Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Diazepam-based covalent modifiers of GPX4 induce ferroptosis in liver cancer cells

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1. General Information:

All chemicals and anhydrous solvents were purchased from commercial sources and used as received unless stated otherwise. Reactions were conducted under argon atmosphere, monitored by thin-layer chromatography (TLC) on a glass plate pre-coated with silica (60 F254) and visualized with UV light. Column chromatography was performed using Merck silica gel (60-120 and 100–200 mesh). ^{1}H and ^{13}C spectra were recorded on JEOL ECS-400 MHz (or ECX-500 MHz for ^{13}C) spectrometers using either residual solvent signals as an internal reference ($CDCl_3$ δ H, 7.24 ppm, δ C 77.1 ppm) or an internal tetramethylsilane (δ H = 0.00, δ C = 0.0). Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. The following abbreviations are used: m (multiplet), s (singlet), br s (broad singlet), d (doublet), t (triplet) dd (doublet of doublet) and dt (doublet of triplet). Protein mass spectral data and high-resolution mass spectra were obtained from HRMS-ESI-Q-Time of Flight LC/MS (Agilent 6546). High performance liquid chromatography (HPLC) was performed on an Agilent 1260 infinity machine model with a ZORBAX SB-C-18 (150 × 4.6 mm, 5 µm)/ Poroshell-120 EC-C18 (150 × 2 mm, 2.7 µm) reverse phase columns and diode array detector (detection wavelength 496, 258 and 315 nm). Absorbance, fluorimetric and luminometric measurements were performed using Promegapc-GloMax® explorer reader. Gels of in-gel fluorescence analysis experiment were visualized using Alexa 546 filter in Chemidoc (ChemiDoc™ MP Imaging System from Bio-Rad). Fluorescence cell imaging was done using Cytation5 cell imaging multimode reader (Biotek) with a blue and red filter and 10x objective. Fluorescence images given in the manuscript has been deposited in FigShare with DOI of 10.6084/m9.figshare.24720258 and 10.6084/m9.figshare.24720375.

X-ray crystallography: Single-crystal of suitable dimensions was used for data collection. Diffraction intensities were collected on a Brucker APEX-II CCD diffractometer, with graphite-monochromated Mo Kα (0.71073 Å) radiation at 100(2) K. Data were corrected for Lorentz and polarization effects; empirical absorption corrections (SADABS v 2.10) were applied. Using Olex2,[1] the structures were solved by ShelXT[2] structure solution program using Intrinsic Phasing and refined with the ShelXL[3] refinement package using Least Squares minimization. The position of the hydrogen atoms was calculated by assuming ideal geometries but not refined. All non-hydrogen atoms were refined with anisotropic thermal parameters by full-matrix least-squares procedures on F2. CCDC number for IITK3101: 2293923, IITK3103: 2293924, IITK3104: 2293925, IITK3105: 2293927; IITK3108: 2293928. This data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk.

2. Supporting Figures:

Figure S1. High performance liquid chromatography traces of lead molecules.

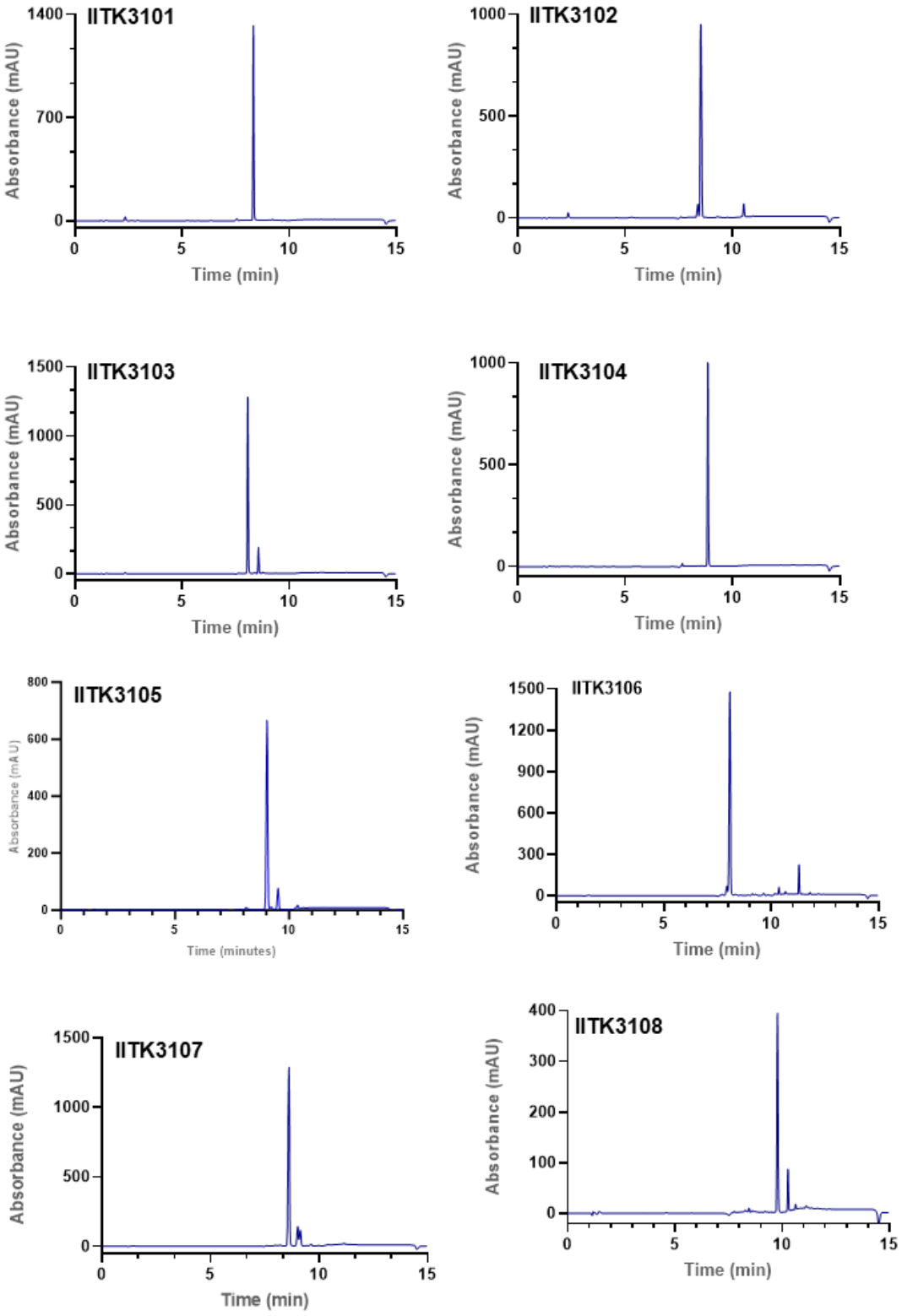


Figure S2. Cell viability data of Huh-7 cells treated with indicated molecules in a dose-dependent fashion is recorded using a resorufin reduction assay.

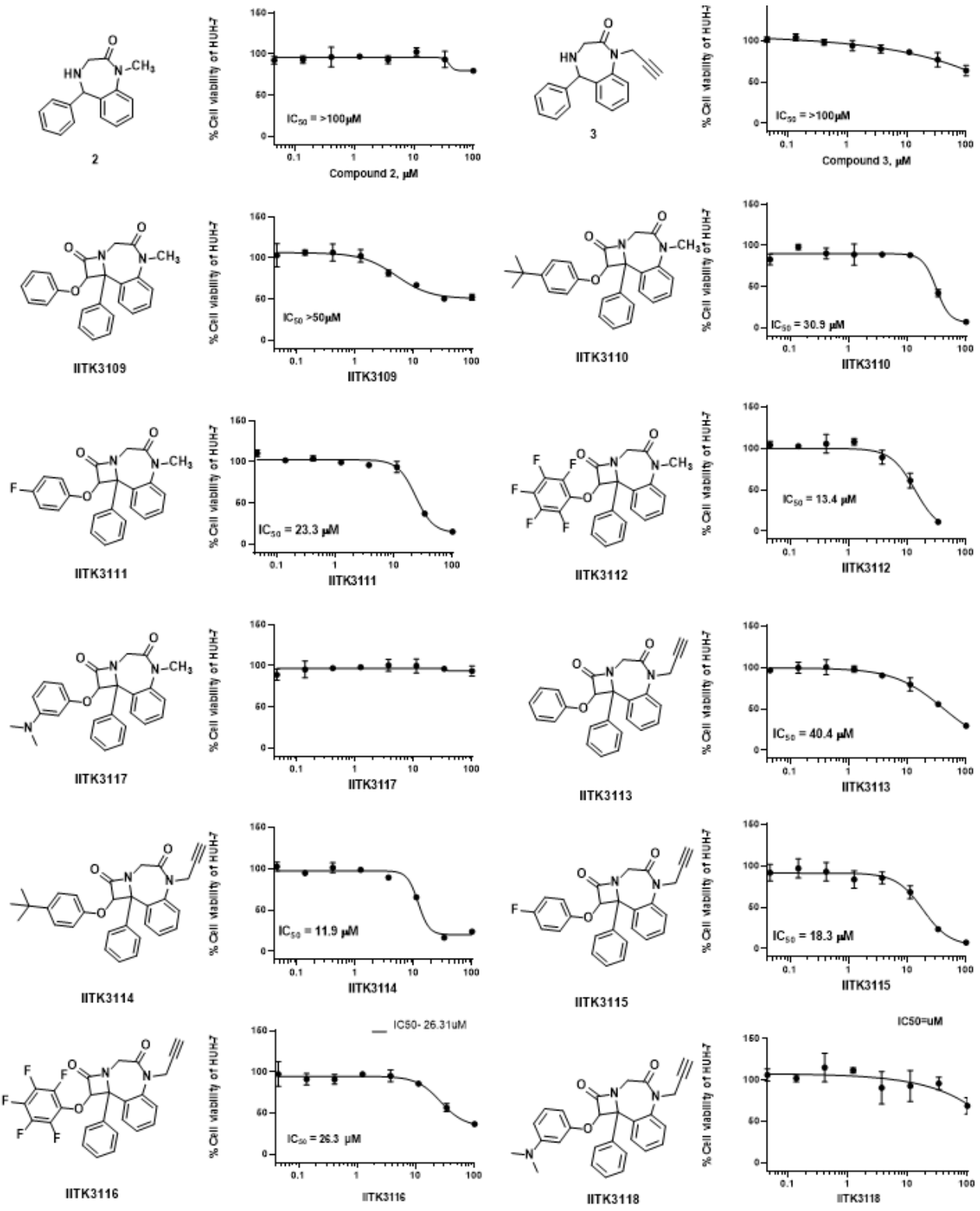


Figure S3. Cell viability data of Huh-7 cells treated with indicated molecules in a dose-dependent fashion is recorded using a resorufin reduction assay.

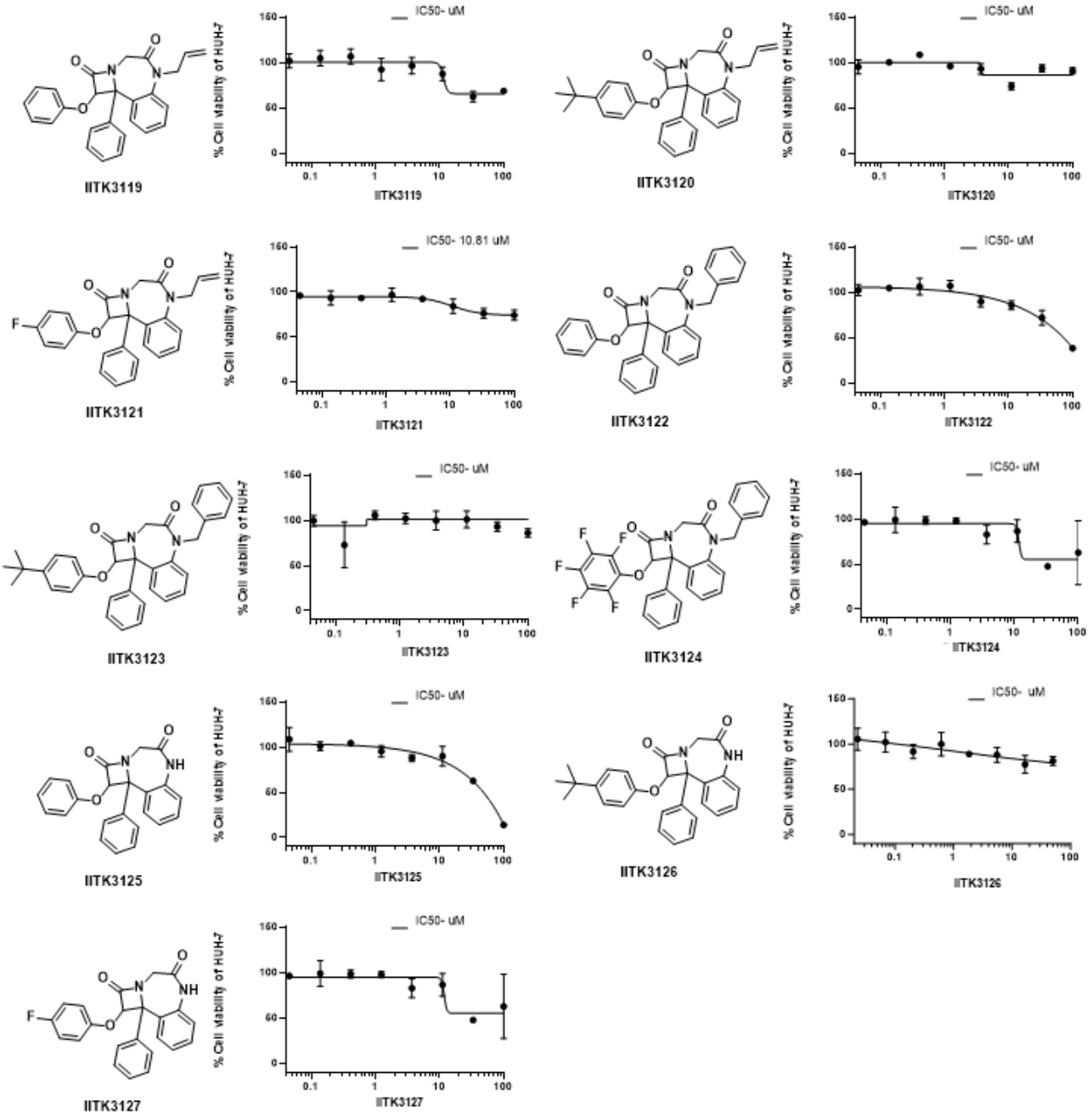
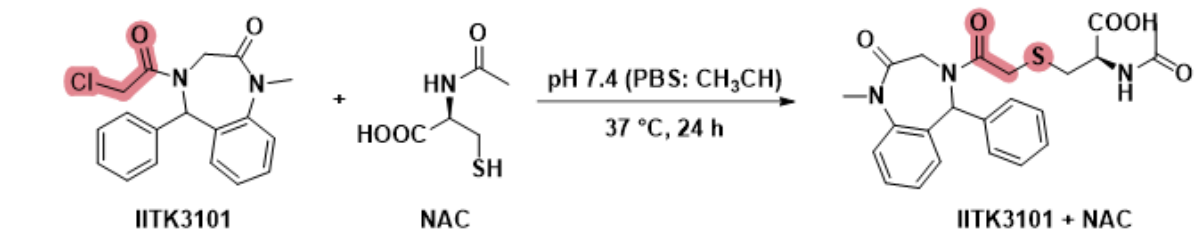
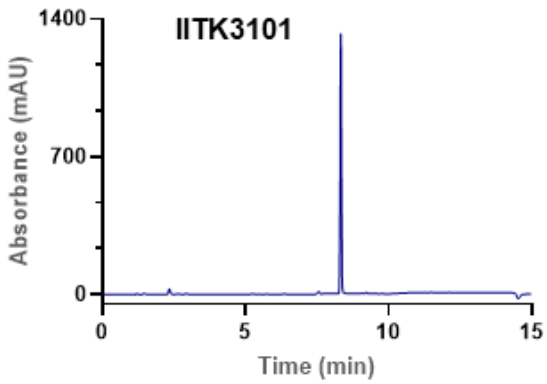
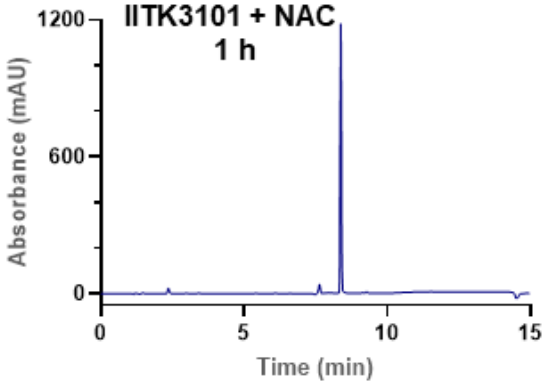
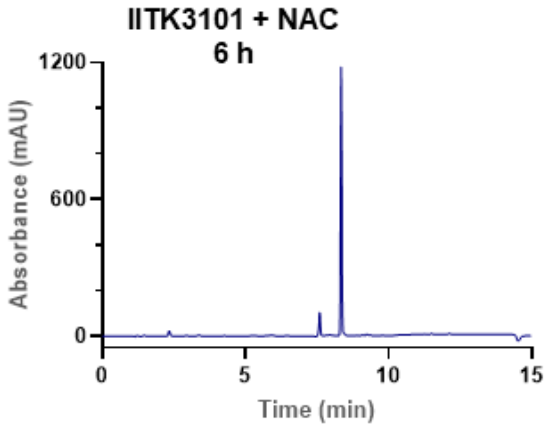


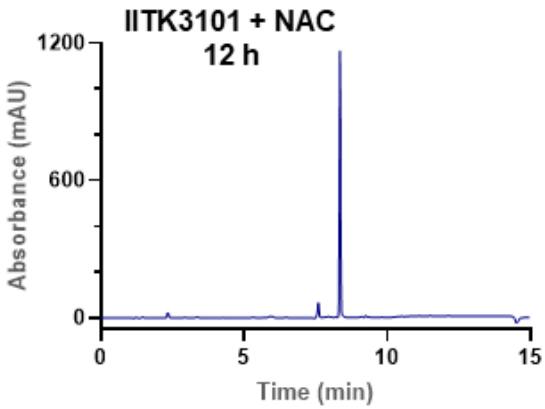
Figure S4. Reactivity of IITK3101 with *N*-acetylcysteine monitored over 24 h at 37 C in pH 7.4 PBS and acetonitrile (1:1 v/v) using high performance liquid chromatography analysis.











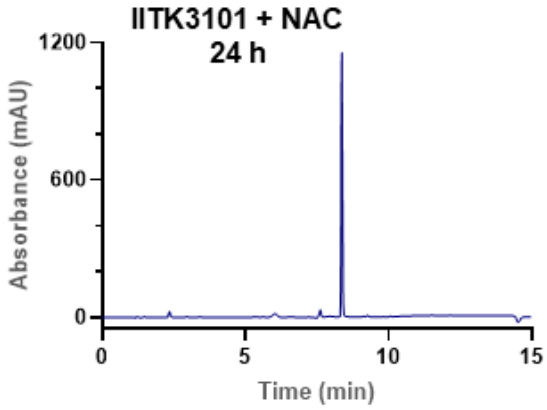
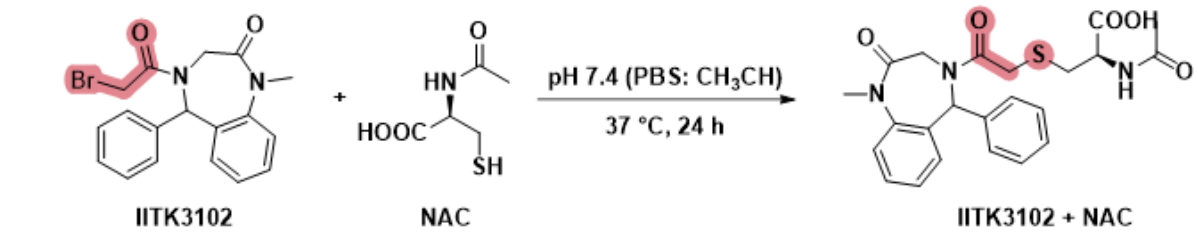
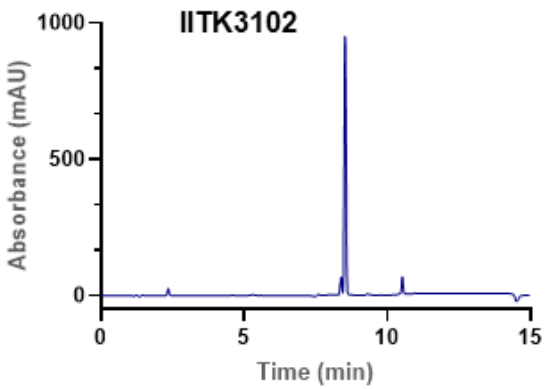
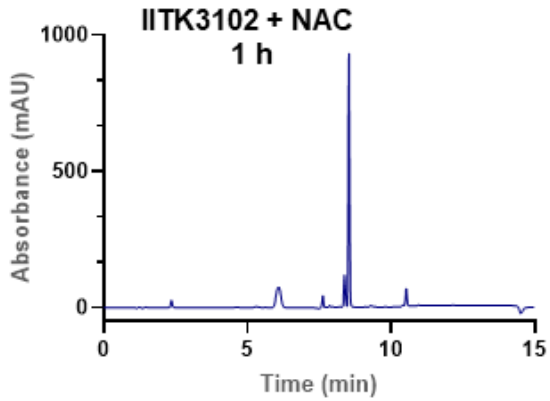
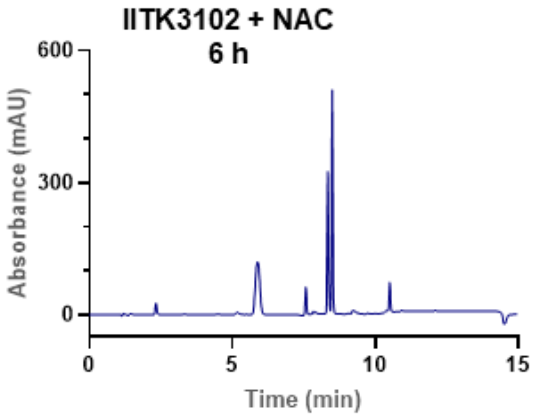


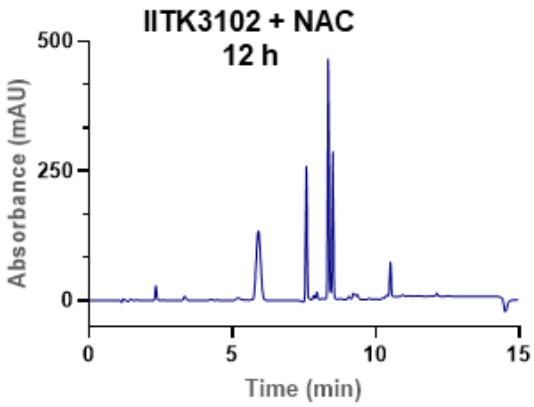
Figure S5. Reactivity of IITK3102 with *N*-acetylcysteine monitored over 24 h at 37 C in pH 7.4 PBS and acetonitrile (1:1 v/v) using high performance liquid chromatography analysis.











