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Supporting Information

# Chemoproteomik-basierte Identifikation von 4-Oxo-β-lactamen als Inhibitoren der Dipeptidylpeptidasen 8 und 9

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Supporting Information

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# Chemoproteomics-enabled Identification of 4-Oxo-β-Lactams as Inhibitors of Dipeptidyl Peptidases 8 and 9

Luís A. R. Carvalho,[+] Breyan Ross,[+] Lorenz Fehr,[+] Oguz Bolgi, Svenja Wöhrle, Kenneth M. Lum, David Podlesainski, Andreia C. Vieira, Reiner Kiefersauer, Rita Félix, Tiago Rodrigues, Susana D. Lucas, Olaf Groß, Ruth Geiss-Friedlander, Benjamin F. Cravatt, Robert Huber, Markus Kaiser,* Rui Moreira*

**Abstract:** Dipeptidyl peptidases 8 and 9 (DPP8/9) have gathered increasing interest as drug targets due to their important roles in diverse biological processes including regulation of immune responses and tumorigenesis. Elucidation of their distinct individual functions remains an ongoing task and could benefit from the availability of novel, chemically diverse and selective chemical tools. So far, all developed DPP8/9 inhibitors are substrate analogues targeting the S1-S2 subsites with limited selectivity. Here, we report the activity-based protein profiling (ABPP)-mediated discovery of 4-oxo-β-lactams as potent, non-substrate-like nanomolar DPP8/9 inhibitors. X-ray crystallographic structures revealed different ligand binding modes for DPP8 and DPP9, including an unprecedented targeting of an extended S2' (eS2') subsite in DPP8. Biological assays confirmed inhibition at both target and cellular levels. Altogether, our integrated chemical proteomics and structure-guided small molecule design approach led to novel DPP8/9 inhibitors that explore previously unaccessed inhibition mechanisms, delivering the highest selectivity index reported to date.Insert abstract text here.

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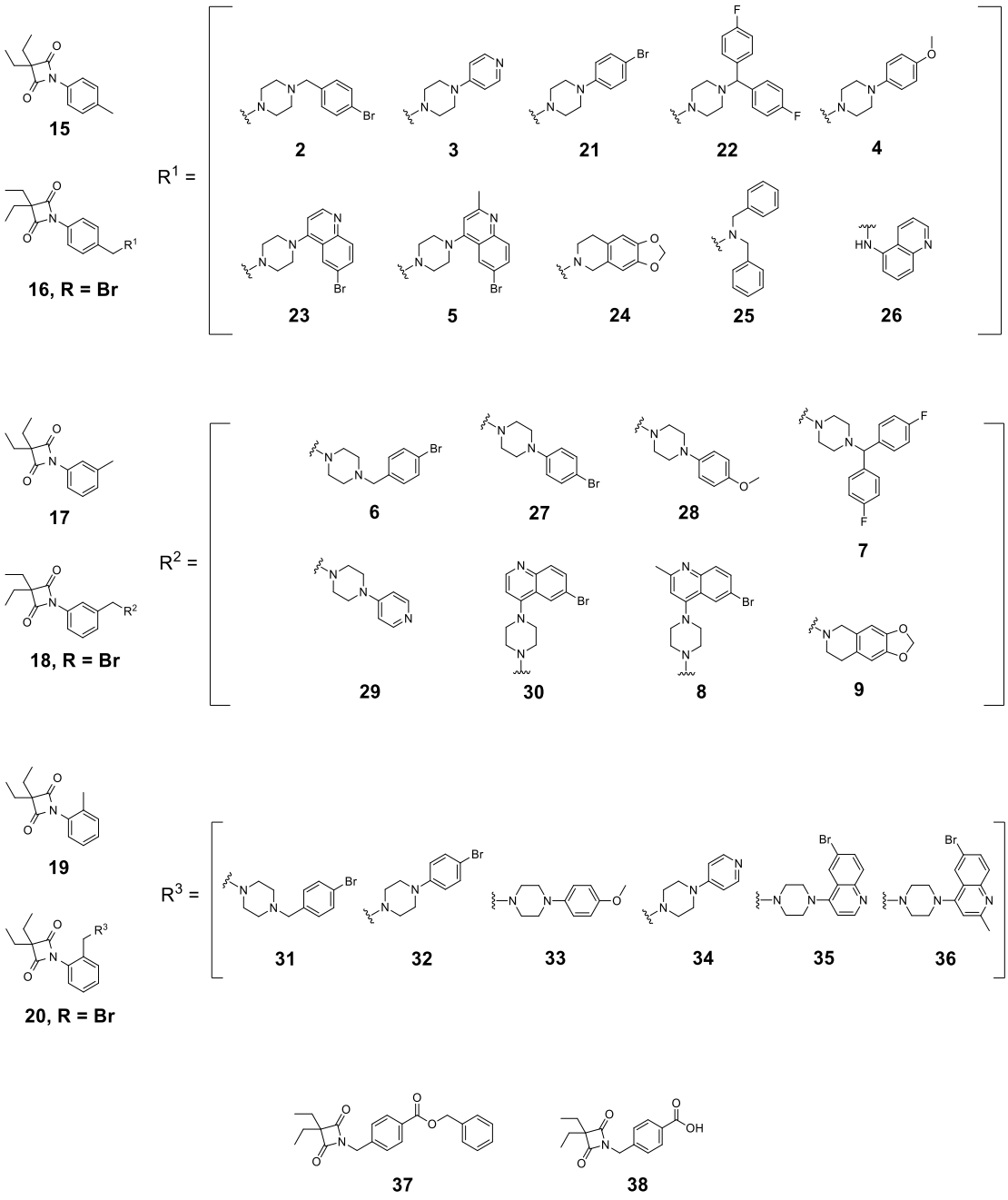
# 1. Supplementary Tables and Figures

