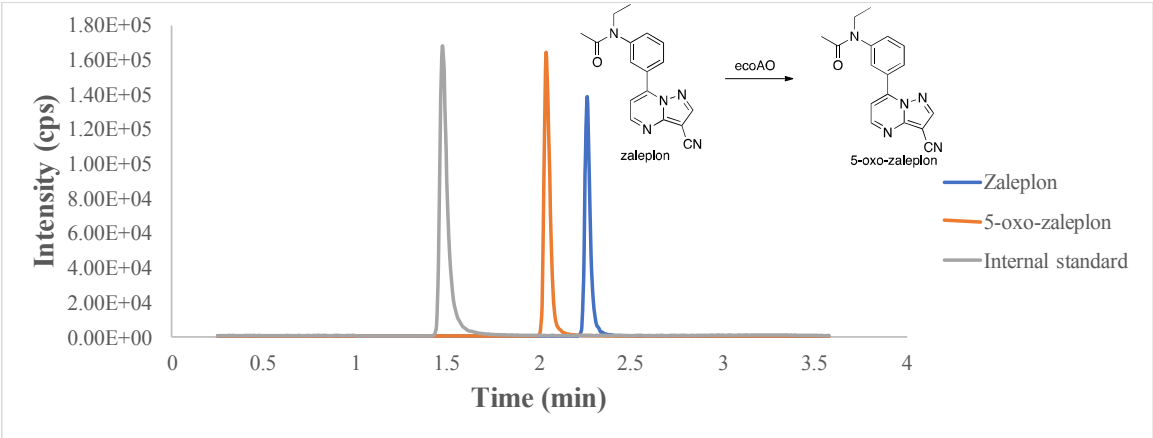


Figure S5. LC-MS/MS chromatogram of zaleplon and 5-oxo-zaleplon.

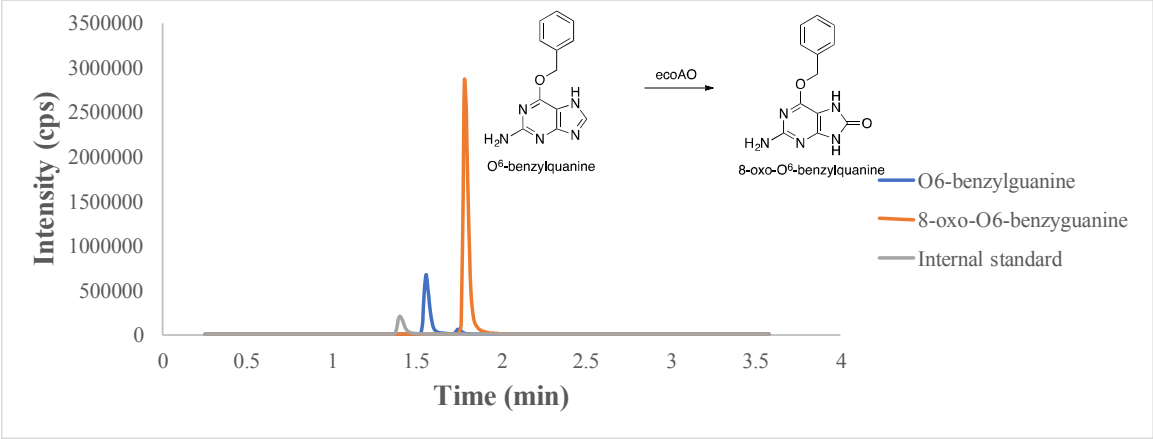
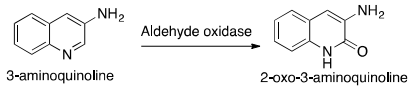


Figure S6. LC-MS/MS chromatogram of O^6-benzylguanine and 8-oxo-O^6-benzyguanine.

3-aminoquinoline metabolism

1 mM (final concentration) of 3-aminoquinoline was incubated with human liver cytosol (0.5 mg/mL), TP1017 cells with (ecoAO, 50 mg/mL) and without pTHco-hAOX1 plasmid for 1 h, 37°C water bath. Reaction was quenched by adding 1 M formic acid containing a known amount of internal standard and centrifuged at 16,100 x *g* for 10 min. The formation of 2-oxo-3-aminoquinoline was monitored in the supernatant using LC-MS/MS.



Scheme S2. AO-mediated oxidation of 3-aminoquinoline.