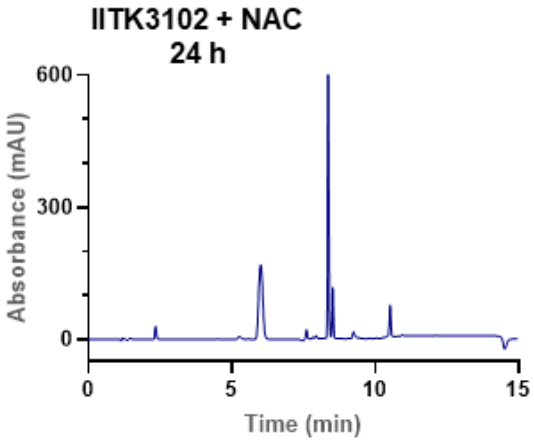
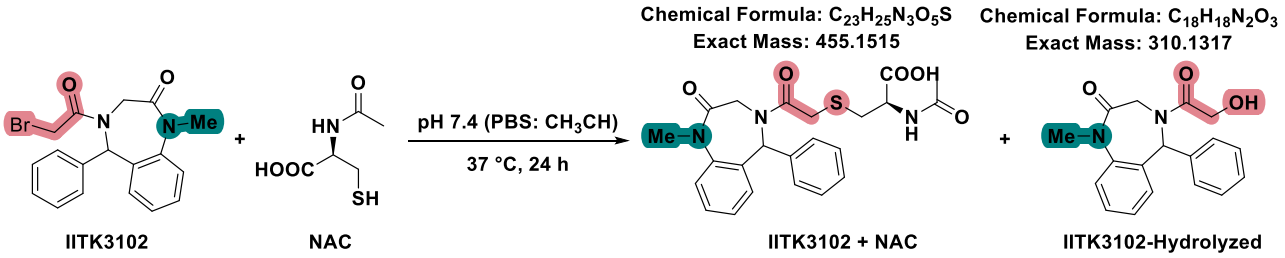
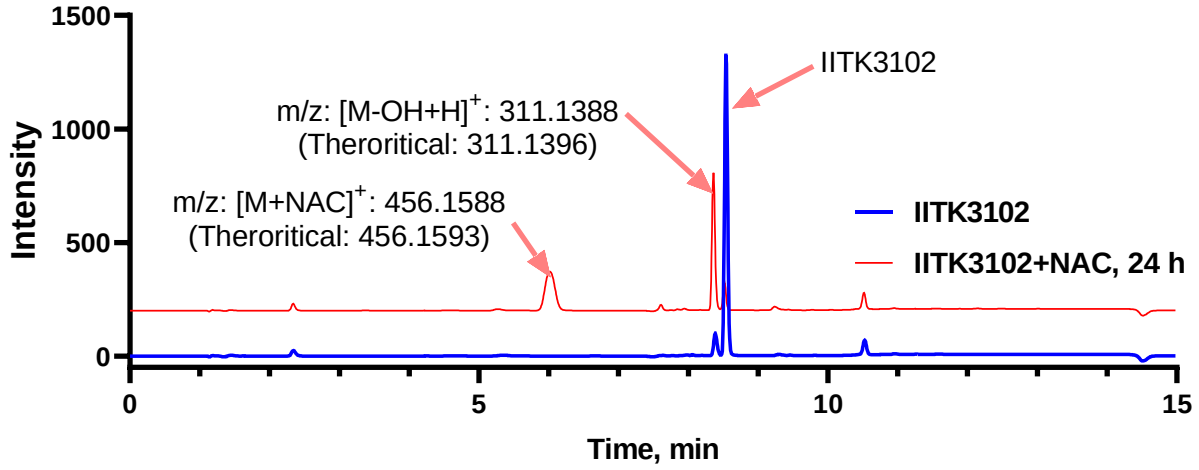


Figure S6. Reactivity of IITK3102 with NAC and in basic medium (K_2CO_3, (1 equiv.)) after 24 h:





Reaction of IITK3102 with K_2CO_3:

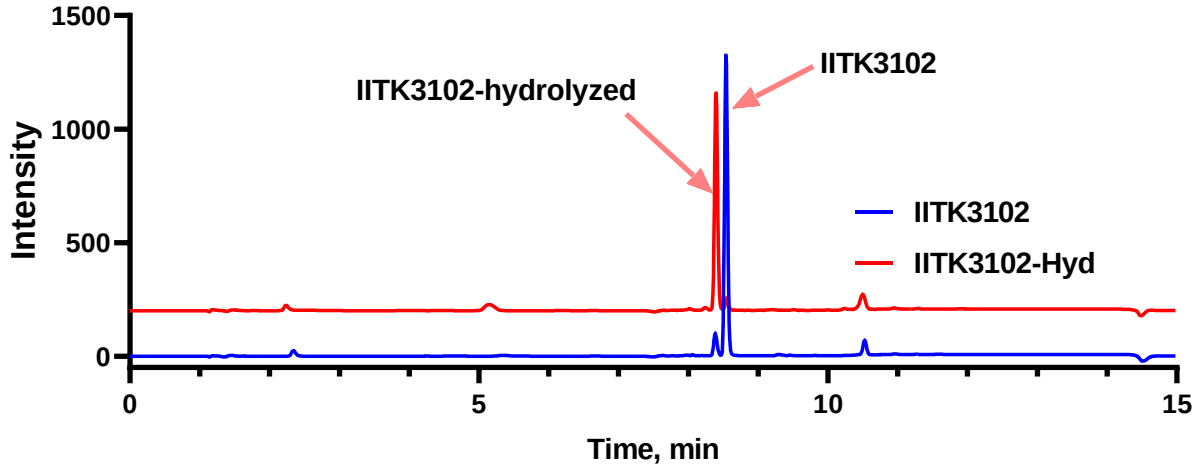
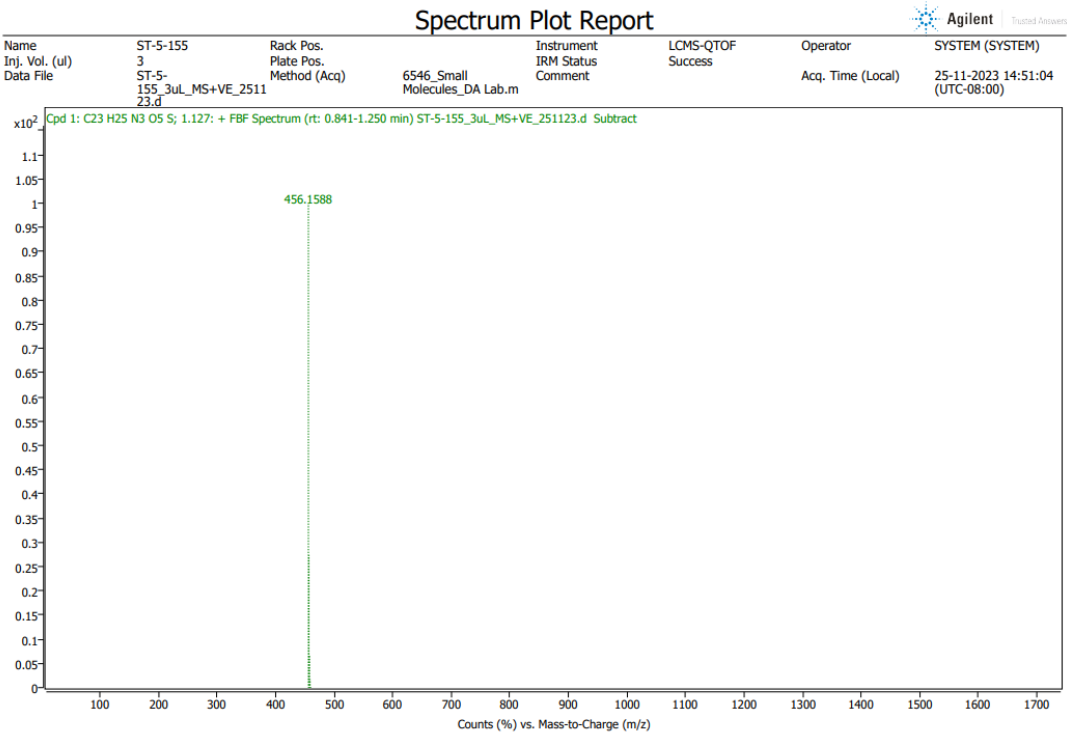


Figure S7. Mass spectral analysis of IITK3102 with NAC reaction mixture:

Mass spectra: For the compound collected at 5.98 min.

Product: $[C_{23}H_{25}N_3O_5S + H]^+$ Expected mass: 456.1593; observed mass: 456.1588



For the compound collected at 8.36 min.

Product: $[C_{18}H_{18}N_2O_3 + H]^+$ Expected mass: 311.1396; observed mass: 311.1388

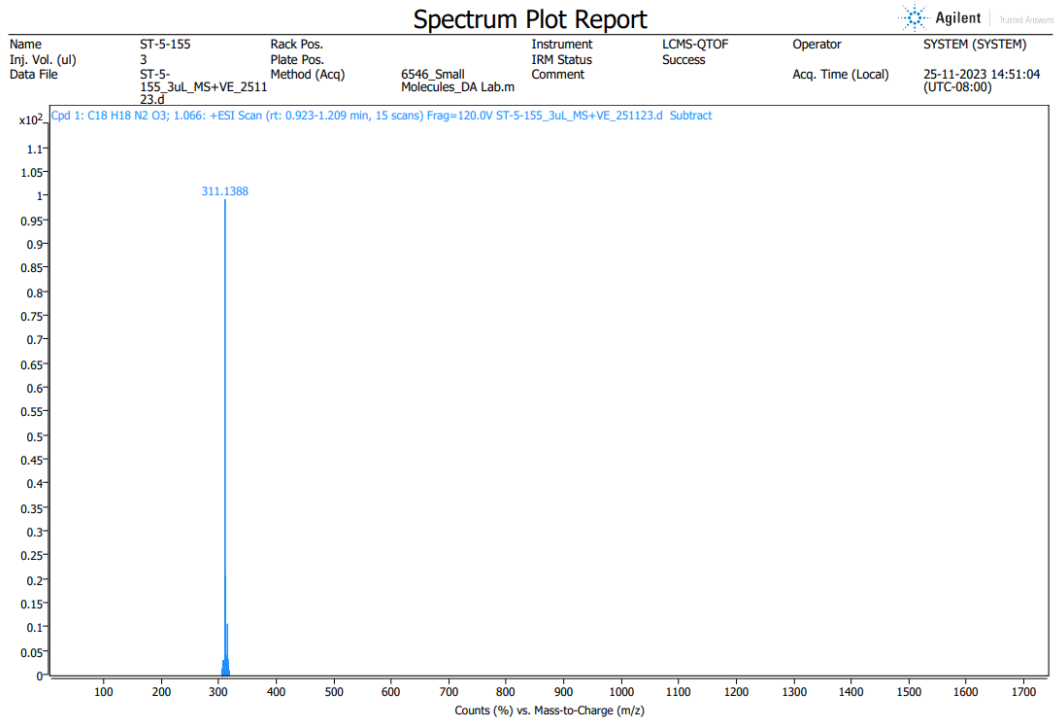
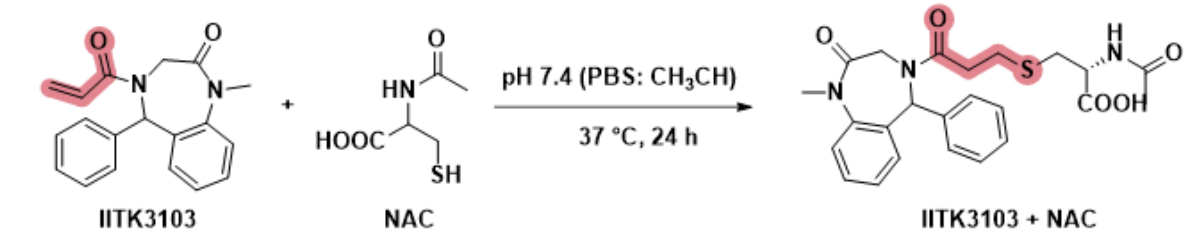


Figure S8. Reactivity of IITK3103 with *N*-acetylcysteine monitored over 24 h at 37 °C in pH 7.4 PBS and acetonitrile (1:1 v/v) using high performance liquid chromatography analysis.



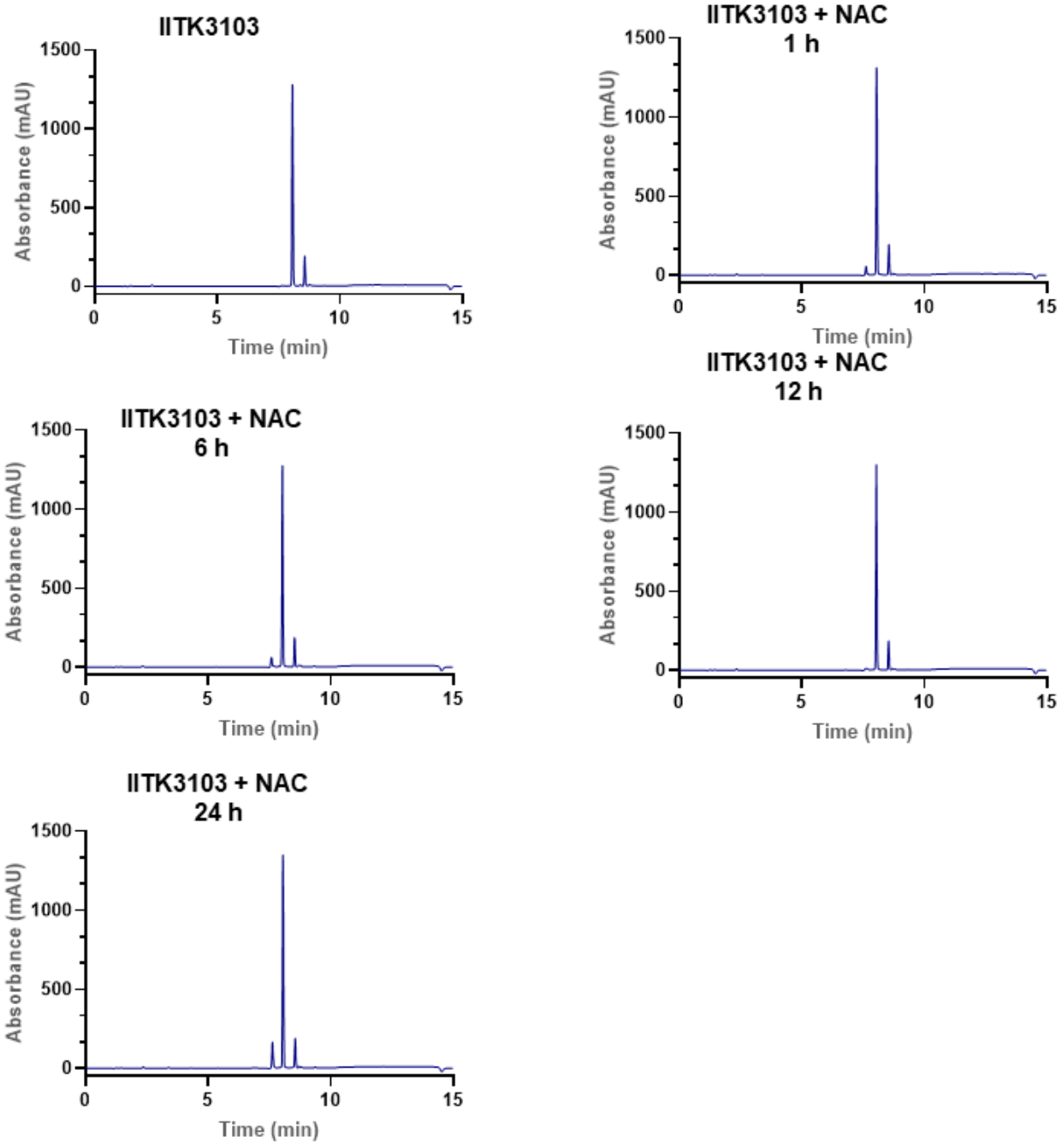


Figure S9. Reactivity of IITK3104 with *N*-acetylcysteine monitored over 24 h at 37 °C in pH 7.4 PBS and acetonitrile (1:1 v/v) using high performance liquid chromatography analysis.

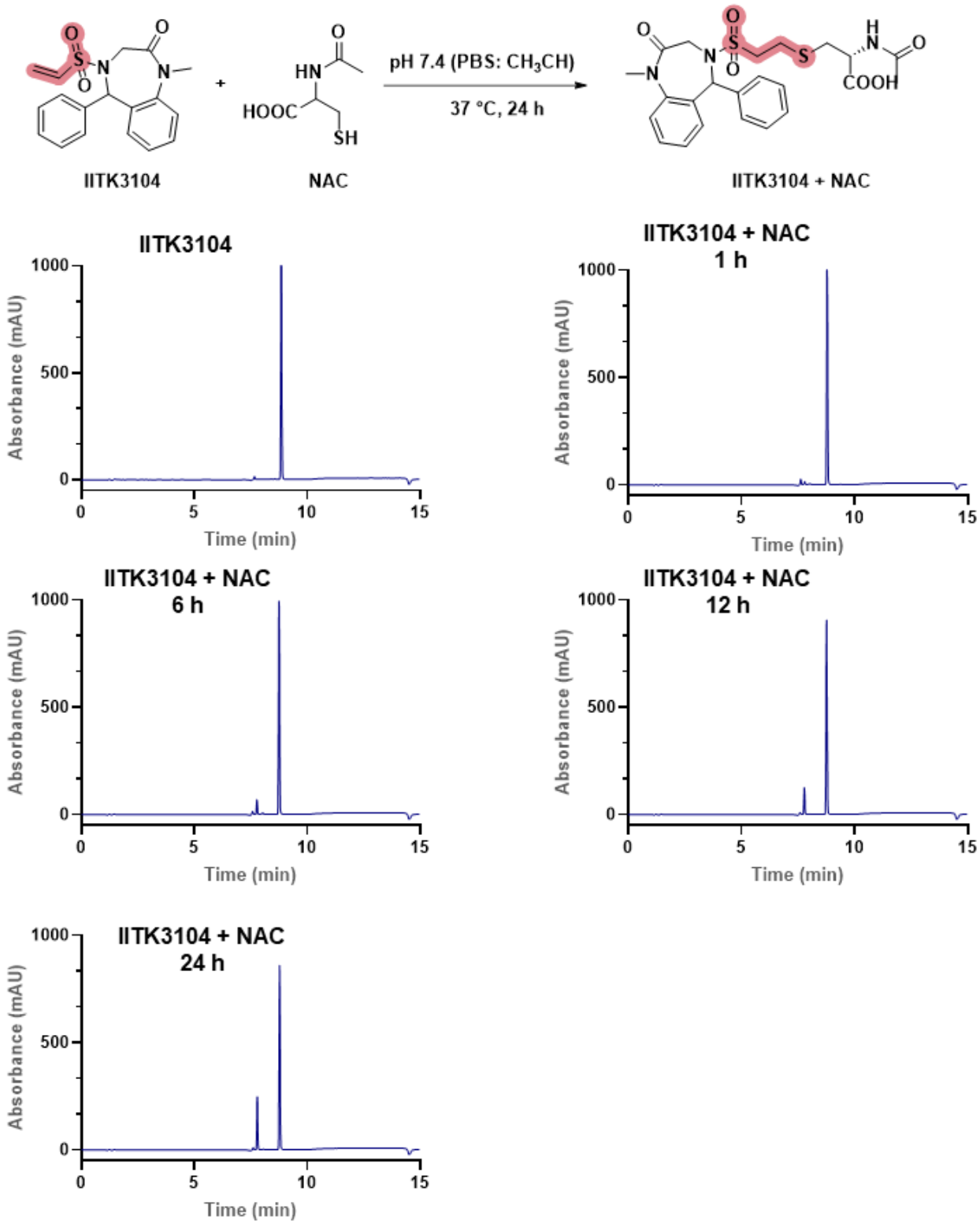
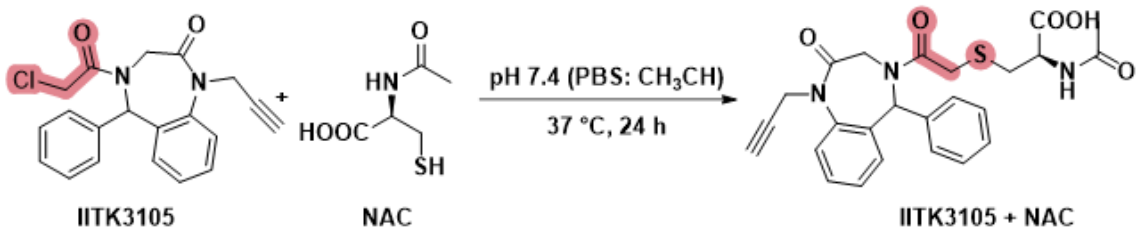


Figure S10. Reactivity of IITK3105 with *N*-acetylcysteine monitored over 24 h at 37 °C in pH 7.4 PBS and acetonitrile (1:1 v/v) using high performance liquid chromatography analysis.