## 1.1. Library of compounds studied by ABPP:




**Scheme S1.1.** Library of 4-Oxo-β-Lactams tested in ABPP.

## 1.2. Full Competitive ABPP Gels

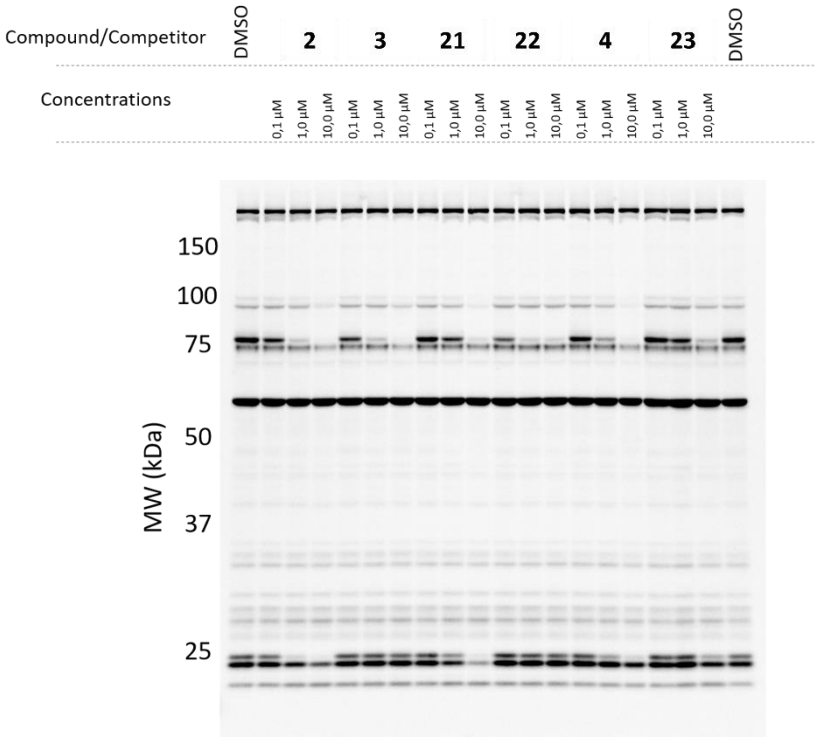



**Figure S1.2.1.** Competitive ABPP; U937 soluble fraction; compounds **2-4**, **21-23**.

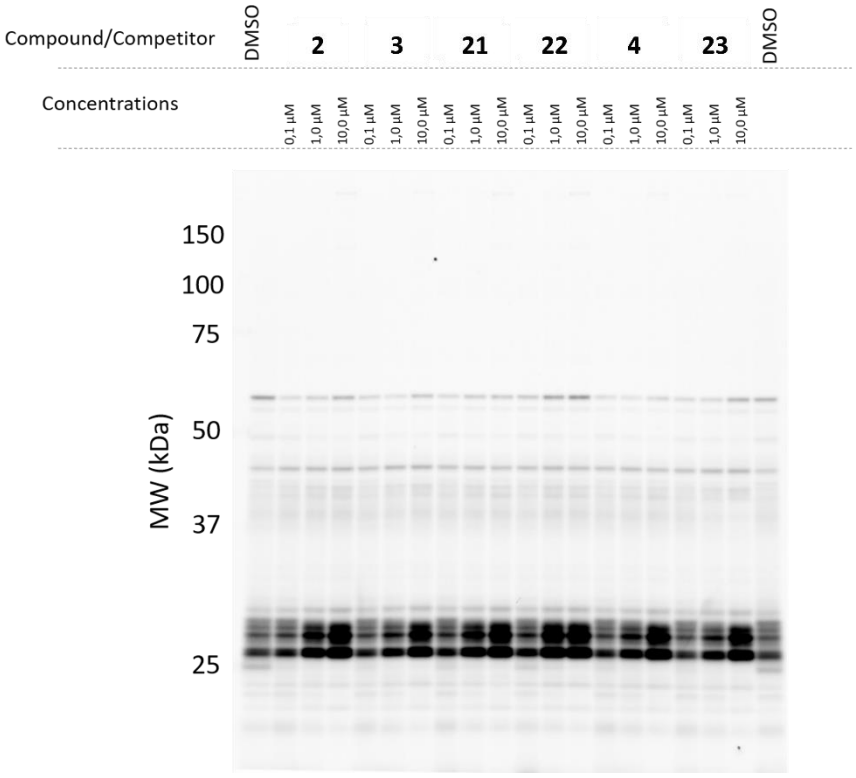



**Figure S1.2.2.** Competitive ABPP; U937 membrane fraction; compounds **2-4**, **21-23**.

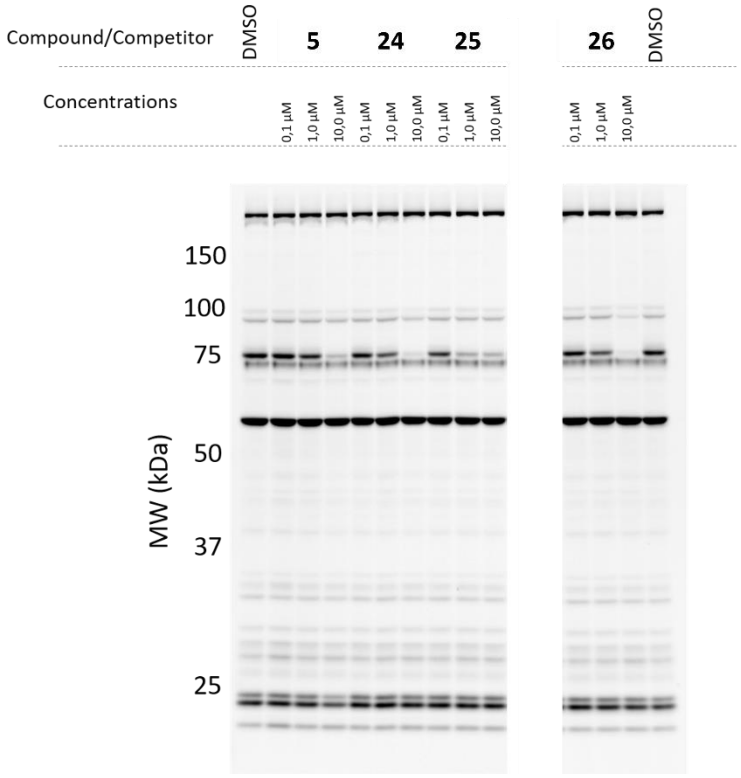



**Figure S1.2.3.** Competitive ABPP; U937 soluble fraction; compounds **5, 24-26**.

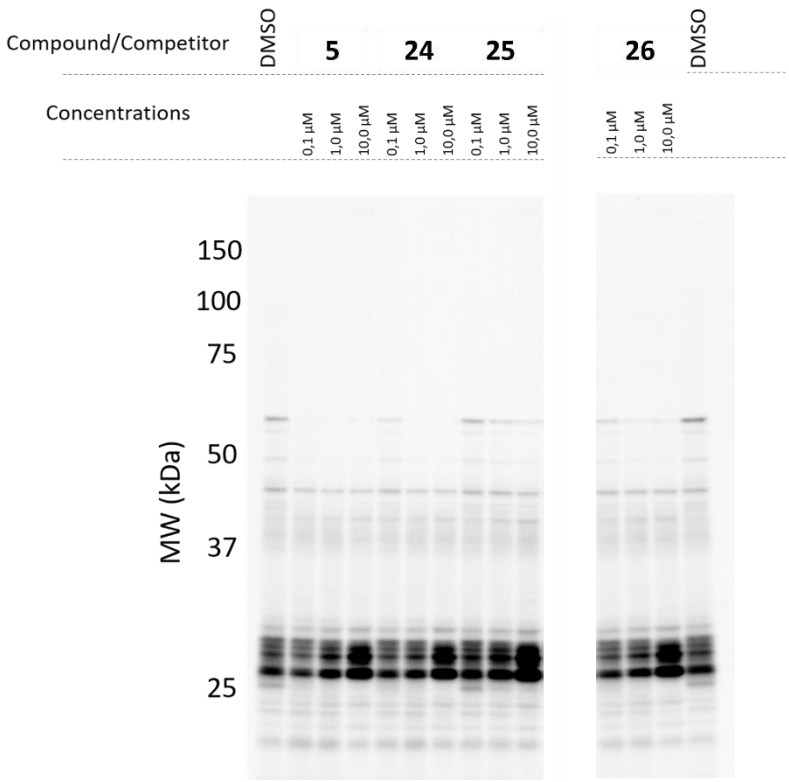



**Figure S1.2.4.** Competitive ABPP; U937 membrane fraction; compounds **5, 24-26**.

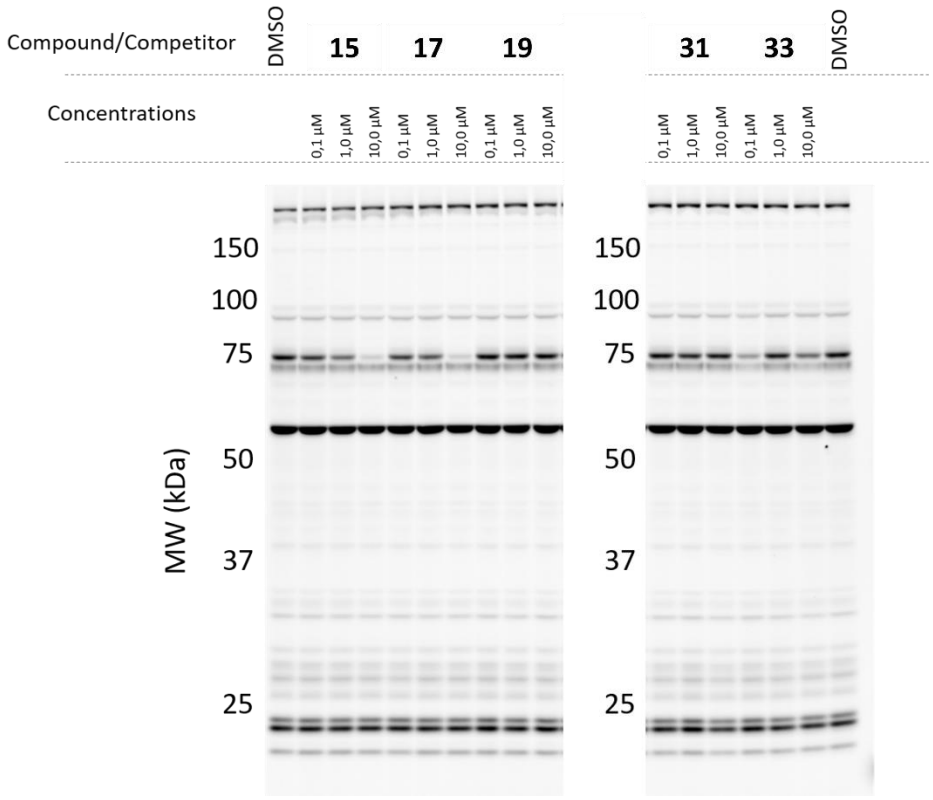



**Figure S1.2.5.** Competitive ABPP; U937 soluble fraction; compounds **15, 17, 19, 31, 33**.

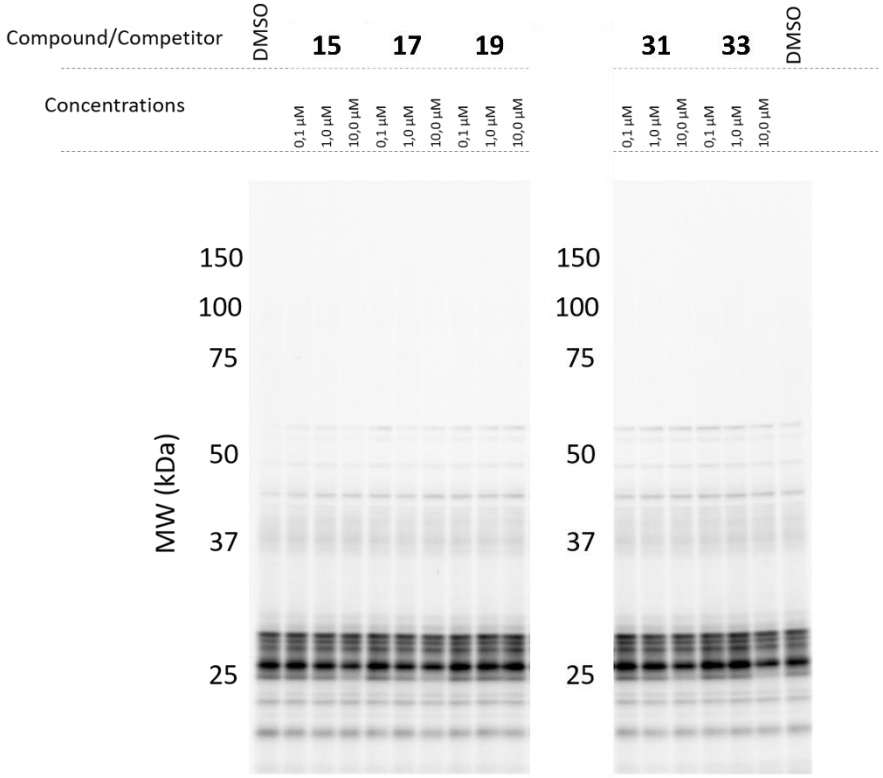



**Figure S1.2.6.** Competitive ABPP; U937 membrane fraction; compounds **15, 17, 19, 31, 33**.

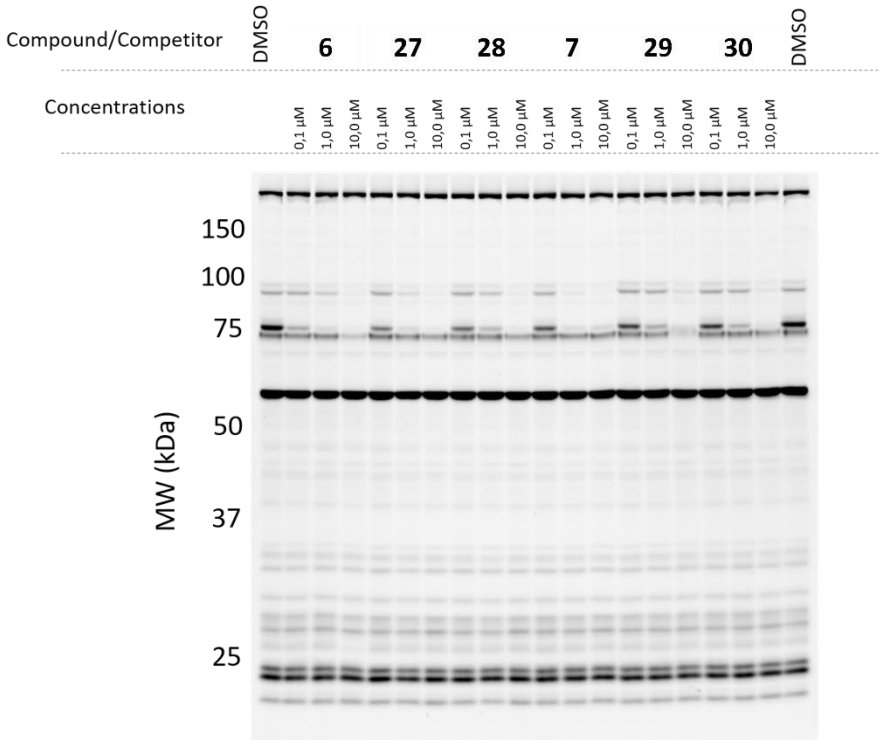



**Figure S1.2.7.** Competitive ABPP; U937 soluble fraction; compounds **6, 7, 27-30**.

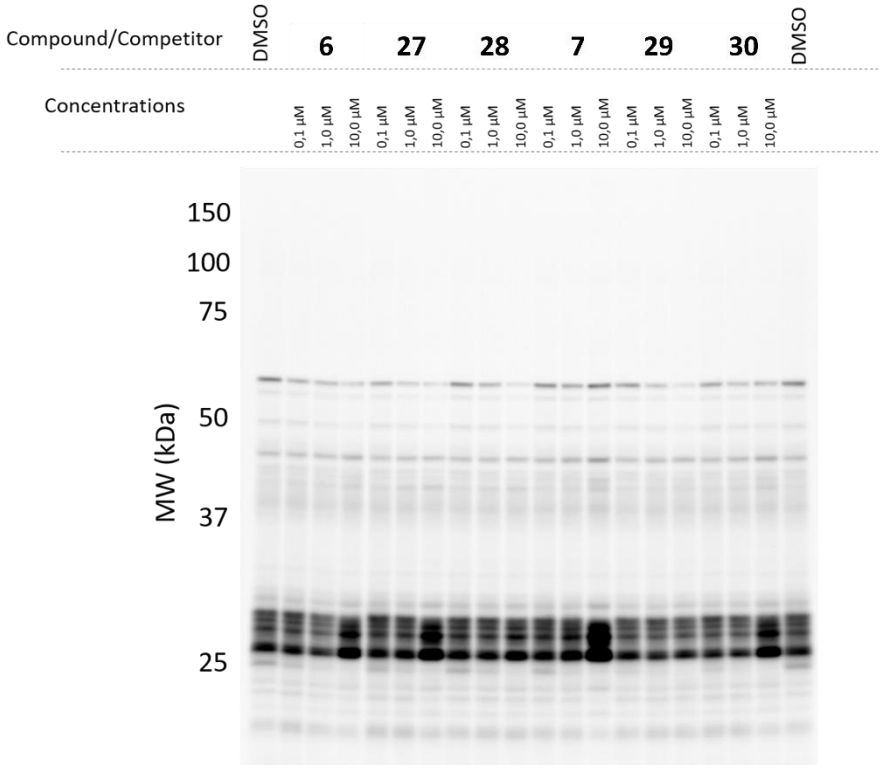



**Figure S1.2.8.** Competitive ABPP; U937 membrane fraction; compounds **6, 7, 27-30**.

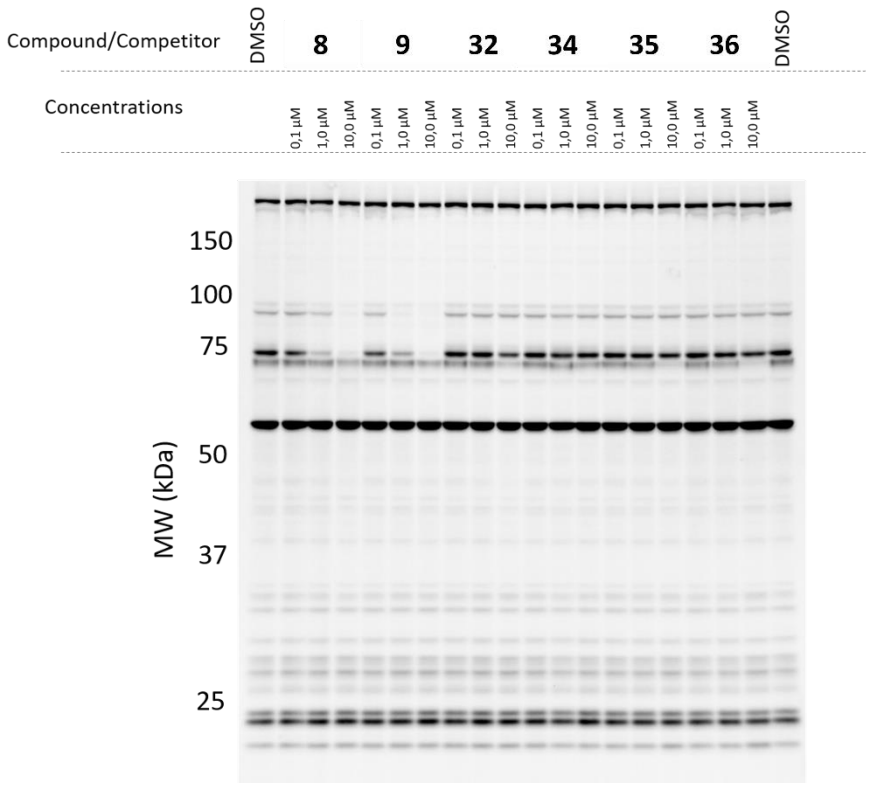



**Figure S1.2.9.** Competitive ABPP; U937 soluble fraction; compounds **8**, **9**, **32**, **34-36**.

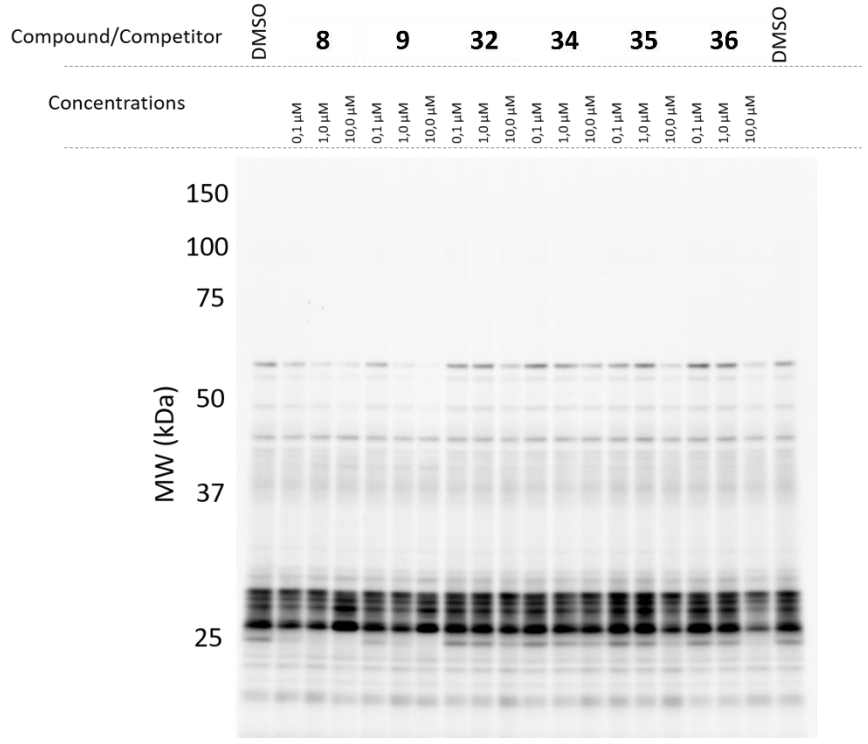



**Figure S1.2.10.** Competitive ABPP; U937 membrane fraction; compounds **8**, **9**, **32**, **34-36**.

## 1.3. Target Validation by Competitive ABPP

### 1.3.1. DPP8

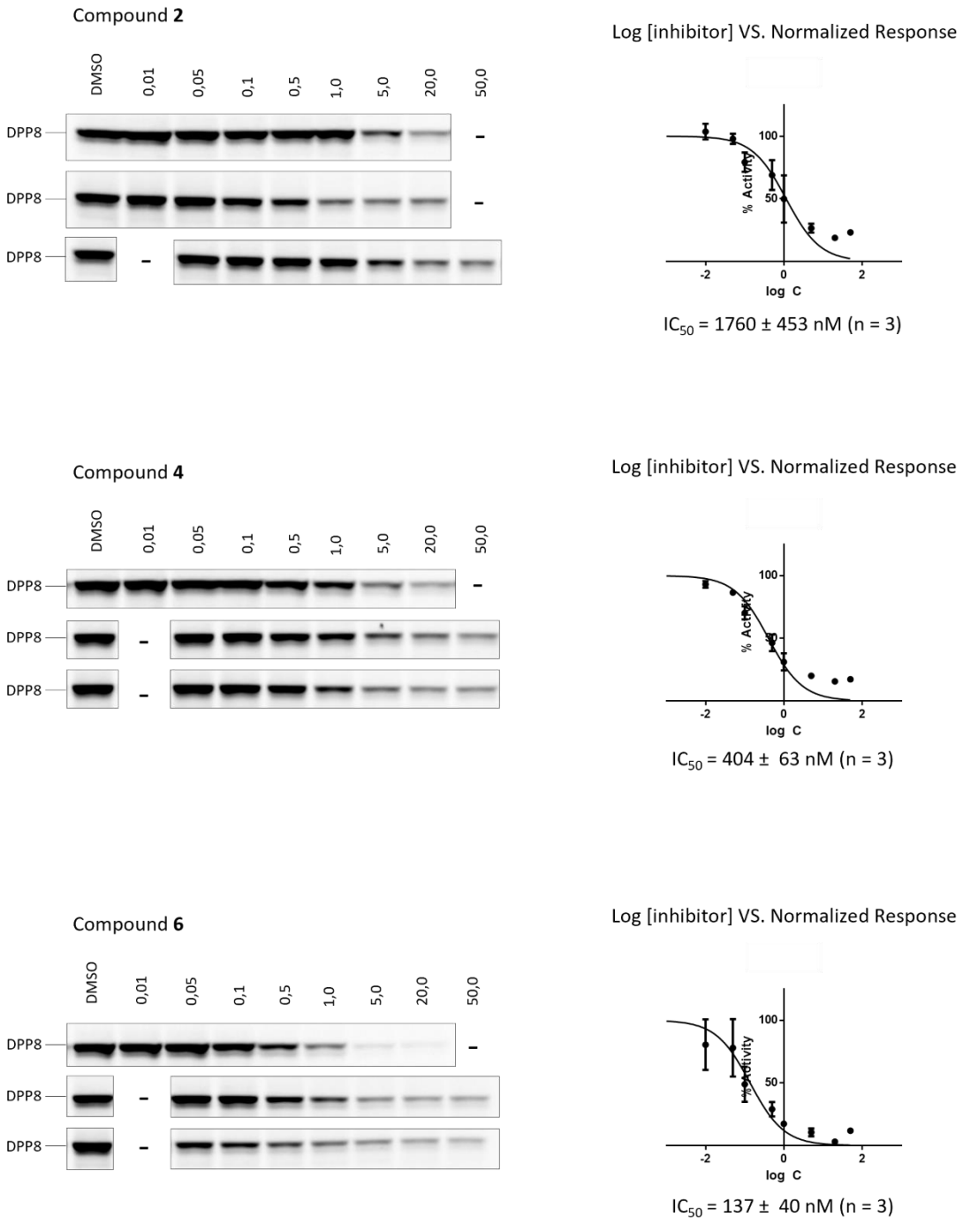



**Figure S1.3.1.1.** DPP8 $IC_{50}$ determination by competitive ABPP for compounds **2**, **4, 6**.

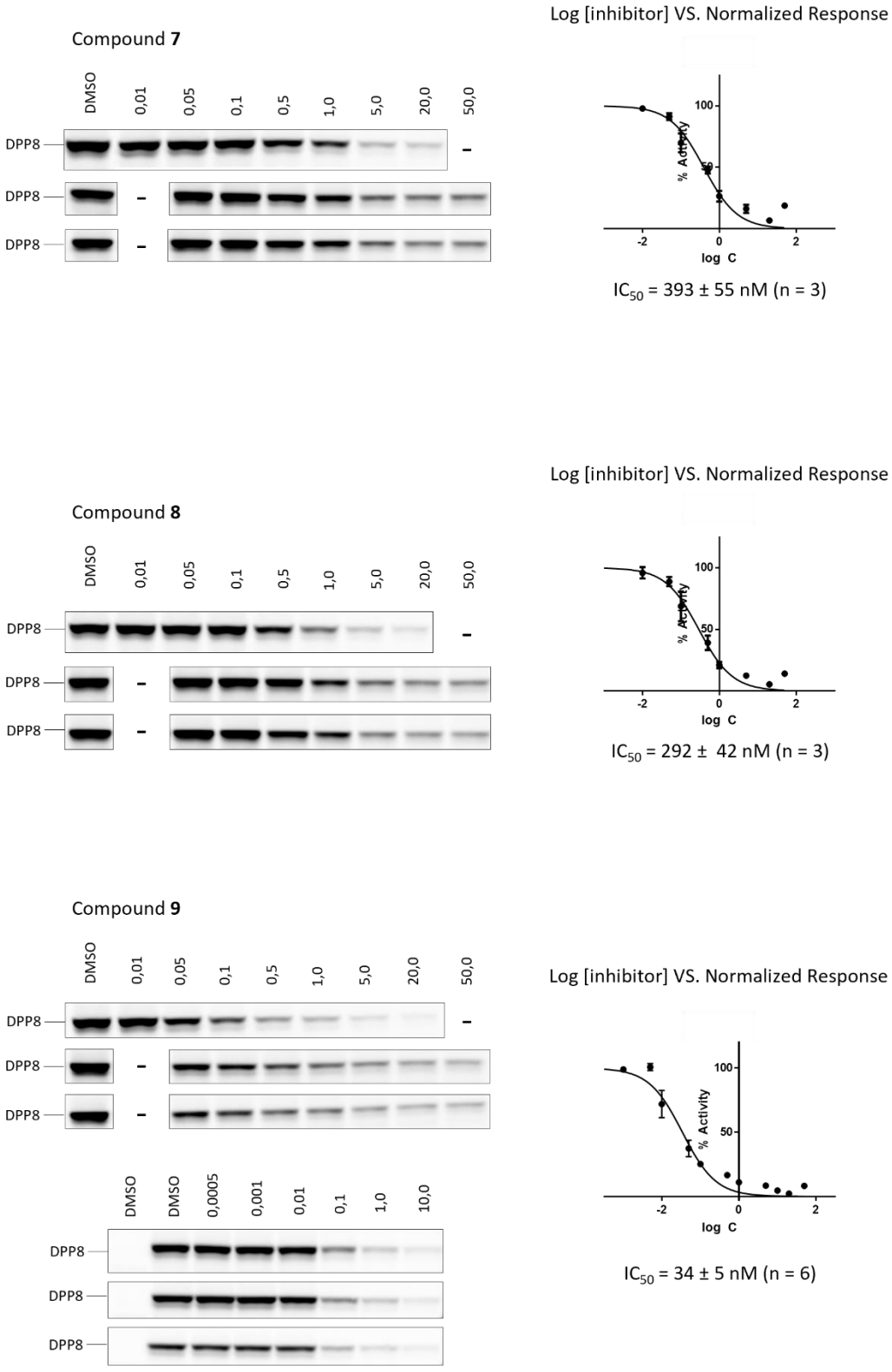



**Figure S1.3.1.2.** DPP8 $IC_{50}$ determination by competitive ABPP for compounds **7**, **8, 9**.

### 1.3.2. DPP9

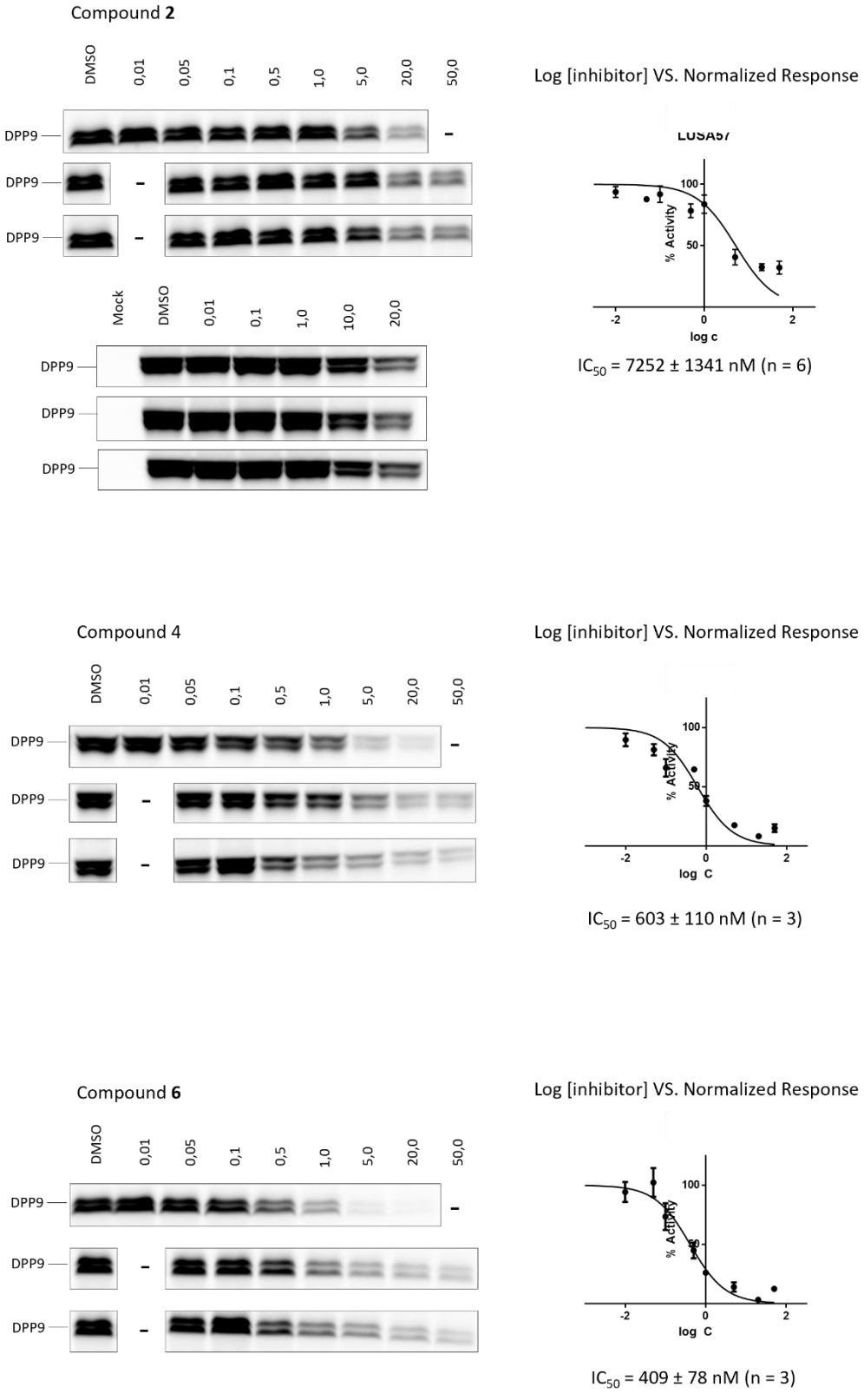



**Figure S1.3.2.1.** DPP9 $IC_{50}$ determination by competitive ABPP for compounds **2**, **4, 6**.

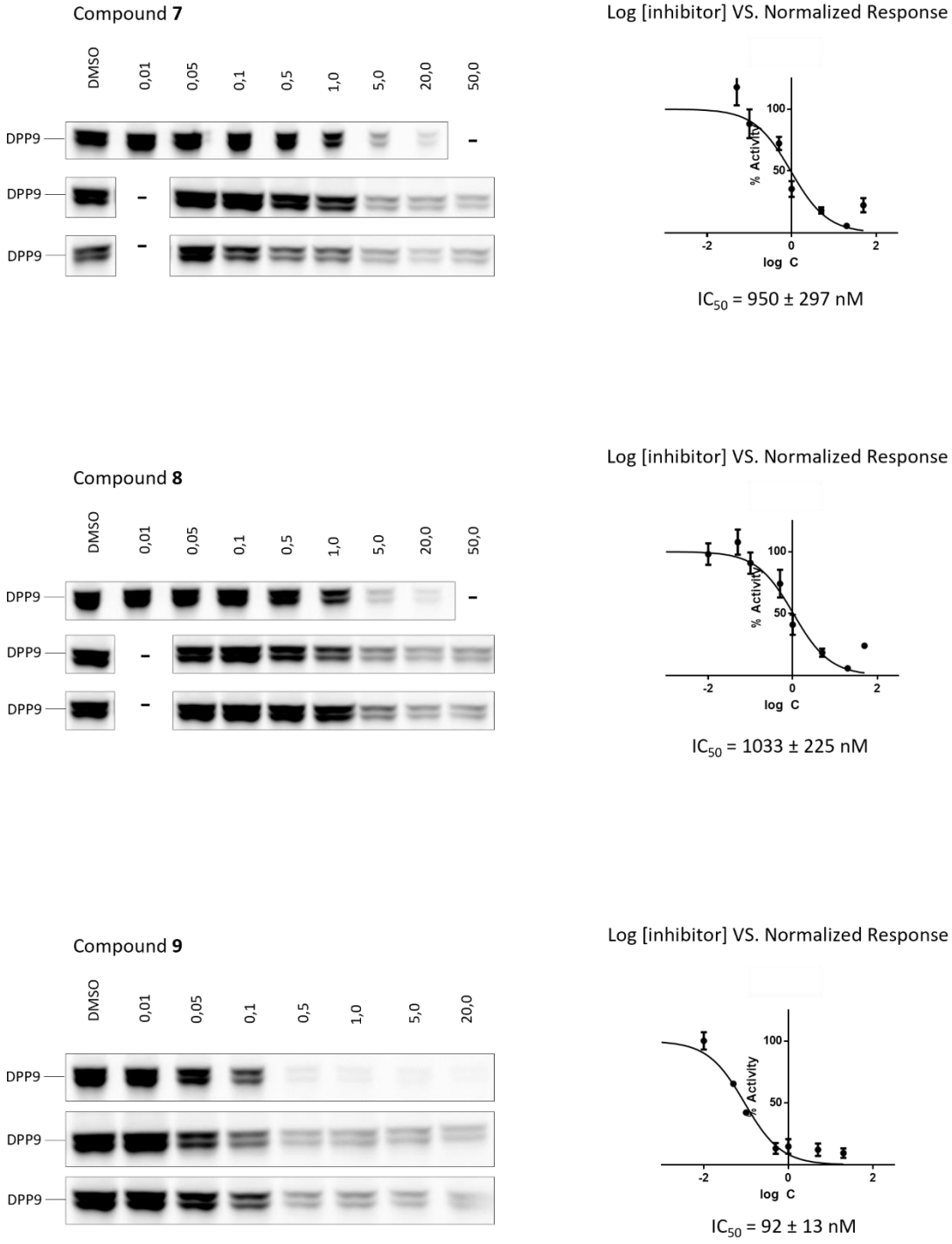



**Figure S1.3.2.2.** DPP9 $IC_{50}$ determination by competitive ABPP for compounds **7**, **8, 9**.

### 1.3.3. DPP4

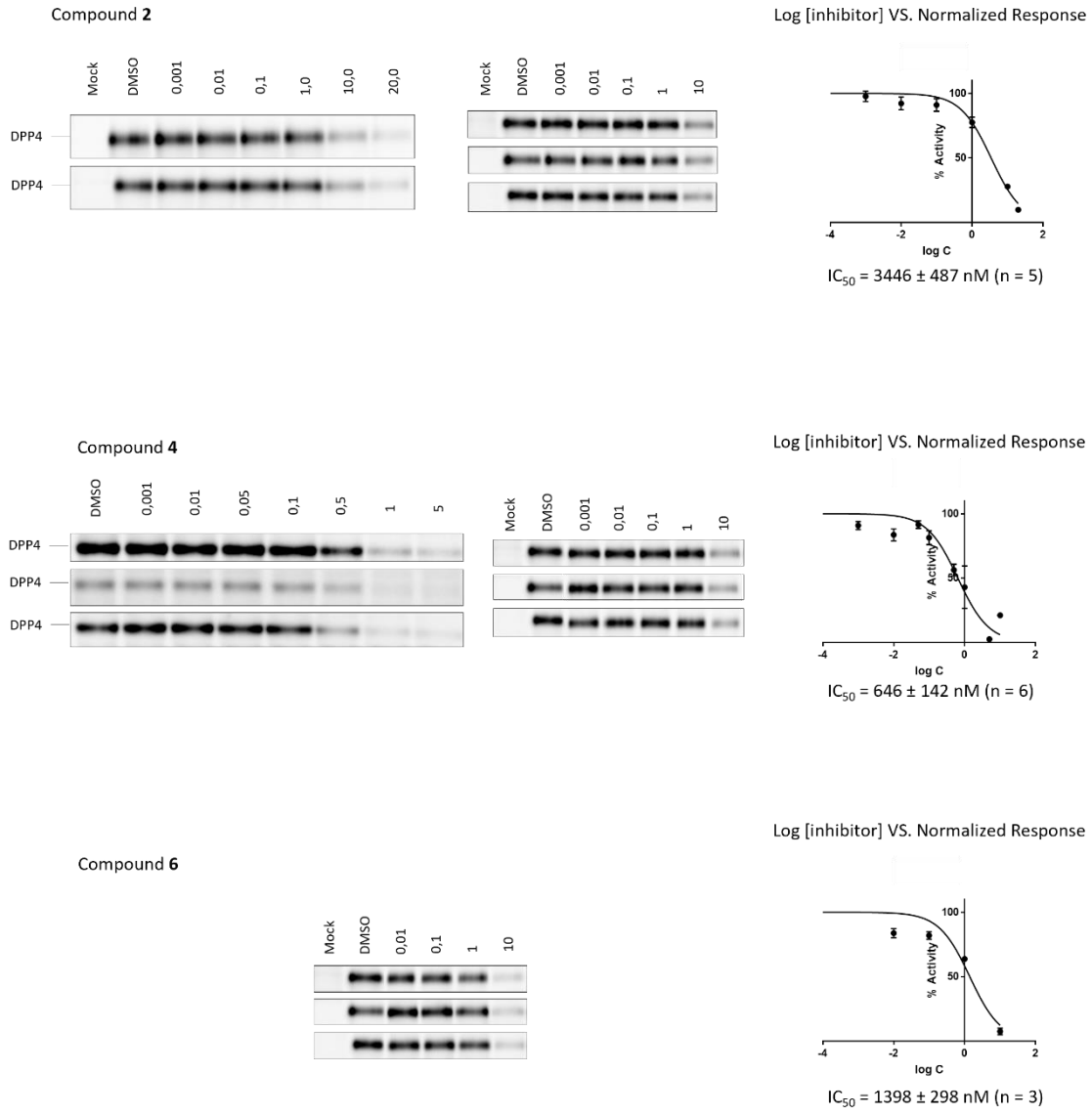
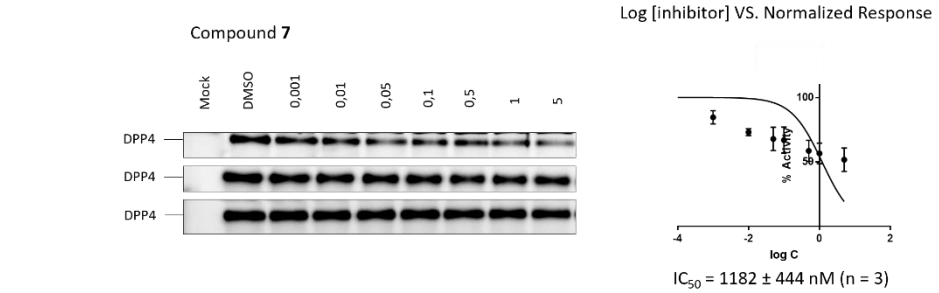
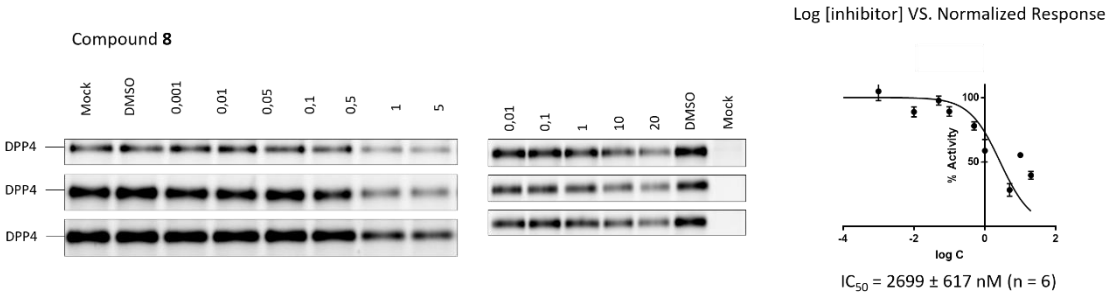