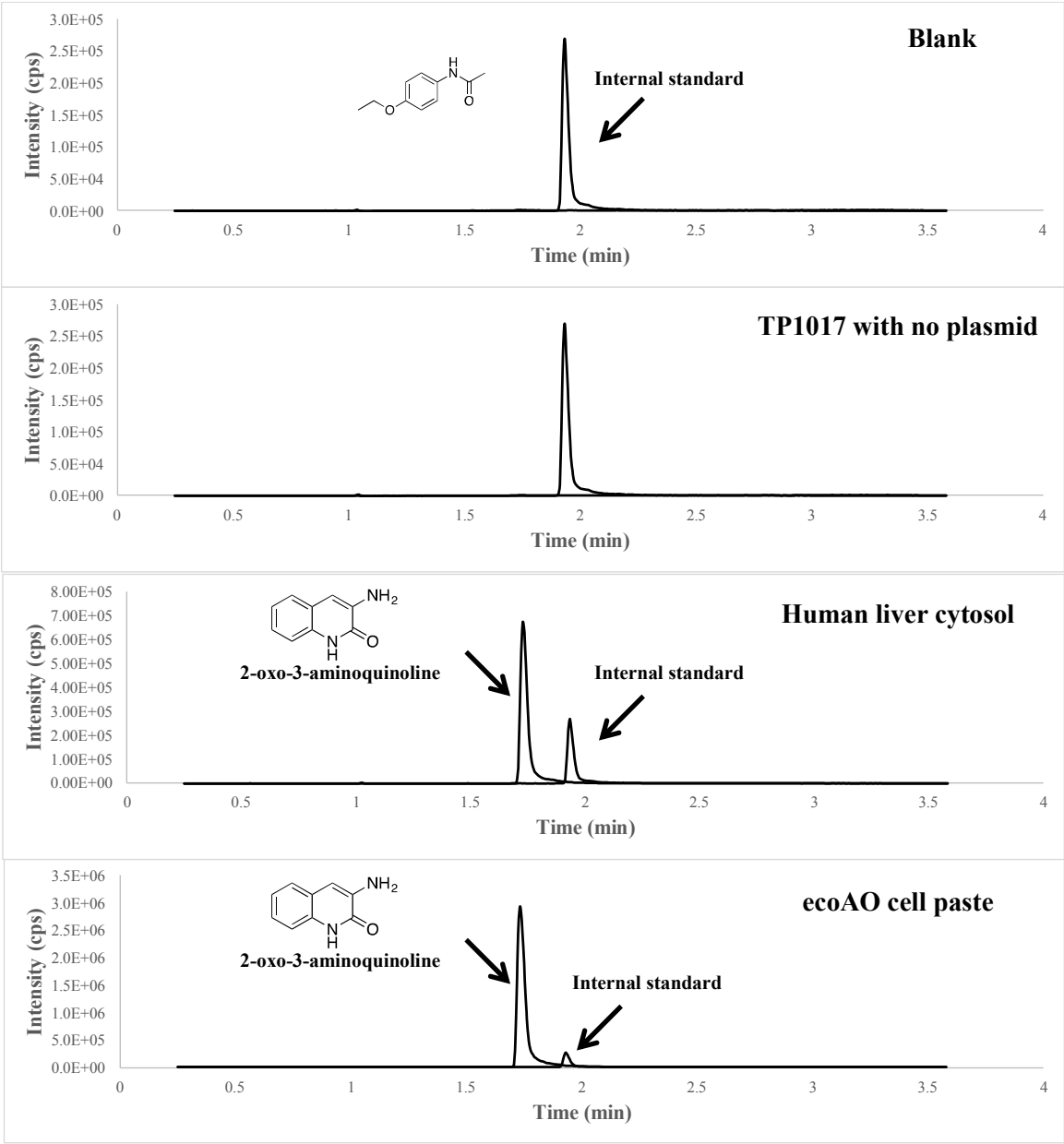


Figure S7. LC-MS/MS chromatogram of 2-oxo-3-aminoquinoine.

NMR spectra of purified AO metabolites

DACA acridone. ^{1}H NMR (300 MHz, Methanol-d_4): δ 8.57 (dd, J = 8.1, 1.4 Hz, 1H), 8.36 (dt, J = 8.4, 0.9 Hz, 1H), 8.24 (dd, J = 7.5, 1.5 Hz, 1H), 7.83 – 7.76 (m, 1H), 7.65 – 7.60 (m, 1H), 7.40 – 7.32 (m, 2H), 2.65 (t, J = 6.7 Hz, 2H), 2.37 (s, 6H). LC-MS (ESI)$^+$: found m/z = 310.2 $[M+H]^+$ (calculated for $C_{18}H_{20}N_3O_2^+$: 310.16).