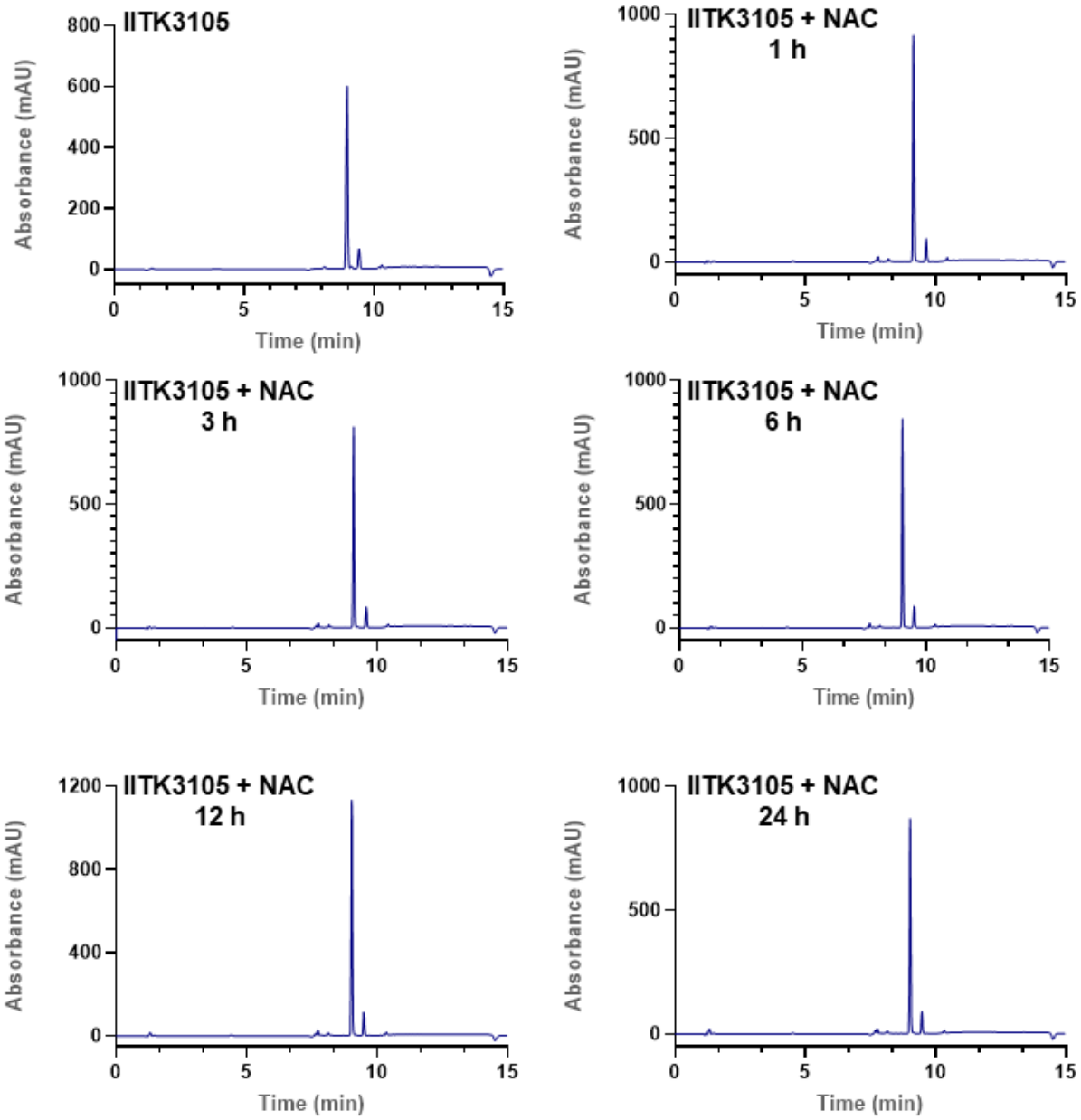


Figure S11. Reactivity of IITK3106 with *N*-acetylcysteine monitored over 24 h at 37 °C in pH 7.4 PBS and acetonitrile (1:1 v/v) using high performance liquid chromatography analysis.

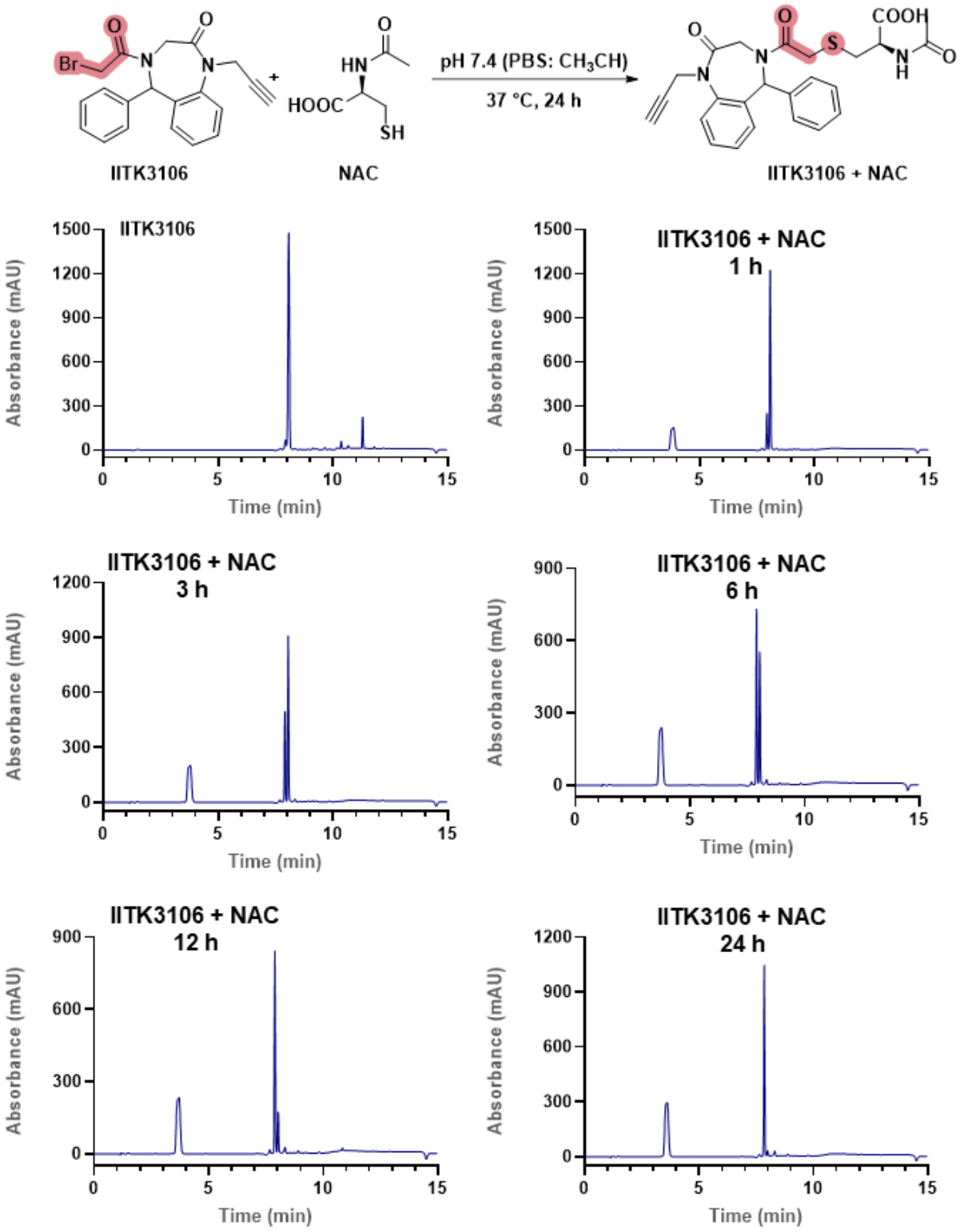
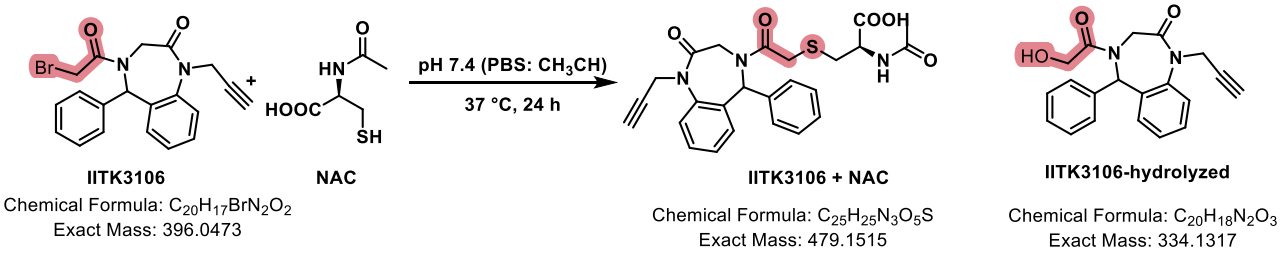


Figure S12 Reactivity of **IITK3106** with NAC and in basic medium (K_2CO_3, (1 equiv.)) after 24 h::

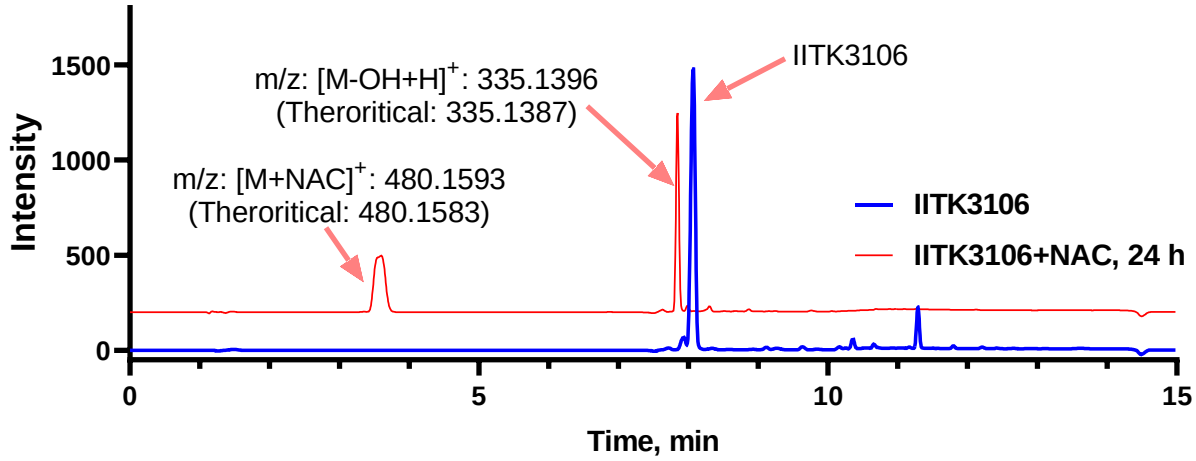


IITK3106
Chemical Formula: $C_{20}H_{17}BrN_2O_2$
Exact Mass: 396.0473

NAC

IITK3106 + NAC
Chemical Formula: $C_{25}H_{25}N_3O_5S$
Exact Mass: 479.1515

IITK3106-hydrolyzed
Chemical Formula: $C_{20}H_{18}N_2O_3$
Exact Mass: 334.1317



Reaction of IITK3102 with K_2CO_3:

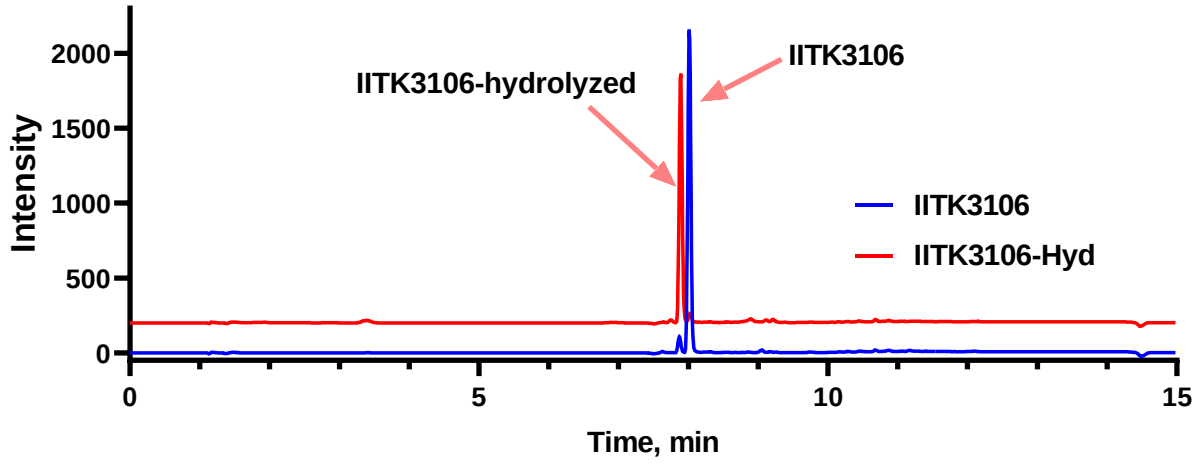
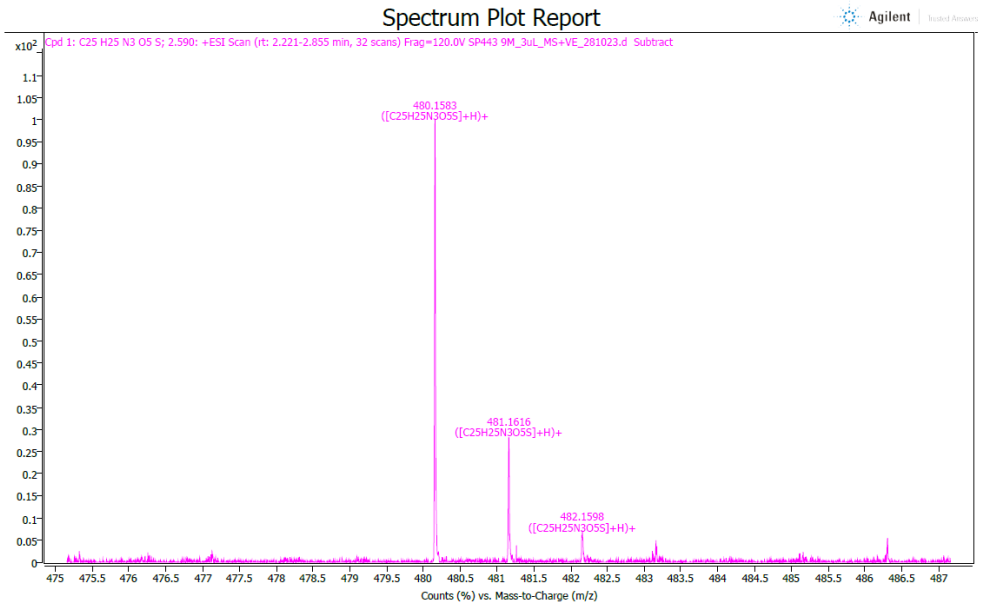


Figure S13. Mass spectral analysis of IITK3106 with NAC reaction mixture:

Mass spectra: For the compound collected at 3.6 min.

Product: $[C_{23}H_{25}N_3O_5S+H]^+$ Expected mass: 480.1593; observed mass: 480.1583



Mass spectra: For the compound collected at 7.9 min.

Product: $[C_{23}H_{25}N_3O_5S + H]^+$ Expected mass: 335.1396; observed mass: 335.1387

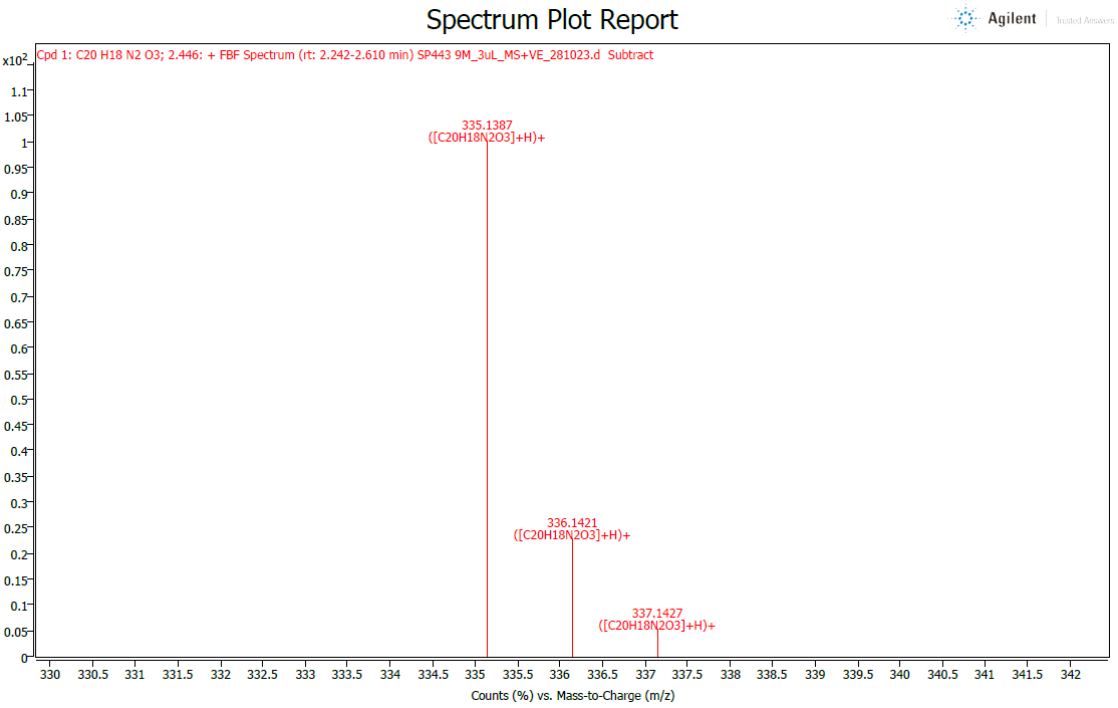
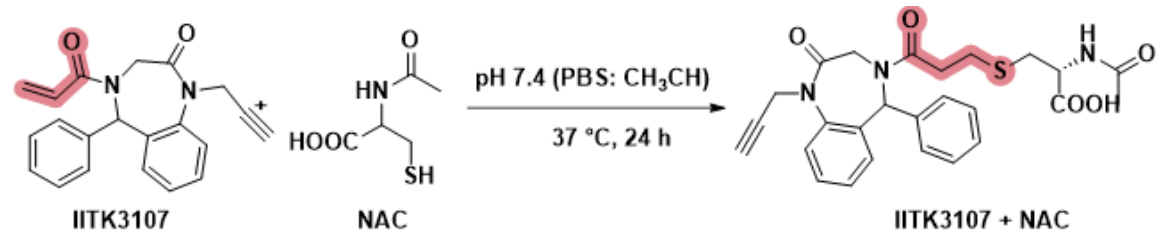
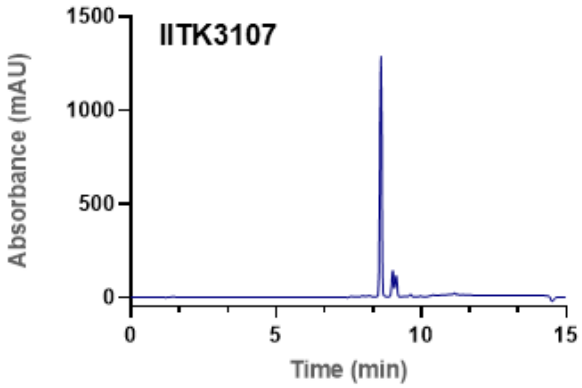
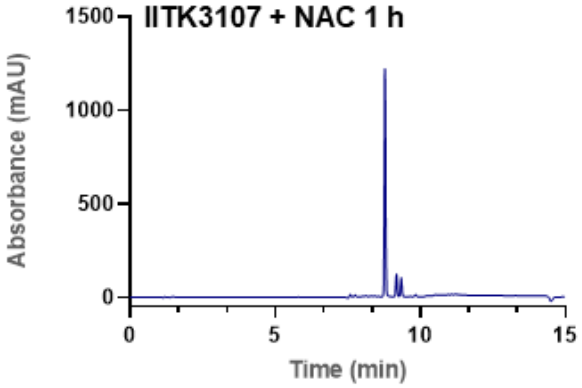
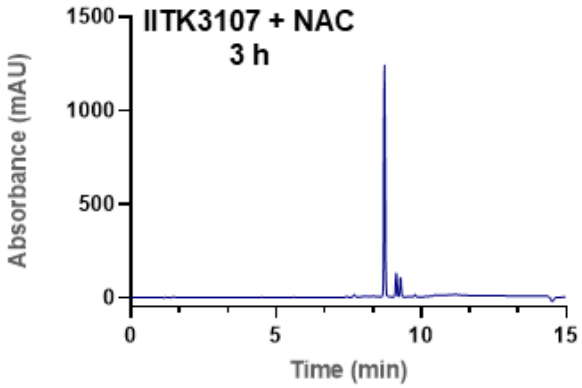


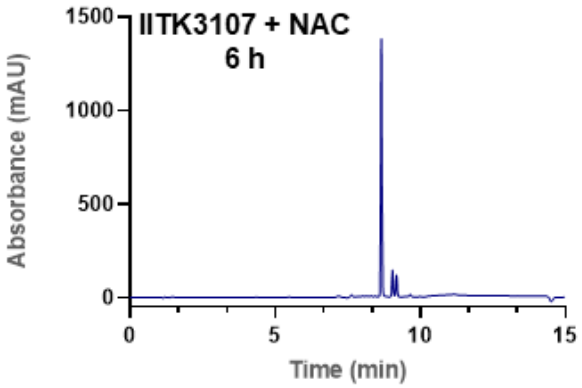
Figure S14. Reactivity of IITK3107 with *N*-acetylcysteine monitored over 24 h at 37 °C in pH 7.4 PBS and acetonitrile (1:1 v/v) using high performance liquid chromatography analysis.

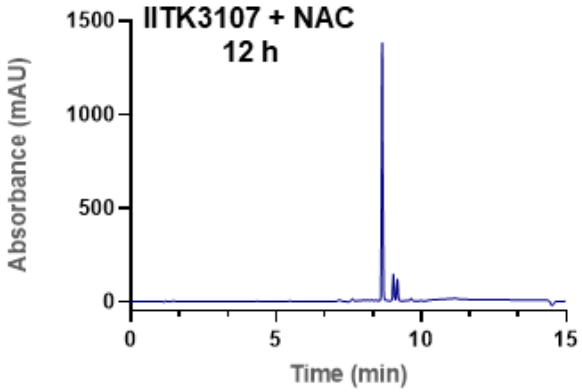












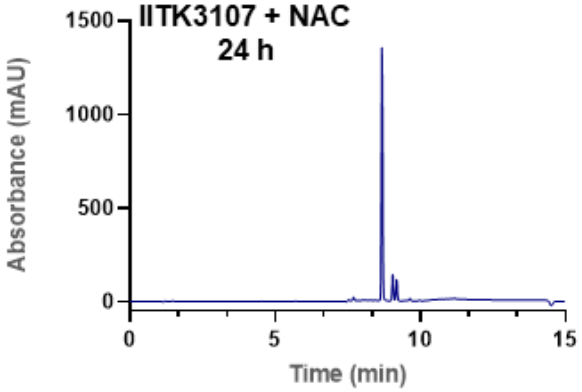


Figure S15. Reactivity of IITK3108 with *N*-acetylcysteine monitored over 24 h at 37 °C in pH 7.4 PBS and acetonitrile (1:1 v/v) using high performance liquid chromatography analysis.