**Figure S1.3.3.1.** DPP4 $IC_{50}$ determination by competitive ABPP for compounds **2**, **4, 6**.



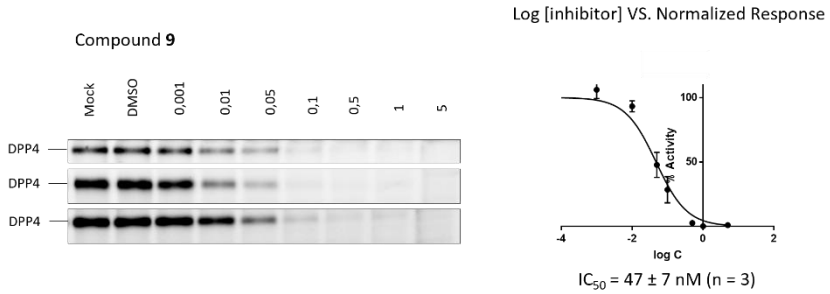




**Figure S1.3.3.2.** DPP4 $IC_{50}$ determination by competitive ABPP for compounds **7**, **8, 9**.

### 1.3.4. DPP7

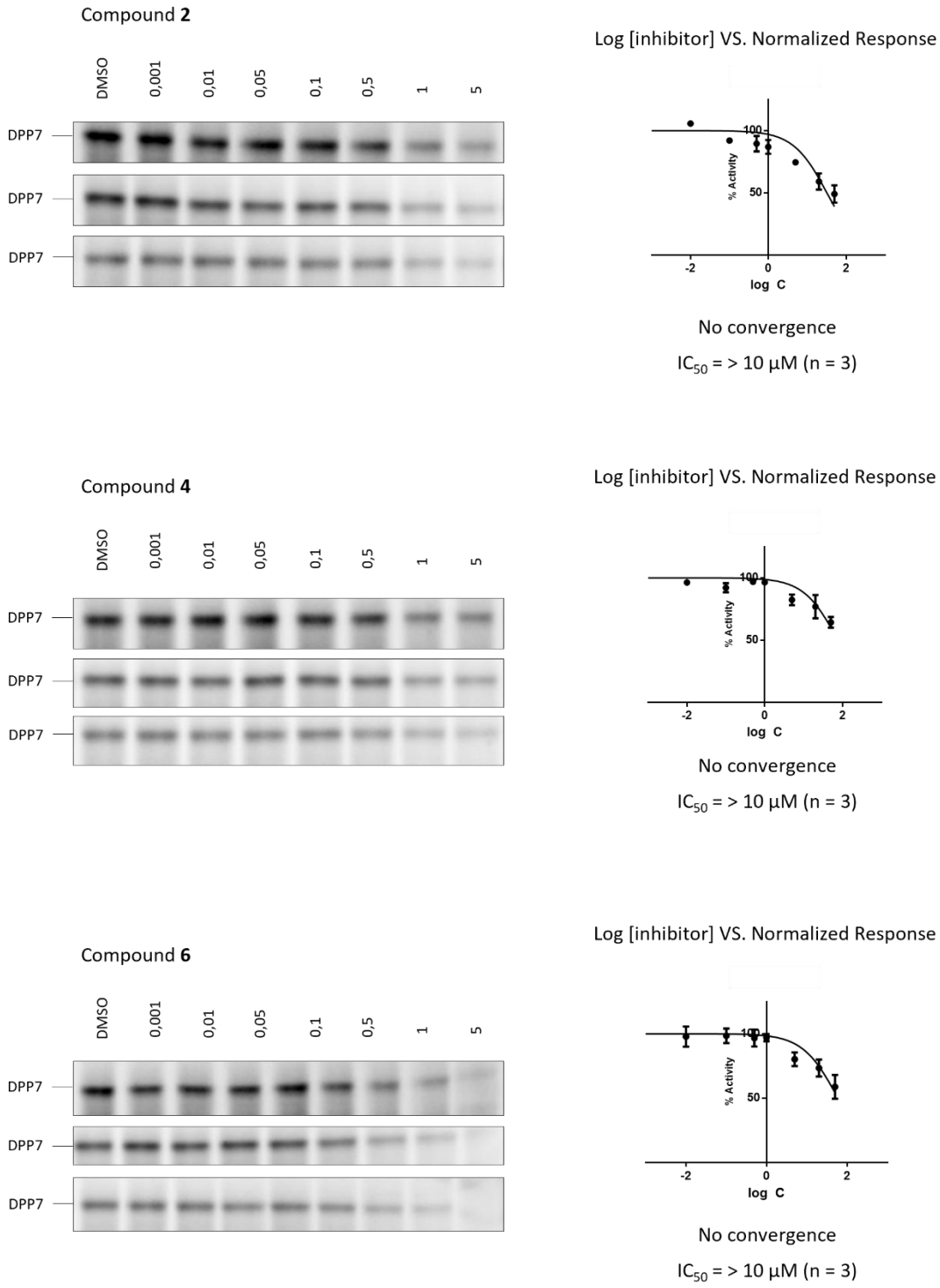



**Figure S1.3.4.1.** DPP7 $IC_{50}$ determination by competitive ABPP for compounds **2**, **4, 6**.

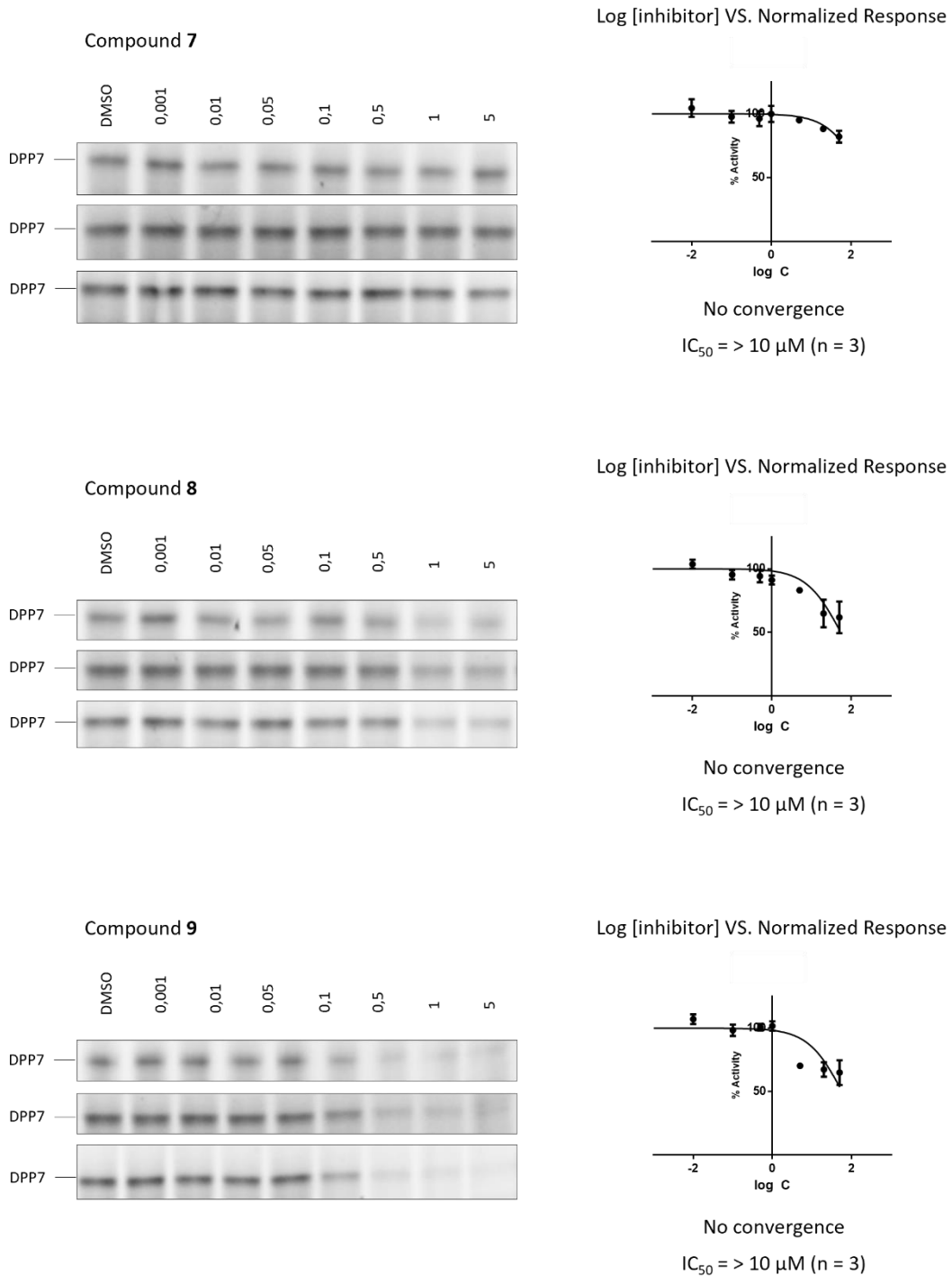



**Figure S1.3.4.2.** DPP7 $IC_{50}$ determination by competitive ABPP for compounds **7**, **8, 9**.

## 1.4. Machine Learning

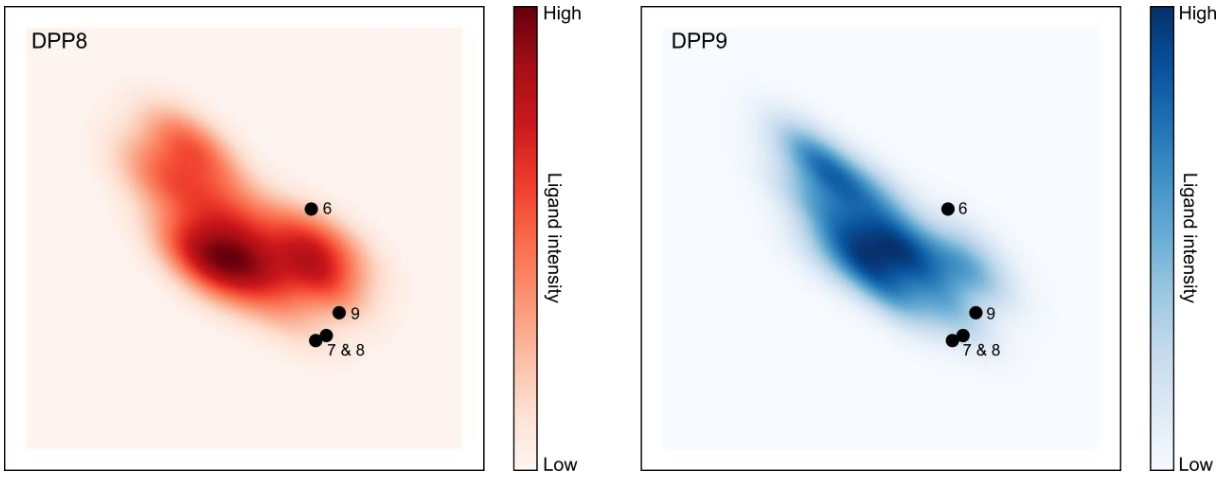
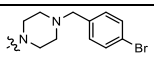
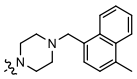
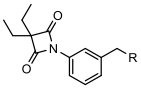
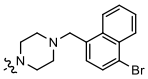
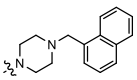
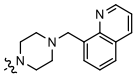
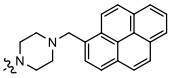
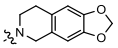
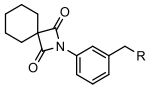
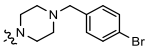
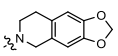
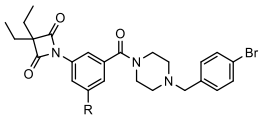
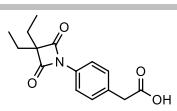
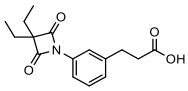
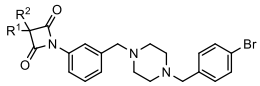



**Figure S1.4.** A Machine learning (t-distributed stochastic neighborhood embedding)-based approach with topological pharmacophore descriptors (CATS2) shows an unprecedented pattern within the DPP8/9 ligand space, as compounds **6-9** do not co-cluster with known inhibitors. 4-oxo-β-lactams thus represent an extension of the so far explored DPP8 and DPP9 inhibitor space.

## 1.5. Inhibition profiles of additional 4-Oxo-β-Lactams

**Table S1.5.** Inhibition Profile of 4-Oxo-β-Lactams (extended table 2).

| # | | R | % residual activity at 5 µM compound concentration | | Ki' [nM] | | DPP8/DPP9 Selectivity |
|---|---|---|---|---|---|---|---|
| | | | DPP8 | DPP9 | DPP8 | DPP9 | |
| **6** | |  | 10 | 61 | 26.3 ± 2.6 | 184 ± 38.4 | 7x |
| **11** | |  | 81 | 90 | 528 ± 175.5 | > 2000 | > 4x |
| **17** | | H | > 90 | > 90 | - | - | - |
| **39** | |  | 12 | 54 | 306 ± 52.1 | - | - |
| **40** |  |  | 7.3 | 79 | 159 ± 14.2 | > 2000 | > 13x |
| **12** | |  | < 5 | 45 | 95.0 ± 15.5 | > 2000 | > 21x |
| **13** | |  | 16 | 39 | 174.1 ± 55.3 | 34.2 ± 30 | 0.2x |
| **14** | |  | > 90 | > 90 | - | - | - |
| **9** | |  | 12 | 31 | 2.7 ± 0.6 | 11.4 ± 3.5 | 4x |
| **41** |  |  | 7.9 | 36 | 17.7 ± 1.4 | 44 ± 11.5 | 3x |
| **42** | |  | 69 | 74 | - | - | - |
| **43** | | H | < 5 | 14 | 9.5 ± 1 | 95 ± 11.9 | 10x |
| **44** |  | OH | < 5 | 6.8 | 7 ± 0.9 | 77.8 ± 7.4 | 11x |
| **45** | | COOMe | 26 | 25 | 30.0 ± 10.4 | 148 ± 23.1 | 5x |
| **46** | | OTBDMS | > 90 | > 90 | - | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **47** |  | | 23 | 22 | 440 ± 71.7 | 919 ± 190 | 2x |
| **48** |  | | 20 | 17 | 316 ± 63.6 | 345 ± 54 | - |
| **49** | | $R^1$ = Me<br>$R^2$ = Et | 62 | 70 | - | - | - |
| **50** | | $R^1$ = Me<br>$R^2$ = Pr | 9.5 | 52 | 315 ± 76.2 | > 2000 | > 6x |
| **51** |  | $R^1$ = Et<br>$R^2$ = Pr | < 5 | 16 | 74.9 ± 18.8 | 1257 ± 203 | 17x |
| **52** | | $R^1$ = Et<br>$R^2$ = Bu | 19 | > 90 | 1313 ± 122 | - | - |
| **53** | | $R^1$ = Pr<br>$R^2$ = Pr | 62 | 67 | - | - | - |
| **54** | | $R^1$ = Me<br>$R^2$ = Bn | < 5 | 13 | 23.3 ± 2 | 202 ± 66.5 | 9x |

## 1.6. Crystal structures of 43 and 45 in complex with DPP8.

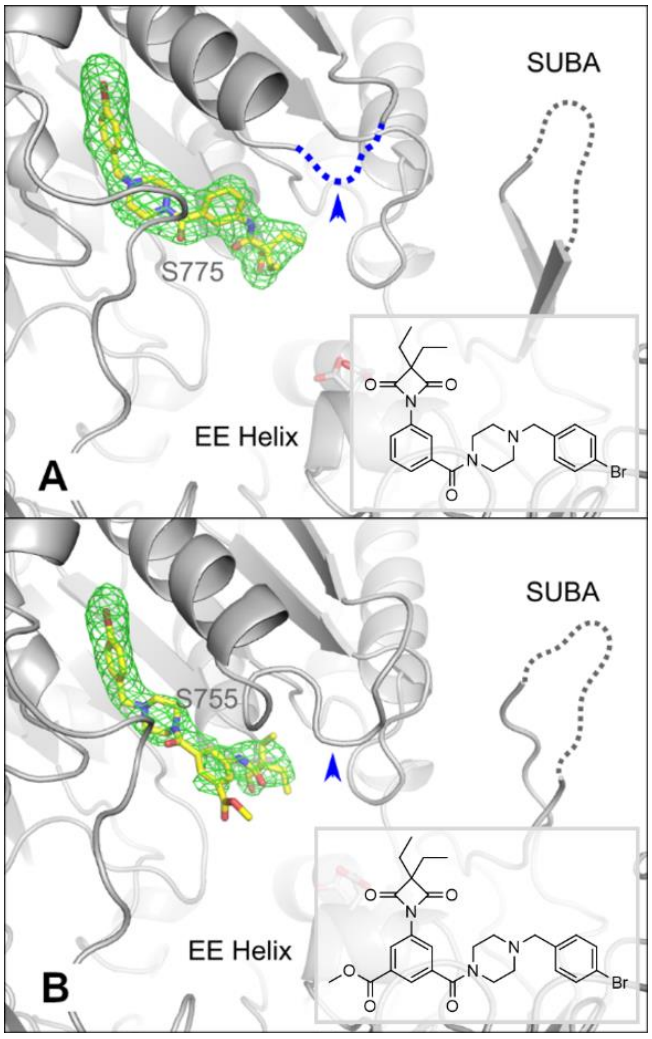



**Figure S.1.6.** X-ray analysis of **43** (PDB ID: 7AYQ) and **45** (PDB ID: 7OR4) in complex with DPP8. A) **43** binds to DPP8 in a similar binding mode as **6**, including the disordering of the H864 segment (blue arrow) and of the SUBA domain. The increase in binding affinity therefore seems to stem from a structural preorganization of the inhibitor structure. B) DPP8-binding by **45** however results in a different binding mode induced by a rotation of the N-phenyl moiety. This rotation of the central unit is associated with an ordering of the H864 segment (blue arrow).

## 1.7. Inflammasome activation

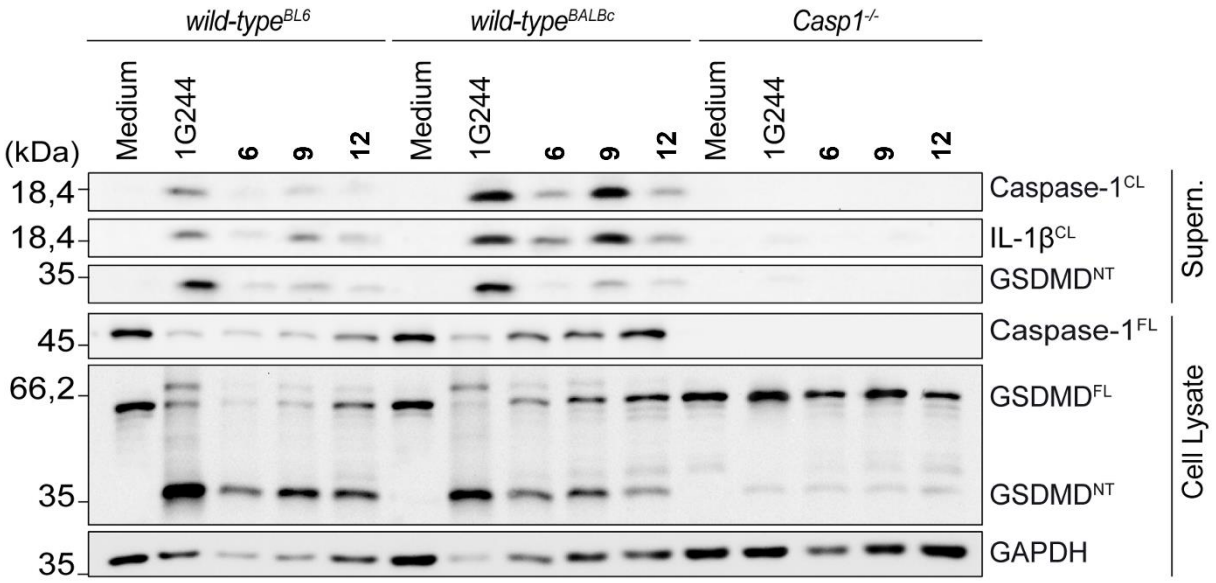



**Figure S1.7.** Inflammasome activation is accompanied by cleavage of the inflammasome effector protease caspase-1 and its pore-forming substrate gasdermin D. Wild-type C57bl/6 and Balb/c and Caspase 1-deficient BMDCs were primed with LPS and then stimulated with 5 µM nigericin, 10 µM VbP, 10 µM 1G244 or 100 µM of compound **6**, **9** or **12** for 16 h. The corresponding cell lysates and supernatants were subsequently analyzed by immunoblotting.