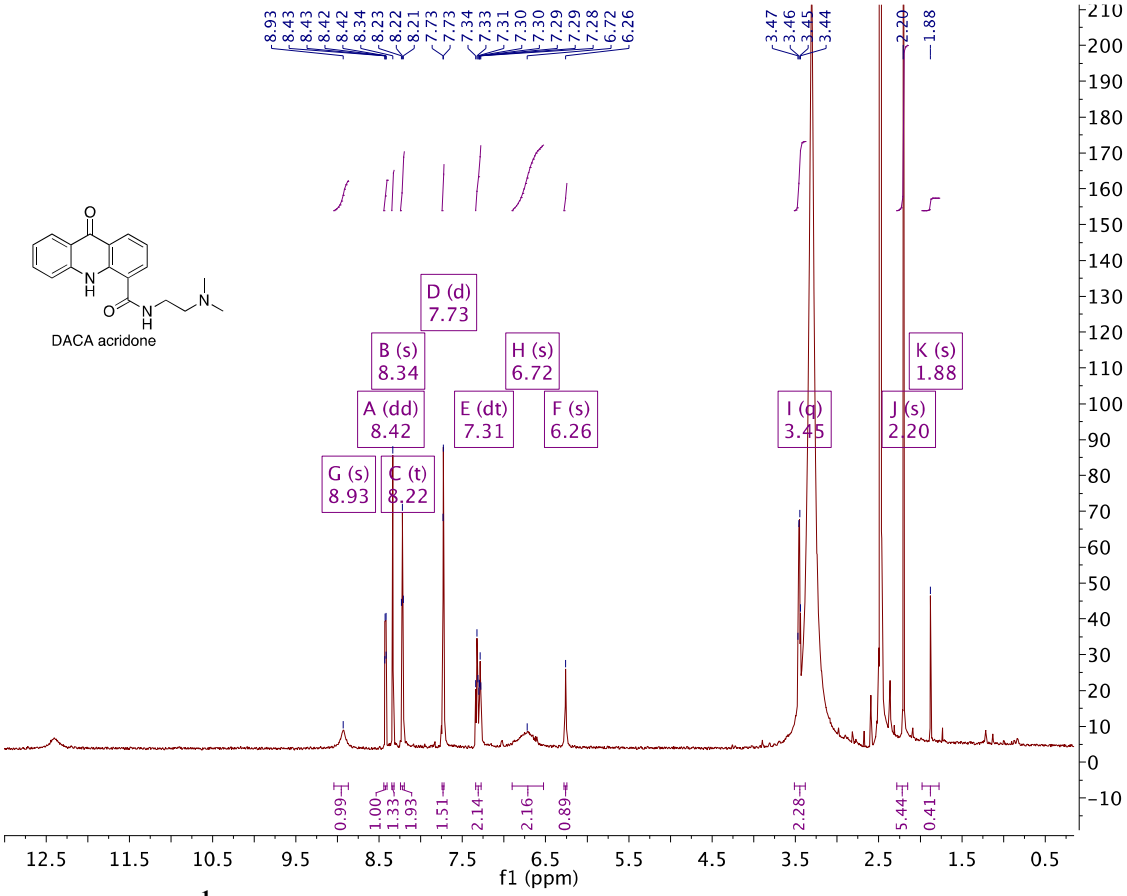


Figure S8. ^{1}H NMR of purified DACA acridone (600 MHz, DMSO-d_6, 20 °C).

2-oxo-zoniporide. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.94 (s, 1H), 7.62 (t, J = 8.0 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 7.6 Hz, 1H), 7.12 (d, J = 9.8 Hz, 1H), 6.53 (d, J = 9.8 Hz, 1H), 6.28 (s, 2H), 1.88 (s, 2H), 1.75 (s, 1H), 0.64 (d, J = 7.3 Hz, 2H). LC-MS (ESI)$^{+}$: found m/z = 337.0 $[M+H]^{+}$, (calculated for $C_{17}H_{17}N_6O_2^{+}$: 337.1).