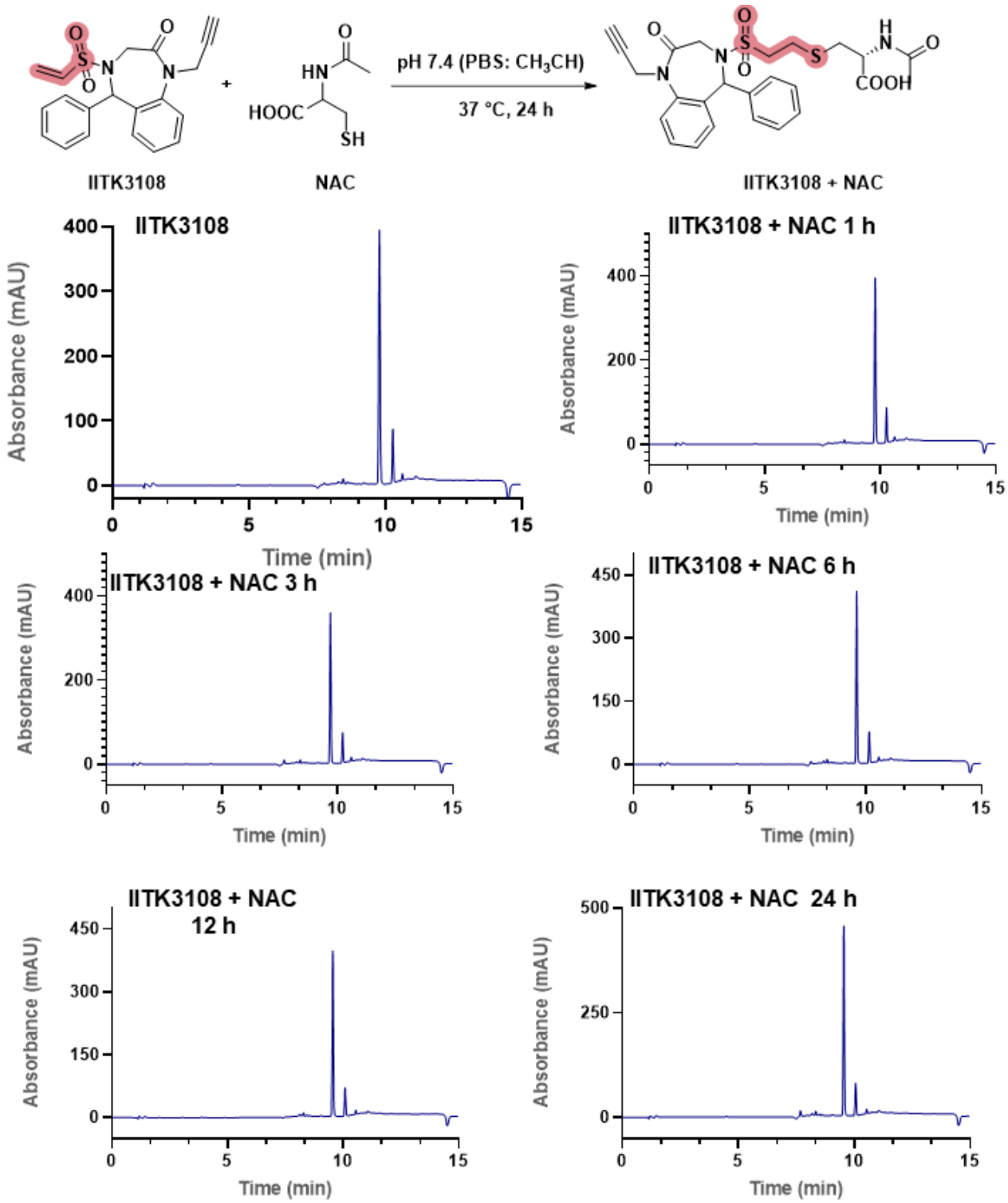
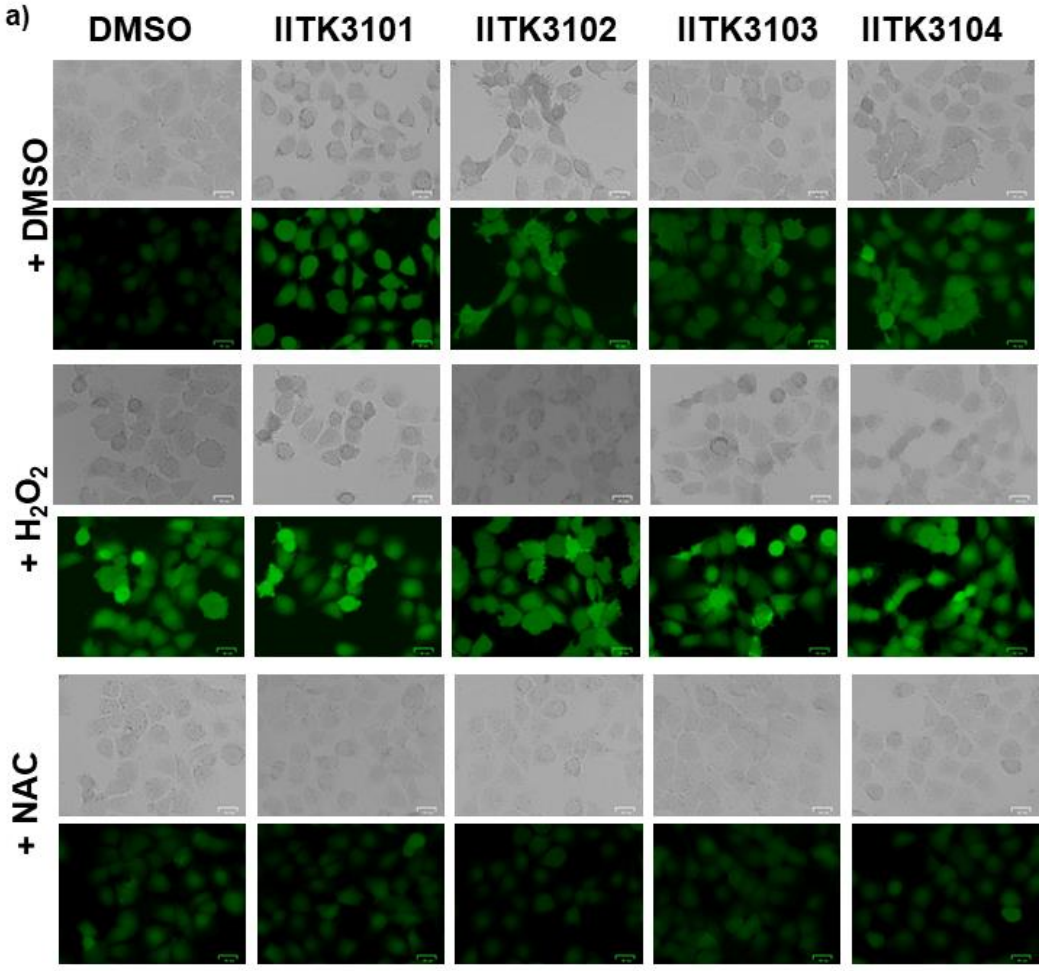
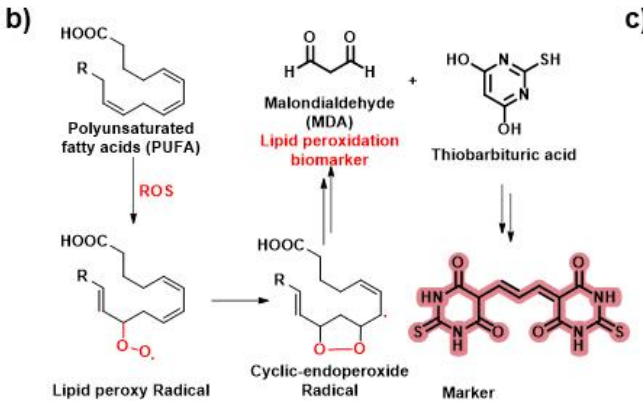


Figure S16. Intracellular ROS accumulation with indicated molecules captured using the oxidized H_2DCF-DA fluorescence imaging and TBARS assay for lipid peroxide quantification in Huh-7 cells. (a) Bright field and fluorescence images of Huh-7 cells after treatment with indicated molecules alone or in combination for 90 mins. (b) Scheme and the data of lipid peroxidation and the quantification of eventual product malondialdhyde (MDA) with thiobarbituric acid in Huh-7 cells after treatment with our lead molecules.





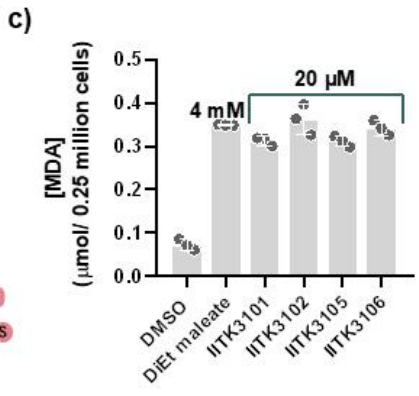


Figure S17. Cell viability data of Huh-7 cells upon combination treatment of IITK3101 and IITK3102 with various cell death pathway modulators such as ferroptosis (Ferrostain-1, liproxstatin-1, *N*-acetylcysteine and RSL3), apoptosis (QVD-OPh) and necroptosis (Necrostatin-1) in a dose-dependent fashion is recorded using a resorufin reduction assay.

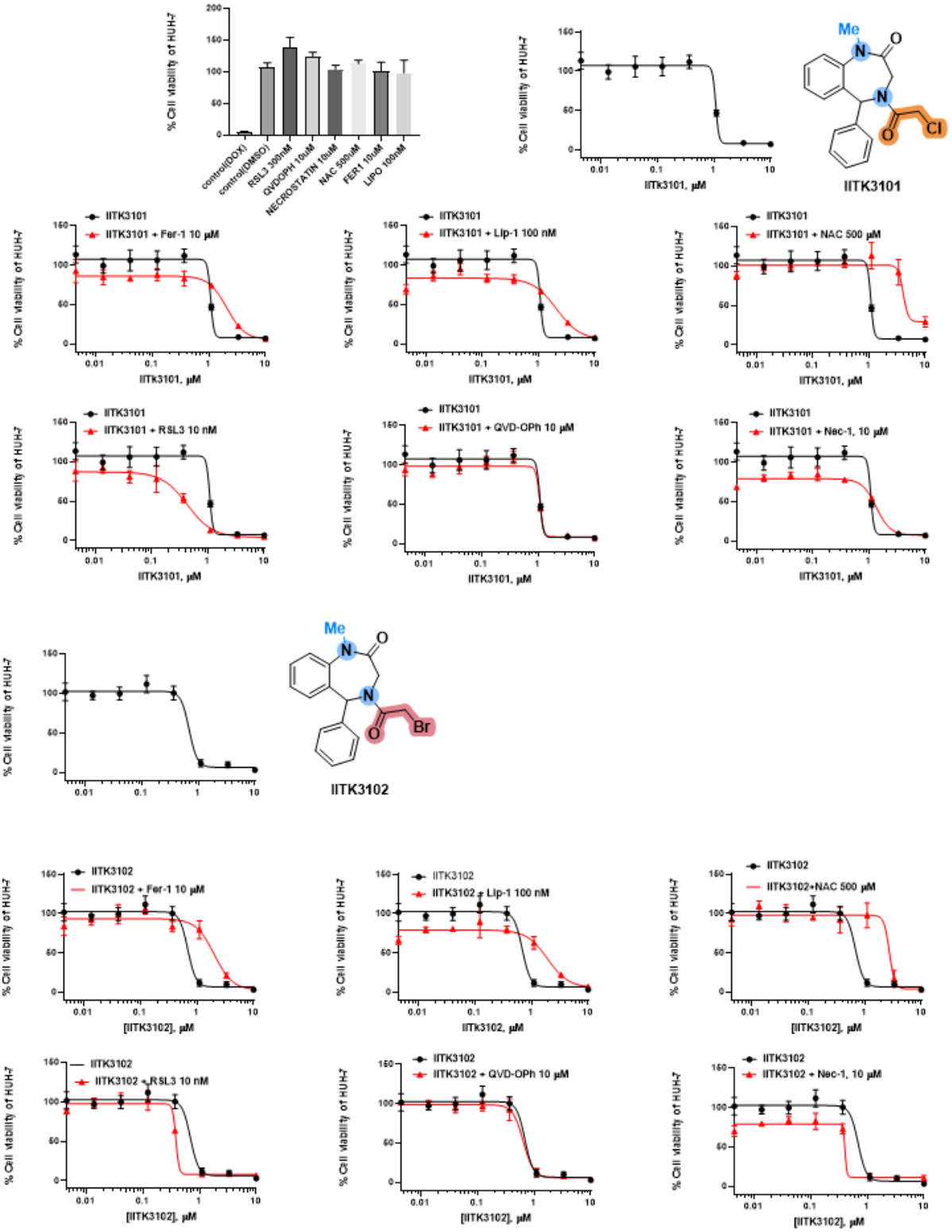


Figure S18. Cell viability data of Huh-7 cells upon combination treatment of IITK3105 and IITK3106 with various cell death pathway modulators such as ferroptosis (Ferrostain-1, liproxstatin-1, *N*-acetylcysteine and RSL3), apoptosis (QVD-OPh) and necroptosis (Necrostatin-1) in a dose-dependent fashion is recorded using a resorufin reduction assay.

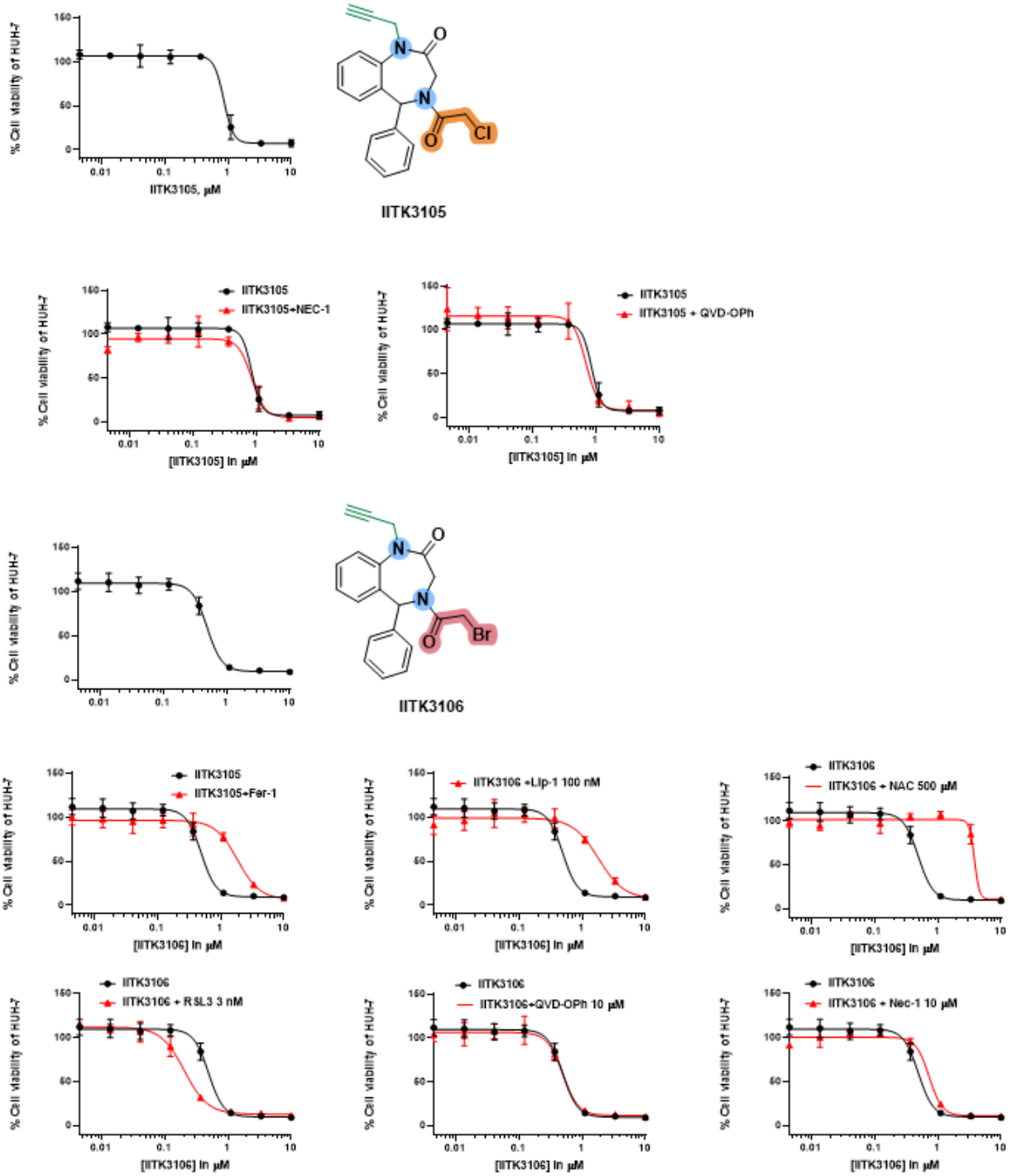
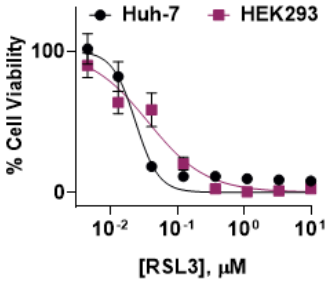
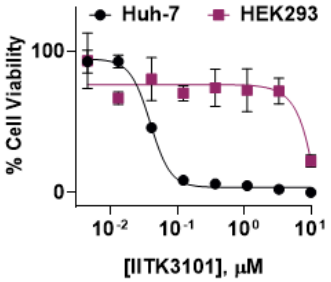
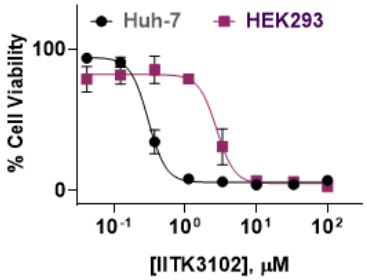


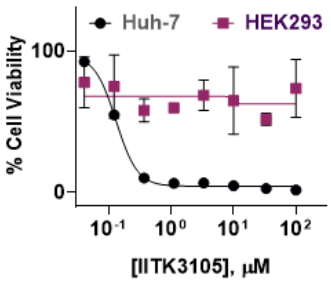
Figure S19. Cell viability was recorded using a resorufin reduction assay for normal kidney (HEK293) cells and Huh-7 cells treated with lead molecules and RSL3. Table shows the EC_{50} values and the calculated selectivity index (SI).



Compds	Huh-7 cells, EC_{50}, µM	HEK293 cells, EC_{50}, µM	Selectivity Index (SI)
RSL3	0.023	0.036	1.56
IITK3101	0.039	>10	>200
IITK3102	0.300	2.750	9.2
IITK3105	0.132	>50	>200
IITK3106	0.167	15	90







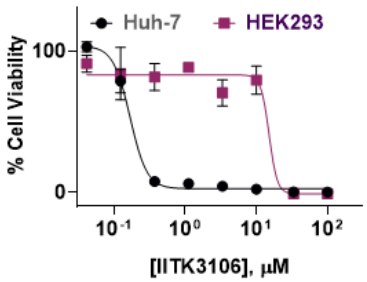


Figure S20. Complete gel images of (a) competitive-ABPP experiment for IITK3105 (0.5 μM) with RSL3 (20 μM) in Huh-7 cells proteome. The Huh-7 cell lysate was pre-treated with RSL3 for 1 h before exposing to IITK3105. (b) Dose-dependent reactivity studies of IITK3105 with Huh-7 cell lysate in pH 7.4 PBS (1x) were captured using TAMRA-N_3 click chemistry mediated in-gel fluorescence analysis. Compound concentrations: 0.125, 0.25, 0.5, 1.0, and 2.0 μM. The Coomassie-stained gel images are given as a loading control.

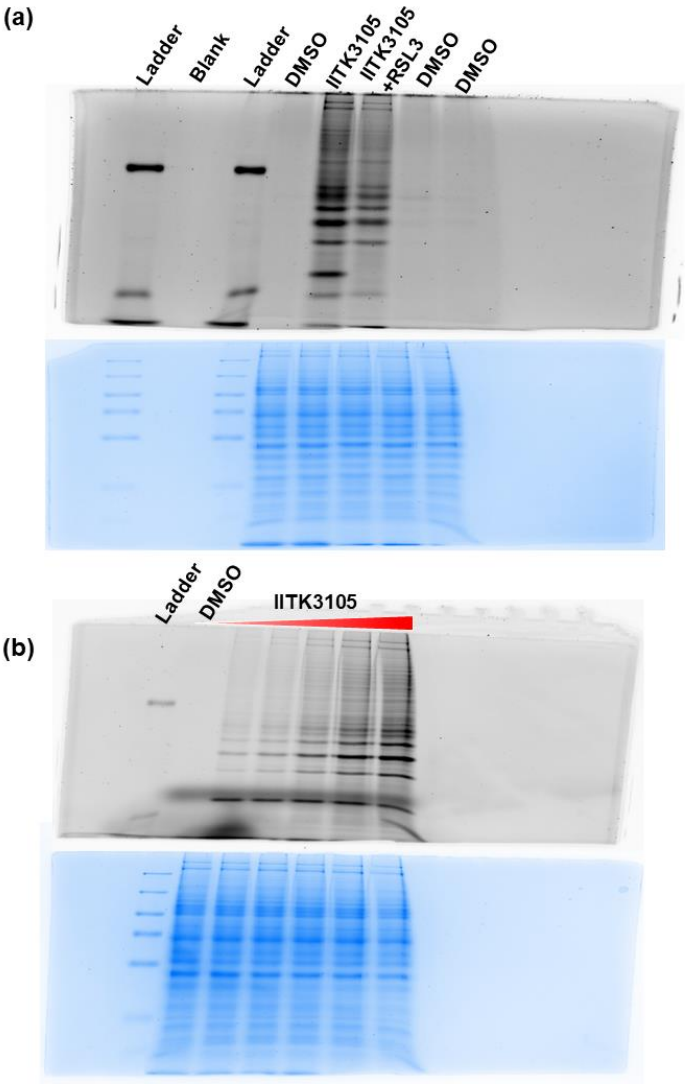
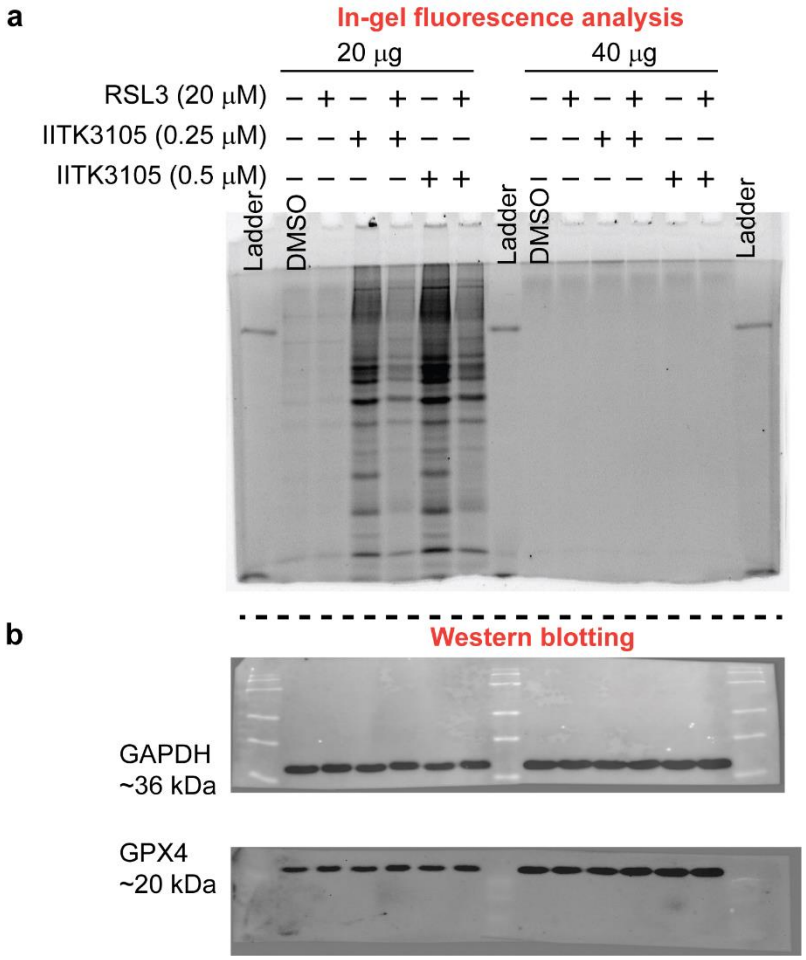


Figure S21. Western blotting data of SDS-PAGE obtained from in-gel fluorescence analysis experiment for GPX4 and GAPDH proteins in Huh-7 cell proteome. (a) In-gel fluorescence analysis of Huh-7 cell lysate pre-treated with RSL3 (20 μM) for 1 h before exposing to IITK3105 at indicated concentrations for 3 h and captured using click chemistry with Azide-fluor-488 dye. (b) Western blotting images of the same SDS-PAGE after labelling for GPX4 and GAPDH proteins using respective monoclonal antibodies.