# 2. Chemical synthesis

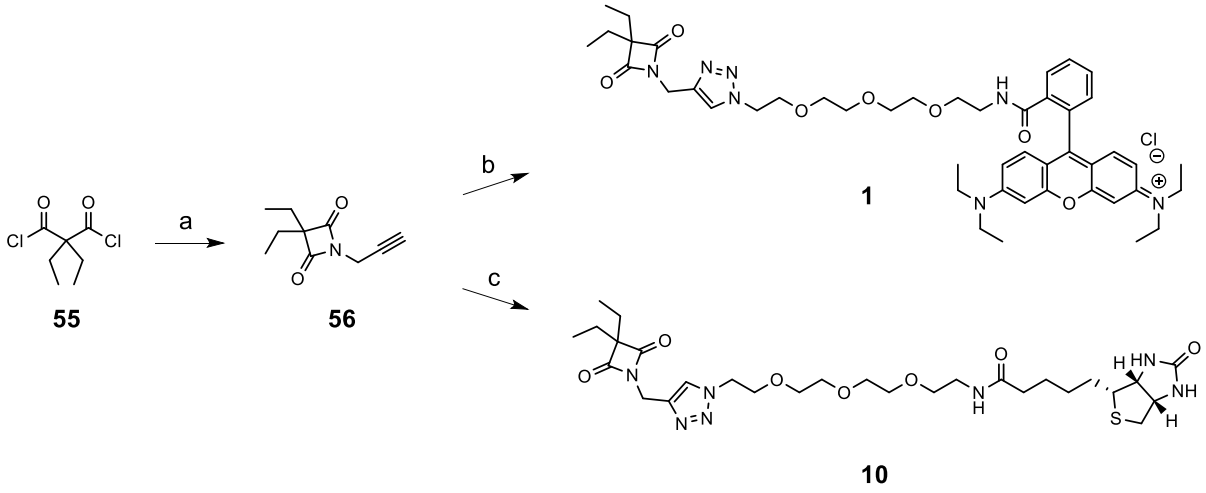
## 2.1. General Information

All reactions were carried out under an inert atmosphere of argon with dry solvents under anhydrous conditions unless otherwise noted.
All reagents were purchased from *Merk*, *abcr*, *Fluorochem*, *Alpha Aesar*, *Acros Organics*, *TCI Chemicals* or *Oakwood Chemicals* and were used without further purification. Culture media and supplements were purchased from *Life Technologies*.
Reactions were monitored by thin-layer chromatography (TLC) carried out on silica coated aluminium plates (60 $F_{254}$) from *Merck* using UV light (wavelength 254 nm or 366 nm) for visualization or a $KMnO_4$ solution and heat as developing agents.
Silica gel (particle size 35 – 70 µm, from *Acros Organics*) was used for compound purification by flash column chromatography. When indicated, flash chromatography was performed in a Combi Flash RF-200 instrument from Teledyne Isco with RediSep normal-phase silica flash columns.
Purification by preparative high performance liquid chromatography (prep. HPLC) was performed on a Prominence UFLC system from *Shimadzu* (peak detection at 210 nm and 254 nm). The system was equipped with a C18 column from *Phenomenex* (*Luna* 5 µm C18(2), 100 x 21.20 mm) and a linear gradient of solvent B (0.1% TFA in ACN) in solvent A (0.1% TFA in $H_2O$) with a flow rate of 20 mL/min was applied.
Freeze-drying of the products was carried out with a lyophilizer (ALPHA 2-4 LD plus) from *CHRIST*.
Liquid chromatography mass spectrometry (LC-MS) analysis was performed on a *Thermo Fisher Scientific* UltiMate 3000 (peak detection at 230 nm and 260 nm) equipped with an Eclipse XDB-C18 column (particle size 5 µm, from *Agilent*) connected to a *Thermo Fisher Scientific* LCQ Fleet electrospray ionization mass spectrometer (ESI-MS) and a linear gradient of solvent B (0.1% FA in ACN) in solvent A (0.1% FA in $H_2O$) with a flow rate of 1 mL/min was used.
High-resolution mass spectrometry (HRMS) spectra were recorded on an Exactive Plus EMR mass spectrometer from *Thermo Fisher Scientific* with a TriVersa NanoMate ESI system from *Advion* or on a *Bruker* maXis II ETD TOF-MS spectrometer.
Nuclear magnetic resonance (NMR) spectra were recorded on a *Bruker* Avance 300 (300 MHz for $^1H$ and 75 MHz for $^{13}C$ NMR), *Bruker* Avance II 400 (400 MHz for $^1H$ and 101 MHz for $^{13}C$ NMR), a *Bruker* AVIII HD 600 NMR spectrometer equipped with a 5 mm CPDCH (C-H) CryoProbe and a *Bruker* AV NEO 500 NMR spectrometer with a 5 mm BBFO Smart Probe.
$^1H$ NMR spectra were reported in the following manner: chemical shifts (δ) in parts per million (ppm) refer to the residual undeuterated solvent signals, multiplicities (s: singlet, d: doublet, t: triplet, dd: doublet of doublet, dt: doublet of triplet; td: triplet of doublet, m: multiplet), coupling constants (*J*) in Hertz (Hz) and number of protons (H).
Protein separations by SDS−PAGE were performed using 10% acrylamide gels, at 300 V and samples were visualized by in-gel fluorescence (broad green excitation [LED] and emission filter 605/50 nm) scanning using a ChemiDoc MP system (Bio-Rad). RAW data was processed using the Image Lab (5.2.1) software (Bio-Rad). Gel images were optimized by tuning contrast and brightness using the same software, with no further modifications performed.

## 2.2. General Synthesis Description

For ABPP experiments, two different 4-oxo-β-lactam-based ABPs, featuring either a rhodamine (**1**) or biotin (**10**) tags were used. These compounds were synthesized via previously reported procedures (Scheme S2.2.1.). Briefly, cyclization of malonyl dichloride with propargylamine in presence of base furnished 4-oxo-β-lactam **56** which was converted into the desired ABPs **1** and **10** by click chemistry with either biotin-$PEG_3$-azide or Rh-$PEG_3$-azide.[1]

**Scheme S2.2.1.** Synthesis of 4-oxo-β-lactam-based ABPs **1** and **10**[a]




[a]Reagents and conditions: (a) propargylamine, 1,4-dioxane, $Et_3N$, 0 °C – rt, o/n, 21%; (b) biotin-$PEG_3$-azide, 1% aqueous $CuSO_4$, 2% aqueous sodium ascorbate, DMSO, rt, 1 h, 41%; (c) Rh-$PEG_3$-azide, 1% aqueous $CuSO_4$, 1% aqueous sodium ascorbate, DMSO, rt, 1 h, 18%.

For the structure-activity relationship studies, systematically derivatized 4-oxo-β-lactam compounds were synthesized, using two different synthetic approaches, differing in the time point of the installation of the 4-oxo-β-lactam moiety (Scheme S2.2.2. and S2.2.3.).

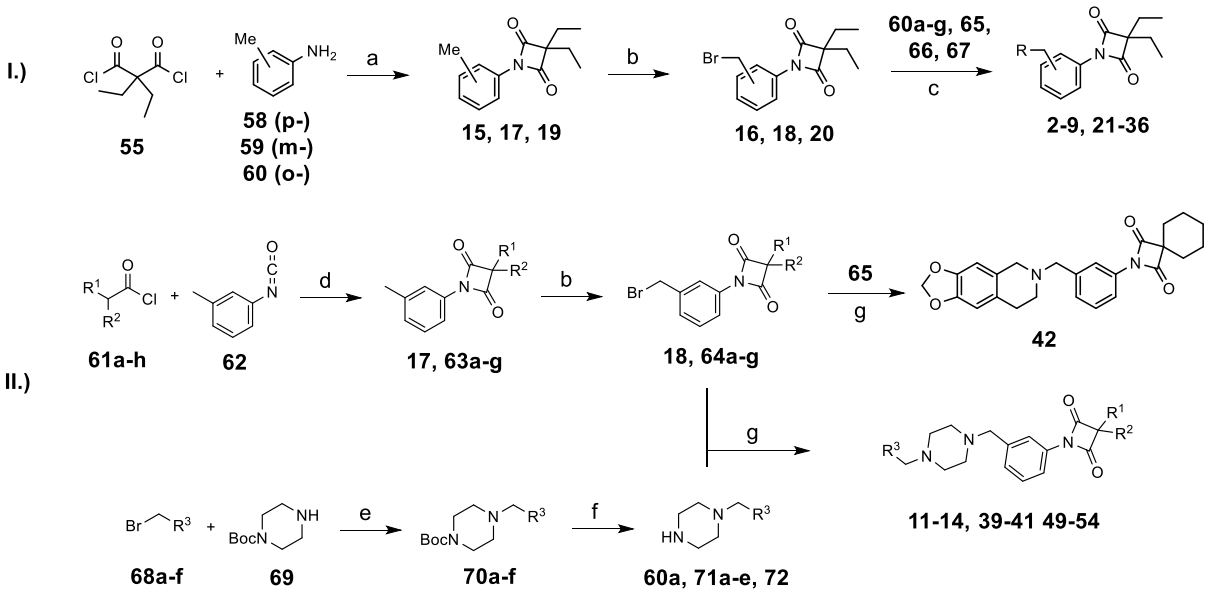
Inhibitor synthesis relied on an installation of the 4-oxo-β-lactam moiety in the initial stage of the synthesis and was achieved via two different pathways (Scheme S2.2.2.).

In synthesis route I, 4-oxo-β-lactams were obtained by a cyclization reaction of **55** with *p*-toluidine, *m*-toluidine or *o*-toluidine as starting materials. In contrast to a previously published synthesis route, DCM instead of 1,4-dioxane was used as a solvent for this reaction. This allowed to perform the reaction at 0 °C instead of rt which led to higher yields of the desired 4-oxo-β-lactams **15, 17, 19** and lower levels of the linear malondiamide. Benzylic bromination and subsequent substitution reactions with different aromatic piperazines (**60a-g**), 6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (TDIQ, **65**), dibenzylamine (**66**) or quinolin-5-amine (**67**) then yielded the desired 4-oxo-β-lactams **2-9, 21-36**.

For the generation of derivatives with various substituents at the 3-position of the central 4-oxo-β-lactam moiety, synthesis route II based on an isocyanate-ketene [2+2] thermal cycloaddition was used. In this procedure, 4-oxo-β-lactams **17** and **63a-g** were obtained from readily available 2,2-disubstituted acid-chlorides (**61a-h**) and *m*-tolyl

isocyanate (**62**) in presence of base at 155 °C. Analogously to route I, subsequent benzylic bromination and nucleophilic substitution, either with **65**, 1-methylpiperazine (**72**) or individually prepared benzylic-piperazine derivatives (**60a** or **71a-e**) gave rise to the final compounds **11-14, 39-42** and **49-54.**

**Scheme S2.2.2.** Synthesis of 4-oxo-β-lactams **2-9, 11-14, 21-36, 39-42, 49-54**[a]




[a]Reagents and conditions: (a) $Et_3N$, DCM, 0 °C – rt, 5 h, 91 – 98%; (b) NBS, benzoyl peroxide, ACN, reflux, o/n; (c) piperazines **60a-g**, **65**, **66** or **67**, ACN, $K_2CO_3$, 60 °C, 4 h, 11 – 57%; (d) $Et_3N$, xylene, 155 °C, 2 h, 24 – 91%; (e) $Et_3N$, THF, rt, 12 h, 55 – 98%; (f) (i) TFA, DCM, rt, 4 h (ii) saturated aqueous $NaHCO_3$, rt, 1 h; (g) **65**, piperazines **60a**, **71a-e** or **72**, $Et_3N$, THF, rt, 12 h, 31 – 63%.

The synthesis of 4-oxo-β-lactam derivatives with an amide bond between the aromatic core and piperazine moiety required an installation of the 4-oxo-β-lactam moiety at a later stage of the synthesis (Scheme S2.2.3.).

In synthesis route I, the amide-linked 4-oxo-β-lactams **43** and **46** were obtained from the benzoic acid derivatives **73** by a HATU-mediated coupling of the piperazine **60a** in presence of base. The so formed intermediates **74** were then Boc-deprotected with TFA, yielding the aniline derivatives **75** which were then transformed into 4-oxo-β-lactams **43** or **46** by cyclization with diethylmalonyl dichloride **55**. The 4-oxo-β-lactam **44** was obtained from **46** by a TBAF-mediated TBDMS deprotection.

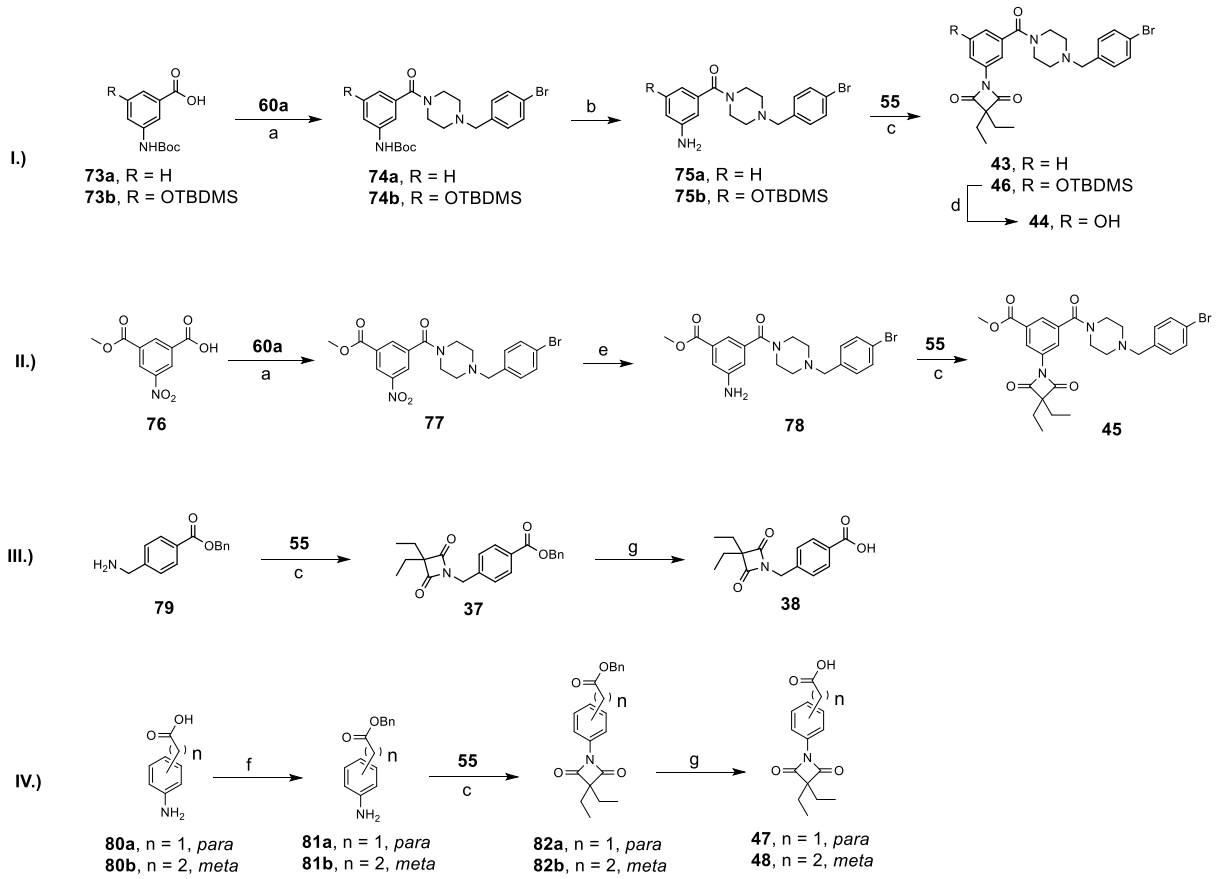
The 4-oxo-β-lactam **45** was obtained by synthesis route II. Commercially available **76** was coupled with piperazine **60a**. The nitro-group of the resulting intermediate **77** was reduced with stannous chloride, delivering aniline **78**. Its cyclization with **55** then resulted in the formation of the desired product **45**.

The carboxylic acid 4-oxo-β-lactam derivatives **38** was synthesized according route III. After cyclization of starting material **79** with **55**, the obtained intermediate **37** was benzyl-deprotected with $H_2$ in presence of catalytic amounts of Pd/C.

In synthesis route IV, carboxylic acid 4-oxo-β-lactam derivatives **47** and **48** were synthesized from starting material **80** that was converted into **81** by a Boc-protection, benzyl ester formation and Boc-deprotection sequence. The

either *meta*- or *para*-substituted **81** was then cyclized with **55**, furnishing intermediate **82**. Deprotection of the benzyl ester with $H_2$ in presence of catalytic amounts of Pd/C then led to the final 4-oxo-β-lactams **47** and **48**.

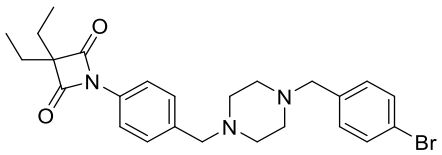
**Scheme S2.2.3.** Synthesis of 4-oxo-β-lactams **37**, **38** and **43-48**[a]




[a]Reagents and conditions: (a) piperazine **60a**, HATU, DIPEA, DMF, 0 °C – rt, 12 h, 38 – 94%; (b) (i) TFA, DCM, rt, 4 h (ii) saturated aqueous $NaHCO_3$, rt, 1 h; (c) **55**, $Et_3N$, DCM, 48 °C or 0 °C to rt, 24 h or 16 h, 11 – 91%; (d) TBAF, THF, 0 °C – rt, 2 h, 68%; (e) $SnCl_2 \cdot 2\ H_2O$, EtOAc, 70 °C, 1 h; (f) (i) $Boc_2O$, $Et_3N$, $H_2O$/1,4-dioxane, rt, 12 h (ii) benzylbromide, $K_2CO_3$, acetone, rt, 12 h (iii) 4 M HCl in 1,4-dioxane, rt, 3 h, 51 – 74%; (g) $H_2$, Pd/C 10 or 20 wt%, rt, 4 h, 31 – 54%.

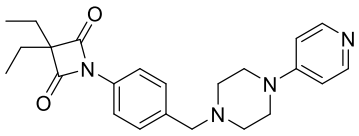
## 2.3. Synthetic Procedures and Characterization – ABPP Compounds

**(2) 1-(4-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



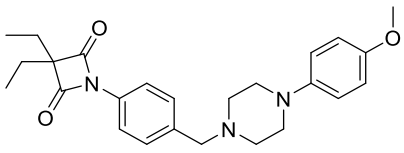
Compound **16** (60 mg, 0.19 mmol) was dissolved in ACN (2 mL). 1-(4-Bromobenzyl)piperazine (54 mg, 0.21 mmol) was added, followed by $K_2CO_3$ (29 mg, 0.21 mmol) and the reaction was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Compound **2** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:2) and obtained as a colorless oil (31 mg, 0.064 mmol, 34%). $^1$H NMR (600 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.5 Hz, 2H), 7.42 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.19 (d, *J* = 8.3 Hz, 2H), 3.50 (s, 2H), 3.45 (s, 2H), 2.46 (s, 4H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.06 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 172.3, 137.3, 137.0, 132.8, 131.4, 130.9, 130.0, 121.0, 119.3, 72.2, 62.5, 62.4, 53.1, 53.1, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{25}H_{31}BrN_3O_2^+$: 484.1594, found: 484.1599.

**(3) 3,3-Diethyl-1-(4-((4-(pyridin-4-yl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione**



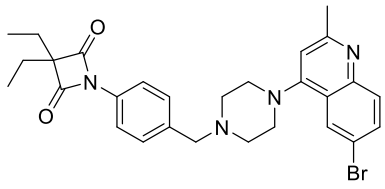
Compound **16** (60 mg, 0.19 mmol) was dissolved in ACN (2 mL). 1-(Pyridin-4-yl)piperazine (34 mg, 0.21 mmol) was added, followed by $K_2CO_3$ (29 mg, 0.21 mmol) and the reaction was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **3** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, MeOH:DCM, 1:9) and obtained as a colorless oil (8.6 mg, 0.022 mmol, 12%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, *J* = 6.0 Hz, 2H), 7.80 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 8.5 Hz, 2H), 6.68 (d, *J* = 6.9 Hz, 2H), 3.55 (s, 2H), 3.41 – 3.30 (m, 4H), 2.66 – 2.47 (m, 4H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.4, 155.9, 146.0, 136.3, 133.1, 130.0, 119.4, 107.9, 72.3, 62.3, 52.4, 46.2, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{23}H_{29}N_4O_2^+$: 393.2285, found: 393.2291.

**(4) 3,3-Diethyl-1-(4-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione**



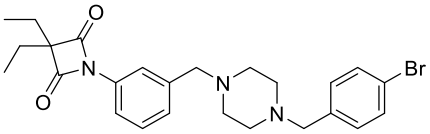
Compound **16** (60 mg, 0.19 mmol) was dissolved in ACN (2 mL). 1-(4-Methoxybenzyl)piperazine (41 mg, 0.21 mmol) was added, followed by $K_2CO_3$ (29 mg, 0.21 mmol) and the reaction was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **4** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:3) and obtained as a colorless oil (37 mg, 0.087 mmol, 46%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.5 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 6.98 – 6.72 (m, 4H), 3.76 (s, 3H), 3.60 (s, 2H), 3.11 (t, *J* = 5.0 Hz, 4H), 2.64 (t, *J* = 5.0 Hz, 4H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.3, 153.9, 145.9, 137.1, 132.80, 130.0, 119.3, 118.3, 114.5, 72.2, 62.6, 55.7, 53.3, 50.8, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{25}H_{32}N_3O_3^+$: 422.2438, found: 422.2444.

**(5) 1-(4-((4-(6-Bromo-2-methylquinolin-4-yl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



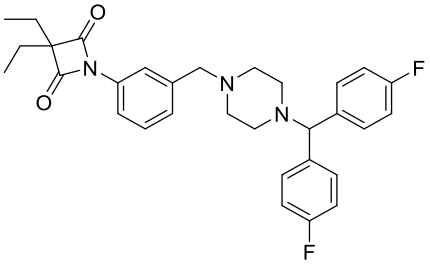
Compound **16** (60 mg, 0.19 mmol) was dissolved in ACN (2 mL). 6-Bromo-2-methyl-4-(piperazin-1-yl)quinoline (64 mg, 0.21 mmol) was added, followed by $K_2CO_3$ (29 mg, 0.21 mmol) and the reaction was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **5** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc) and obtained as a white solid (37 mg, 0.070 mmol, 37%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, *J* = 2.2 Hz, 1H), 7.92 (s, 1H), 7.82 (d, *J* = 8.5 Hz, 2H), 7.69 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 2H), 6.74 (s, 1H), 3.65 (s, 2H), 3.26 (s, 4H), 2.76 – 2.73 (m, 4H), 2.69 (s, 3H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.3, 160.1, 156.3, 148.0, 136.8, 132.9, 132.5, 131.1, 130.0, 126.0, 123.4, 119.4, 118.5, 110.4, 72.3, 62.6, 53.1, 52.3, 29.7, 25.7, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{28}H_{32}BrN_4O_2^+$: 535.1703, found: 535.1709.

**(6) 1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (synthetic route I)**



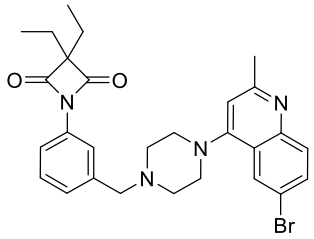
Compound **18** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 1-(4-Bromobenzyl)piperazine (42 mg, 0.16 mmol) was added, followed by $K_2CO_3$ (23 mg, 0.16 mmol) and the reaction was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **6** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:2) and obtained as a colorless oil (26 mg, 0.054 mmol, 36%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.77 (t, *J* = 1.9 Hz, 1H), 7.72 (d, *J* = 8.2, 1.6 Hz, 1H), 7.46 – 7.41 (m, 2H), 7.36 (t, *J* = 7.8 Hz, 1H), 7.29 – 7.25 (m, 2H), 7.23 (d, *J* = 8.1 Hz, 2H), 3.56 (s, 2H), 3.51 (s, 2H), 2.55 (s, 8H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.4, 140.0, 137.4, 133.9, 131.4, 130.9, 129.2, 127.5, 121.0, 119.9, 118.1, 72.1, 62.6, 62.4, 53.1, 31.1, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{25}H_{31}BrN_3O_2^+$: 484.1594, found: 484.1599.