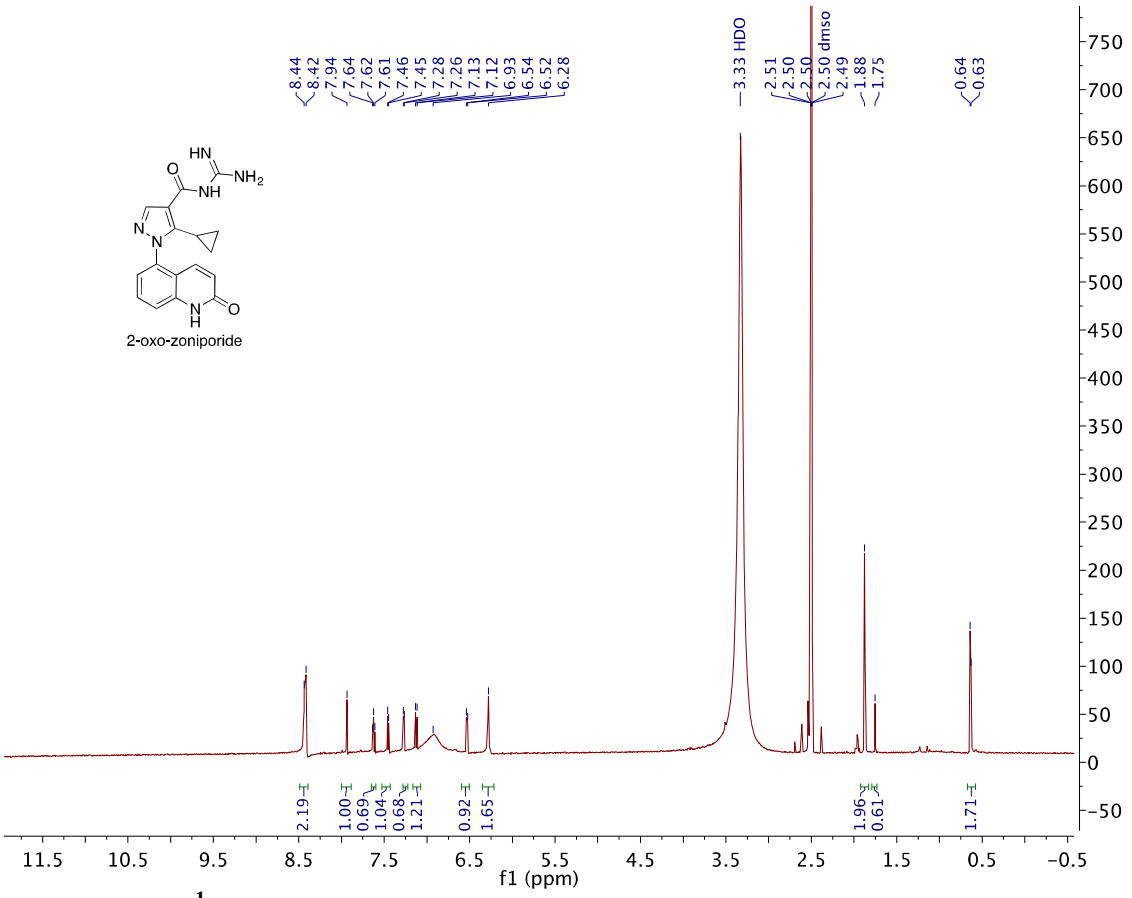


Figure S9. ^{1}H NMR of purified 2-oxo-zoniporide (600 MHz, DMSO-d_6, 20 °C).

5-oxo-zaleplon. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.92 (s, 1H), 7.79 (d, J = 7.7 Hz, 1H), 7.72 (s, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.40 (d, J = 8.0 Hz, 1H), 5.94 (s, 1H), 3.67 (q, J = 7.0 Hz, 2H), 1.77 (s, 3H), 1.02 (t, J = 7.2 Hz, 3H). LC-MS (ESI)$^{+}$: found m/z = 322.3 $[M+H]^{+}$ (calculated for $C_{17}H_{18}N_5O_2^{+}$: 322.1).

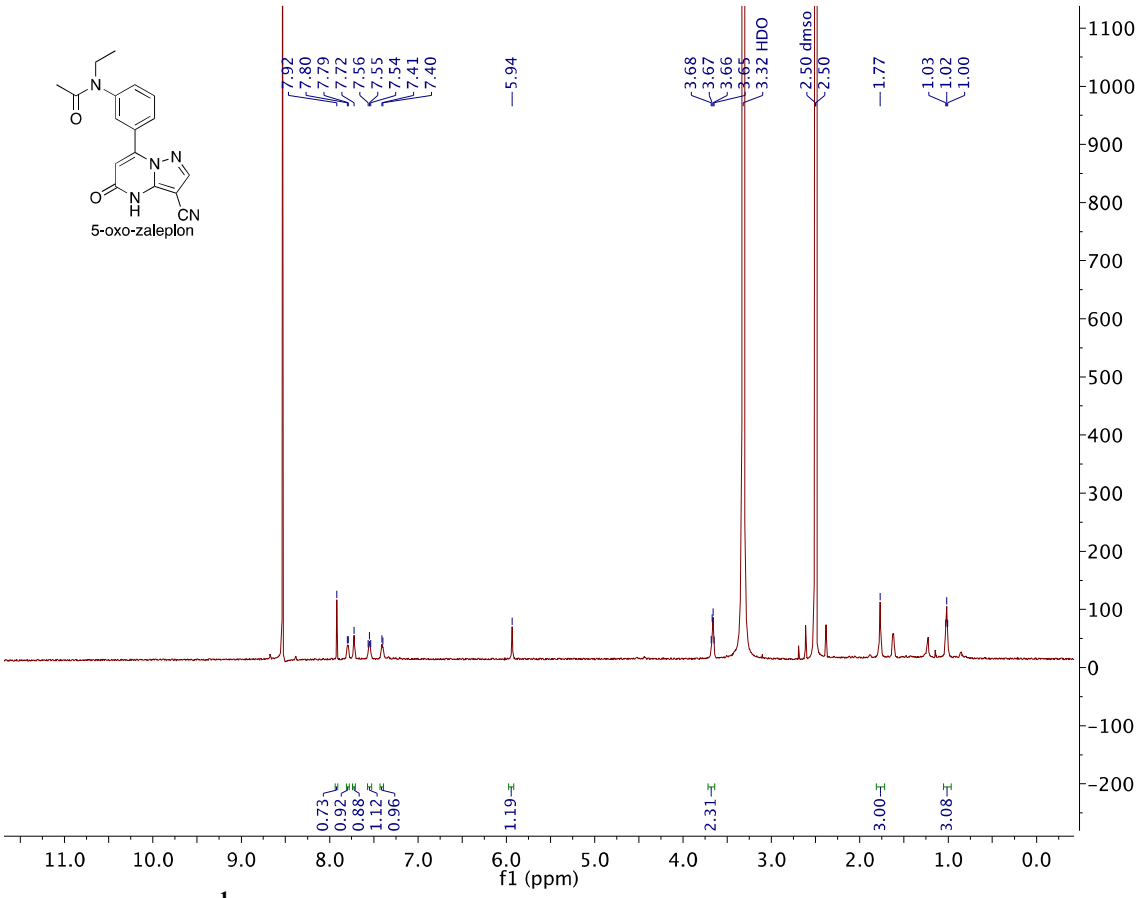


Figure S10. ^{1}H NMR of purified 5-oxo-zaleplon (600 MHz, DMSO-d_6, 20 °C).