3. **Materials and Methods:**

HPLC experiment protocol:

HPLC reactions were performed at 4 mM concentration of both electrophile and nucleophile in 1:1 ratio. Stock solutions of the electrophile (10 mM) was made in acetonitrile (HPLC Grade obtained from FINAR) and nucleophile in 1:1 ACN: PBS (pH=7.4, vol/vol). For kinetic studies, reaction was conducted in a medium of ACN: PBS (1: 1. Vol/vol, 500 µL) containing 4 mM concentration of both electrophile and nucleophile as final concentration. Reaction mixture was incubated at 37 °C and analysed using HPLC at various time points. The reaction mixture was diluted to 0.8 mM final concentration in 1:1 ACN: Buffer and the injection volume was 10 µL. For the HPLC analysis, gradient method (shown below) was used with a constant flow rate of 0.5 mL/minute and 258 nm wavelength was used.

Time (minutes)	Acetonitrile (0.1% trifluoroacetic acid)	Water (0.1% trifluoroacetic acid)
5	30%	70%
5.10	60%	40%
8.00	60%	40%
8.10	90%	10%
12.00	90%	10%
12.10	30%	70%
15.00	30%	70%

Cell culture: Huh-7 and HEK293 cells were grown in DMEM (Dulbecco's Modified Eagle Medium Gibco). supplemented with 10% FBS (Foetal Bovine Serum- Gibco) and 1% PenStrep (Pencillin Streptomycin- Gibco) at 37 °C in a humidified CO_2 incubator. Cells were cultured in T75 flasks (Thermo) until they attain 70%-80% confluency. Cells were trypsinized with 4 mL of trypsin (0.25% Trypsin-EDTA - Gibco) and counted by using hemocytometer before using for further experiments.

Dose-dependent and Combination cell viability studies in Huh-7 cell line by Resazurin assay: Cells were cultured and processed as discussed above. In 384 well plate, 500 cells/well with 75 µL media were plated into 360 wells of the plate (Thermo). The remaining 24 wells were plated with only media. Plates were incubated at 37 ^{o}C, 5% CO_2 (Forma steri cycle CO_2 incubator - Thermo Fisher Scientific) for 24 hours. A 8-point-three-fold dilution series of our molecules starting from 100 or 10 µM was used to assess the dose-dependent cell viability. **For combination experiment**: Huh-7 cells were treated with 10 uM to 0.0045 uM of IITK3101, IITK3102, IITK3105 and IITK3106 alone or in combination with NAC: 500 µM, QVD-OPh: 10 µM, RSL3: 500 nM, ferrostatin-1: 10 µM, Liproxstatin-1: 100 nM necrostatin-1: 10 µM. Here, 0.1% DMSO was used as negative control and 533 nM doxorubicin positive control conditions were used in the treatment. Cells were incubated for 72 hours at 37 ^{o}C, 5% CO_2 after treatment. After 72 hours of incubation, media was discarded from the wells and washed with 1X PBS. Then, 0.02 mg/mL of resazurin (SRL) was made in DMEM media and 50 µL of resazurin was added to every well of 384 well plate. After addition of resazurin, cells were incubated in the incubator for 6 hours and fluorescence readings were recorded (excitation wavelength - 520 nm and emission wavelength – 580 nm) in multimode plate reader. Percentage cell viability was calculated as follows, ((fluorescence of treated cells - fluorescence of media control)/ (fluorescence of DMSO controls – fluorescence of media control)) * 100. Graphs were plotted by using non-linear regression curve fit programme of Graphpad Prism 9.0. EC_{50} was calculated using Graphpad Prism software.

ROS quantification by using DCFDA in Huh-7 cell lines: For measurement of total intracellular ROS levels in Huh-7 cells, 25000 cells per well were plated in clear flat bottom 96 well plates in 200 µL DMEM. Plates were incubated at 37 ^{o}C

and 5% CO_2 for 24 hours. Post 24 hours, cells were treated for 90 minutes at 37 ^oC and 5% CO_2 with 0.02% DMSO, H_2O_2 - 100 µM, NAC - 500 µM, IITK3101, IITK3102, IITK3105 and IITK3106 - 20 µM and their combinations. Post 90 minutes, 2 uM of 2',7'-Dichlorodihydrofluorescein 3',6'-diacetate (H_2-DCFDA) (Thermo) (dissolved in DMSO) was directly added to cells and incubated in the incubator for 5 minutes. Media was removed from the wells and cells were washed with 100 µL of 1X PBS for twice, cells were observed under microscope and images were captured using fluorescent microscope (Bio-Rad ZOE fluorescent cell imager).

TBARS - Lipid Peroxidation Assay: For lipid peroxidation assay in Huh-7 cells, 0.35 million cells per well were plated in clear bottom 12 well plate (NEST) and incubated in the incubator (5% CO_2, 37 ^OC) for 24 hours. They were treated in triplicate with DMSO, 20 µM of IITK3101, IITK3102, IITK3105 and IITK3106 and 4 mM of Diethyl maleate for 6 hours in the incubator. Post treatment, cells were washed with ice cold 1X PBS. Cells were scrapped by adding 200 µL of 2.5% trichloroacetic acid (SRL) and centrifuged for 15 minutes at 13,000g. The supernatant was collected and 200 µL of 15% TCA and 500 µL of 0.67% thiobarbituric acid (TBA) (GLR) are added to the same. They were vortexed are heated at 95 ^oC for 30 minutes and allowed to cool down to room temperature. To all the samples, 500 µL of n-butanol (Rankem) was added and let the aqueous and organic phases got separated. Then, 200 µL of organic phase of all the samples are added to various wells of clear bottom 96-well plate and fluorescence readings were recorded at excitation wavelength 530 nm and emission wavelength 550 nm in multimode plate reader. For the quantification of MDA, malondialdehyde tetrabutyl ammonium salt (sigma) standard curve generation experiment was performed. We used five different concentrations of MDA were prepared in 2.5% TCA in triplicates as follows, 2.5 µM, 1.25 µM, 0.625 µM, 0.312 µM, 0 µM . To these, 100 µL of 0.67% TBA was added and heated at 95 ^oC for 30 minutes. The samples were allowed to cool down and fluorescence readings were recorded as described above. Concentration of MDA in the samples was calculated by using Y= mX + C equation of MDA standard curve, where Y was fluorescence reading, m was slope, X was concentration of unknown MDA and C was constant. The data provided are representative of three or four independent experiments.

Activity-Based Protein Profiling (ABPP) experiment protocol[4]

Huh-7 cells were cultured in DMEM medium supplemented with 10% FBS and 1% PenStrep under 5% CO2 atmosphere as stated above. Then, the media was removed, and cells were washed with 5 mL of cold 1XPBS and stored in -80 °C freezer overnight. Next, the cells were scrapped by adding 1 mL of the 1X protease phosphatase inhibitor (diluted with 1XPBS) on ice and lysed using a probe sonicator with 2 sec on and 1 sec off pulse rate for 60 sec. The lysis mixture was centrifuged at 20,000g for 45 minutes at 4 °C and collected the supernatant. Bradford assay was used for protein quantification and from this, 10 µg/reaction of protein in the lysate was taken for a click chemistry mediated in-gel fluorescence analysis. For the click reaction, 4.26 µL of the click reaction mixture (components are given below) was added in each sample and incubated for 60 minutes at RT in dark. Then, 9.26 µL of 5X protein loading/reducing buffer was added and incubated for 10 minutes in dark at RT. Samples were loaded in a 4-12% SDS-PAGE gel and resolved at 145 V for 80 minutes. The labelled proteins bands were visualized using Alexa 546/488 filter in chemidoc (ChemiDoc™ MP Imaging System from Bio-Rad). Then, the gels were stained with gel staining solution (0.5% w/v brilliant blue R-250 dissolved in 45% v/v ethanol and 10% v/v glacial acetic acid in dH_2O) overnight and destained with destaining solution (10% glacial acetic acid in dH_2O) before imaging with chemidoc instrument for visualizing total protein loading.

Procedure for the preparation of click cocktail per sample tube:

- 1 mM Tris((1-benzyl-4-triazolyl)methyl)amine (TBTA) prepared in 1:4 DMSO: tert-Butyl alcohol = 1.25 µL
- 20% Sodium Dodecyl Sulfate (SDS) prepared in dH2O = 1.5 µL
- 50 mM Tris(2-carboxyethyl)phosphine) (TCEP) prepared in dH2O = 0.5 µL
- CuSO4 (50 mM in dH2O) = 0.5 µL

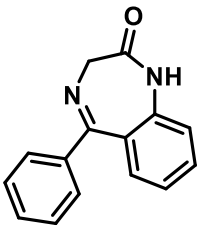
- Azide fluor 545/488 (5 mM in DMSO) = 0.5 µL

Western blotting protocol

The SDS-PAGE from ABPP-in gel fluorescence analysis experiment was taken forward for western blotting. Initially, the proteins were transferred to a PVDF membrane at 40 V and 4 °C overnight. The protein transfer was confirmed using Ponceau staining for 3-5 min at RT with the standard Ponceau reagent (8 mL) and the blot was washed with water for three times (5mL each) before visual inspection. Following the confirmation of protein transfer, the blot was washed with TBST (1X) buffer for 10 min each for three times. Next, the membrane was blocked using 5% skimmed milk for 2 h at RT, then labeled with primary antibodies (GPX4, Rabbit monoclonal antibody, ARC0558, Thermofisher (1: 500) and Anti-GAPDH monoclonal antibody; Clone: ABM22C5 (1: 2500)) in 1% skimmed milk at 4 °C for overnight. Then, the blot was washed with TBST buffer (1X) for 10 min each for three times. Subsequently, peroxidase conjugated Goat anti Rabbit IgG (H+L) secondary antibody (Abgenex) was used at 1:5000 dilution in 1% skimmed milk and incubated for 2 h at RT. Again, the blot was washed with TBST (1X) three times (10 min each). Finally, the blot was developed using enhanced chemiluminescence in a ChemiDoc MP instrument using chemiluminescence application.

4. Synthesis and Characterizations:

Compound 1 (5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one)



The compound was synthesized by following the reported synthesis protocol[5] and purified using silica gel column chromatography to obtain compound **1** in 87% yield (2.10 g, R_f = 0.3 (30% EtOAc: Hexane) as a yellow solid.**^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.79 (s, 1H), 7.53 – 7.48 (m, 2H), 7.48 – 7.43 (m, 1H), 7.43 – 7.38 (m, 1H), 7.35 (t, *J* = 7.4 Hz, 2H), 7.31 – 7.27 (m, 1H), 7.20 (d, *J* = 7.9 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 4.31 (s, 2H).

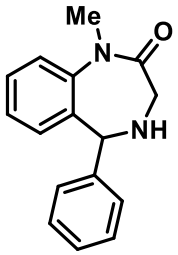
Table S1. Condition optimization for the reduction reaction:

Entry	Catalyst or reagent	Equivalent	Solvent	Temperature	Time	Observation
1	$NaCNBH_3$	3	EtOH	RT	>36 h	No reaction
2	$NaBH_4$	2.5	EtOH	RT	>36 h	<5% conversion
3	$NaBH_4$	5	EtOH	RT	>36 h	<5% conversion
3	$LiAlH_4$	2	Dry THF	0 °C	1 h	nseparable mixture of products
4	Pd/C, H_2	10 mol%	Dry MeOH	RT	3 h	nseparable mixture of products
5	Pd/C, H_2	5 mol%	Dry MeOH	RT	3 h	nseparable mixture of products
6	Zn dust	10 equiv.	acetic acid	RT	1 h	Quantitative product formation

General protocol for the synthesis of compound 2 and 3

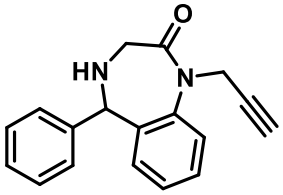
Starting material (500 mg, 2.92 mmol) and preactivated Zn dust (1 g, 29.16 mmol) was suspended in acetic acid (5 mL) and the reaction was stirred at RT for 1 hour. Reaction mixture was diluted with ice-cold water (5 mL) and neutralized by adding 2 M NaOH (15 mL). The reaction mixture was extracted with CH_2Cl_2 (30 mL x 3), washed with brine solution (10 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure in a rotary evaporator.

Compound 2 (1-methyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one)



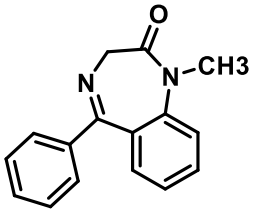
Yellow-colored jelly liquid product was obtained in a quantitative yield. R_f = 0.2 in 30% ethyl acetate in hexane. **FT-IR** (v, cm^{-1}); 3297, 1652, 1452, 1129. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.44 (d, *J* = 7.3 Hz, 2H), 7.40 – 7.28 (m, 4H), 7.21 – 7.16 (m, 1H), 7.09 (dd, *J* = 11.3, 4.2 Hz, 1H), 6.75 (d, *J* = 7.6 Hz, 1H), 5.25 (s, 1H), 3.41 (t, *J* = 13.2 Hz, 2H), 3.34 (s, *J* = 14.2 Hz, 3H), 2.63 (s, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 126 MHz): δ 170.95, 143.5, 140.9, 134.2, 129.4, 128.7, 128.9, 128.1, 127.8, 126.4, 121.5, 60.0, 50.2, 34.6, 29.8. **HRMS** (ESI +ve-TOF) of [$C_{16}H_{17}N_2O+H^+$] calculated 253.1341, found., 253.1378.

Compound 3 (5-phenyl-1-(prop-2-yn-1-yl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one)



Yellow-colored oily liquid product was obtained in a quantitative yield. R_f = 0.3 in 30% ethyl acetate in hexane (522 mg, quantitative yield. **FT-IR** (v, cm^{-1}); 3291, 2118, 1658, 1450,1378.**^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.47 – 7.27 (m, 7H), 7.12 (t, *J* = 7.3 Hz, 1H), 6.72 (d, *J* = 7.8 Hz, 1H), 5.35 (s, 1H), 4.71 (dd, *J* = 17.2, 2.4 Hz, 1H), 4.44 (dd, *J* = 17.4, 2.3 Hz, 1H), 3.47 – 3.31 (m, 2H), 2.67 (s (*br*), 1H), 2.25 (t, *J* = 2.4 Hz; 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 126 MHz): 170.7, 141.9, 140.8, 134.8, 129.4, 128.7, 128.5, 128.2, 127.9, 127.1, 121.6, 79.2, 71.9, 59.6, 50.0, 36.7. **HRMS** (ESI +ve-TOF) of [$C_{18}H_{17}N_2O+H^+$] calculated 277.1335, found., 277.1344.

Compound 4 (1-methyl-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one)



Procedure 1- In an oven-dried round bottom flask, 5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (2.12 mmol) was dissolved in dry THF under argon atmosphere. To this, a solution of sodium hydride (2.35 mmol) in THF was added dropwise at 0 ˚C and stirred for 10 min before the addition of methyl iodide (3.17 mmol) in a dropwise manner. The reaction was continued for another 6 h at RT to notice near complete consumption of the starting material monitored using thin layer chromatography. Then, the reaction was quenched with water, extracted with multiple portions of ethyl acetate (3 x 15 mL). The combined organic layer was washed with brine solution (20 mL) and dried over sodium sulphate to obtain the crude product. This was purified using silica gel column chromatography to obtain *N*-methyl-2-aminonebzophenone in 50 % yield (265 mg, R_f = 0.3 (20 % EtOAC: Hexane)) as a yellow amorphous solid.

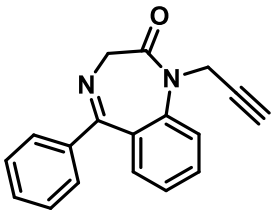
Procedure 2: In an oven-dried round bottom flask, (*N*-methyl-2-aminonebzophenone (4.73 mmol) was dissolved in dry DCM and cooled in an ice bath. To this cold solution, 2-chloroacetyl chloride (7.10 mmol) and sodium bicarbonate (18.93 mmol) were added dropwise and stirred for 6 h at room temperature. After complete consumption of the starting material, monitored using thin layer chromatography, the reaction was quenched with water, extracted with multiple portions of DCM (3 x 15 mL). The combined organic layer was washed with brine solution (~20 mL) and dried over anhydrous sodium sulphate (~10 g) before evaporating to dryness in a rotary evaporator to obtain crude product. This was purified using a silica gel column chromatography to obtain the chloroacetmide compound in 82% yield (1.1 g, R_f = 0.3 (20 % EtOAC: Hexane)) as a white solid. Next, the chloroacetmide compound (1 g, 3.43 mmol) was dissolved in methanol: ammonia solution (3:1) at 0 ˚C in an RB and stirred for 5 minutes at RT then the reaction flask was shifted to 65 ºC for 4 hours. The complete consumption of the starting material was monitored using thin layer chromatography, then the solvent was evaporated under reduced pressure, resuspended with water and extracted with multiple portions of DCM (3 x15 mL). The combined organic layer was washed with brine solution (~20 mL), dried over sodium sulphate (~10 g) and evaporated to dryness under reduced pressure. This crude product was purified using silica gel column chromatography to obtain compound **4** in 70 % yield (600 mg, R_f = 0.3 (20 % EtOAC: Hexane)) as a yellow amorphous solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.60 (d, *J* = 7.5 Hz, 2H), 7.56 – 7.51 (m, 1H), 7.44 (t, *J* = 7.3 Hz, 1H), 7.41 – 7.27 (m, 4H), 7.18 (t, *J* = 7.4 Hz, 1H), 4.80 (d, *J* = 10.7 Hz, 1H), 3.77 (d, *J* = 10.7 Hz, 1H), 3.41 (s, 3H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 101 MHz): δ 170.4, 170.2, 144.1, 138.9, 131.4, 130.6, 130.4, 129.6, 128.8, 128.2, 123.8, 121.0, 57.0, 34.8. **IR** (*v*, cm^{-1}): 2917, 2852, 1660, 1604, 1439, 1317, 1260, 1123, 1070, 983, 915, 736, 694. **HRMS** (ESI +Ve-TOF) for $[C_{16}H_{14}N_2O+H]^+$: calculated.,451.1179 Found: 251.1195

General procedure for N-alkylation of benzodiazepine:

In an oven-dried round bottom flask, 5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (2.12 mmol) a dissolved in dry DMF, followed by potassium carbonate (7.62 mmol) and alkyl bromide (3.05 mmol) were added. Then, the reaction mixture was stirred for 6 h at room temperature. After complete consumption of the starting material, monitored using thin layer chromatography, the reaction was quenched with water, extracted with multiple portions of DCM (3 x 15 mL). The combined organic layer was washed with brine solution (~20 mL) and dried over anhydrous sodium sulphate (~10 g) before evaporating to dryness in a rotary evaporator to obtain crude product. This was purified using a silica gel column chromatography to obtain pure product.

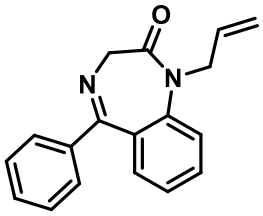
Compound 5 (5-phenyl-1-(prop-2-yn-1-yl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one)



Following the general procedure, we have synthesized compound **5.** Yield: 86 % (600 mg, R_f = 0.5 (30 % EtOAC: Hexane)) as a yellow sticky solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, *J* = 7.7 Hz, 1H), 7.59 (dd, *J* = 6.9, 1.6 Hz, 3H), 7.49 – 7.42 (m, 1H), 7.39 (dd, *J* = 8.1, 6.6 Hz, 2H), 7.32 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.25 – 7.18 (m, 1H), 4.84 (d, *J* = 10.6 Hz, 1H), 4.70 (dd, *J* = 17.6, 2.4 Hz, 1H), 4.51 (dd, *J* = 17.5, 2.5 Hz, 1H), 3.82 (d, *J* = 10.8 Hz, 1H), 2.27 (t, *J* = 2.4 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 125 MHz): δ 170.5, 169.3, 142.4, 138.9, 131.5, 130.6, 130.5, 129.6, 129.3, 128.3, 124.5, 121.2, 79.0, 72.5, 56.8, 36.9.