**(7) 1-(3-((4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



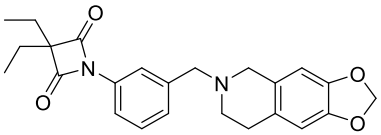
Compound **18** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 1-(Bis(4-fluorophenyl)methyl)piperazine (47 mg, 1.6 mmol) was added, followed by $K_2CO_3$ (23 mg, 1.6 mmol) and the reaction was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **7** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:4) and obtained as a colorless oil (43 mg, 0.083 mmol, 55%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.75 (t, *J* = 1.7 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.39 – 7.30 (m, 6H), 6.95 (t, *J* = 8.7 Hz, 4H), 4.22 (s, 1H), 3.55 (s, 2H), 2.56 – 2.34 (m, 8H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.06 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.4, 162.9, 160.9, 139.9, 138.5, 138.4, 133.9, 129.4, 129.3, 129.2, 127.6, 119.9, 118.1, 115.6, 115.4, 74.6, 72.1, 62.6, 53.4, 51.8, 24.0, 9.4; HRMS (ESI): *m/z* calcd for $C_{31}H_{34}F_2N_3O_2^+$: 518.2614, found: 518.2621.

**(8) 1-(3-((4-(6-Bromo-2-methylquinolin-4-yl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



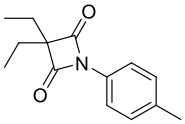
Compound **18** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 6-Bromo-2-methyl-4-(piperazin-1-yl)quinoline (49 mg, 0.16 mmol) was added, followed by $K_2CO_3$ (22 mg, 0.16 mmol) and the reaction was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **8** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc) and obtained as a white solid (47 mg, 0.088 mmol, 59%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, *J* = 2.2 Hz, 1H), 7.92 – 7.85 (m, 2H), 7.76 (ddd, *J* = 8.0, 2.1, 1.2 Hz, 1H), 7.68 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.33 – 7.28 (m, 1H), 6.75 (s, 1H), 3.67 (s, 2H), 3.26 (s, 4H), 2.77 (s, 4H), 2.67 (s, 3H), 1.87 (q, *J* = 7.5 Hz, 4H), 1.08 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.4, 160.1, 156.3, 148.0, 139.9, 134.0, 132.5, 131.1, 129.4, 127.5, 126.0, 123.5, 119.8, 118.5, 118.2, 110.4, 72.2, 62.6, 53.1, 52.3, 25.8, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{28}H_{32}BrN_4O_2^+$: 535.1703, found: 535.1706.

**(9) 1-(3-((7,8-Dihydro-[1,3]dioxolo[4,5-g]isoquinolin-6(5H)-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



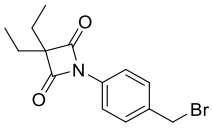
Compound **18** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 5,6,7,8-Tetrahydro-[1,3]dioxolo[4,5-g]isoquinoline (28 mg, 0.16 mmol) was added, followed by $K_2CO_3$ (22 mg, 0.16 mmol) and the reaction was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **9** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:4) and obtained as a yellow oil (34 mg, 0.084 mmol, 56%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.82 – 7.81 (m, 1H), 7.78 – 7.73 (m, 1H), 7.41 (d, *J* = 7.4 Hz, 2H), 6.58 (s, 1H), 6.46 (s, 1H), 5.88 (s, 2H), 3.74 (s, 2H), 3.61 (s, 2H), 2.82 (d, *J* = 26.2 Hz, 4H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.4, 146.2, 145.8, 140.2, 133.9, 129.3, 127.6, 127.5, 127.4, 119.9, 118.2, 108.6, 106.6, 100.7, 72.1, 62.2, 56.2, 50.7, 29.2, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{24}H_{27}N_2O_4^+$: 407.1965, found: 407.1973.

**(15) 3,3-Diethyl-1-(*p*-tolyl)azetidine-2,4-dione**



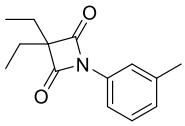
Diethylmalonyl dichloride (1.50 g, 7.61 mmol) was dissolved in DCM (15 mL) and the mixture was cooled to 0 °C. *p*-Toluidine (612 mg, 5.71 mmol) and $Et_3N$ (2.121 mL, 15.22 mmol) were mixed with DCM (30 mL) and added dropwise. The reaction mixture was stirred for 1 h at 0 °C and then allowed to reach rt and stirred for additional 4 h. The solvent was evaporated in vacuo. The solid residue was purified by flash column chromatography (silica gel, EtOAc:hexane, 1:9) to yield compound **15** as a colorless oil (1.202 g, 5.200 mmol, 91%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.4 Hz, 2H), 7.21 (dt, *J* = 8.1, 0.7 Hz, 2H), 2.35 (s, 3H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.06 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.4, 136.8, 131.5, 129.8, 119.3, 72.1, 24.1, 21.3, 9.4.

**(16) 1-(4-(Bromomethyl)phenyl)-3,3-diethylazetidine-2,4-dione**



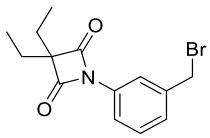
The toluidine-derivatized 4-oxo-β-lactam **15** (1.00 g, 4.32 mmol) was dissolved in ACN (40 mL). NBS (846 mg, 4.76 mmol) and benzoyl peroxide (105 mg, 0.432 mmol) were added. The reaction mixture was refluxed overnight. The solvent was evaporated in vacuo. The brominated compound was purified by flash column chromatography (silica gel, EtOAc:hexane, 1:9) to remove reaction side products, yielding compound **16** with a trace amount of co-eluting starting material **15**. The mixture was used without further purification in the next step.

**(17) 3,3-Diethyl-1-(*m*-tolyl)azetidine-2,4-dione (synthetic route I)**



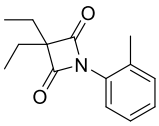
Diethylmalonyl dichloride (1.5 g, 7.61 mmol) was dissolved in DCM (15 mL) and the mixture was cooled to 0 °C. *m*-Toluidine (612 mg, 5.71 mmol) and $Et_3N$ (2.121 mL, 15.22 mmol) were mixed with DCM (30 mL) and added dropwise. The reaction mixture was stirred for 1 h at 0 °C and then allowed to reach rt and stirred for additional 4 h. The solvent was evaporated in vacuo. The solid residue was purified by flash column chromatography (silica gel, EtOAc:hexane, 1:9) to yield compound **17** as a colorless oil (1.294 g, 5.590 mmol, 98%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.08 (d, *J* = 7.6 Hz, 1H), 2.38 (d, *J* = 0.7 Hz, 3H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.2, 139.4, 133.8, 129.1, 127.6, 119.7, 116.4, 72.0, 24.0, 21.4, 9.3.

**(18) 1-(3-(Bromomethyl)phenyl)-3,3-diethylazetidine-2,4-dione**



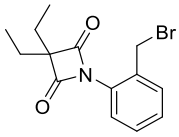
The toluidine-modified 4-oxo-β-lactam **17** (1.00 g, 4.32 mmol) was dissolved in ACN (40 mL). NBS (846 mg, 4.76 mmol) and benzoyl peroxide (105 mg, 0.432 mmol) were added. The reaction mixture was refluxed overnight. The solvent was evaporated in vacuo. The brominated compound was purified by flash column chromatography (silica gel, EtOAc:hexane, 1:9) to remove reaction side products, yielding compound **18** with a trace amount of co-eluting starting material **17**. The mixture was used without further purification in the next step.

**(19) 3,3-Diethyl-1-(*o*-tolyl)azetidine-2,4-dione**



Diethylmalonyl dichloride (1.50 g, 7.61 mmol) was dissolved in DCM (15 mL) and the mixture was cooled to 0 °C. *o*-Toluidine (612 mg, 5.71 mmol) and $Et_3N$ (2.121 mL, 15.22 mmol) were mixed with DCM (30 mL) and added dropwise. The reaction mixture was stirred for 1 h at 0 °C and then allowed to reach rt and stirred for additional 4h. The solvent was evaporated in vacuo. The solid residue was purified by flash column chromatography (silica gel, EtOAc:hexane, 1:9) to yield compound **19** as a colorless oil (1.056 g, 4.570 mmol, 80%).$^1$H NMR (500 MHz, $CDCl_3$) δ 7.29 – 7.27 (m, 2H), 7.25 – 7.23 (m, 2H), 2.35 (s, 3H), 1.88 (q, J = 7.5 Hz, 4H), 1.14 (t, J = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.9, 134.0, 131.6, 130.0, 129.1, 126.8, 125.7, 70.90, 24.2, 19.0, 9.6.

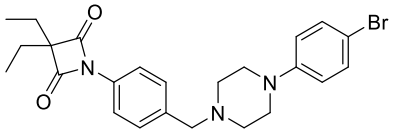
**(20) 1-(2-(Bromomethyl)phenyl)-3,3-diethylazetidine-2,4-dione**



The toluidine-derivatized 4-oxo-β-lactam **19** (781 mg, 3.38 mmol) was dissolved in ACN (31 mL). NBS (661 mg, 3.71 mmol) and benzoyl peroxide (82 mg, 0.34 mmol) were added and the reaction mixture was refluxed overnight. The solvent was evaporated in vacuo. The brominated compound was purified by flash column chromatography (silica

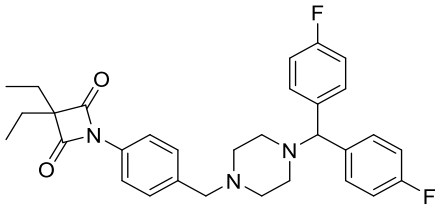
gel, EtOAc:hexane, 1:9) to remove reaction side products, yielding compound **20** with a trace amount of co-eluting starting material **19**. The mixture was used without further purification in the next step.

**(21) 1-(4-((4-(4-Bromophenyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



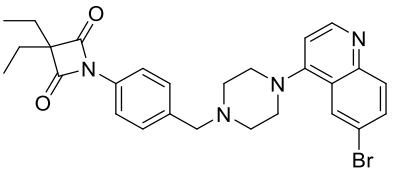
Compound **16** (60 mg, 0.19 mmol) was dissolved in ACN (2 mL). 1-(4-Bromophenyl)piperazine (51 mg, 0.21 mmol) was added, followed by $K_2CO_3$ (29 mg, 0.21 mmol) and the reaction was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **21** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:4) and obtained as a colorless oil (50 mg, 0.11 mmol, 56%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.5 Hz, 2H), 7.42 (d, *J* = 8.1 Hz, 2H), 7.35 – 7.30 (m, 2H), 6.79 – 6.75 (m, 2H), 3.58 (s, 2H), 3.18 (s, 4H), 2.61 (s, 4H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.3, 150.4, 132.9, 132.0, 130.0, 119.3, 117.7, 111.9, 72.3, 62.5, 53.0, 49.0, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{24}H_{29}BrN_3O_2^+$: 470.1438, found: 470.1444.

**(22) 1-(4-((4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



Compound **16** (60 mg, 0.19 mmol) was dissolved in ACN (2 mL). 1-(Bis(4-fluorophenyl)methyl)piperazine (109 mg, 0.210 mmol) was added, followed by $K_2CO_3$ (61 mg, 0.21 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **22** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:4) and obtained as a colorless oil (50 mg, 0.097 mmol, 51%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.0 Hz, 2H), 7.39 – 7.35 (m, 2H), 7.32 (dd, *J* = 8.6, 5.5 Hz, 6H), 6.95 (t, *J* = 8.7 Hz, 4H), 4.23 (s, 1H), 3.53 (s, 2H), 2.47 (s, 8H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.06 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 172.3, 137.3, 137.1, 132.8, 131.4, 130.9, 130.0, 120.9, 119.3, 72.2, 62.6, 62.4, 53.1, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{31}H_{34}F_2N_3O_2^+$: 518.2614, found: 518.2619.

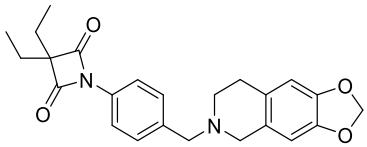
**(23) 1-(4-((4-(6-Bromoquinolin-4-yl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



Compound **16** (60 mg, 0.19 mmol) was dissolved in ACN (2 mL). 6-Bromo-4-(piperazin-1-yl)quinoline (61 mg, 0.21 mmol) was added, followed by $K_2CO_3$ (29 mg, 0.21 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **23** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc) and obtained as a white solid (22 mg, 0.042 mmol, 22%). $^1$H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, *J* = 5.1 Hz, 1H), 8.00 (d, *J* = 8.9 Hz, 1H), 7.87 – 7.79 (m, 2H), 7.74 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.47 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 5.2 Hz, 1H), 3.71 (s, 2H), 3.47 – 3.17 (m, 4H), 2.97 – 2.63 (m, 4H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.3, 156.3, 151.1, 148.1, 136.5, 133.0, 132.7, 131.8, 130.1, 126.2, 124.9,

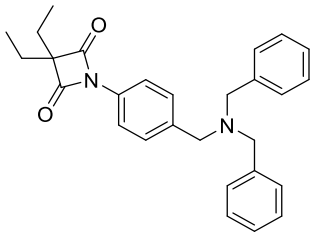
119.6, 119.4, 109.7, 72.3, 62.5, 53.0, 52.2, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{27}H_{30}BrN_4O_2^+$: 521.1547, found: 521.1552.

**(24) 1-(4-((7,8-Dihydro-[1,3]dioxolo[4,5-g]isoquinolin-6(5H)-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



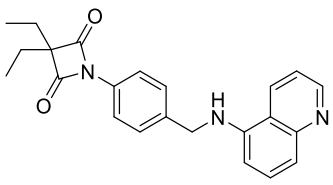
Compound **16** (46 mg, 0.15 mmol) was dissolved in ACN (0.5 mL). 5,6,7,8-Tetrahydro-[1,3]dioxolo[4,5-g]isoquinoline (28 mg, 0.16 mmol) was added, followed by $K_2CO_3$ (22 mg, 0.16 mmol) and the reaction was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **24** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:4) and obtained as a yellow oil (22 mg, 0.054 mmol, 36%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.5 Hz, 2H), 7.44 (d, *J* = 8.5 Hz, 2H), 6.56 (s, 1H), 6.44 (s, 1H), 5.87 (s, 2H), 3.67 (s, 2H), 3.54 (s, 2H), 2.75 (dt, *J* = 43.4, 5.8 Hz, 4H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.4, 146.3, 145.9, 132.9, 130.0, 127.2, 119.4, 108.6, 106.6, 100.8, 72.3, 62.0, 56.1, 50.6, 29.8, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{24}H_{27}N_2O_4^+$: 407.1965, found: 407.1970.

**(25) 1-(4-((Dibenzylamino)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



Compound **16** (25 mg, 0.081 mmol) was dissolved in ACN (0.5 mL). Dibenzylamine (17 mg, 0.089 mmol) was added, followed by $K_2CO_3$ (12 mg, 0.089 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **25** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 3:2) and obtained as a white solid (11 mg, 0.026 mmol, 32%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.5 Hz, 2H), 7.45 (d, *J* = 8.2 Hz, 2H), 7.41 – 7.37 (m, 4H), 7.32 (t, *J* = 7.5 Hz, 4H), 7.26 – 7.22 (m, 2H), 3.55 (s, 6H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.06 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.4, 139.5, 138.6, 132.7, 129.6, 128.9, 128.4, 127.1, 119.2, 72.2, 58.0, 57.5, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{28}H_{31}N_2O_2^+$:427.2380, found: 427.2388.

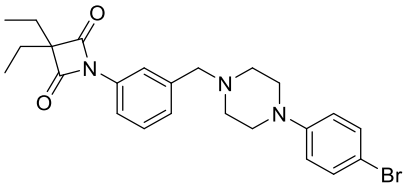
**(26) 3,3-Diethyl-1-(4-((quinolin-5-ylamino)methyl)phenyl)azetidine-2,4-dione**



Compound **16** (50 mg, 0.16 mmol) was dissolved in ACN (1 mL). Quinolin-5-amine (26 mg, 0.18 mmol) was added, followed by $K_2CO_3$ (24 mg, 0.18 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **26** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc) and obtained as a yellow oil (17 mg, 0.046 mmol, 28%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.60 (dd, *J* = 4.3, 1.6 Hz, 1H), 7.90 (d, *J* = 9.1 Hz, 1H), 7.88 (dd, *J* = 7.2, 1.1 Hz, 1H), 7.82 (d, *J* = 8.5 Hz, 2H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.14 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.66 (d, *J* = 2.6 Hz, 1H), 4.51 (s, 1H), 4.44 (s, 2H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.06 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz,

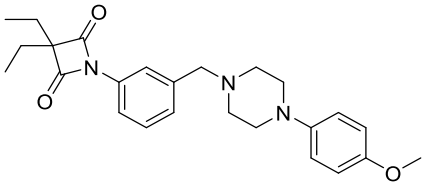
$CDCl_3$) δ 172.3, 146.2, 145.9, 143.1, 137.5, 134.3, 133.1, 130.3, 130.2, 128.3, 121.6, 121.5, 119.7, 103.6, 72.3, 47.9, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{23}H_{24}N_3O_2^+$: 374.1863, found: 374.1868.

**(27) 1-(3-((4-(4-Bromophenyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



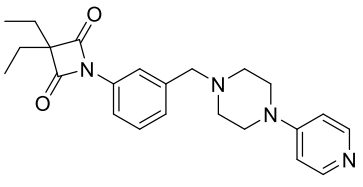
Compound **18** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 1-(4-Bromophenyl)piperazine (39 mg, 0.16 mmol) was added, followed by $K_2CO_3$ (22 mg, 0.16 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **27** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:4) and obtained as a colorless oil (40 mg, 0.085 mmol, 57%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.39 (t, *J* = 7.7 Hz, 1H), 7.35 – 7.30 (m, 3H), 6.78 (d, *J* = 9.0 Hz, 2H), 3.62 (s, 2H), 3.20 (s, 4H), 2.65 (s, 4H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.08 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.4, 150.5, 139.8, 134.0, 132.0, 129.3, 127.48, 119.9, 118.2, 117.7, 111.8, 72.2, 62.6, 53.0, 49.1, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{24}H_{29}BrN_3O_2^+$: 470.1438, found: 470.1443.

**(28) 3,3-Diethyl-1-(3-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione**



Compound **18** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 1-(4-Methoxybenzyl)piperazine (31 mg, 1.6 mmol) was added, followed by $K_2CO_3$ (23 mg, 1.6 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **28** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:3) and obtained as a colorless oil (29 mg, 0.069 mmol, 46%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.74 (dt, *J* = 7.5, 1.8 Hz, 1H), 7.43 – 7.30 (m, 2H), 6.97 – 6.73 (m, 4H), 3.76 (s, 3H), 3.62 (s, 2H), 3.13 (t, *J* = 4.9 Hz, 4H), 2.66 (t, *J* = 5.0 Hz, 4H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.08 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.4, 153.9, 145.9, 140.0, 133.9, 129.3, 127.6, 119.9, 118.3, 114.5, 72.2, 62.6, 55.7, 53.3, 50.7, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{25}H_{32}N_3O_3^+$: 422.2438, found: 422.2444.

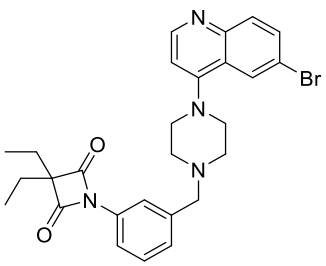
**(29) 3,3-Diethyl-1-(3-((4-(pyridin-4-yl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione**



Compound **18** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 1-(Pyridin-4-yl)piperazine (28 mg, 0.17 mmol) was added, followed by $K_2CO_3$ (23 mg, 0.17 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **29** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, MeOH:DCM, 1:9) and obtained as a yellow oil (22 mg, 0.056 mmol, 37%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.25 (d, *J* = 6.7 Hz, 2H), 7.82 (t, *J* = 1.9 Hz, 1H), 7.79 – 7.73 (m, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.26 – 7.23 (m, 1H), 6.82 – 6.76 (m, 2H), 3.59 (s, 2H), 3.57 – 3.51 (m, 4H), 2.63 – 2.60 (m, 4H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz,

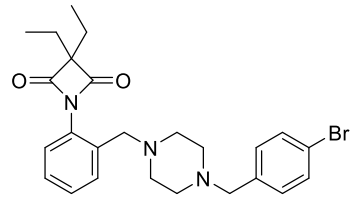
$CDCl_3$) δ 172.4, 156.6, 143.3, 139.1, 134.1, 129.5, 127.4, 119.7, 118.5, 107.6, 72.3, 62.2, 52.3, 46.2, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{23}H_{29}N_4O_2^+$: 393.2285, found: 393.2290.

**(30) 1-(3-((4-(6-Bromoquinolin-4-yl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



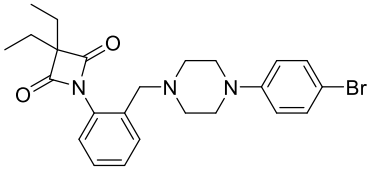
Compound **18** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 6-Bromo-4-(piperazin-1-yl)quinoline (23 mg, 0.16 mmol) was added, followed by $K_2CO_3$ (48 mg, 0.16 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **30** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc) and obtained as a white solid (44 mg, 0.084 mmol, 56%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.69 (d, *J* = 5.2 Hz, 1H), 8.14 (d, *J* = 2.2 Hz, 1H), 8.02 (d, *J* = 8.9 Hz, 1H), 7.86 (d, *J* = 1.9 Hz, 1H), 7.79 – 7.76 (m, 1H), 7.74 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 7.8 Hz, 1H), 6.89 (d, *J* = 5.2 Hz, 1H), 3.71 (s, 2H), 3.36 (d, *J* = 5.3 Hz, 4H), 2.81 (s, 4H), 1.87 (q, *J* = 7.5 Hz, 4H), 1.08 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.4, 156.3, 151.3, 148.3, 139.8, 134.1, 132.6, 132.0, 129.4, 127.5, 126.2, 125.0, 119.8, 119.5, 118.3, 109.8, 72.2, 62.6, 53.1, 52.3, 24.1, 9.4; HRMS (ESI): *m/z* calcd for $C_{27}H_{30}BrN_4O_2^+$: 521.1547, found: 521.1552.

**(31) 1-(2-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



Compound **20** (50 mg, 0.16 mmol) was dissolved in ACN (1 mL). 1-(4-Bromobenzyl)piperazine (45 mg, 0.18 mmol) was added, followed by $K_2CO_3$ (24 mg, 0.18 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **31** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:2) and obtained as a colorless oil (18 mg, 0.037 mmol, 23%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.49 – 7.39 (m, 3H), 7.36 – 7.23 (m, 3H), 7.20 – 7.14 (m, 2H), 3.58 (s, 2H), 3.43 (s, 2H), 2.39 (s, 8H), 1.91 (q, *J* = 7.5 Hz, 4H), 1.13 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 172.9, 137.3, 134.3, 131.4, 131.3, 130.9, 130.0, 129.0, 128.1, 126.2, 121.0, 70.1, 62.4, 60.0, 53.4, 53.0, 23.7, 9.5; HRMS (ESI): *m/z* calcd for $C_{25}H_{31}BrN_3O_2^+$: 484.1594, found: 484.1602.