8-oxo-O^6-benzylguanine.
LC-MS $(ESI)^+$: found m/z = 258.2 $[M+H]^+$ (calculated for $C_{12}H_{12}N_5O_2^+$: 258.1).

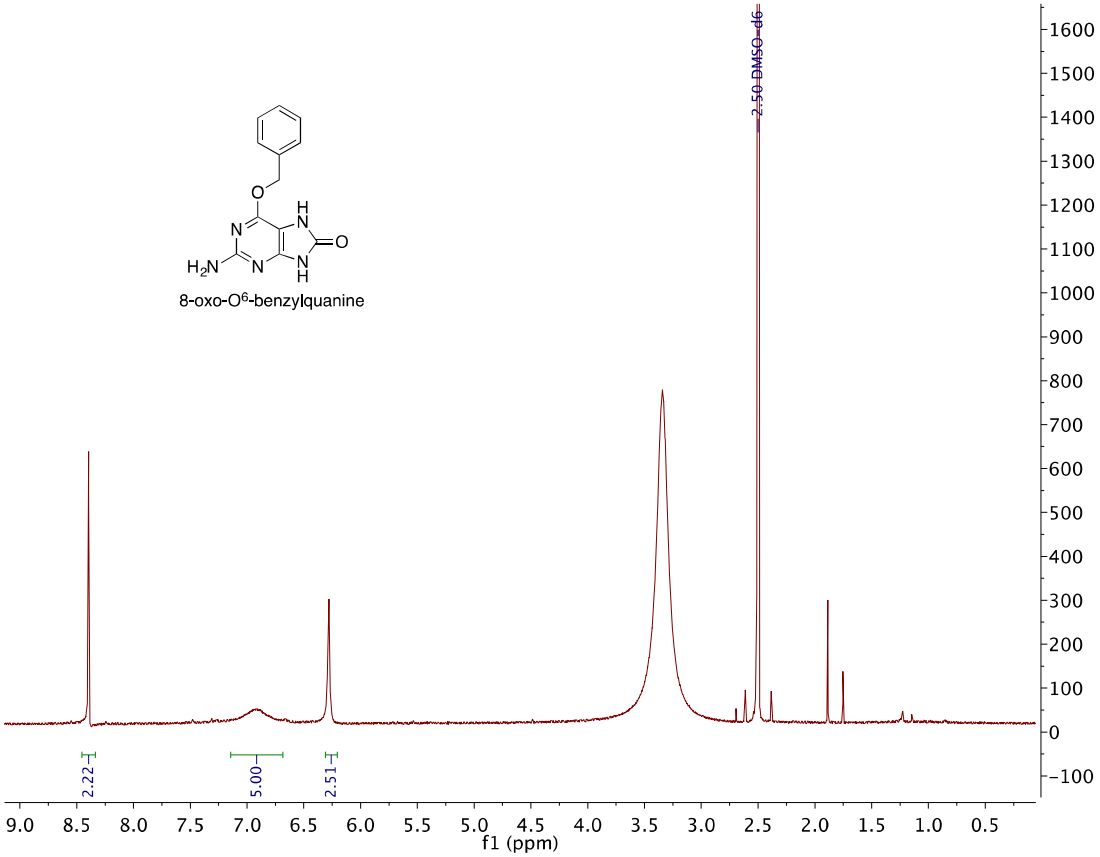


Figure S11. ^{1}H NMR of purified 8-oxo-O^6-benzylguanine (600 MHz, DMSO-d_6, 20 °C).

Extinction coefficient

A set of DMSO stock was prepared by serial dilution ranging from 0 - 25 mM. 2 µL of this DMSO stock was added to 998 µL of KPi buffer to make 1000 µL (final volume) making 1.56 - 50 µM (0.2% v/v DMSO) final concentration. The absorbance was measured four times at specified wavelengths (n=2 sets) for each concentration using Agilent 8453 UV-vis (Agilent Technologies, Sta. Clara, CA, USA). The absorbance measured were < 1.0 absorbance units (AU). The extinction coefficient was calculated from the slope using linear regression.