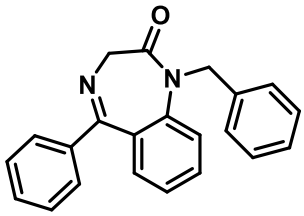
Compound 6 (1-allyl-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one)



Following the general procedure, we have synthesized compound **6**. Yield: 68 % (400 mg, R_f = 0.5 (30 % EtOAC: Hexane)) as a yellow sticky liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 – 7.53 (m, 2H), 7.53 – 7.46 (m, 1H), 7.45 – 7.38 (m, 2H), 7.35 (ddd, *J* = 7.2, 5.6, 2.7 Hz, 2H), 7.27 – 7.23 (m, 1H), 7.20 – 7.11 (m, 1H), 5.95 – 5.69 (m, 1H), 5.17 – 4.99 (m, 2H), 4.78 (dd, *J* = 10.4, 3.3 Hz, 1H), 4.64 – 4.51 (m, 1H), 4.49 – 4.32 (m, 1H), 3.79 (dd, *J* = 10.4, 2.8 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz): δ 170.5, 169.1, 143.0, 138.9, 133.1, 131.4, 130.5, 129.6, 128.3, 124.3, 121.8, 117.1, 77.6, 77.2, 76.9, 57.1, 49.9.

Compound 7 (1-benzyl-5-phenyl-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one)



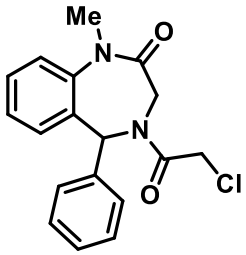
Following the general procedure, we have synthesized compound **7**. Yield: 40 % (290 mg, R_f = 0.5 (30 % EtOAc: Hexane)) as a yellow sticky solid.

^{1}H NMR (400 MHz, $CDCl_3$):δ 7.43 (dd, *J* = 5.4, 4.3 Hz, 1H), 7.41 (d, *J* = 1.6 Hz, 1H), 7.39 (s, 1H), 7.37 (dd, *J* = 5.9, 4.7 Hz, 2H), 7.32 (d, *J* = 7.9 Hz, 1H), 7.17 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.15 – 7.09 (m, 3H), 7.04 (dd, *J* = 6.4, 3.0 Hz, 2H), 5.61 (d, *J* = 15.4 Hz, 1H), 4.87 (d, *J* = 10.4 Hz, 1H), 4.78 (d, *J* = 15.5 Hz, 1H), 3.88 (d, *J* = 10.4 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz): δ 170.7, 169.5, 142.2, 138.8, 136.8, 131.2, 130.5, 130.4, 130.3, 129.5, 128.6, 128.2, 127.4, 127.3, 124.4, 122.3, 57.0, 49.8.

General protocol for the synthesis of IITK3101, IITK3102, IITK3105 and IITK3106:

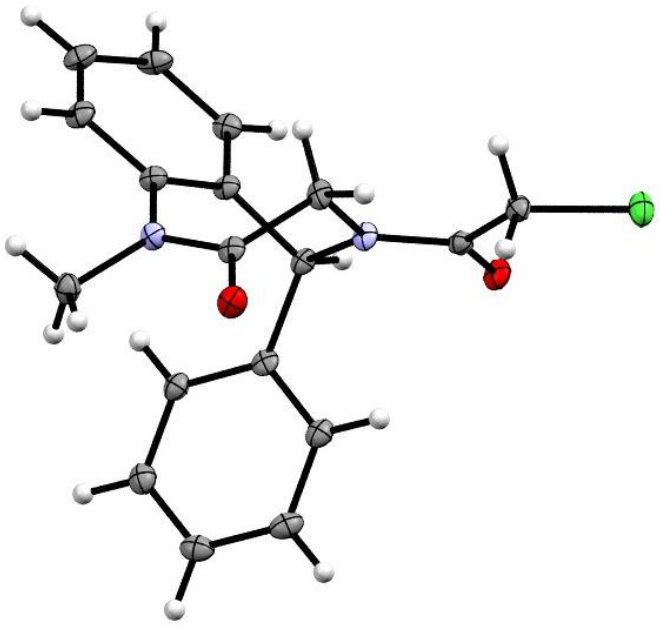
To an ice-cold solution of compound 2 (0.2 mmol) in dry CH_2Cl_2 (5 mL), 0.3 mmol of chloro-acetyl chloride (IITK3101 & IITK3105) or bromo-acetyl bromide (IITK3102 & IITK3106) was added slowly followed by the addition of sodium bicarbonate (0.6 mmol). Then, the reaction mixture was stirred at RT for 4 h and after completion of the reaction, the reaction was quenched with 10 mL of water. The extraction was done in CH_2Cl_2 (10 mL X 3 times) and collected organic layer was dried over sodium sulafte and evaporated in a rotary evaporator. Purity of the compound was found to be more than 95% by UHPLC and TLC.

IITK3101 4-(2-chloroacetyl)-1-methyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one



Obtained crude was purified by column chromatography (40% ethyl acetate in hexane) as a yellow-coloured jelly liquid with 85% yield. *R*f= 0.8 in 30% ethyl acetate in hexane. FT-IR (v, cm^{-1}); 1653, 1453, 1127, 739.**^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.58 – 6.88 (m, 9H) aromatic protons, 6.38 (s, 1H) benzylic -CH proton, 5.08 – 3.38 (m, 4H) aliphatic protons, 2.60, 2.59 (s, 3H) methyl protons. **^{13}C{^{1}H} NMR** (126 MHz, $CDCl_3$) δ 165.7, 164.9, 142.3, 138.7, 132.7, 131.9, 131.2, 130.5, 130.3, 128.9, 128.4, 127.3, 127.1, 125.0, 124.1, 123.8, 63.9, 61.5, 50.5, 47.0, 41.4, 41.1, 34.3. In both ^{1}H and ^{13}C NMR spectra, each one of the peaks were found to be doubling. We observe from NMR that there are two rotamers in the reaction mixture, however only single spot was observed in TLC. Based on the integration, we could assign the presence of two rotamers in 1: 1.5 ratio. HRMS (ESI +ve-TOF) of [$C_{18}H_{17}ClN_2O_2$+H^+] calculated 329.1057, found 329.1055.

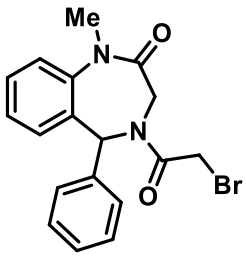
Table S2: Crystal data and structure refinement for IITK3101



Identification code	IITK3101
Empirical formula	$C_{18}H_{17}N_2O_2$
Formula weight	328.8
Temperature/K	296.15
Crystal system	Orthorhombic
Space group	Pccn
a/Å	21.1441(11)
b/Å	11.5844(7)
c/Å	12.8398(7)

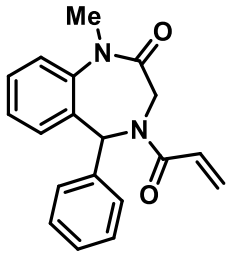
α/°	90
β/°	90
γ/°	90
Volume/Å^3	3145.0(3)
Z	8
ρ_{calc} g/cm^3	1.389
μ/mm^{-1}	0.254
F(000)	1378.0
Crystal size/mm^3	0.29 x 0.26 x0.21
Radiation	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	3.86 to 56.64
Index ranges	-28 ≤ h ≤ 28, -15 ≤ k ≤ 15, -17 ≤ l ≤ 17
Reflections collected	64122
Independent reflections	3918 [R_{int} = 0.0585, R_{sigma} = 0.0226]
Data/restraints/parameters	3918/0/209
Goodness-of-fit on F^2	1.052
Final R indexes [I>=2σ (I)]	R_1 = 0.0411, wR_2 = 0.1117
Final R indexes [all data]	R_1 = 0.0452, wR_2 = 0.1166
Largest diff. peak/hole / e Å^{-3}	0.51/-0.41

IITK3102 4-(2-bromoacetyl)-1-methyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one



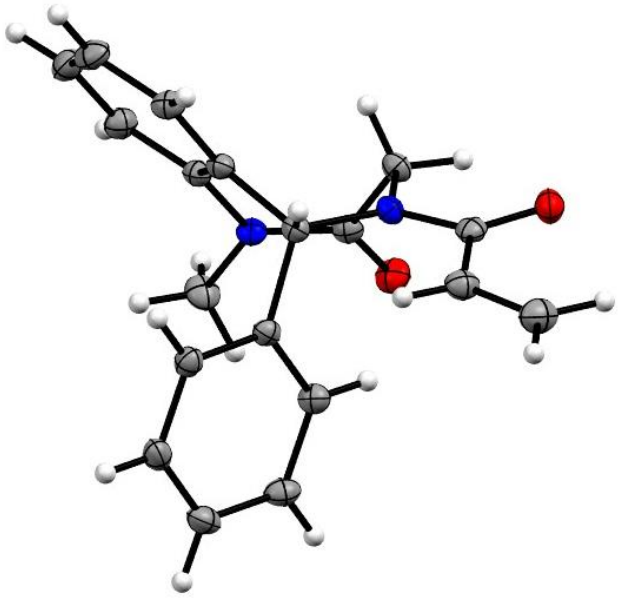
Obtained crude was purified by column chromatography (40% ethyl acetate in hexane) as a yellow-coloured jelly liquid with 65% yield. *R*f= 0.8 in 50% ethyl acetate-hexane. FT-IR (v, cm^{-1}); 1647, 1450, 1201, 741. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.65 – 6.90 (m, 9H) aromatic protons, 6.39 (s, 1H) benzylic -CH proton, 5.07- 3.38 (m, 4 H) aliphatic protons, 2.61, 2.58 (s, 3H) methyl protons. **^{13}C{^{1}H} NMR** (126 MHz, $CDCl_3$) δ 166.1, 165.8, 142.2, 139.1, 138.8, 132.7, 131.9, 131.4, 130.5, 130.3, 128.8, 128.4, 127.9, 127.2, 127.0, 124.9, 124.1, 123.8, 64.2, 61.4, 51.0, 46.9, 34.3, 26.1. In both ^{1}H and ^{13}C NMR spectra, each one of the peaks were found to be doubling. We observe from NMR that there are two rotamers in the reaction mixture, however only a single spot was observed in TLC. Based on the integration, we could assign the presence of two rotamers in 1: 1.5 ratio. **HRMS** (ESI +ve-TOF) of [$C_{18}H_{17}BrN_2O_2$+H^+] calculated, 373.0552, found 373.0549.

IITK3103 4-acryloyl-1-methyl-5-phenyl-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one



Obtained crude was purified by column chromatography (40% ethyl acetate in hexane) as a yellow-coloured jelly liquid with 90% yield. R_f= 0.7 in 50% ethyl acetate in hexane. **FT-IR** (v, cm^{-1}); 1656, 1415, 1174. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.55 – 5.71 (m, 13H) aromatic protons 9, olefinic proton 3 and 1 benzylic -CH proton, 5.10 -3.38 (m, 2H) aliphatic protons, 2.60-2.59 (s, 3H) methyl protons. **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$) δ 166.3, 165.8, 165.3, 142.4, 139.2, 133.1, 131.9, 131.2, 130.6, 130.0, 129.7, 128.6, 128.3, 127.7, 127.4, 127.1, 126.8, 125.2, 123.9, 64.9, 61.1, 50.0, 46.8, 34.3. In both ^{1}H and ^{13}C NMR spectra, each one of the peaks were found to be doubling. We observe from NMR that there are two rotamers in the reaction mixture, however only single spot was observed in TLC. Based on the integration, we could assign the presence of two rotamers in ~1: 1 ratio. HRMS (ESI +ve-TOF) of [$C_{19}H_{18}N_2O_2$+H^+] calculated 307.1447, found 307.1441.

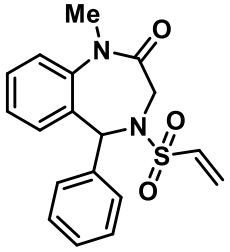
Table S3: Crystal data and structure refinement for IITK3103



Identification code	IITK3103
Empirical formula	$C_{19}H_{16}N_2O_2$
Formula weight	304.351
Temperature/K	296.15
Crystal system	Monoclinic
Space group	$P2_1/n$
a/Å	9.1979(4)

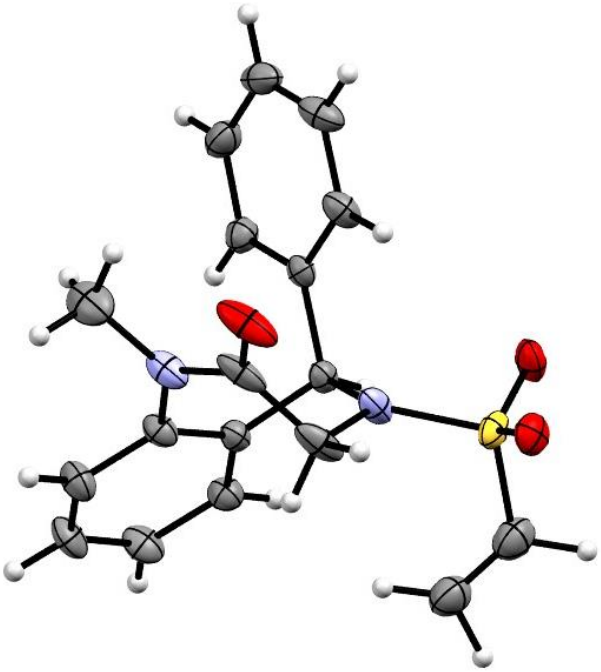
b/Å	15.6759(8)
c/Å	10.7636(5)
α/°	90
β/°	95.704(2)
γ/°	90
Volume/Å^3	1544.27(13)
Z	4
ρ_{calc} g/cm^3	1.309
μ/mm^{-1}	0.086
F(000)	640.4
Crystal size/mm^3	0.29 x 0.26 x0.21
Radiation	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	4.6 to 56.74
Index ranges	-12 ≤ h ≤ 12, -20 ≤ k ≤ 20, -14 ≤ l ≤ 14
Reflections collected	32415
Independent reflections	3859 [R_{int} = 0.0427, R_{sigma} = 0.0205]
Data/restraints/parameters	3859/0/217
Goodness-of-fit on F^2	1.036
Final R indexes [I>=2σ (I)]	R_1 = 0.0373, wR_2 = 0.0966
Final R indexes [all data]	R_1 = 0.0422, wR_2 = 0.0997
Largest diff. peak/hole / e Å^{-3}	0.34/-0.22

IITK3104 1-methyl-5-phenyl-4-(vinylsulfonyl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one



Obtained crude was purified by column chromatography (40% ethyl acetate in hexane) as a yellow-colored jelly liquid with 60% yield. *R*f= 0.8 in 50% ethyl acetate in hexane. FT-IR (v, cm^{-1}); 1653, 1327, 1141. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.4-7.49 (m, 2H), 7.40 – 7.31 (m, 1H), 7.29 – 7.11 (m, 6H), 6.43 (dd, *J* = 16.5, 9.8 Hz, 1H), 6.28 (d, *J* = 16.5 Hz, 1H), 6.09 (s, 1H), 5.99 (d, *J* = 9.8 Hz, 1H), 4.18 (d, *J* = 11.3 Hz, 1H), 3.37 (d, *J* = 11.2 Hz, 1H), 2.56 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.4, 142.1, 139.9, 133.6, 132.1, 131.2, 130.4, 128.8, 128.3, 127.5, 127.2, 125.3, 124.1, 77.4, 77.1, 76.8, 63.4, 48.8, 34.5. HRMS (ESI +ve-TOF) of [$C_{18}H_{18}N_2O_3S+H^+$] calculated 343.1116, found 343.1110.

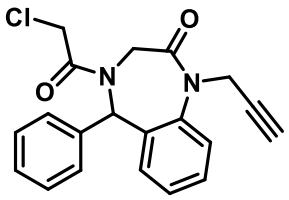
Table S4: Crystal data and structure refinement for IITK3104



Identification code	IITK3104
Empirical formula	$C_{18}H_{18}N_2O_3S$
Formula weight	342.40
Temperature/K	296.15
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	13.7896(3)
b/Å	9.0412(2)
c/Å	13.8981(4)
α/°	90
β/°	108.8700(10)
γ/°	90
Volume/Å^3	1639.61(7)
Z	4
ρ_{calc} g/cm^3	1.387
μ/mm^{-1}	0.216
F(000)	720.0
Crystal size/mm^3	0.33 x 0.28 x 0.23
Radiation	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	5.468 to 50.098
Index ranges	-16 ≤ h ≤ 16, -10 ≤ k ≤ 10, -16 ≤ l ≤ 16
Reflections collected	29271
Independent reflections	2903 [R_{int} = 0.0391, R_{sigma} = 0.0171]
Data/restraints/parameters	2903/0/277
Goodness-of-fit on F^2	1.072

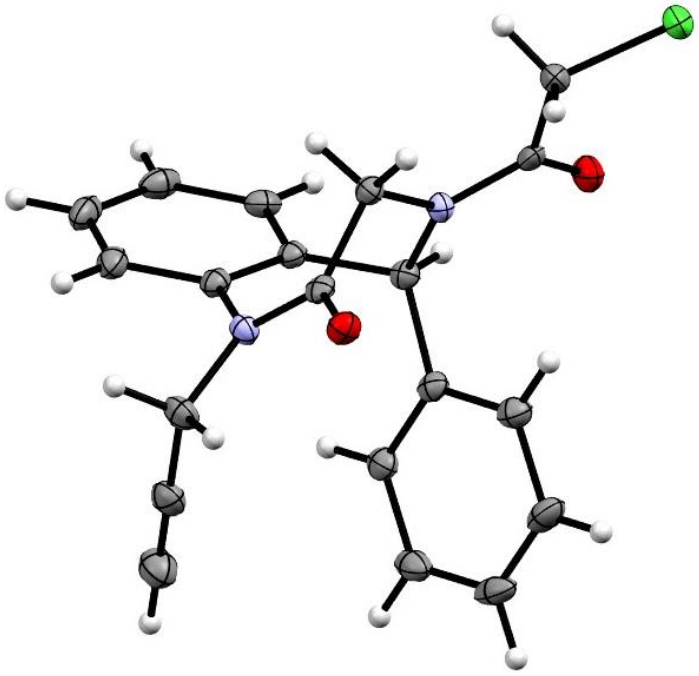
Final R indexes [I>=2σ (I)]	R_1 = 0.0330, wR_2 = 0.0874
Final R indexes [all data]	R_1 = 0.0378, wR_2 = 0.0897
Largest diff. peak/hole / e Å^{-3}	0.22/-0.34

IITK3105 4-(2-chloroacetyl)-5-phenyl-1-(prop-2-yn-1-yl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one



Obtained crude was purified by column chromatography (30% ethyl acetate in hexane) as a yellow-coloured jelly liquid with 85% yield. *R*f= 0.5 in 30% ethyl acetate in hexane. **FT-IR** (v, cm^{-1}); 3275, 1957, 1660, 1384, 1188, 746. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.77 – 6.79 (m, 9H) aromatic protons, 6.38 (s, 1H) benzylic -CH proton, 5.14 – 3.19 (m, 6H) aliphatic protons, 2.16-2.11 (t, 1H) propargyl -CH proton. **^{13}C{^{1}H} NMR** ($CDCl_3$, 126 MHz): 164.4, 138.6, 131.9, 131.3, 130.4, 128.7, 128.5, 128.3, 128.1, 127.7, 127.5, 127.3, 124.9, 123.6, 78.5, 72.4, 61.5, 50.5, 41.4, 36.9. In both ^{1}H and ^{13}C NMR spectra, each one of the peaks were found to be doubling. We observe from NMR that there are two rotamers in the reaction mixture, however only single spot was observed in TLC. Based on the integration, we could assign the presence of two rotamers in 1: 1.35 ratio. **HRMS** (ESI +ve-TOF) of [$C_{20}H_{17}ClN_2O_2$+H^+] calculated, 353.1051, found., 353.1024.

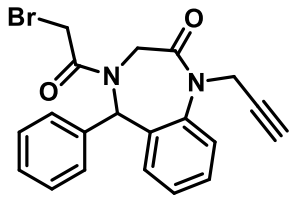
Table S5: Crystal data and structure refinement for IITK3105



Identification code	IITK3105
Empirical formula	$C_{20}H_{17}ClN_2O_2$
Formula weight	352.823
Temperature/K	296.15
Crystal system	Orthorhombic
Space group	$Pna2_1$
a/Å	10.2787(5)
b/Å	10.2787(5)
c/Å	27.5592(15)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	3375.1(3)
Z	4
ρ_{calc} g/cm^3	0.694
μ/mm^{-1}	0.121
F(000)	737.0
Crystal size/mm^3	0.29 × 0.26 × 0.21
Radiation	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	5.44 to 56.64
Index ranges	-13 ≤ h ≤ 13, -15 ≤ k ≤ 15, -36 ≤ l ≤ 36
Reflections collected	52444
Independent reflections	8372 [R_{int} = 0.0491, R_{sigma} = 0.0332]
Data/restraints/parameters	8372/1/451
Goodness-of-fit on F^2	1.050

Final R indexes [I>=2σ (I)]	R_1 = 0.0352, wR_2 = 0.0809
Final R indexes [all data]	R_1 = 0.0352, wR_2 = 0.0809
Largest diff. peak/hole / e Å^{-3}	0.34/-0.22

IITK3106 (4-(2-bromoacetyl)-5-phenyl-1-(prop-2-yn-1-yl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one)

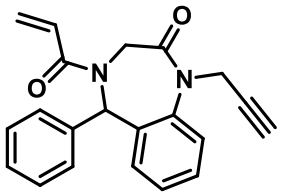


Obtained crude was purified by column chromatography (30% ethyl acetate in hexane) as a yellow-colored jelly liquid with 85% yield. R_f = 0.5 in 30% ethyl acetate in hexane**. FT-IR** (v, cm^{-1}); 3290, 2248, 1652, 1492, 1397, 726. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.73 – 6.81 (m, 9H) aromatic protons, 6.39 (s, 1H) benzylic -CH proton, 5.13 – 3.19 (m, 6H) aliphatic protons, 2.16-2.11 (t, 1H) propargyl -CH proton. **^{13}C{^{1}H} NMR** ($CDCl_3$, 126 MHz): δ 166.1, 165.9, 164.4, 141.3, 138.7, 132.4, 131.9, 131.4, 130.6, 130.4, 129.0, 128.5, 128.1, 127.3, 126.9, 127.0, 126.9, 124.9, 123.8, 123.5, 78.5, 72.4, 64.2, 61.4, 51.0, 46. 9, 36.9, 36.6. In both ^{1}H and ^{13}C NMR spectra, each one of the peaks were found to be doubling. We observe from NMR that there are two rotamers in the reaction mixture, however only single spot was observed in TLC. Based on the integration, we could assign the presence of two rotamers in ~1:1 ratio. **HRMS** (ESI +ve-TOF) of [$C_{20}H_{18}BrN_2O_2$+H^+], calculated, 397.0546, found 397.0797.