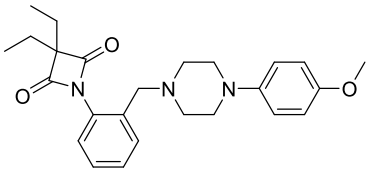
**(32) 1-(2-((4-(4-Bromophenyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



Compound **20** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 1-(4-Bromophenyl)piperazine (39 mg, 1.6 mmol) was added, followed by $K_2CO_3$ (22 mg, 1.6 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **32** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:4) and obtained as a colorless oil (20 mg, 0.043 mmol, 28%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.53 – 7.48 (m, 1H), 7.37 – 7.27 (m, 5H), 6.75 (d, *J* = 9.0 Hz, 2H), 3.65 (s, 2H), 3.15 – 3.05 (m, 4H), 2.56 – 2.45 (m, 4H), 1.91 (q, *J* = 7.5 Hz, 4H), 1.14 (t,

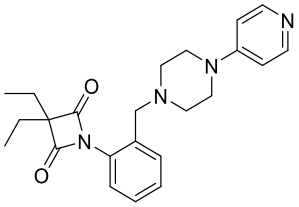
*J* = 7.5 Hz, 6H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 172.9, 150.4, 133.9, 132.0, 131.4, 130.1, 129.0, 128.3, 126.3, 117.7, 111.9, 70.2, 60.0, 53.3, 48.9, 23.8, 9.5; HRMS (ESI): *m/z* calcd for $C_{24}H_{29}BrN_3O_2^+$: 470.1438, found: 470.1442.

**(33) 3,3-Diethyl-1-(2-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione**



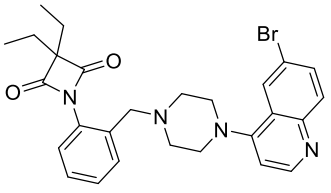
Compound **20** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 1-(4-Methoxybenzyl)piperazine (31 mg, 0.16 mmol) was added, followed by $K_2CO_3$ (23 mg, 0.16 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **33** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc:hexane, 1:3) and obtained as a colorless oil (23 mg, 0.055 mmol, 36%). $^1$H NMR (600 MHz, $CDCl_3$) δ 7.54 – 7.50 (m, 1H), 7.37 – 7.32 (m, 2H), 7.31 – 7.29 (m, 1H), 6.90 – 6.85 (m, 2H), 6.85 – 6.81 (m, 2H), 3.76 (s, 3H), 3.65 (s, 2H), 3.10 – 2.93 (m, 4H), 2.60 – 2.45 (m, 4H), 1.92 (q, *J* = 7.5 Hz, 4H), 1.14 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 172.9, 153.9, 145.8, 134.1, 131.4, 130.0, 129.0, 128.2, 126.2, 118.2, 114.5, 70.2, 60.1, 55.7, 53.6, 50.6, 23.7, 9.6; HRMS (ESI): *m/z* calcd for $C_{25}H_{32}N_3O_3^+$: 422.2438, found: 422.2444.

**(34) 3,3-Diethyl-1-(2-((4-(pyridin-4-yl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione**



Compound **20** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 1-(Pyridin-4-yl)piperazine (27 mg, 0.16 mmol) was added, followed by $K_2CO_3$ (22 mg, 0.16 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **34** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, MeOH:DCM, 1:9) and obtained as a colorless oil (13 mg, 0.033 mmol, 22%). $^1$H NMR (600 MHz, $CDCl_3$) δ 7.48 – 7.45 (m, 1H), 7.43 – 7.40 (m, 2H), 7.34 – 7.28 (m, 2H), 7.27 – 7.25 (m, 1H), 7.18 – 7.15 (m, 2H), 3.58 (s, 2H), 3.42 (s, 2H), 2.38 (s, 8H), 1.91 (q, *J* = 7.5 Hz, 4H), 1.13 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 172.8, 137.3, 134.3, 131.4, 131.3, 130.9, 129.9, 128.9, 128.0, 126.2, 120.9, 70.1, 62.4, 60.0, 53.4, 53.0, 23.7, 9.5; HRMS (ESI): *m/z* calcd for $C_{23}H_{29}N_4O_2^+$: 393.2285, found: 393.2284.

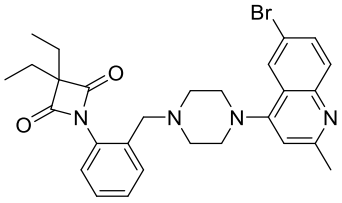
**(35) 1-(2-((4-(6-Bromoquinolin-4-yl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



Compound **20** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 6-Bromo-4-(piperazin-1-yl)quinoline (48 mg, 0.16 mmol) was added, followed by $K_2CO_3$ (24 mg, 0.16 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **35** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc) and obtained as a white solid (26 mg, 0.050 mmol, 34%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.73 (d, *J* = 5.5 Hz, 1H), 8.11 (d, *J* = 2.2 Hz, 1H), 8.03 (d, *J* = 9.0 Hz, 1H), 7.76 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.56 – 7.49 (m, 1H), 7.41 – 7.29 (m, 3H), 6.86 (d, *J* = 5.5 Hz, 1H), 3.75 (s, 2H), 3.35 – 3.33 (m, 4H), 2.69 (t, *J* = 4.7 Hz, 4H), 1.93 (q, *J* = 7.5 Hz, 4H), 1.16 (t, *J* = 7.5 Hz, 6H); $^{13}$C

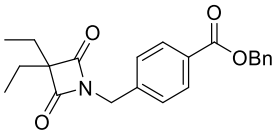
NMR (126 MHz, $CDCl_3$) δ 172.9, 157.6, 148.6, 134.0, 133.4, 131.4, 130.1, 129.5, 129.2, 128.6, 126.6, 126.5, 123.9, 120.1, 108.8, 70.3, 59.8, 53.1, 52.1, 23.8, 9.6; HRMS (ESI): *m/z* calcd for $C_{27}H_{30}BrN_4O_2^+$: 521.1547, found: 521.1551.

**(36) 1-(2-((4-(6-Bromo-2-methylquinolin-4-yl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



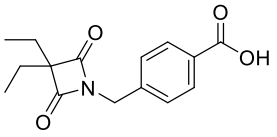
Compound **20** (46 mg, 0.15 mmol) was dissolved in ACN (1 mL). 6-Bromo-2-methyl-4-(piperazin-1-yl)quinoline (49 mg, 0.16 mmol) was added, followed by $K_2CO_3$ (22 mg, 0.16 mmol) and the reaction mixture was heated to 60 °C for 4 h. $H_2O$ (5 mL) was added. The compound was extracted with 3 x 3 mL of EtOAc. The organic fractions were collected, combined, dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Product **36** was purified from the crude mixture by preparative thin-layer chromatography (silica gel, EtOAc) and obtained as a white solid (28 mg, 0.052 mmol, 35%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.06 – 7.98 (m, 2H), 7.74 (d, *J* = 8.9 Hz, 1H), 7.54 (d, *J* = 6.8 Hz, 1H), 7.42 – 7.34 (m, 2H), 7.31 (d, *J* = 7.0 Hz, 1H), 6.70 (s, 1H), 3.74 (s, 2H), 3.33 (s, 4H), 2.72 (s, 3H), 2.68 (s, 4H), 1.93 (q, *J* = 7.4 Hz, 4H), 1.16 (t, *J* = 7.4 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.9, 134.1, 133.5, 131.4, 130.1, 129.2, 128.5, 126.5, 126.4, 122.4, 119.4, 109.5, 70.3, 59.8, 53.1, 52.1, 23.8, 9.6; HRMS (ESI): *m/z* calcd for $C_{28}H_{32}BrN_4O_2^+$: 535.1703, found: 535.1712.

**(37) Benzyl 4-((3,3-diethyl-2,4-dioxoazetidin-1-yl)methyl)benzoate**



Diethylmalonyl dichloride (389 mg, 1.97 mmol) was dissolved in DCM (3 mL) and the mixture was cooled to 0 °C. The amine handle benzyl 4-(aminomethyl)benzoate (356 mg, 1.48 mmol) and $Et_3N$ (413 µL, 2.96 mmol) were dissolved in DCM (6 mL) and added to the reaction mixture dropwise. The reaction mixture was stirred for 1 h at 0 °C and then allowed to reach rt and stirred for additional 4 h. The solvent was evaporated in vacuo and the crude mixture was purified by flash column chromatography (silica gel, EtOAc:hexane, 1:9 → 1:1) to yield the final product **37** as a colorless oil (126 mg, 0.340 mmol, 23%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, *J* = 8.4 Hz, 2H), 7.51 – 7.29 (m, 7H), 5.36 (s, 2H), 4.51 (s, 2H), 1.73 (q, *J* = 7.5 Hz, 4H), 0.92 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 174.2, 166.0, 140.1, 136.0, 130.5, 130.2, 128.8, 128.5, 128.42, 128.37, 71.2, 67.0, 42.5, 23.8, 9.3.

**(38) 4-((3,3-Diethyl-2,4-dioxoazetidin-1-yl)methyl)benzoic acid**

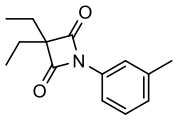


Compound **37** (100 mg, 0.270 mmol) was dissolved in MeOH (1 mL). Pd/C (20 mg, 20 wt%) was added. Triethylsilane (318 mg, 2.70 mmol) was slowly added dropwise while the reaction mixture was stirred vigorously. The mixture was stirred at rt for 5 minutes. Then, the mixture was diluted with EtOAc and filtered. The filtrate was evaporated in vacuo. The crude mixture was purified by flash column chromatography and the final product **38** was obtained as a white solid (40 mg, 0.15 mmol, 54%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.3 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 4.54 (s, 2H), 1.74 (q, *J* = 7.5 Hz, 4H), 0.93 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 174.2, 171.2, 140.9, 131.0, 129.3, 128.5, 71.3, 42.5, 23.8, 9.3. HRMS (ESI): *m/z* calcd for $C_{15}H_{18}NO_4^+$: 276.1230, found: 276.1238.

## 2.4. Synthetic Procedures and Characterization – SAR Studies Compounds

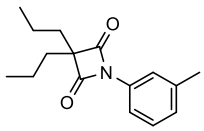
*General method for the formation of 4-oxo-β-lactams **17** and **63a-g** via thermal [2+2]-cycloadditions*
*m*-Tolyl isocyanate (1 equiv) and the respective acid chloride (2 equiv) were dissolved in xylene (0.6 M with respect to the limiting reagent) and heated to 110 °C. A solution of $Et_3N$ (3.6 equiv) in xylene (2.4 M) was added dropwise to the mixture, before it was heated to 155 °C and stirred at this temperature for 2 h. Then, the mixture was allowed to cool to rt, filtrated, and the filtrate was concentrated in vacuo. Purification of the crude product by flash column chromatography (silica gel, EtOAc:cyclohexane, 1:30), afforded 4-oxo-β-lactams **17** and **63a-g** in pure form.

**(17) 3,3-Diethyl-1-(*m*-tolyl)azetidine-2,4-dione (synthetic route II)**



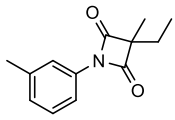
Colorless oil; yield: 72% (1.21 g, 5.23 mmol); $R_f$ = 0.39 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.61 (m, 2H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.07 (d, *J* = 7.2 Hz, 1H), 2.38 (s, 3H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.6 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.2, 139.4, 133.8, 129.1, 127.6, 119.7, 116.4, 72.0, 24.0, 21.4, 9.3; LC-MS (ESI) $R_t$ = 11.25 min; *m/z* calcd for $C_{14}H_{18}NO_2^+$: 232.1, found: 231.9.

**(63a) 3,3-Dipropyl-1-(*m*-tolyl)azetidine-2,4-dione**



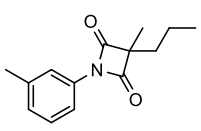
Colorless oil; yield: 83% (1.57 g, 6.03 mmol); $R_f$ = 0.43 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.62 (m, 2H), 7.29 (t, *J* = 7.3 Hz, 1H), 7.07 (d, *J* = 7.7 Hz, 1H), 2.38 (s, 3H), 1.82 – 1.73 (m, 4H), 1.55 – 1.43 (m, 4H), 1.00 – 0.91 (m, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.4, 139.3, 133.9, 129.0, 127.5, 119.6, 116.3, 70.8, 34.0, 33.3, 20.4, 18.3, 14.1; LC-MS (ESI) $R_t$ = 12.88 min; *m/z* calcd for $C_{16}H_{22}NO_2^+$: 260.2, found: 260.0.

**(63b) 3-Ethyl-3-methyl-1-(*m*-tolyl)azetidine-2,4-dione**



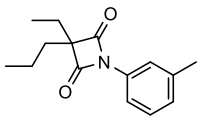
Yellowish oil; yield: 38% (829 mg, 3.82 mmol); $R_f$ = 0.29 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.58 (m, 2H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.07 (d, *J* = 6.8 Hz, 1H), 2.38 (s, 3H), 1.85 (q, *J* = 7.5 Hz, 2H), 1.47 (s, 3H), 1.08 (t, *J* = 7.5 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.6, 139.4, 134.1, 129.1, 127.5, 119.7, 116.3, 66.6, 25.5, 21.5, 16.1, 9.3; LC-MS (ESI) $R_t$ = 10.69 min; *m/z* calcd for $C_{13}H_{16}NO_2^+$: 218.1, found: 217.9.

**(63c) 3-Methyl-3-propyl-1-(*m*-tolyl)azetidine-2,4-dione**



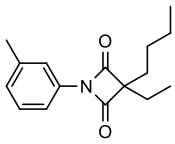
Yellowish oil; yield: 48% (997 mg, 4.31 mmol); $R_f$ = 0.41 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.62 (m, 2H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.07 (d, *J* = 7.8 Hz, 1H), 2.38 (s, 3H), 1.82 – 1.73 (m, 2H), 1.58 – 1.44 (m, 5H), 0.99 (t, *J* = 7.3 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.5, 139.2, 134.1, 129.0, 127.4, 119.5, 116.2, 65.9, 34.3, 21.3, 18.3, 16.3, 14.0; LC-MS (ESI) $R_t$ = 11.28 min; *m/z* calcd for $C_{14}H_{18}NO_2^+$: 232.1, found: 231.9.

**(63d) 3-Ethyl-3-propyl-1-(*m*-tolyl)azetidine-2,4-dione**



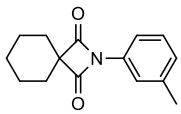
Yellowish oil; yield: 78% (440 mg, 1.79 mmol); $R_f$ = 0.40 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.61 (m, 2H), 7.28 (t, *J* = 7.8 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 2.37 (s, 3H), 1.85 (q, *J* = 7.5 Hz, 2H), 1.80 – 1.74 (m, 2H), 1.54 – 1.43 (m, 2H), 1.07 (t, *J* = 7.6 Hz, 3H), 1.01 – 0.94 (m, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.3, 139.3, 133.8, 129.0, 127.5, 119.7, 116.3, 71.4, 33.0, 24.3, 21.4, 18.4, 14.2, 9.2; LC-MS (ESI) $R_t$ = 11.82 min; *m/z* calcd for $C_{15}H_{20}NO_2^+$: 246.2, found: 246.0.

**(63e) 3-Butyl-3-ethyl-1-(*m*-tolyl)azetidine-2,4-dione**



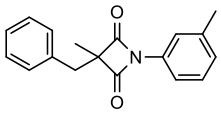
Yellowish oil; yield: 91% (1.71 g, 6.58 mmol); $R_f$ = 0.45 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.69 – 7.61 (m, 2H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.08 (d, *J* = 7.7 Hz, 1H), 2.38 (s, 3H), 1.85 (q, *J* = 7.5 Hz, 2H), 1.81 – 1.75 (m, 2H), 1.38 – 1.29 (m, 4H), 0.90 (t, *J* = 7.1 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.5, 139.5, 133.9, 129.2, 127.6, 119.8, 116.4, 71.5, 30.7, 27.1, 24.5, 22.9, 13.9, 9.3; LC-MS (ESI) $R_t$ = 12.23 min; *m/z* calcd for $C_{16}H_{22}NO_2^+$: 260.2, found: 260.0.

**(63f) 2-(*m*-Tolyl)-2-azaspiro[3.5]nonane-1,3-dione**



Yellowish solid; ca. 1:4 yield: 79% (1.72 g, 7.37 mmol); $R_f$ = 0.41 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.57 (m, 2H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 2.38 (s, 3H), 2.03 – 1.88 (m, 4H), 1.84 – 1.73 (m, 4H), 1.61 – 1.48 (m, 2H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.9, 139.4, 134.4, 129.1, 127.4, 119.7, 116.3, 65.6, 29.0, 28.4, 25.1, 22.8, 22.1, 21.5; LC-MS (ESI) $R_t$ = 11.52 min; *m/z* calcd for $C_{15}H_{18}NO_2^+$: 244.1, found: 243.9.

**(63g) 3-Benzyl-3-methyl-1-(*m*-tolyl)azetidine-2,4-dione**

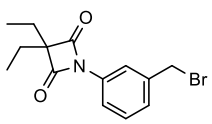


Yellowish oil; yield: 38% (647 mg, 2.32 mmol); $R_f$ = 0.32 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.37 (m, 2H), 7.33 – 7.27 (m, 4H), 7.26 – 7.19 (m, 1H), 7.18 – 7.11 (m, 1H), 7.03 (d, *J* = 7.7 Hz, 1H), 3.11 (s, 2H), 2.33 (s, 3H), 1.55 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.8, 139.2, 135.0, 133.3, 129.6, 128.9, 128.6, 127.6, 127.4, 119.9, 116.6, 67.6, 38.6, 21.3, 16.5; LC-MS (ESI) $R_t$ = 11.06 min; *m/z* calcd for $C_{18}H_{18}NO_2^+$: 280.1, found: 280.0.

*General method for the benzylic bromination leading to intermediates **18, 64a-g***

The respective 4-oxo-β-lactam **17, 63a-g** (1 equiv), NBS (1.1 equiv) and benzoyl peroxide (0.1 equiv) were suspended in ACN (0.3 M with respect to the limiting reagent), heated to 75 °C and stirred at this temperature for 16 h. After filtration of the mixture, the filtrate was concentrated in vacuo and purified by flash column chromatography (silica gel, EtOAc:cyclohexane, 1:30), affording the brominated 4-oxo-β-lactams **18, 64a-g** in pure form.

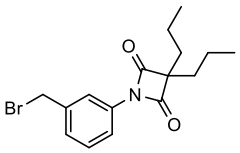
**(18) 1-(3-(Bromomethyl)phenyl)-3,3-diethylazetidine-2,4-dione**



Colorless oil; yield: 93% (2.95 g, 9.51 mmol); $R_f$ = 0.32 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.82 (d, *J* = 8.3 Hz, 1H), 7.58 – 7.36 (m, 1H), 7.31 (d, *J* = 9.5 Hz, 1H), 4.50 (s, 2H), 1.88 (q, *J* = 6.5, 6.1 Hz, 4H), 1.09 (t, *J*

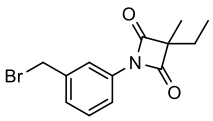
= 7.6 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.9, 139.2, 134.1, 129.7, 127.3, 119.3, 118.9, 72.1, 32.4, 23.8, 9.2.

**(64a) 1-(3-(Bromomethyl)phenyl)-3,3-dipropylazetidine-2,4-dione**



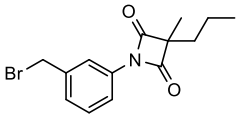
Yellowish oil; yield: 80% (1.46 g, 4.32 mmol); $R_f$ = 0.55 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.82 (d, *J* = 8.3 Hz, 1H), 7.50 – 7.37 (m, 1H), 7.32 (d, *J* = 7.9 Hz, 1H), 4.50 (s, 2H), 1.85 – 1.76 (m, 4H), 1.57 – 1.46 (m, 4H), 1.05 – 0.92 (m, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.1, 139.2, 134.2, 129.8, 127.3, 119.3, 118.9, 71.1, 33.2, 32.4, 18.3, 14.2.

**(64b) 1-(3-(Bromomethyl)phenyl)-3-ethyl-3-methylazetidine-2,4-dione**



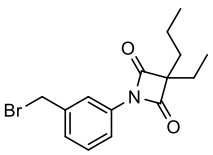
Colorless oil; 64% (725 mg, 2.45 mmol); $R_f$ = 0.26 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.82 (d, *J* = 8.6 Hz, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 4.50 (s, 2H), 1.88 (q, *J* = 7.5 Hz, 2H), 1.50 (s, 3H), 1.10 (t, *J* = 7.6 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.3, 139.2, 134.4, 129.7, 127.2, 119.3, 118.9, 66.7, 32.4, 25.4, 16.0, 9.3; LC-MS (ESI) $R_t$ = 10.59 min; *m/z* calcd for $C_{13}H_{14}NO_2^+$: 216.1, found: 216.0.

**(64c) 1-(3-(Bromomethyl)phenyl)-3-methyl-3-propylazetidine-2,4-dione**



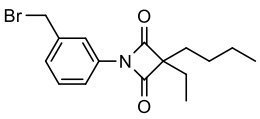
Colorless oil; yield: 64% (854 mg, 2.75 mmol); $R_f$ = 0.26 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.81 (d, *J* = 8.1 Hz, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 4.50 (s, 2H), 1.85 – 1.76 (m, 2H), 1.56 – 1.45 (m, 5H), 1.00 (t, *J* = 7.4 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.4, 139.2, 134.5, 129.7, 127.3, 119.3, 118.9, 66.2, 34.3, 32.4, 18.4, 16.4, 14.1; LC-MS (ESI) $R_t$ = 11.11 min; *m/z* calcd for $C_{14}H_{16}NO_2^+$: 230.1, found: 230.0.

**(64d) 1-(3-(Bromomethyl)phenyl)-3-ethyl-3-propylazetidine-2,4-dione**



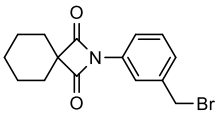
Colorless oil; yield: 65% (506 mg, 1.56 mmol); $R_f$ = 0.44 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 7.9 Hz, 1H), 4.50 (s, 2H), 1.88 (q, *J* = 7.5 Hz, 2H), 1.83 – 1.76 (m, 2H), 1.56 – 1.45 (m, 2H), 1.09 (t, *J* = 7.5 Hz, 3H), 0.99 (t, *J* = 7.3 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.1, 139.2, 134.3, 129.8, 127.4, 119.4, 119.0, 71.7, 33.0, 32.4, 24.3, 18.4, 14.2, 9.3; LC-MS (ESI) $R_t$ = 11.59 min; *m/z* calcd for $C_{15}H_{18}BrNO_2^+$: 324.1, found: 324.0.

**(64e) 1-(3-(Bromomethyl)phenyl)-3-butyl-3-ethylazetidine-2,4-dione**



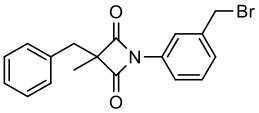
Yellowish oil; yield: 68% (1.42 g, 4.19 mmol); $R_f$ = 0.36 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.82 (d, *J* = 8.1 Hz, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 7.9 Hz, 1H), 4.50 (s, 2H), 1.92 – 1.76 (m, 4H), 1.50 – 1.32 (m, 4H), 1.13 – 1.04 (m, 3H), 0.96 – 0.87 (m, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.2, 139.3, 134.3, 129.8, 127.4, 119.4, 119.0, 71.7, 32.4, 30.6, 27.0, 24.4, 22.9, 13.8, 9.3.