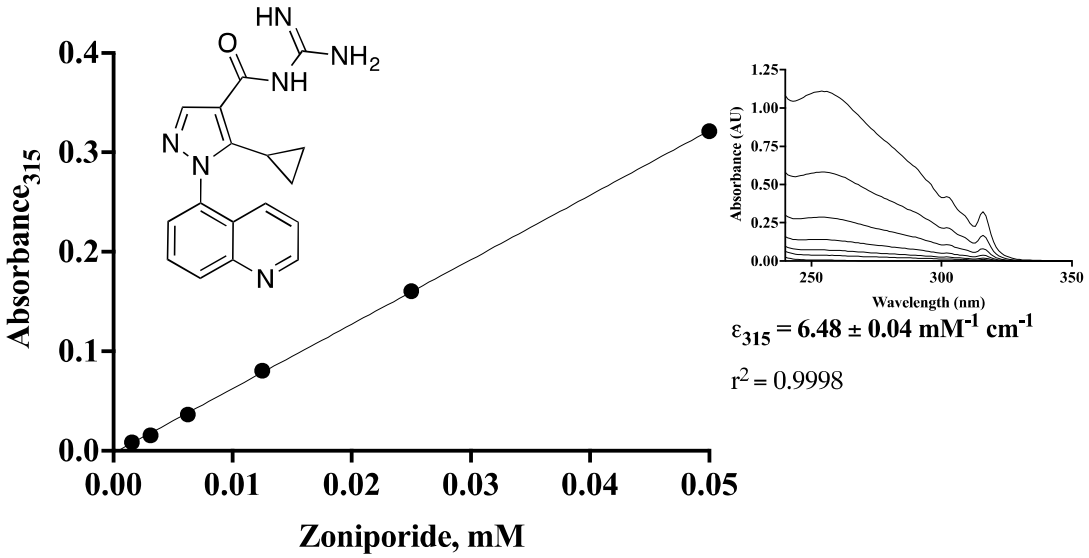
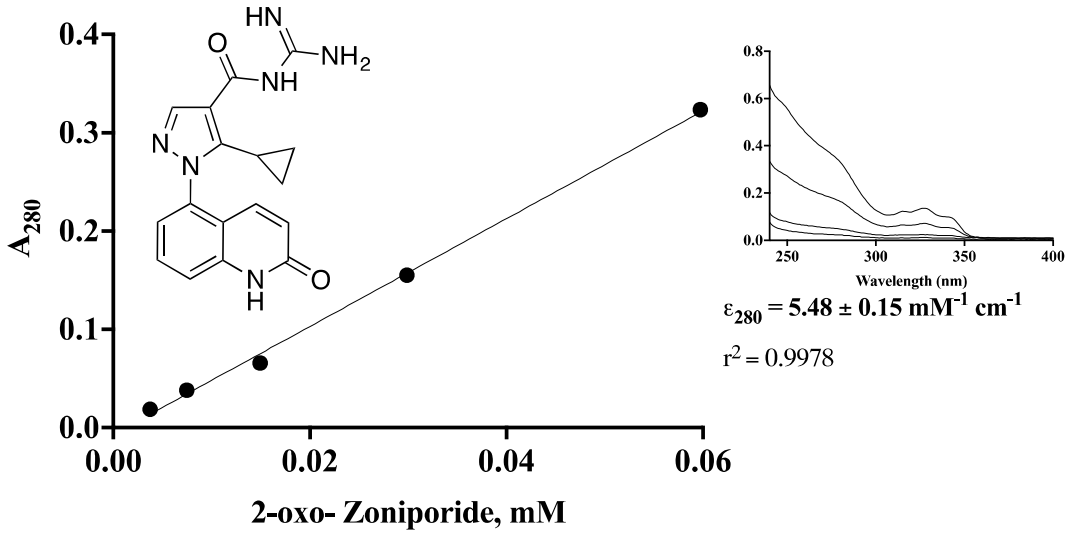


Figure S12. Extinction coefficient and UV spectra of zoniporide.



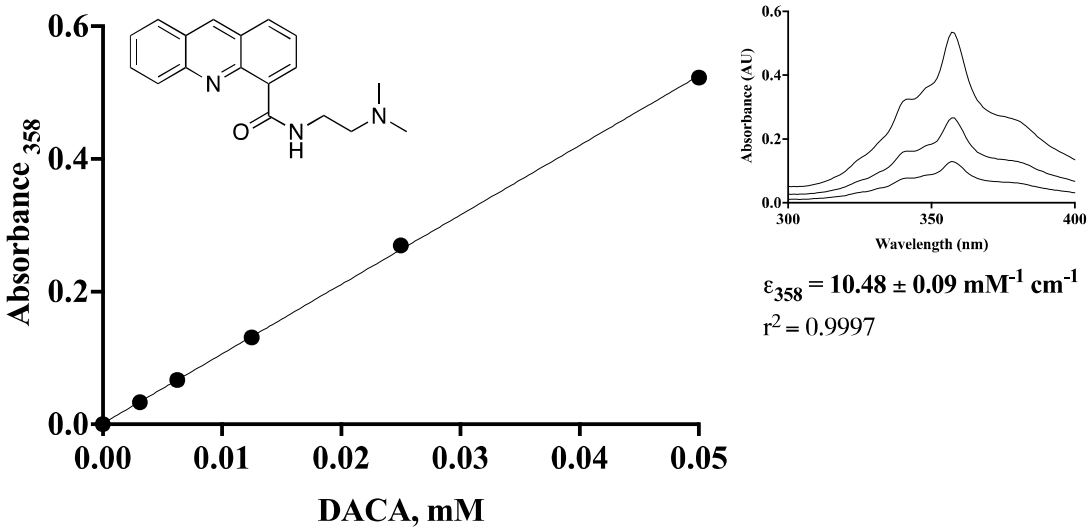


Figure S15. Extinction coefficient and UV spectra of DACA.