General protocol for the synthesis of IITK3103, IITK3104, IITK3107, IITK3108

To an ice-cold solution of compound 6 and 7 (0.2 mmol) in dry dcm (5 mL), acryloyl chloride and 2-chloroethane sulfonyl chloride (0.3 mmol) was added slowly followed by the addition of triethylamine (0.4 mmol for IITK3103 and IITK3107 and 0.8 mmol for IITK3104 and IITK3108). Then, reaction mixture was stirred at rt for 4 h and after completion of the reaction, reaction was quenched with 10 mL of water. Extraction was done in dcm (10 mL X 3 times) and collected organic layer was dried over sodium sulafte and the solvent was removed in rotary evaporator.

IITK3107 4-acryloyl-5-phenyl-1-(prop-2-yn-1-yl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4]diazepin-2-one

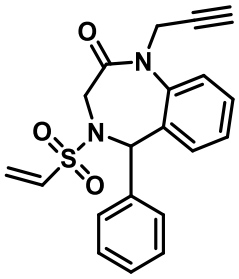


Obtained crude was purified by column chromatography (30% ethyl acetate in hexane) as a yellow-colored jelly liquid with 90% yield. *R*f= 0.3 in 30% ethyl acetate in hexane. **FT-IR** (v, cm^{-1}); 3281, 2119, 1662, 1452, 1233. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.73 – 6.56 (m, 10H) aromatic 9 and 1 olefinic protons, 6.31 (m, 2H) benzylic -CH proton and 1 olefinic proton, 5.82 (m, 1H) olefinic proton, 5.16 – 3.17 (m, 4H) aliphatic protons, 2.16-2.11 (s, 1H) propargyl -CH proton. **^{13}C{^{1}H} NMR** ($CDCl_3$, 126 MHz): δ 166.2, 165.8, 164.8, 141.5, 141.3, 139.5, 139.2, 132.8, 131.9, 131.2, 130.4, 130.3, 130.2, 129.9,

128.7, 128.4, 127.8, 127.6, 127.3, 127.1, 125.2, 125.0, 123.7, 123.4, 117.4, 78.6, 72.2, 61.1, 60.7, 50.0, 46.7, 36.9, 36.5, 32.9, 29.7.

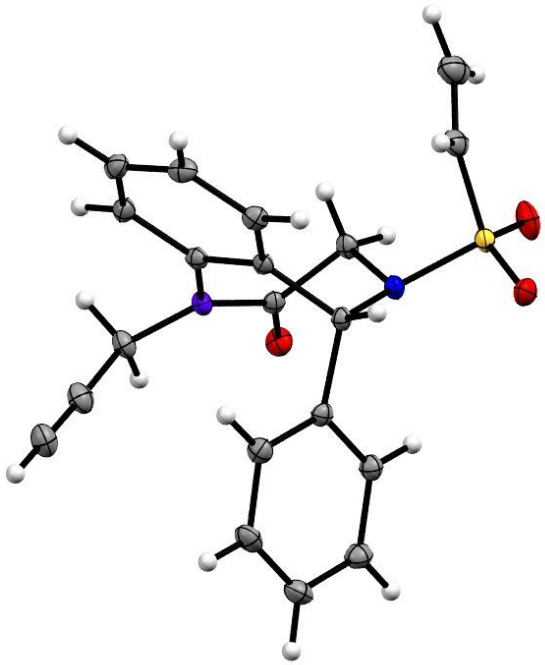
In both ^{1}H and ^{13}C NMR spectra, each one of the peaks were found to be doubling. We observe from NMR that there are two rotamers in the reaction mixture, however only single spot was observed in TLC. Based on the integration, we could assign the presence of two rotamers in ~1: 1 ratio. **HRMS** (ESI +ve-TOF) of [$C_{21}H_{18}N_2O_2$+H^+] calculated 331.1441, found 331.1113.

IITK3108 (5-phenyl-1-(prop-2-yn-1-yl)-4-(vinylsulfonyl)-1,3,4,5-tetrahydro-2H-benzo[e][1,4] diazepin-2-one)



Obtained crude was purified by column chromatography (40% ethyl acetate in hexane) as a yellow-colored jelly liquid with 60% yield. *R*f= 0.8 in 50% ethyl acetate in hexane. **FT-IR** (v, cm^{-1}); 3284, 2120, 1652, 1451, 1186. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.60 (dd, J = 8.0, 1.1 Hz, 1H), 7.51 (d, J = 1.7 Hz, 1H), 7.47 – 7.44 (m, 1H), 7.41 (dd, J = 7.4, 1.2 Hz, 1H), 7.24 – 7.17 (m, 3H), 7.15 – 7.10 (m, 2H), 6.42 (d, J = 9.8 Hz, 1H), 6.30 (d, J = 16.5 Hz, 1H), 6.10 (s, 1H), 6.00 (d, J = 9.8 Hz, 1H), 4.25 (dd, J = 11.4, 0.9 Hz, 1H), 3.89 (dd, J = 17.6, 2.5 Hz, 1H), 3.41 (d, J = 11.4 Hz, 1H), 3.15 (dd, J = 17.6, 2.5 Hz, 1H).), 2.17 (). **^{13}C{^{1}H} NMR** ($CDCl_3$, 126 MHz): δ 163.8, 141.2, 139.9, 133.6, 131.9, 131.2, 130.5, 128.9, 128.5, 127.7,127.6, 125.2, 123.9, 78.7, 72.4, 63.4, 48.8, 37.0. **HRMS** (ESI +ve-TOF) of [$C_{20}H_{19}N_2O_3S$+H^+] calculated 367.1111, found. 367.1118.

Table S6: Crystal data and structure refinement for IITK3108



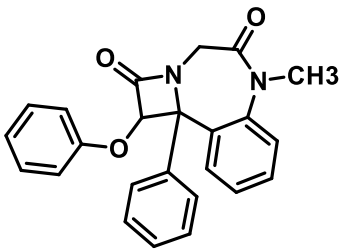
Identification code	IITK3108
Empirical formula	$C_{20}H_{16}N_2O_3S$
Formula weight	364.428
Temperature/K	296.15
Crystal system	Monoclinic
Space group	$P2_1/n$
a/Å	8.3439(2)
b/Å	13.2708(3)
c/Å	15.3297(3)
α/°	90
β/°	94.487(1)
γ/°	90
Volume/Å^3	1692.26(7)
Z	4
ρ_{calc}g/cm^3	1.430
μ/mm^{-1}	0.215
F(000)	761.0
Crystal size/mm^3	0.3 × 0.25 × 0.22
Radiation	Mo Kα (λ = 0.71073)

2Θ range for data collection/°	4.06 to 56.8
Index ranges	-11 ≤ h ≤ 11, -17 ≤ k ≤ 17, -20 ≤ l ≤ 16
Reflections collected	28346
Independent reflections	4214 [R_{int} = 0.0314, R_{sigma} = 0.0209]
Data/restraints/parameters	4214/0/243
Goodness-of-fit on F^2	1.006
Final R indexes [I>=2σ (I)]	R_1 = 0.0380, wR_2 = 0.0971
Final R indexes [all data]	R_1 = 0.0433, wR_2 = 0.1006

General procedure for the synthesis of β-lactam derivatives:

The β-lactam derivatives were synthesized following reported protocol[5] with minor modifications. Briefly, in an oven-dried round bottom flask, 2-phenoxyacetic acids (1.5 equiv.) was dissolved in dry CH_2Cl_2 under argon atmosphere and added BOP-Cl (1.5 equiv.). After stirring the reaction mixture for 10 minutes at RT, substituted-benzodiazipinones (1 equiv.) and triethyl amine (1.5 equiv. dropwise) were added at 0 ˚C and stirring continued for 6 h at 40 ˚C. After near complete consumption of the starting material, followed using thin layer chromatography, the reaction was quenched with water, extracted with multiple portions of DCM (3 x 15 mL). The combined organic layer was washed with brine solution (20 mL) and dried over sodium sulphate to obtain the crude product. This was purified using silica gel column chromatography to obtain the final pure product.

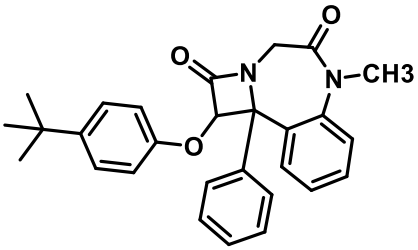
IITK3109 (6-methyl-1-phenoxy-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3109 in 65% yield (50 mg, R_f = 0.3 (20 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.64 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.53 – 7.49 (m, 1H), 7.45 (dd, *J* = 7.6, 1.3 Hz, 1H), 7.28 (m, 3H), 7.26 – 7.16 (m, 4H), 6.98 (s, 1H), 6.88 – 6.80 (m, 2H), 5.81 (s, 1H), 4.43 (d, *J* = 13.6 Hz, 1H), 3.82 (d, *J* = 13.5 Hz, 1H), 2.57 (s, 3H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 125 MHz): δ 165.5, 163.9, 157.2, 141.6, 137.4, 133.4, 130.2, 129.5, 128.1, 127.5, 126.6, 126.4, 126.3, 122.8, 116.8, 86.0, 70.9, 45.4, 36.5. **IR** (v, cm^{-1}):3741, 1750, 1688, 1490, 1289, 1235, 747, 689. **HRMS** (ESI +ve-TOF) for $[C_{24}H_{20}N_2O_3+H]^{+.}$ calculated,385.1547 Found: 385.1563

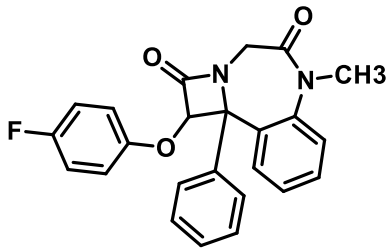
IITK3110 (1-(4-(tert-butyl)phenoxy)-6-methyl-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we synthesized IITK3110 and purified using silica gel column chromatography to obtain IITK3110 in 61 % yield (128 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (400 MHz, CHLOROFORM-D) δ 7.68 – 7.60 (m, 1H), 7.56 – 7.41 (m, 2H), 7.27 (m, 2H), 7.26 – 6.97 (m, 5H), 6.82 – 6.72 (m, 2H), 5.78 (s, 1H), 4.42 (d, *J* = 13.6 Hz, 1H), 3.82 (d, *J* = 13.5 Hz, 1H), 2.56 (s, 3H), 1.26 (s, 9H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 101 MHz): δ 165.8, 163.9, 155.0, 145.6, 141.6, 137.5, 133.5, 130.2, 128.1, 127.5, 126.6, 126.5, 126.3, 116.3, 86.2, 70.9, 45.4, 36.5, 34.2, 31.5. **IR** (v, cm^{-1}): 2959, 1770, 1665, 1498, 1375, 1218, 1083, 823, 756, 693**. HRMS** (ESI +ve-TOF) for $[C_{16}H_{14}ClNO_2+H]^+$: calculated.,441.2173. Found: 441.2118

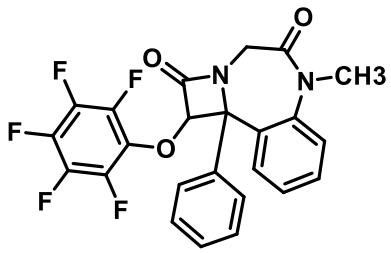
IITK3111 (1-(4-fluorophenoxy)-6-methyl-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3111 in 90 % yield (72 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (500 MHz, $CDCl_3$):δ 7.63 – 7.56 (m, 1H), 7.55 – 7.38 (m, 3H), 7.28 (m, 3H), 7.24 (d, *J* = 7.9 Hz, 2H), 6.94 – 6.84 (m, 2H), 6.82 – 6.73 (m, 2H), 5.72 (s, 1H), 4.42 (d, *J* = 13.6 Hz, 1H), 3.82 (d, *J* = 13.6 Hz, 1H), 2.57 (s, 3H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 125 MHz): δ 165.3, 163.8, 159.4, 159.0, 157.5, 153.2, 150.6, 141.6, 137.4, 133.3, 130.3, 128.2, 127.5, 126.5, 126.4, 126.3, 118.3, 116.0, 115.9, 86.6, 70.9, 45.4, 36.5. **IR** (v, cm^{-1}): 3738, 3211, 3075, 2954, 2918, 2854, 2360, 2324, 1760, 1663, 1585, 1499, 1442, 1378, 754, 690, 522. **HRMS** (ESI +ve-TOF) for $[C_{24}H_{19}FN_2O_3+H]^{+:}$ calculated,403.1452 Found: 403.1440

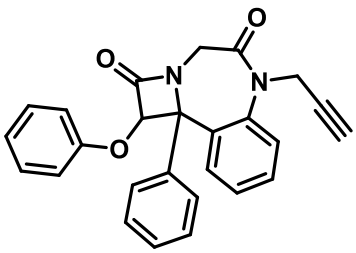
IITK3112 (6-methyl-1-(perfluorophenoxy)-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3112 in 53 % yield (50 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.65 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.54 (td, *J* = 7.7, 1.7 Hz, 1H), 7.50 (td, *J* = 7.5, 1.4 Hz, 1H), 7.29 (dd, *J* = 15.7, 10.0 Hz, 4H), 7.25 (d, *J* = 1.3 Hz, 1H), 5.80 (s, 1H), 4.40 (d, *J* = 13.7 Hz, 1H), 3.81 (d, *J* = 13.7 Hz, 1H), 2.55 (s, 3H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 125 MHz): δ 163.5, 163.3, 141.4, 136.5, 132.8, 130.5, 128.6, 128.4, 127.7, 126.5, 126.4, 126.0, 87.9, 70.8, 45.4, 36.5. **IR** (v, cm^{-1}): 2357, 1764, 1654, 1511, 1371, 1285, 1169, 987, 755, 697, 609. **HRMS** (ESI +ve-TOF) for $[C_{24}H_{15}F_2N_2O_3+H]^{+:}$ calculated,475.1076 Found: 475.1098

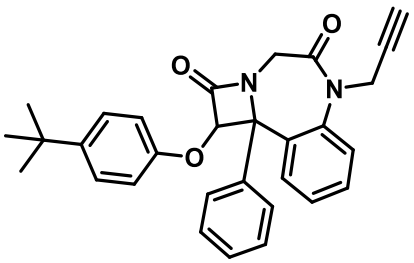
IITK3113 (1-phenoxy-10b-phenyl-6-(prop-2-yn-1-yl)-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3113 in 81% yield (50 mg, R_f = 0.4 (30 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (dd, *J* = 7.9, 1.0 Hz, 1H), 7.64 (dd, *J* = 7.4, 1.6 Hz, 1H), 7.51 (dtd, *J* = 18.2, 7.4, 1.3 Hz, 2H), 7.26 (s, 3H), 7.25 – 7.12 (m, 3H), 6.98 (t, *J* = 7.3 Hz, 1H), 6.83 (d, *J* = 8.3 Hz, 2H), 5.82 (s, 1H), 4.49 (d, *J* = 13.6 Hz, 1H), 3.94 – 3.82 (m, 2H), 3.07 (dd, *J* = 17.5, 2.5 Hz, 1H), 2.18 (t, *J* = 2.5 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 125 MHz): δ 165.5, 163.5, 157.1, 140.6, 137.4, 133.2, 130.3, 129.5, 128.2, 128.1, 126.5, 125.8, 122.9, 116.8, 86.1, 78.8, 72.3, 71.0, 45.4, 38. 8. **IR** (v, cm^{-1}) 3282, 2920, 1754, 1677, 1223, 748, 683, 566. **HRMS** (ESI +ve-TOF) for $[C_{26}H_{20}N_2O_3+H]^{+:}$ calculated,409.1547 Found: 409.1562

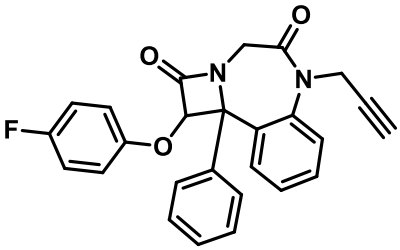
IITK3114 (1-(4-(tert-butyl)phenoxy)-10b-phenyl-6-(prop-2-yn-1-yl)-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3114 in 92 % yield (72 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (ddd, *J* = 20.1, 7.6, 1.5 Hz, 2H), 7.48 (ddd, *J* = 11.0, 7.5, 1.4 Hz, 2H), 7.38 – 7.04 (m, 7H), 6.79 – 6.68 (m, 2H), 5.77 (s, 1H), 4.46 (d, *J* = 13.6 Hz, 1H), 3.93 – 3.78 (m, 2H), 3.05 (dd, *J* = 17.5, 2.5 Hz, 1H), 2.16 (t, *J* = 2.5 Hz, 1H), 1.24 (s, 9H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 125 MHz): δ 165.7, 163.6, 155.0, 145.7, 140.6, 137.5, 133.3, 130.2, 128.2, 128.1, 126.6,126.5, 126.5, 126.3, 125.8, 116.3, 86.4, 78.8, 72.3, 71.0, 45.4, 38.8, 34.2, 31.5. **IR** (v, cm^{-1}): 3406, 3252, 2957, 1760, 1675, 1235, 828, 693. **HRMS** (ESI +ve-TOF) for $[C_{30}H_{28}N_2O_3+H]^{+:}$ calculated,465.2173 Found: 465.2174

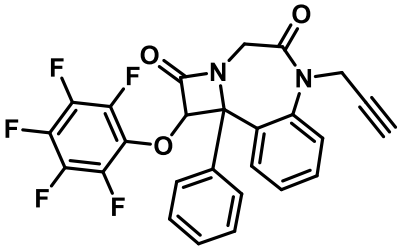
IITK3115 (1-(4-fluorophenoxy)-10b-phenyl-6-(prop-2-yn-1-yl)-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3115 in 64% yield (50 mg, R_f = 0.3 (30 % EtOAC: Hexane)) as a white amorphous solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.61 – 7.44 (m, 3H), 7.25 (d, *J* = 5.9 Hz, 3H), 6.91 – 6.84 (m, 2H), 6.79 – 6.73 (m, 2H), 5.72 (s, 1H), 4.47 (d, *J* = 13.6 Hz, 1H), 3.93 – 3.81 (m, 2H), 3.06 (dd, *J* = 17.4, 2.5 Hz, 1H), 2.17 (t, *J* = 2.5 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz): δ 165.3, 163.5, 153.2, 140.5, 137.3, 133.0, 130.4, 128.3, 128.1, 126.4, 125.8, 118.3, 118.2, 116.1, 115.9, 86.6, 78.7, 72.4, 71.0, 45.4, 38.9. **IR** (v, cm^{-1}): 3280, 1765, 1672, 1494, 1381, 1285, 1194, 753, 565**. HRMS** (ESI +ve-TOF) for $[C_{26}H_{19}FN_2O_3+H]^{+\cdot}$ calculated,427.1452 Found: 427.1470

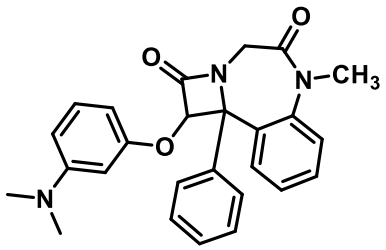
IITK3116 (1-(perfluorophenoxy)-10b-phenyl-6-(prop-2-yn-1-yl)-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3116 in 44% yield (40 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a white amorphous solid

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 – 7.68 (m, 1H), 7.68 – 7.63 (m, 1H), 7.56 (d, *J* = 2.0 Hz, 1H), 7.56 – 7.44 (m, 2H), 7.30 (s, 3H), 5.80 (s, 1H), 4.46 (d, *J* = 13.7 Hz, 1H), 3.91 – 3.80 (m, 2H), 3.06 (dd, *J* = 17.5, 2.5 Hz, 1H), 2.18 (dd, *J* = 5.2, 2.7 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz): δ 165.3, 163.5, 153.2, 140.5, 137.3, 133.0, 130.4, 128.3, 128.1, 126.4, 125.9, 118.3, 118.2, 116.1, 115.9, 86.6, 78.7,72.4, 71.0, 45.4, 38.9. **IR** (v, cm^{-1}): 3260, 1766, 1671, 1511, 984, 757, 522. **HRMS** (ESI +ve-TOF) for $[C_{26}H_{15}F_5N_2O_3+H]^{+\cdot}$ calculated,499.1076 Found: 499.1090

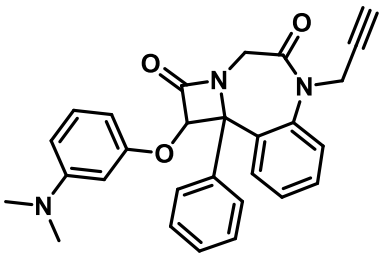
IITK3117 (1-(3-(dimethylamino)phenoxy)-6-methyl-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3117 in 59 % yield (50 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a brown solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.67 (d, *J* = 7.0 Hz, 1H), 7.50 (td, *J* = 7.7, 1.3 Hz, 1H), 7.43 (dd, *J* = 7.5, 6.8 Hz, 1H), 7.28 (m, 3H), 7.23 (d, *J* = 7.9 Hz, 2H), 7.08 (t, *J* = 8.2 Hz, 1H), 6.51 – 6.24 (m, 3H), 6.10 (s, 1H), 5.82 (s, 1H), 4.42 (d, *J* = 13.6 Hz, 1H), 3.81 (d, *J* = 13.5 Hz, 1H), 2.85 (s, 6H), 2.57 (s, 3H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 101 MHz): δ 165.8, 163.9, 158.5, 141.6, 137.6, 133.5, 130.1, 129.7, 128.1, 127.5, 126.7, 126.2, 107.5, 102.1, 86.6, 71.1, 45.3, 40.6, 36.5, 29.7**. IR** (v, cm^{-1}): 2921, 2855, 1760, 1666, 1603, 1494, 1449, 1367, 1284, 1227, 1162, 1000, 907, 823, 734, 691, 603, 529. **HRMS** (ESI +ve-TOF) for $[C_{26}H_{25}N_3O_3+H]^+$: calculated,428.1969. Found: 428.1991

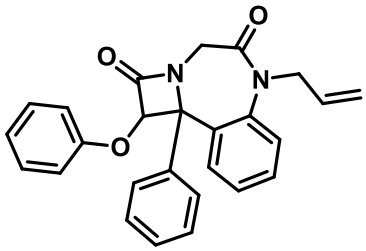
IITK3118 (1-(3-(dimethylamino)phenoxy)-10b-phenyl-6-(prop-2-yn-1-yl)-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3118 in 40% yield (33 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a white amorphous solid