**(64f) 2-(3-(Bromomethyl)phenyl)-2-azaspiro[3.5]nonane-1,3-dione**



Yellowish solid; yield: 69% (1.58 g, 4.90 mmol); $R_f$ = 0.37 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.78 (d, *J* = 8.2 Hz, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.29 (d, *J* = 7.6 Hz, 1H), 4.48 (s, 2H), 1.98 – 1.92 (m, 4H), 1.82 – 1.73 (m, 4H), 1.59 – 1.51 (m, 2H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.6, 139.2, 134.8, 129.8, 127.2, 119.4, 119.0, 65.9, 32.6, 28.3, 25.1, 22.7; LC-MS (ESI) $R_t$ = 11.35 min; *m/z* calcd for $C_{15}H_{17}BrNO_2^+$: 322.0, found: 321.9.

**(64g) 3-Benzyl-1-(3-(bromomethyl)phenyl)-3-methylazetidine-2,4-dione**

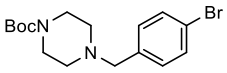


Yellowish oil; yield: 30% (189 mg, 0.528 mmol); $R_f$ = 0.22 (silica gel, EtOAc:cyclohexane, 1:30); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.32 – 7.26 (m, 5H), 7.25 – 7.24 (m, 1H), 7.11 (d, *J* = 7.0 Hz, 1H), 4.41 (s, 2H), 3.11 (s, 2H), 1.55 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.6, 139.1, 134.8, 130.2, 129.7, 129.6, 128.6, 128.5, 127.5, 127.5, 119.7, 119.2, 67.9, 38.7, 32.3, 16.5; LC-MS (ESI) $R_t$ = 10.96 min; *m/z* calcd for $C_{18}H_{17}BrNO_2^+$: 358.0, found: 357.8.

*General method for the formation of benzylic piperazines leading to intermediates 70a-f*

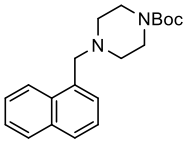
The corresponding benzyl bromide (1 equiv) and *tert*-butyl piperazine-1-carboxylate (1.1 equiv) were dissolved in THF (0.4 M with respect to the limiting reagent) and $Et_3N$ (1.1 equiv) was subsequently added dropwise to the mixture. The resulting mixture was stirred at rt for 12 h until it was filtrated, and the filtrate was concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, EtOAc:cyclohexane, 1:4 for **69a** and **69d**, 1:7 for **69b**, **69e-f** and 2:1 for **69c**), affording piperazines **70a-f** in pure form.

**(70a) *tert*-Butyl 4-(4-bromobenzyl)piperazine-1-carboxylate**



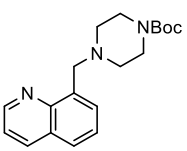
Colorless oil; yield 98% (1.45 g, 4.45 mmol); $R_f$ = 0.24 (silica gel, EtOAc:cyclohexane, 1:4); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 8.4 Hz, 2H), 7.18 (d, *J* = 8.2 Hz, 2H), 3.47 – 3.36 (m, 6H), 2.36 (s, 4H), 1.43 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 154.8, 136.9, 131.5, 130.8, 121.1, 79.7, 62.3, 52.9, 28.5; LC-MS (ESI) $R_t$ = 6.31 min; *m/z* calcd for $C_{16}H_{24}BrN_2O_2^+$: 355.1, found: 355.0.

**(70b) *tert*-Butyl 4-(naphthalen-1-ylmethyl)piperazine-1-carboxylate**



Colorless oil; yield: 98% (1.45 g, 4.45 mmol); $R_f$ = 0.27 (silica gel, EtOAc:cyclohexane, 1:7); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 7.7 Hz, 1H), 7.82 – 7.76 (m, 1H), 7.57 – 7.46 (m, 2H), 7.44 – 7.38 (m, 2H), 3.89 (s, 2H), 3.45 (s, 4H), 2.46 (s, 4H), 1.52 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 154.6, 133.8, 133.5, 132.4, 128.3, 128.0, 127.3, 125.6, 125.5, 125.0, 124.6, 79.3, 61.1, 52.9, 28.3; LC-MS (ESI) $R_t$ = 6.33 min; *m/z* calcd for $C_{20}H_{27}N_2O_2^+$: 327.2, found: 327.0.

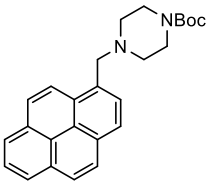
**(70c) *tert*-Butyl 4-(quinolin-8-ylmethyl)piperazine-1-carboxylate**



Yellowish oil; yield: 86% (631 mg, 1.93 mmol); $R_f$ = 0.46 (silica gel, EtOAc:cyclohexane, 2:1); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.96 – 8.90 (m, 1H), 8.18 – 8.09 (m, 1H), 7.89 (d, J = 7.1 Hz, 1H), 7.72 (d, J = 9.7 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.42 – 7.34 (m, 1H), 4.32 (s, 2H), 3.53 (s, 4H), 2.60 (s, 4H), 1.49 (s, 9H); $^{13}$C NMR (101

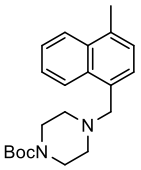
MHz, $CDCl_3$) δ 154.7, 149.3, 146.8, 136.2, 135.9, 129.0, 128.1, 126.7, 126.1, 120.8, 79.3, 56.9, 53.2, 28.3; LC-MS (ESI) $R_t$ = 5.70 min; *m/z* calcd for $C_{19}H_{26}N_3O_2^+$: 328.2, found: 328.0.

**(70d) *tert*-Butyl 4-(pyren-1-ylmethyl)piperazine-1-carboxylate**



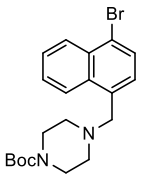

White solid; yield: 55% (0.373 g, 0.931 mmol); $R_f$ = 0.32 (silica gel, EtOAc:cyclohexane, 1:4); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 9.3 Hz, 1H), 8.18 (t, *J* = 7.2 Hz, 2H), 8.11 (d, *J* = 7.9 Hz, 2H), 8.04 (s, 2H), 8.04 – 7.93 (m, 2H), 4.18 (s, 2H), 3.44 (s, 4H), 2.53 (s, 4H), 1.47 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 154.9, 131.4, 131.0, 131.0, 130.1, 128.4, 127.5, 127.4, 126.0, 125.2, 124.9, 124.5, 124.2, 79.7, 53.2, 28.6; LC-MS (ESI) $R_t$ = 7.91 min; *m/z* calcd for $C_{26}H_{29}N_2O_2^+$: 401.2, found: 400.9.

**(70e) *tert*-Butyl 4-((4-methylnaphthalen-1-yl)methyl)piperazine-1-carboxylate**




Colorless oil; yield: 82% (1.46 g, 4.28 mmol); $R_f$ = 0.28 (silica gel, EtOAc:cyclohexane, 1:7); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.39 – 8.33 (m, 1H), 8.06 – 7.98 (m, 1H), 7.59 – 7.50 (m, 2H), 7.32 – 7.21 (m, 2H), 3.86 (s, 2H), 3.45 (s, 4H), 2.69 (s, 3H), 2.45 (s, 4H), 1.52 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 154.6, 134.0, 133.0, 132.5, 131.6, 127.2, 125.7, 125.4, 125.3, 125.3, 124.3, 79.2, 61.2, 52.89, 28.35, 19.41; LC-MS (ESI) $R_t$ = 6.66 min; *m/z* calcd for $C_{21}H_{29}N_2O_2^+$: 341.2, found: 341.0.

**(70f) *tert*-Butyl 4-((4-bromonaphthalen-1-yl)methyl)piperazine-1-carboxylate**

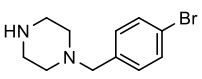



Colorless oil; yield: 96% (1.30 g, 3.21 mmol); $R_f$ = 0.26 (silica gel, EtOAc:cyclohexane, 1:7); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.29 (t, *J* = 7.4 Hz, 2H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.57 (p, *J* = 6.9 Hz, 2H), 7.23 (d, *J* = 7.7 Hz, 1H), 3.82 (s, 2H), 3.43 (s, 4H), 2.44 (s, 4H), 1.49 (s, 9H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 154.7, 133.7, 133.6, 132.1, 129.1, 127.7, 127.5, 127.0, 126.5, 125.1, 122.7, 79.4, 60.8, 53.0, 28.4; LC-MS (ESI) $R_t$ = 7.86 min; *m/z* calcd for $C_{20}H_{26}BrN_2O_2^+$: 405.1, found: 405.0.

*General Boc-deprotection method leading to intermediates **60a**, **71a-e***

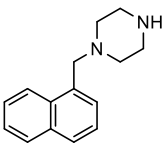
The corresponding Boc-protected amine **69a-f** or the corresponding Boc-protected aniline (1 equiv) was dissolved in DCM (0.2 M) and cooled to 0 °C. Afterwards, TFA (10 equiv) was added to the solution and the mixture was stirred at rt for 16 h or for the indicated time. Upon quenching the mixture through addition of sat. aqueous $NaHCO_3$ (until a pH of 6 was reached), it was stirred at rt for 1 h. Then, the organic layer was separated, and the aqueous layer was extracted two times with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The crude products **60a**, **71a-e** were used as TFA salts without further purification in the next step.

**(60a) 1-(4-Bromobenzyl)piperazine**



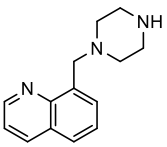

LC-MS (ESI) $R_t$ = 4.14 min; *m/z* calcd for $C_{11}H_{16}BrN_2^+$: 255.1, found: 255.2.

**(71a) 1-(Naphthalen-1-ylmethyl)piperazine**



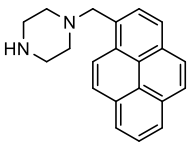
LC-MS (ESI) $R_t$ = 4.98 min; *m/z* calcd for $C_{15}H_{19}N_2^+$: 227.2, found: 227.0.

**(71b) 8-(Piperazin-1-ylmethyl)quinolone**



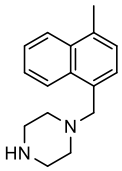
LC-MS (ESI) $R_t$ = 2.18 min; *m/z* calcd for $C_{14}H_{18}N_3^+$: 228.2, found: 228.1

**(71c) 1-(Pyren-1-ylmethyl)piperazine**



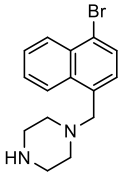
LC-MS (ESI) $R_t$ = 6.23 min; *m/z* calcd for $C_{21}H_{21}N_2^+$: 301.2, found: 300.9.

**(71d) 1-((4-Methylnaphthalen-1-yl)methyl)piperazine**



LC-MS (ESI) $R_t$ = 5.27 min; *m/z* calcd for $C_{16}H_{21}N_2^+$: 241.2, found: 241.0.

**(71e) 1-((4-Bromonaphthalen-1-yl)methyl)piperazine**

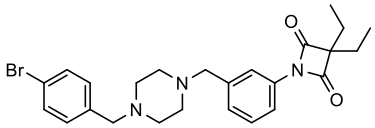


LC-MS (ESI) $R_t$ = 6.30 min; *m/z* calcd for $C_{15}H_{18}BrN_2^+$: 305.1, found: 305.2.

*General method for the substitution of benzylic bromides leading to the final products* ***6, 11-14, 39-42, 49-54***

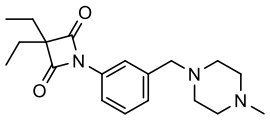
The corresponding brominated 4-oxo-β-lactam **18** or **64a-g** (1 equiv) and the corresponding piperazine **60a, 71a-e** or 1-methylpiperazine (**72**) (1.1 equiv) were suspended or dissolved in THF (0.4 M with respect to the limiting reagent) and $Et_3N$ (6 equiv) was subsequently added dropwise to the mixture. The resulting solution was stirred at rt for 12 h until it was filtrated, and the filtrate was concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, EtOAc:cyclohexane, 1:3 for **6**, 1:2 for **41, 49-53** and MeOH:DCM, 20:1 for **11**) or by prep. HPLC (**12-14, 39-40, 42** and **54**) affording the final 4-oxo-β-lactams **6**, **11-14, 39-42** and **49-54** in pure form.

**(6) 1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (synthetic route II)**



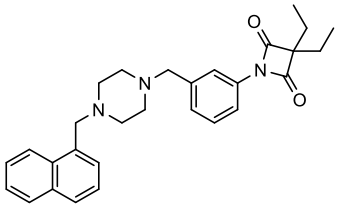
Colorless oil; yield: 49% (0.379 g, 0.782 mmol); $R_f$ = 0.22 (silica gel, EtOAc:cyclohexane, 1:3); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.71 (d, *J* = 8.1 Hz, 1H), 7.44 – 7.40 (m, 2H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.25 – 7.17 (m, 3H), 3.54 (s, 2H), 3.49 (s, 2H), 2.52 (s, 8H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.06 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.4, 133.9, 131.5, 131.0, 129.3, 127.6, 121.2, 119.9, 118.2, 72.1, 62.4, 62.2, 53.0, 52.9, 24.1, 9.4; LC-MS (ESI) $R_t$ = 6.99 min; *m/z* calcd for $C_{25}H_{31}N_3O_2^+$: 484.2, found: 484.3; HRMS (ESI): calcd for $C_{25}H_{30}N_3O_2$: 483.1521, found (deconvoluted): 483.1517.

**(11) 3,3-Diethyl-1-(3-((4-methylpiperazin-1-yl)methyl)phenyl)azetidine-2,4-dione**



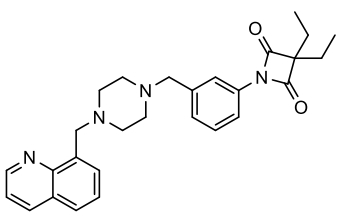
Colorless oil; yield: 59% (0.877 g, 2.66 mmol); $R_f$ = 0.10 (silica gel, DCM:MeOH, 20:1); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.69 (d, *J* = 8.2 Hz, 1H), 7.32 (t, *J* = 7.9 Hz, 1H), 7.20 (d, *J* = 7.7 Hz, 1H), 3.52 (s, 2H), 2.57 (s, 8H), 2.38 (s, 3H), 1.82 (q, *J* = 7.5 Hz, 4H), 1.04 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.3, 139.7, 133.9, 129.2, 127.4, 119.7, 118.1, 72.1, 62.2, 54.8, 52.2, 45.4, 24.0, 9.3; LC-MS (ESI) $R_t$ = 6.17 min; *m/z* calcd for $C_{19}H_{28}N_3O_2^+$: 330.2, found: 330.2; HRMS (ESI): calcd for $C_{19}H_{27}N_3O_2$: 329.2103, found: (deconvoluted) 329.2105.

**(12) 3,3-Diethyl-1-(3-((4-(naphthalen-1-ylmethyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione**



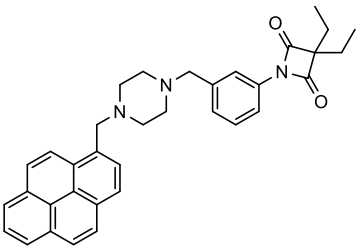
White solid (TFA salt); yield: 50% (0.450 g, 0.988 mmol); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 8.4 Hz, 1H), 7.97 – 7.85 (m, 4H), 7.67 – 7.40 (m, 5H), 7.31 (d, *J* = 7.7 Hz, 1H), 4.66 (s, 2H), 4.15 (s, 2H), 3.57 (s, 4H), 3.46 (s, 4H), 1.83 (q, *J* = 7.7 Hz, 4H), 1.03 (t, *J* = 7.3 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.0, 134.8, 134.0, 132.1, 131.6, 131.3, 130.7, 129.7, 129.4, 128.9, 127.9, 126.8, 125.5, 124.4, 122.7, 121.3, 120.9, 72.6, 60.5, 57.3, 48.8, 48.7, 24.0, 9.2; LC-MS (ESI) $R_t$ = 7.97 min; *m/z* calcd for $C_{29}H_{34}N_3O_2^+$: 456.3, found: 456.3; HRMS (ESI): calcd for $C_{29}H_{33}N_3O_2$: 455.2573, found: (deconvoluted) 455.2578.

**(13) 3,3-Diethyl-1-(3-((4-(quinolin-8-ylmethyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione**



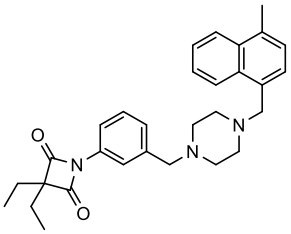
White solid (TFA salt); yield: 56% (0.324 g, 0.710 mmol); $^1$H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.65 (d, *J* = 8.3 Hz, 1H), 8.11 (d, *J* = 8.3 Hz, 1H), 8.01 – 7.90 (m, 3H), 7.87 – 7.81 (m, 1H), 7.78 (d, *J* = 7.0 Hz, 1H), 7.46 (t, *J* = 8.2 Hz, 1H), 7.32 (d, *J* = 7.8 Hz, 1H), 4.71 (s, 2H), 4.29 (s, 2H), 3.59 (s, 4H), 3.44 (s, 4H), 1.83 (q, *J* = 7.2, 6.8 Hz, 4H), 1.03 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.9, 148.3, 142.7, 141.4, 135.7, 134.8, 130.8, 130.7, 129.3, 129.1, 128.9, 128.6, 125.6, 122.5, 121.5, 121.2, 117.1, 114.3, 72.6, 60.5, 58.1, 53.0, 49.5, 49.3, 24.0, 9.2; LC-MS (ESI) $R_t$ = 7.43 min; *m/z* calcd for $C_{28}H_{33}N_4O_2^+$: 457.3, found: 457.2; HRMS (ESI): *m/z* calcd for $C_{28}H_{33}N_4O_2^+$: 457.2598 found: 457.2593.

**(14) 3,3-Diethyl-1-(3-((4-(pyren-1-ylmethyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione**



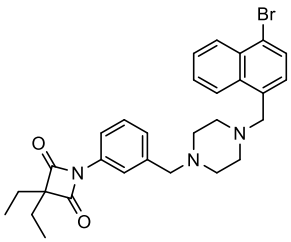
White solid (TFA salt); yield: 63% (0.300 g, 0.566 mmol); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.30 (d, *J* = 9.3 Hz, 1H), 8.22 – 8.17 (m, 2H), 8.17 – 8.11 (m, 2H), 8.08 (d, *J* = 8.9 Hz, 1H), 8.06 – 7.97 (m, 3H), 7.83 – 7.76 (m, 2H), 7.37 (t, *J* = 8.2 Hz, 1H), 7.28 – 7.22 (m, 1H), 4.81 (s, 2H), 4.02 (s, 2H), 3.37 (s, 4H), 3.20 (s, 4H), 1.78 (q, *J* = 7.5 Hz, 4H), 0.98 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.1, 131.7, 129.7, 129.2, 128.8, 126.5, 125.0, 122.1, 121.0, 120.3, 72.4, 57.7, 49.6, 49.4, 49.2, 23.9, 9.2; LC-MS (ESI) $R_t$ = 8.92 min; *m/z* calcd for $C_{35}H_{36}N_3O_2^+$: 530.3, found: 530.0; HRMS (ESI): calcd for $C_{35}H_{35}N_3O_2$: 529.2729, found: (deconvoluted) 529.2722.

**(39) 3,3-Diethyl-1-(3-((4-((5-methylnaphthalen-1-yl)methyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione**



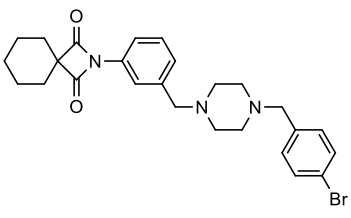
White solid (TFA salt); yield: 52% (0.480 g, 0.847 mmol); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.11 – 7.97 (m, 2H), 7.97 – 7.88 (m, 2H), 7.63 – 7.56 (m, 2H), 7.52 (d, *J* = 7.2 Hz, 1H), 7.46 (t, *J* = 7.0 Hz, 1H), 7.32 (dd, *J* = 15.7, 7.4 Hz, 2H), 4.75 (s, 2H), 4.25 (s, 2H), 3.70 (s, 4H), 3.60 (s, 4H), 2.70 (s, 3H), 1.83 (q, *J* = 6.6, 5.7 Hz, 4H), 1.03 (t, *J* = 6.6 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.9, 139.1, 134.9, 133.2, 131.9, 131.4, 130.8, 129.0, 128.7, 127.7, 126.8, 126.3, 125.5, 122.9, 121.34, 121.25, 121.21, 117.2, 114.3, 72.6, 60.5, 57.7, 48.5, 48.5, 23.9, 19.8, 9.2; LC-MS (ESI) $R_t$ = 8.34 min; *m/z* calcd for $C_{30}H_{36}N_3O_2^+$: 470.3, found: 470.2; HRMS (ESI): calcd for $C_{30}H_{35}N_3O_2$: 469.2729, found: (deconvoluted) 469.2731.

**(40) 1-(3-((4-((4-Bromonaphthalen-1-yl)methyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione**



White solid (TFA salt); yield: 58% (0.585 g, 1.09 mmol); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.37 – 8.30 (m, 1H), 8.13 – 8.06 (m, 1H), 7.92 (d, *J* = 12.4 Hz, 2H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.69 – 7.62 (m, 2H), 7.52 – 7.42 (m, 2H), 7.32 (d, *J* = 7.7 Hz, 1H), 4.58 (s, 2H), 4.18 (s, 2H), 3.53 (s, 4H), 3.46 (s, 4H), 1.84 (q, *J* = 6.6, 5.7 Hz, 4H), 1.04 (t, *J* = 7.2 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.0, 134.8, 133.2, 132.5, 131.2, 130.8, 129.7, 129.4, 129.0, 128.7, 128.6, 128.2, 123.4, 121.3, 121.1, 72.6, 60.5, 57.4, 49.1, 48.8, 24.0, 9.2; LC-MS (ESI) $R_t$ = 8.66 min; *m/z* calcd for $C_{29}H_{33}BrN_3O_2^+$: 534.2, found: 534.3; HRMS (ESI): *m/z* calcd for $C_{29}H_{33}BrN_3O_2^+$: 534.1751, found: 534.1746.

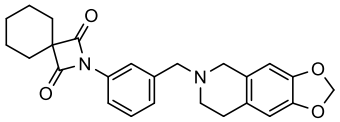
**(41) 2-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-2-azaspiro[3.5]nonane-1,3-dione**



White solid; yield: 55% (503 mg, 1.01 mmol); $R_f$ = 0.31 (silica gel, EtOAc:cyclohexane, 1:2); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.71 (d, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 2H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.27 – 7.18 (m, 3H), 3.54 (s, 2H), 3.48 (s, 2H), 2.51 (s, 8H), 1.99 – 1.91 (m, 4H), 1.81 – 1.72 (m, 4H), 1.59 – 1.50 (m, 2H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.8, 139.6, 137.0, 134.4, 131.5, 131.0, 129.2, 127.4, 121.1, 119.8, 118.0,

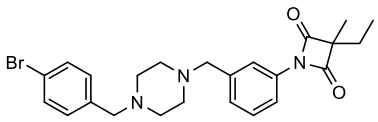
65.7, 62.5, 62.3, 53.98, 52.92, 28.4, 25.1, 22.8; LC-MS (ESI) $R_t$ = 8.24 min; *m/z* calcd for $C_{26}H_{31}BrN_3O_2^+$: 496.2, found: 496.4; HRMS (ESI): *m/z* calcd for $C_{26}H_{31}BrN_3O_2^+$: 496.1594, found: 496.1599.