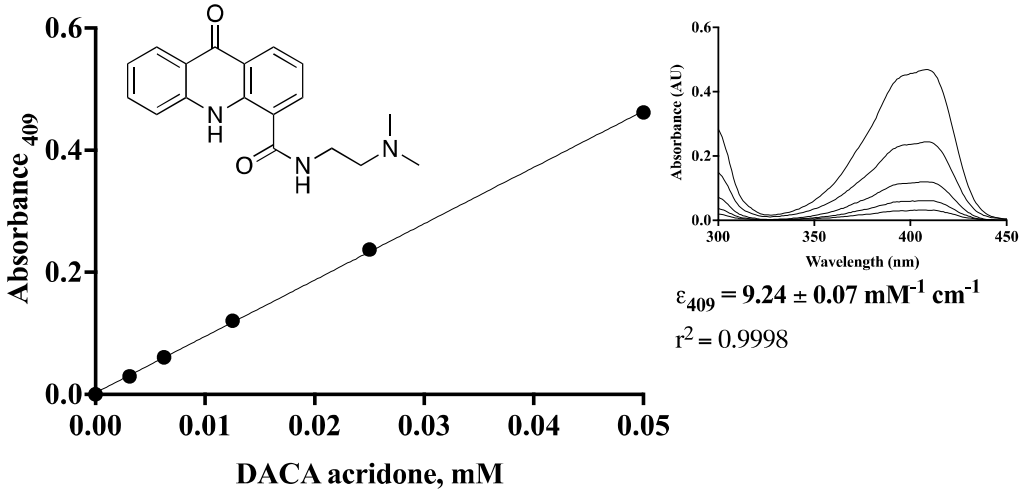
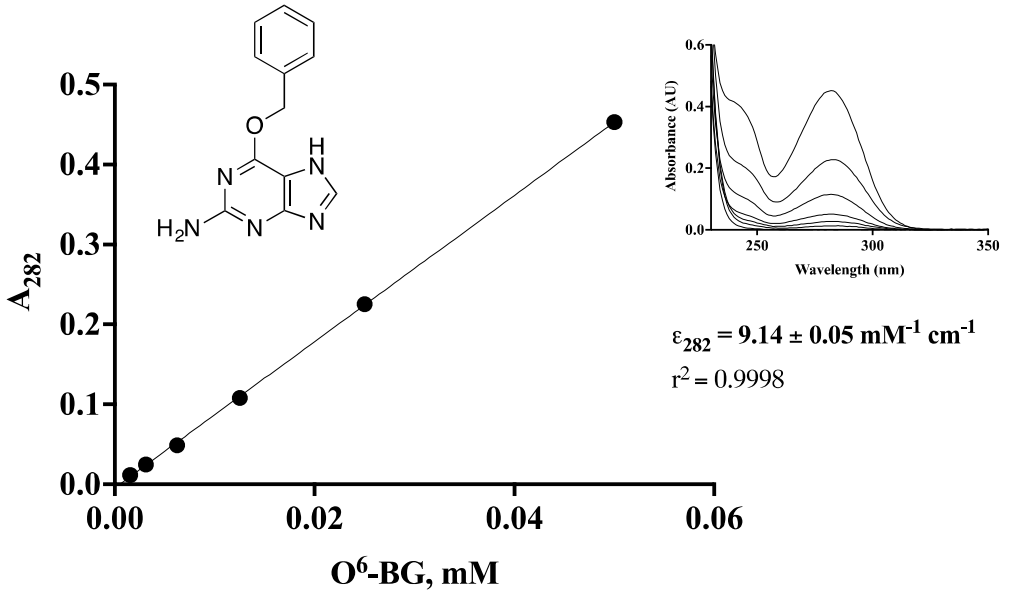


Figure S16. Extinction coefficient and UV spectra of DACA acridone.