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (dd, *J* = 12.3, 4.4 Hz, 2H), 7.48 (tdd, *J* = 15.2, 10.6, 4.3 Hz, 2H), 7.24 (s, 4H), 7.06 (t, *J* = 8.2 Hz, 1H), 6.26 (dd, *J* = 63.0, 55.7 Hz, 3H), 5.81 (s, 1H), 4.46 (d, *J* = 13.6 Hz, 1H), 3.92 – 3.79 (m, 2H), 3.05 (dd, *J* = 17.5, 2.5 Hz, 1H), 2.84 (s, 6H), 2.17 (t, *J* = 2.4 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz): δ 165.7, 163.6, 158.4, 140.6, 137.5, 133.3, 130.2, 129.8, 128.2, 128.1, 126.7, 126.6, 125.7, 86.6, 78.8, 72.3, 71.2, 45.4, 40.6, 38.9. **IR** (v, cm^{-1}): 3254, 1755, 1683, 1608, 1566, 1356, 1227, 1064, 749, 684, 529. **HRMS** (ESI +ve-TOF) for $[C_{28}H_{25}N_3O_3+H]^{+:}$ calculated,452.1969 Found: 452.1989

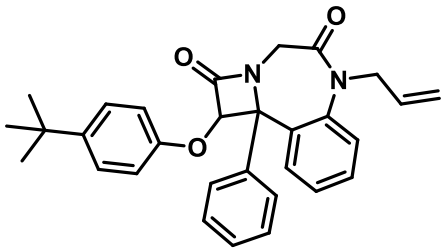
IITK3119 (6-allyl-1-phenoxy-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3119 in 90% yield (66 mg, R_f = 0.4 (30 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (dd, *J* = 7.0, 2.0 Hz, 1H), 7.42 (td, *J* = 7.5, 3.9 Hz, 2H), 7.34 (dd, *J* = 7.3, 1.9 Hz, 1H), 7.26 (s, 2H), 7.23 – 7.04 (m, 3H), 6.96 (t, *J* = 7.4 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 2H), 5.80 (s, 1H), 5.14 (ddt, *J* = 16.2, 10.0, 6.0 Hz, 1H), 4.97 – 4.86 (m, 2H), 4.45 (d, *J* = 13.4 Hz, 1H), 3.85 (d, *J* = 13.4 Hz, 1H), 3.67 (dd, *J* = 15.5, 5.7 Hz, 1H), 3.41 (dd, *J* = 15.5, 6.4 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz): δ 165.7, 163.7, 157.2, 140.9, 137.5, 133.3, 132.8, 130.0, 129.5, 128.2, 127.5, 126.7, 126.6, 125.9, 122.8, 117.6, 116.8, 86.3, 71.1, 52.7, 45.7. **IR** (v, cm^{-1}): 2923, 1750, 1665, 1485, 1377, 1231, 934, 754, 695. **HRMS** (ESI +ve-TOF) for $[C_{26}H_{21}N_2O_3+H]^{+:}$ calculated,411.1703 Found: 411.1687

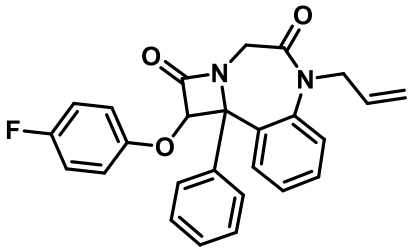
IITK3120 (6-allyl-1-(4-(tert-butyl)phenoxy)-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3120 in 70% yield (60 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 – 7.50 (m, 2H), 7.47 – 7.41 (m, 2H), 7.41 – 7.38 (m, 1H), 7.33 (dd, *J* = 7.3, 1.8 Hz, 1H), 7.26 (d, *J* = 5.0 Hz, 2H), 7.22 – 7.14 (m, 3H), 6.80 – 6.71 (m, 2H), 5.77 (s, 1H), 5.19 – 5.08 (m, 2H), 5.00 – 4.85 (m, 2H), 4.44 (d, *J* = 13.4 Hz, 1H), 3.83 (t, *J* = 12.0 Hz, 1H), 3.67 (dd, *J* = 15.4, 5.6 Hz, 1H), 3.41 (dd, *J* = 15.4, 6.3 Hz, 1H), 1.24 (s, 9H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz): δ 165.5, 163.6, 159.7, 153.2, 140.9, 137.4, 133.1, 132.7, 130.0, 128.3, 127.6, 126.7, 126.6, 125.9, 118.4, 118.3, 117.7, 116.1, 115.8, 86.9, 71.1, 52.7, 45.7. **IR** (v, cm^{-1}): 3743, 2957, 1764, 1671,1374, 1233, 755, 697. **HRMS** (ESI +ve-TOF) for $[C_{30}H_{30}N_2O_3+H]^{+:}$ calculated,467.2329 Found: 467.2359

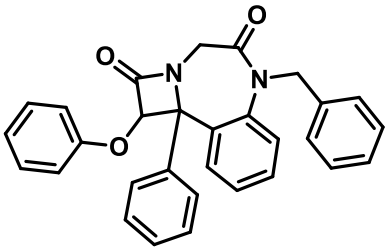
IITK3121 (6-allyl-1-(4-fluorophenoxy)-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3121 in 68% yield (53 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.58 (dd, *J* = 7.3, 1.8 Hz, 1H), 7.47 (d, *J* = 1.8 Hz, 1H), 7.46 – 7.44 (m, 1H), 7.43 (d, *J* = 1.5 Hz, 1H), 7.36 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.30 (s, 1H), 7.29 (s, 2H), 6.92 – 6.85 (m, 2H), 6.82 – 6.76 (m, 2H), 5.72 (s, 1H), 5.22 – 5.11 (m, 1H), 4.99 – 4.89 (m, 2H), 4.46 (d, *J* = 13.5 Hz, 1H), 3.86 (d, *J* = 13.4 Hz, 1H), 3.69 (ddt, *J* = 15.4, 5.7, 1.4 Hz, 1H), 3.43 (ddt, *J* = 15.5, 6.4, 1.2 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 125 MHz): δ 165.5, 163.6, 159.4, 157.5, 153.3, 140.9, 137.4, 133.2, 132.7, 130.0, 128.3, 127.6, 126.6, 125.9, 118.4, 118.3, 117.7, 116.0, 115.9, 86.9, 71.1, 52.7, 45.7. **IR** (v, cm^{-1}): 3741, 1755, 1657, 1502, 1212, 825, 752, 758. **HRMS** (ESI +ve-TOF) for $[C_{26}H_{21}FN_2O_3+H]^{+:}$ calculated,429.1609 Found: 429.1621

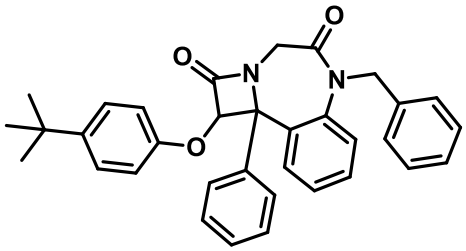
IITK3122 (6-benzyl-1-phenoxy-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3122 in 89% yield (66 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (400 MHz, $CDCl_3$):δ 7.62 (d, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 7.30 (t, *J* = 7.0 Hz, 4H), 7.24 (dd, *J* = 6.1, 2.5 Hz, 2H), 7.19 (d, *J* = 8.0 Hz, 3H), 7.08 – 7.01 (m, 2H), 7.01 – 6.93 (m, 2H), 6.83 (d, *J* = 8.1 Hz, 2H), 5.82 (s, 1H), 4.53 (dd, *J* = 14.3, 11.4 Hz, 2H), 3.92 (d, *J* = 13.3 Hz, 1H), 3.17 (d, *J* = 15.6 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz): δ 165.6, 164.4, 157.2, 141.3, 137.4, 133.1, 129.9, 129.5, 128.6, 128.3, 127.9, 127.6, 127.3, 126.6, 126.0, 122.9, 116.8, 86.3, 71.1, 53.4, 45.7. **IR** (v, cm^{-1}): 3742, 1768, 1688, 1293, 1231, 762, 698. **HRMS** (ESI +ve-TOF) for $[C_{30}H_{24}N_2O_3+H]^{+:}$ calculated,461.1860 Found: 461.1729

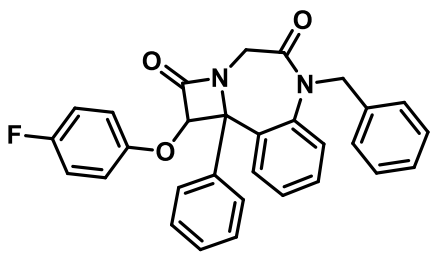
IITK3123 (6-benzyl-1-(4-(tert-butyl)phenoxy)-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3123 in 60% yield (48 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (400 MHz, $CDCl_3$):δ 7.62 (d, *J* = 7.3 Hz, 1H), 7.54 – 7.34 (m, 2H), 7.28 (s, 4H), 7.20 (t, *J* = 8.2 Hz, 6H), 7.04 (d, *J* = 6.1 Hz, 2H), 6.97 (d, *J* = 7.8 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 2H), 5.78 (s, 1H), 4.52 (t, *J* = 13.9 Hz, 2H), 3.91 (d, *J* = 13.2 Hz, 1H), 3.17 (d, *J* = 15.5 Hz, 1H), 1.24 (s, 9H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz): δ 165.8, 164.5, 155.0, 145.71, 141.3, 137.5, 137.4, 133.2, 129.9, 128.6, 128.2, 127.9, 127.6, 127.3, 126.7, 126.6, 126.3, 126.0, 116.3, 86.6, 71.1, 53.4, 45.7, 34.2, 31.5. **IR** (v, cm^{-1}): 2955, 1762, 1667, 1500, 1362, 1233, 755, 708. **HRMS** (ESI +ve-TOF) for $[C_{34}H_{32}N_2O_3+H]^{+:}$ calculated,517.2486 Found: 517.2502

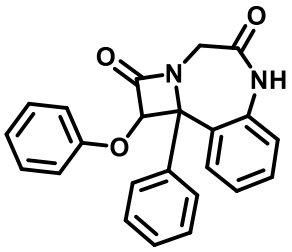
IITK3124 (6-benzyl-1-(4-fluorophenoxy)-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3124 in 89% yield (65 mg, R_f = 0.3 (30 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (400 MHz, $CDCl_3$):δ 7.52 (d, *J* = 7.3 Hz, 1H), 7.35 (t, *J* = 7.3 Hz, 1H), 7.25 (t, *J* = 7.1 Hz, 4H), 7.20 – 7.15 (m, 3H), 7.15 (s, 1H), 7.02 – 6.97 (m, 2H), 6.94 (d, *J* = 7.9 Hz, 1H), 6.87 – 6.77 (m, 2H), 6.76 – 6.66 (m, 2H), 5.67 (s, 1H), 4.47 (t, *J* = 14.3 Hz, 2H), 3.86 (d, *J* = 13.3 Hz, 1H), 3.12 (d, *J* = 15.5 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz): δ 165.4, 164.3, 153.2, 141.2, 137.3, 132.9, 129.9, 128.6, 128.4, 127.90, 127.5, 127.3, 126.5, 126.5, 126.0, 118.3, 118.2, 116.0, 115.8, 86.9, 71.0, 53.4, 45.6. **IR** (v, cm^{-1}): 3744, 1766, 1670, 1499, 1381, 1195, 829, 749. **HRMS** (ESI +ve-TOF) for $[C_{30}H_{23}FN_2O_3+H]^{+.}$ calculated,479.1765 Found: 479.1780.

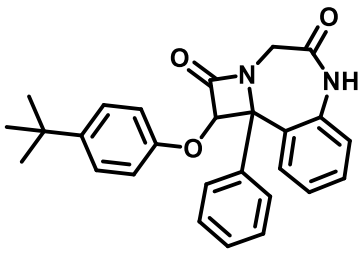
IITK3125 (1-phenoxy-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)



Following the general synthesis protocol, we obtained IITK3125 in 65% yield (128 mg, R_f = 0.3 (40 % EtOAc: Hexane)) as a white amorphous solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.42 – 7.35 (m, 1H), 7.35 – 7.27 (m, 4H), 7.23 (dd, *J* = 12.8, 5.1 Hz, 3H), 7.18 – 7.10 (m, 2H), 7.08 – 6.94 (m, 2H), 6.83 (d, *J* = 8.1 Hz, 2H), 5.75 (s, 1H), 4.52 (d, *J* = 17.0 Hz, 1H), 4.21 (d, *J* = 17.0 Hz, 1H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 100 MHz): δ 167.09, 164.83, 157.20, 135.52, 135.02, 130.40, 129.86, 129.65, 128.96, 128.76, 128.51, 128.02, 125.03, 123.23, 122.86, 117.38, 90.13, 71.05, 44.38. **IR** (v, cm^{-1}): 3216, 1739, 1682, 1587, 1484, 1234, 1111, 744, 687, 522. **HRMS** (ESI +ve-TOF) for $[C_{23}H_{18}N_2O_3+H]^{+.}$ calculated,371.1390 Found: 371.1394

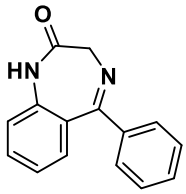
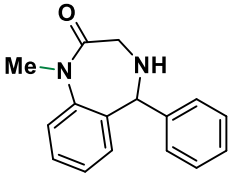
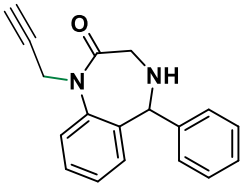
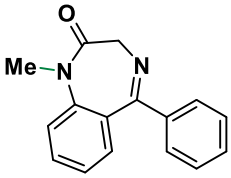
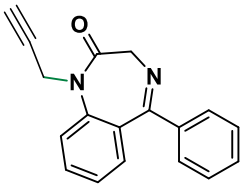
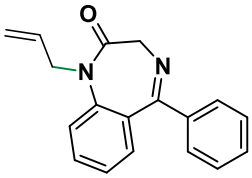
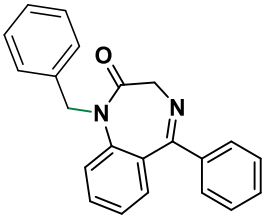
IITK3126 (1-(4-(tert-butyl)phenoxy)-10b-phenyl-6,10b-dihydroazeto[1,2-d]benzo[f][1,4]diazepine-2,5(1H,4H)-dione)

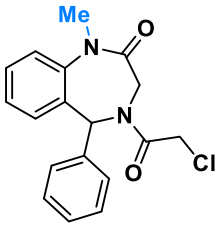
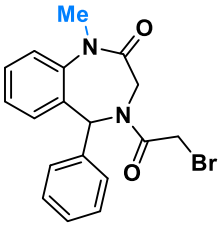
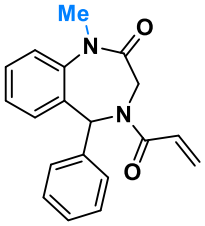
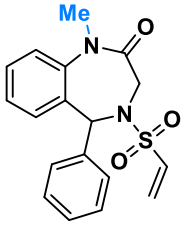
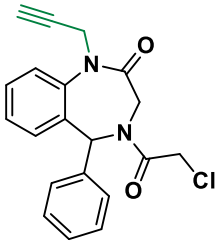
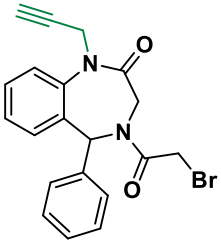
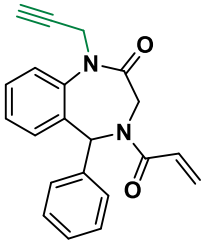


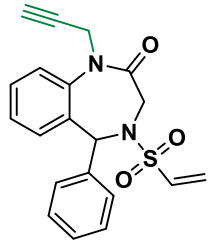
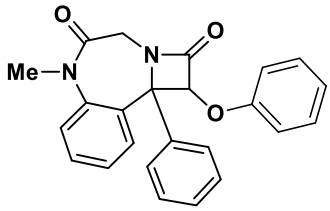
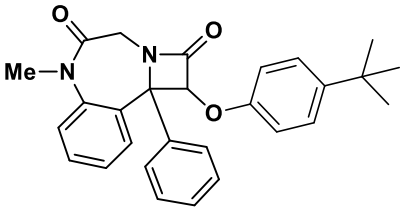
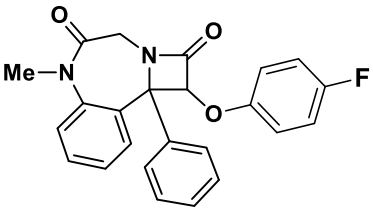
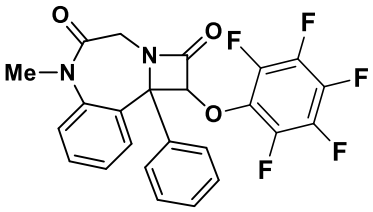
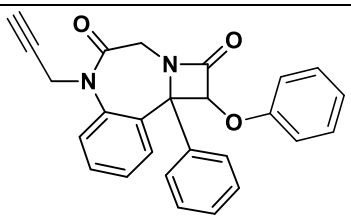
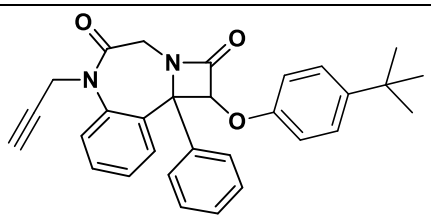
Following the general synthesis protocol, we obtained IITK3126 in 61% yield (138 mg, R_f = 0.3 (40 % EtOAc: Hexane)) as a white amorphous solid.

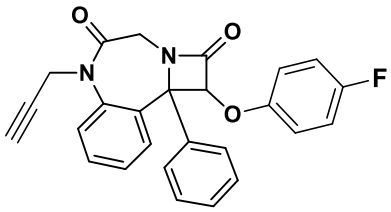
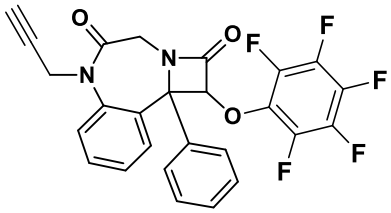
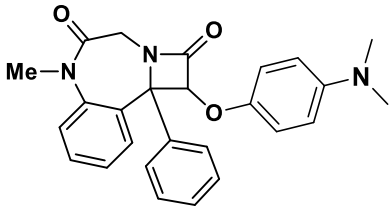
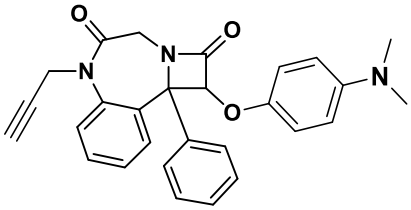
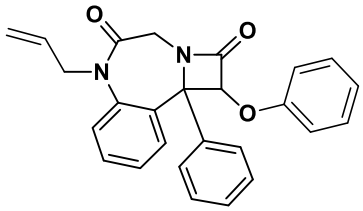
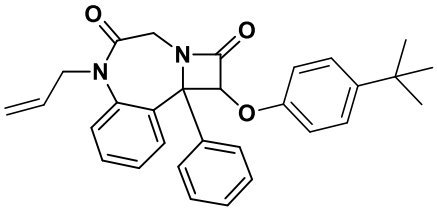
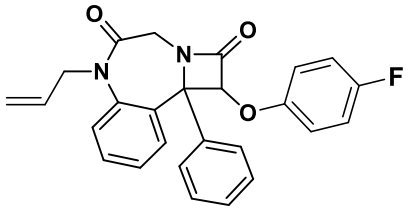
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.40 – 7.36 (m, 1H), 7.35 – 7.31 (m, 3H), 7.31 – 7.29 (m, 1H), 7.25 – 7.21 (m, 3H), 7.16 (dd, *J* = 6.7, 3.0 Hz, 2H), 7.02 (d, *J* = 7.6 Hz. 1H), 6.81 – 6.75 (m, 2H), 5.71 (s, 1H), 4.53 (d, *J* = 17.0 Hz, 1H), 4.21 (d, *J* = 17.0 Hz, 1H), 1.27 (s, 9H). **^{13}C{^{1}H} NMR** ($CDCl_3$, 125 MHz): δ 165.39, 163.83, 159.47, 153.27, 141.63, 137.43, 133.32, 130.31, 128.29, 127.59, 126.59, 126.43, 126.37, 118.39, 118.32, 116.08, 115.90, 86.64, 70.96, 68.11, 45.43, 36.57. **IR** (v, cm^{-1}): 2954, 1760, 1663, 1499, 1442, 1378, 1234, 951, 754, 690, 522. **HRMS** (ESI +ve-TOF) for $[C_{27}H_{26}N_2O_3+H]^{+.}$ calculated,427.2016 Found: 427.2020

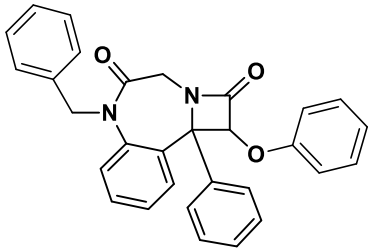
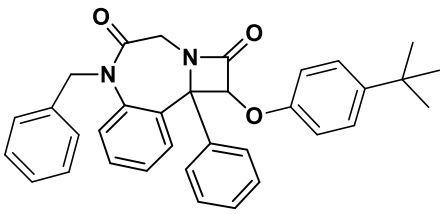
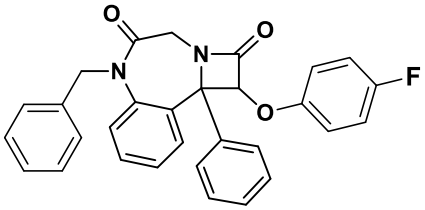
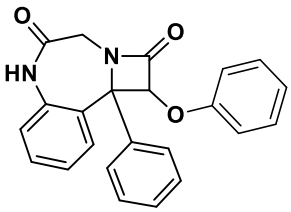
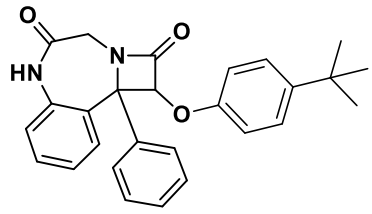
Table S7: List of molecules, their structure, code, yield and their EC_{50} in Huh-7 cells.

S. No	Structure	Code	Yield	Huh-7, EC_{50} in µM
1		1	70	>100
2		2	Quantitative	>100
3		3	Quantitative	>100
4		4	50	>100
5		5	86	>100
6		6	68	>100
7		7	40	>100

8		IITK3101	98	0.36
9		IITK3102	Quantitative	0.30
10		IITK3103	Quantitative	25.0
11		IITK3104	53	>50
12		IITK3105	85	0.132
13		IITK3106	65	0.167
14		IITK3107	89	17.0

15		IITK3108	58	>50
16		IITK3109	65	>50
17		IITK3110	61	30.9
18		IITK3111	90	23.3
19		IITK3112	53	13.4
20		IITK3113	81	>100
21		IITK3114	92	40.4

22		IITK3115	64	11.9
22		IITK3116	44	18.3
23		IITK3117	59	26.3
24		IITK3118	40	>100
25		IITK3119	90	>100
26		IITK3120	70	>100
27		IITK3121	68	>100

28		IITK3122	89	>50
29		IITK3123	60	>100
30		IITK3124	89	>50
31		IITK3125	65	>50
32		IITK3126	61	>100

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