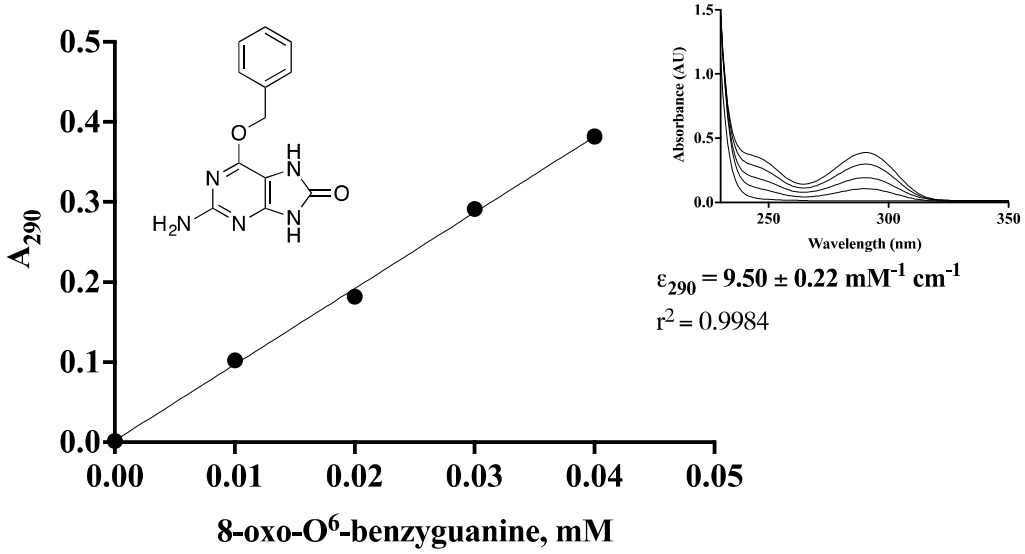
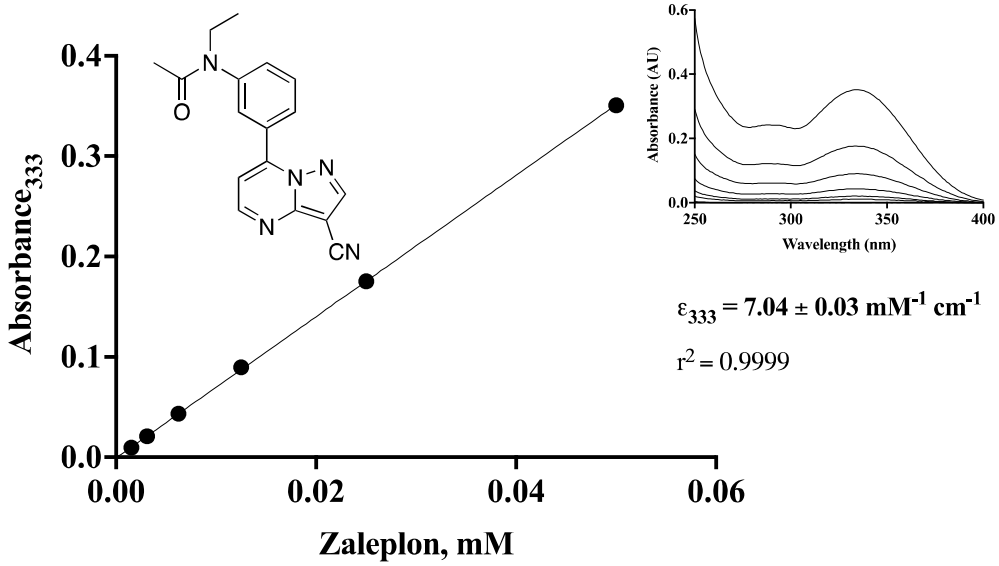
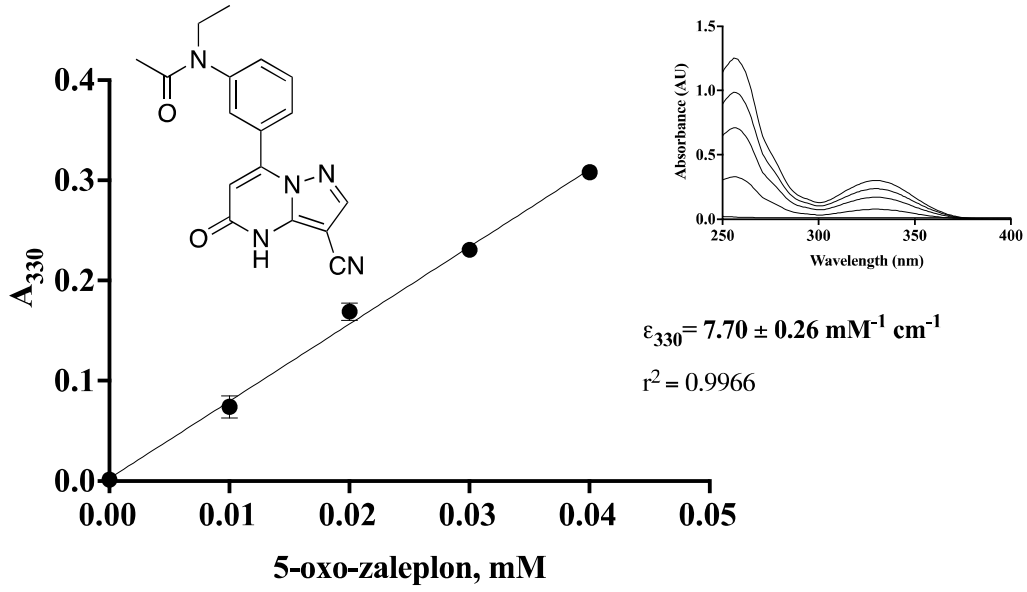


Figure S18. Extinction coefficient and UV spectra of 8-oxo-O^6-benzylguanine.


Absorbance$_{333}$
Zaleplon, mM
Absorbance (AU)
Wavelength (nm)
ε_{333} = 7.04 ± 0.03 mM^{-1} cm^{-1}
r^2 = 0.9999


A_{330}
5-oxo-zaleplon, mM
Absorbance (AU)
Wavelength (nm)
ε_{330}= 7.70 ± 0.26 mM^{-1} cm^{-1}
r^2 = 0.9966