**(42) 2-(3-((7,8-Dihydro-[1,3]dioxolo[4,5-g]isoquinolin-6(5H)-yl)methyl)phenyl)-2-azaspiro[3.5]nonane-1,3-dione**



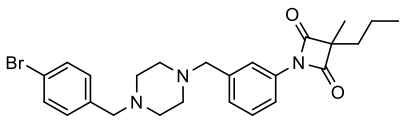
White solid (TFA salt); yield: 56% (222 mg, 0.431 mmol); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.95 – 7.87 (m, 2H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 6.60 (s, 1H), 6.48 (s, 1H), 5.93 (s, 2H), 4.41 – 4.22 (m, 3H), 3.99 (s, 1H), 3.71 (s, 1H), 3.36 – 3.10 (m, 2H), 2.98 (s, 1H), 1.98 – 1.90 (m, 4H), 1.80 – 1.69 (m, 4H), 1.58 – 1.48 (m, 2H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.5, 148.3, 147.4, 135.2, 130.7, 129.8, 128.8, 123.7, 121.2, 120.7, 119.2, 108.5, 106.7, 101.6, 66.2, 57.9, 52.0, 48.8, 28.3, 25.0, 24.5, 22.7; LC-MS (ESI) $R_t$ = 7.05 min; *m/z* calcd for $C_{25}H_{27}N_2O_4^+$: 419.2, found: 419.2; HRMS (ESI): calcd for $C_{25}H_{26}N_2O_4$: 418.1893, found: (deconvoluted) 418.1894.

**(49) 1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3-ethyl-3-methylazetidine-2,4-dione**



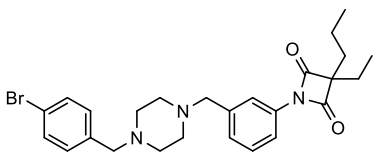
Yellowish oil; yield: 58% (271 mg, 0.576 mmol); $R_f$ = 0.28 (silica gel, EtOAc:cyclohexane, 1:2); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.36 (t, *J* = 7.8 Hz, 1H), 7.25 (d, *J* = 7.6 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 3.54 (s, 2H), 3.47 (s, 2H), 2.50 (s, 8H), 1.86 (q, *J* = 7.5 Hz, 2H), 1.48 (s, 3H), 1.09 (t, *J* = 7.5 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.6, 139.8, 137.1, 134.1, 131.4, 130.9, 129.2, 127.5, 120.9, 119.7, 118.0, 66.6, 62.5, 62.2, 53.0, 53.0, 25.5, 16.1, 9.4; LC-MS (ESI) $R_t$ = 6.93 min; *m/z* calcd for $C_{24}H_{29}BrN_3O_2^+$: 470.1, found: 470.3; HRMS (ESI): calcd for $C_{24}H_{28}BrN_3O_2$: 469.1365, found: (deconvoluted) 469.1366.

**(50) 1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3-methyl-3-propylazetidine-2,4-dione**



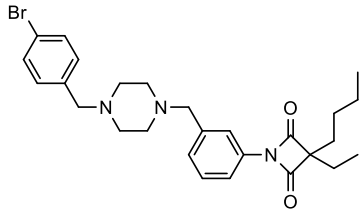
Yellowish oil; yield: 60% (233 mg, 0.481 mmol); $R_f$ = 0.29 (silica gel, EtOAc:cyclohexane, 1:2); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.72 (d, *J* = 8.9 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 2H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.25 (d, *J* = 7.8 Hz, 1H), 7.21 (d, *J* = 8.1 Hz, 2H), 3.54 (s, 2H), 3.47 (s, 2H), 2.50 (s, 8H), 1.84 – 1.73 (m, 2H), 1.59 – 1.41 (m, 5H), 0.98 (t, *J* = 7.3 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.7, 139.8, 137.2, 134.1, 131.4, 130.9, 129.2, 127.5, 120.9, 119.7, 118.0, 66.1, 62.5, 62.3, 53.0, 52.97, 34.5, 18.5, 16.5, 14.2; LC-MS (ESI) $R_t$ = 7.28 min; *m/z* calcd for $C_{25}H_{31}BrN_3O_2^+$: 484.2, found: 484.3; HRMS (ESI): calcd for $C_{25}H_{30}BrN_3O_2$: 483.1521, found: (deconvoluted) 483.1520.

**(51) 1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3-ethyl-3-propylazetidine-2,4-dione**



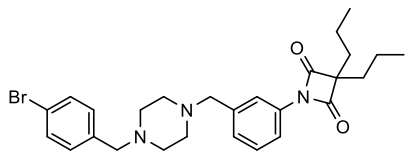
Yellowish oil; yield: 48% (149 mg, 0.299 mmol); $R_f$ = 0.37 (silica gel, EtOAc:cyclohexane, 1:2); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.25 (d, *J* = 7.7 Hz, 1H), 7.21 (d, *J* = 8.2 Hz, 2H), 3.54 (s, 2H), 3.47 (s, 2H), 2.50 (s, 8H), 1.86 (q, *J* = 7.5 Hz, 2H), 1.82 – 1.74 (m, 2H), 1.55 – 1.42 (m, 2H), 1.08 (t, *J* = 7.5 Hz, 3H), 0.97 (t, *J* = 7.3 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.4, 139.8, 137.1, 133.9, 131.4, 130.9, 129.2, 127.5, 121.0, 119.8, 118.0, 71.5, 62.5, 62.3, 53.0, 53.0, 33.0, 24.4, 18.4, 14.3, 9.3; LC-MS (ESI) $R_t$ = 7.35 min; *m/z* calcd for $C_{26}H_{33}BrN_3O_2^+$: 498.2, found: 498.2; HRMS (ESI): calcd for $C_{26}H_{32}BrN_3O_2$: 497.1678, found: (deconvoluted) 497.1673.

**(52) 1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3-butyl-3-ethylazetidine-2,4-dione**



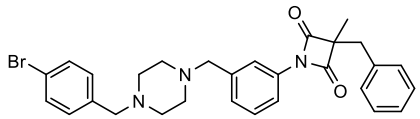
Colorless oil; yield: 44% (402 mg, 0.784 mmol); $R_f$ = 0.37 (silica gel, EtOAc:cyclohexane, 1:2); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.74 (d, *J* = 8.2 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 2H), 7.36 (t, *J* = 7.8 Hz, 1H), 7.26 (d, *J* = 7.7 Hz, 1H), 7.21 (d, *J* = 8.1 Hz, 2H), 3.54 (s, 2H), 3.47 (s, 2H), 2.50 (s, 8H), 1.87 (q, *J* = 7.5 Hz, 2H), 1.83 – 1.77 (m, 2H), 1.51 – 1.31 (m, 4H), 1.08 (t, *J* = 7.5 Hz, 3H), 0.91 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.3, 139.8, 137.2, 133.8, 131.3, 130.8, 129.1, 127.4, 120.9, 119.7, 117.9, 71.3, 62.4, 62.2, 53.0, 30.6, 27.0, 24.3, 22.9, 13.8, 9.3; LC-MS (ESI) $R_t$ = 8.16 min; *m/z* calcd for $C_{27}H_{35}BrN_3O_2^+$: 512.2, found: 512.4; HRMS (ESI): calcd for $C_{27}H_{34}BrN_3O_2$: 511.1834, found: (deconvoluted) 511.1829.

**(53) 1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3,3-dipropylazetidine-2,4-dione**



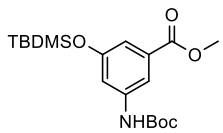
White solid; yield: 56% (1.25 g, 2.44 mmol); $R_f$ = 0.42 (silica gel, EtOAc:cyclohexane, 1:2); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.74 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.26 (d, *J* = 7.8 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 3.55 (s, 2H), 3.49 (s, 2H), 2.52 (s, 8H), 1.84 – 1.74 (m, 4H), 1.57 – 1.45 (m, 4H), 0.98 (t, *J* = 7.4 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.3, 139.6, 136.9, 133.9, 131.4, 130.9, 129.1, 127.4, 121.0, 119.7, 117.9, 70.9, 62.3, 62.1, 52.8, 52.8, 33.3, 29.5, 18.3, 14.2; LC-MS (ESI) $R_t$ = 7.91 min; *m/z* calcd for $C_{27}H_{35}BrN_3O_2^+$: 512.2, found: 512.3; HRMS (ESI): calcd for $C_{27}H_{34}BrN_3O_2$: 511.1834, found: (deconvoluted) 511.1830.

**(54) 3-Benzyl-1-(3-((4-(4-bromobenzyl)piperazin-1-yl)methyl)phenyl)-3-methylazetidine-2,4-dione**



White solid (TFA salt); yield: 31% (94 mg, 0.149 mmol); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 1H), 7.66 – 7.60 (m, 2H), 7.59 – 7.54 (m, 3H), 7.44 – 7.35 (m, 2H), 7.34 – 7.25 (m, 8H), 7.21 – 7.16 (m, 1H), 4.11 (s, 2H), 4.07 (s, 2H), 3.47 – 3.36 (m, 8H), 3.10 (s, 2H), 1.55 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.5, 143.7, 141.4, 134.9, 134.2, 132.8, 132.5, 130.5, 129.7, 128.9, 128.7, 127.5, 121.4, 121.0, 68.2, 60.4, 60.2, 48.8, 38.7, 16.5; LC-MS (ESI) $R_t$ = 7.60 min; *m/z* calcd for $C_{29}H_{31}BrN_3O_2^+$: 532.2, found: 532.3; HRMS (ESI): calcd for $C_{29}H_{30}BrN_3O_2$: 531.1521, found: (deconvoluted) 531.1517.

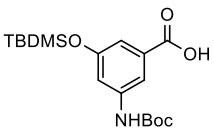
**(73b-precursor) Methyl 3-((*tert*-butoxycarbonyl)amino)-5-((*tert*-butyldimethylsilyl)oxy)benzoate**



*tert*-Butyl(chlor)dimethylsilan (1.3 equiv) was added to a solution of methyl 3-amino-5-hydroxybenzoate (1 equiv) and imidazole (1.5 equiv) in THF (0.27 M with respect to the limiting reagent). The resulting mixture was stirred at rt for 12 h and then quenched with $H_2O$. THF was removed in vacuo and the remaining aqueous layer was extracted three times with $Et_2O$. The combined organic layers were washed with brine, dried over $Na_2SO_4$, concentrated in vacuo and the crude product (8.764 g) was directly used in the next step without further purification. The crude silyl-protected hydroxybenzoate (1 equiv) and DIPEA (4 equiv) were dissolved in $H_2O$/Dioxane (1:1, 0.39 M with respect to the limiting reagent) and the resulting solution was cooled to 0 °C. $Boc_2O$ (1.1 equiv) was added and the final mixture was stirred at rt for 18 h. Then, the mixture was extracted three times with EtOAc, the combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography

(silica gel, EtOAc:cyclohexane, 1:9), affording the titled compound in pure form. White solid; yield: 72% over two steps (8.23 g, 21.6 mmol); $R_f$ = 0.48 (silica gel, EtOAc:cyclohexane, 1:9); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.60 (m, 1H), 7.31 (s, 1H), 7.20 – 7.14 (m, 1H), 7.04 (s, 1H), 3.89 (s, 3H), 1.51 (s, 9H), 0.98 (s, 9H), 0.22 (s, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 166.9, 156.3, 152.7, 140.0, 131.6, 115.4, 114.8, 112.7, 80.7, 52.3, 28.4, 25.7, 18.2, -4.39; LC-MS (ESI) $R_t$ = 12.80 min; *m/z* calcd for $C_{15}H_{24}NO_5Si^+$ [-$^t$Bu$^+$, +$H_2$]: 326.1, found: 325.9.

**(73b) 3-((*tert*-Butoxycarbonyl)amino)-5-((*tert*-butyldimethylsilyl)oxy)benzoic acid**

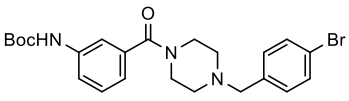



Methylbenzoate **73b-precursor** (1 equiv) was dissolved in DCE (0.13 M with respect to the limiting reagent) and trimethyltin hydroxide (10 equiv) was subsequently added. The resulting mixture was stirred at 80 °C for 6 h. Then, the solvent was removed in vacuo, the remaining residue was suspended in EtOAc, washed with 0.1 M aqueous $KHSO_4$, brine, dried over $Na_2SO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, EtOAc:cyclohexane, 1:2), affording the titled compound **73b** in pure form. White solid; yield: 57% (1.09 g, 2.97 mmol); $R_f$ = 0.21 (silica gel, EtOAc:cyclohexane, 1:2); $^1$H NMR (400 MHz, $CD_3OD$) δ 10.59 (s, 1H), 9.22 (s, 1H), 8.88 (s, 1H), 8.70 – 8.65 (m, 1H), 3.06 (s, 9H), 2.55 (s, 9H), 1.78 (s, 6H); $^{13}$C NMR (101 MHz, $CD_3OD$) δ 169.5, 157.3, 155.0, 154.9, 142.1, 142.0, 133.3, 132.6, 116.0, 115.8, 115.7, 114.2, 114.1, 81.0, 28.7, 26.1, 19.0, -4.3; LC-MS (ESI) $R_t$ = 11.52 min; *m/z* calcd for $C_{13}H_{22}NO_3Si^+$ [-Boc$^+$, +$H_2$]: 268.1, found: 268.3.

*General method for the amide bond formation leading to intermediates **74a-b** and **77***

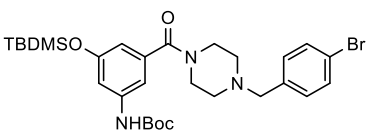
1-(4-Bromobenzyl)piperazine **60a** (1 equiv) and DIPEA (2.2 equiv) were dissolved in DMF (0.16 M with respect to the limiting reagent) and the mixture was cooled to 0 °C. Then, the corresponding benzoic acid **73a**, **73b** or **76** (1 equiv) and HATU (1.1 equiv) were added, and the mixture was stirred at rt for 12 h. The solvent was removed in vacuo and the remaining residue was dissolved in EtOAc. The resulting organic layer was washed with sat. aqueous $KHSO_4$, sat. aqueous $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, EtOAc:cyclohexane, 1:1 for **74b**, MeOH:DCM, 1:14 for **74a** and **77**), affording intermediates **74a-b** and **77** in pure form.

**(74a) *tert*-Butyl (3-(4-(4-bromobenzyl)piperazine-1-carbonyl)phenyl)carbamate**




White solid; yield: 99% (1.97 g, 4.15 mmol); $R_f$ = 0.46 (silica gel, MeOH:DCM, 1:14); $^1$H NMR (400 MHz, $CD_3OD$) δ 7.53 (s, 1H), 7.49 – 7.42 (m, 3H), 7.32 (t, *J* = 7.9 Hz, 1H), 7.25 (d, *J* = 8.1 Hz, 2H), 7.02 – 6.98 (m, 1H), 3.74 (s, 2H), 3.50 (s, 2H), 3.44 (s, 2H), 2.51 (s, 2H), 2.39 (s, 2H), 1.51 (s, 9H); $^{13}$C NMR (101 MHz, $CD_3OD$) δ 172.2, 155.0, 141.1, 138.0, 137.2, 132.5, 132.2, 130.2, 122.1, 121.8, 120.9, 117.8, 81.1, 62.8, 54.1, 53.7, 43.2, 38.9, 28.7; LC-MS (ESI) $R_t$ = 7.03 min; *m/z* calcd for $C_{23}H_{29}BrN_3O_3^+$: 474.1, found: 474.0.

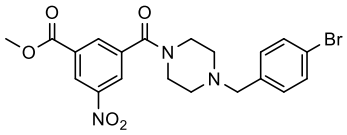
**(74b) *tert*-Butyl (3-(4-(4-bromobenzyl)piperazine-1-carbonyl)-5-((*tert*-butyldimethylsilyl)oxy)phenyl)carbamate**




Yellowish solid; yield: 38% (661 mg, 1.09 mmol); $R_f$ = 0.32 (silica gel, EtOAc:cyclohexane, 1:1); $^1$H NMR (400 MHz, $CD_3OD$) δ 9.05 – 8.99 (m, 2H), 8.84 – 8.79 (m, 2H), 8.71 – 8.68 (m, 1H), 8.62 – 8.60 (m, 1H), 8.03 – 8.00 (m, 1H), 5.34 – 5.24 (m, 2H), 5.08 (s, 2H), 5.00 (s, 2H), 4.07 (s, 2H), 3.97 (s, 2H), 3.07 (s, 9H), 2.55 (s, 9H), 1.78 (s, 6H); $^{13}$C NMR (101 MHz, $CD_3OD$) δ 171.9, 157.6, 154.8, 142.5, 138.1, 137.9,

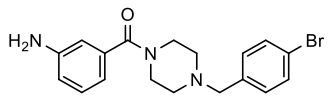
132.5, 132.3, 122.1, 113.3, 112.4, 110.8, 81.1, 62.7, 28.7, 26.1, 19.1, -4.3; LC-MS (ESI) $R_t$ = 9.50 min; *m/z* calcd for $C_{29}H_{43}BrN_3O_4Si^+$: 604.2, found: 604.0.

**(77) Methyl 3-(4-(4-bromobenzyl)piperazine-1-carbonyl)-5-nitrobenzoate**



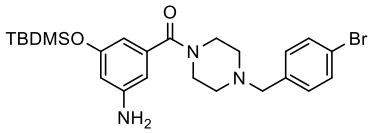
Yellowish solid; yield: 94% (3.847 g, 8.321 mmol); $R_f$ = 0.35 (silica gel, EtOAc:cyclohexane, 2:1); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.85 – 8.82 (m, 1H), 8.41 – 8.38 (m, 1H), 8.33 – 8.31 (m, 1H), 7.43 – 7.38 (m, 2H), 7.18 – 7.14 (m, 2H), 3.95 (s, 3H), 3.84 – 3.72 (m, 2H), 3.52 (s, 2H), 3.39 (s, 2H), 2.64 – 2.35 (m, 4H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 166.9, 164.4, 148.3, 137.6, 133.6, 131.7, 131.0, 126.2, 125.5, 61.9, 53.1; LC-MS (ESI) $R_t$ = 6.50 min; *m/z* calcd for $C_{20}H_{21}BrN_3O_5^+$: 462.1, found: 462.2.

**(75a) (3-Aminophenyl)(4-(4-bromobenzyl)piperazin-1-yl)methanone**



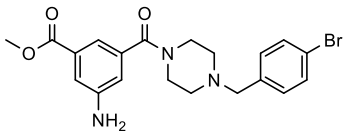
Following the general boc-deprotection method described above with carbamate **74a** and a reaction time of 16 h, delivered the titled compound **75a**, which was used in the next step without further purification. White solid; 99% (1.39 g, 3.71 mmol); $^1$H NMR (400 MHz, $CD_3OD$) δ 7.46 (d, *J* = 8.2 Hz, 2H), 7.25 (d, *J* = 8.1 Hz, 2H), 7.14 (t, *J* = 7.8 Hz, 1H), 6.78 – 6.73 (m, 1H), 6.69 – 6.66 (m, 1H), 6.62 (d, *J* = 7.5 Hz, 1H), 3.72 (s, 2H), 3.51 (s, 2H), 3.44 (s, 2H), 2.50 (s, 2H), 2.38 (s, 2H); $^{13}$C NMR (101 MHz, $CD_3OD$) δ 173.0, 149.6, 137.9, 137.4, 132.5, 132.3, 130.4, 122.1, 117.5, 116.6, 114.0, 62.8, 54.2, 53.7, 43.1, 38.9; LC-MS (ESI) $R_t$ = 5.10 min; *m/z* calcd for $C_{18}H_{21}BrN_3O^+$: 374.1, found: 374.2.

**(75b) (3-Amino-5-((*tert*-butyldimethylsilyl)oxy)phenyl)(4-(4-bromobenzyl)piperazin-1-yl)methanone**



Following the general boc-deprotection method described above with carbamate **74b** and a reaction time of 3 h, delivered the crude product **75b** (365 mg), which was used in the next step without further purification. LC-MS (ESI) $R_t$ = 7.69 min; *m/z* calcd for $C_{24}H_{35}BrN_3O_2Si^+$: 504.2, found: 504.1.

**(78) Methyl 3-amino-5-(4-(4-bromobenzyl)piperazine-1-carbonyl)benzoate**



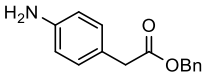
Nitrobenzoate **77** (1 equiv, 200 mg, 0.433 mmol) and Tin(II) chloride dihydrate (5 equiv, 489 mg, 2.17 mmol) were dissolved in dry EtOAc (3 mL) and the resulting mixture was stirred at 70 °C for 1 h. Then, the mixture was poured on a solution of 5% aqueous $NaHCO_3$ (10 mL) and EtOAc (5 mL) was added. The organic layer was separated and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product **78** (192 mg) was used without further purification in the next step. LC-MS (ESI) $R_t$ = 5.54 min; *m/z* calcd for $C_{20}H_{23}BrN_3O_3^+$: 432.1, found: 432.2.

*General method for the formation of O-protected (aminophenyl)acetates **81a** and **81b***

The corresponding aminophenylacetic acid **80a** or **80b** (1 equiv) and $Et_3N$ (2 equiv) were dissolved in $H_2O$/Dioxane (3:7, 0.3 M with respect to the limiting reagent). After the solution was stirred for a few minutes, $Boc_2O$ (2 equiv) was added and the resulting mixture was stirred at rt for 12 h. Then, it was neutralized with 2 M aqueous HCl, diluted with $H_2O$ and extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $Na_2SO_4$, concentrated in vacuo and the crude product was directly used in the next step without further purification. The crude boc-protected aminophenylacetic acid (1 equiv), benzylbromide (1.1 equiv) and $K_2CO_3$ (2 equiv) were suspended in acetone (0.5 M with respect to the limiting reagent) and stirred at rt for 12 h. After evaporation of the solvent in vacuo, the

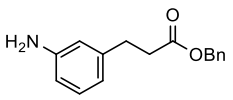
residue was dissolved in EtOAc, washed with $H_2O$ and concentrated in vacuo. The crude, fully protected product was dissolved in 4 M HCl in dioxane (0.5 M with respect to the limiting reagent) and stirred at rt for 3 h. Then, the mixture was concentrated in vacuo and the residue was dissolved in EtOAc/saturated aqueous $NaHCO_3$ (1:5, 0.4 M with respect to the limiting reagent) and it was stirred for 4 h before the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated in vacuo. Purification of the crude product by flash column chromatography (silica gel, EtOAc:cyclohexane, 1:3) afforded the *O*-protected (aminophenyl)acetates **81a** and **81b** in pure form.

**(81a) Benzyl 2-(4-aminophenyl)acetate**



Yellowish oil; yield: 74% (1.42 g, 5.89 mmol); $R_f$ = 0.36 (silica gel, EtOAc:cyclohexane, 1:3); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.34 (m, 5H), 7.13 (d, *J* = 8.0 Hz, 2H), 6.66 (d, *J* = 8.7 Hz, 2H), 5.19 (s, 2H), 3.69 (s, 2H), 3.62 (s, 2H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.9, 145.5, 135.9, 130.0, 128.4, 128.0, 128.0, 123.4, 115.1, 66.3, 40.4; LC-MS (ESI) $R_t$ = 7.05 min; *m/z* calcd for $C_{15}H_{16}NO_2^+$: 242.1, found: 242.0.

**(81b) Benzyl 3-(3-aminophenyl)propanoate**

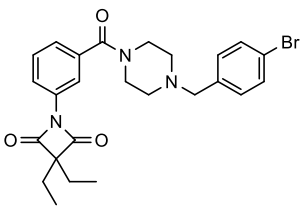


Yellowish oil; yield: 51% (1.58 g, 6.19 mmol); $R_f$ = 0.34 (silica gel, EtOAc:cyclohexane, 1:3); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.30 (m, 5H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.63 (d, *J* = 7.6 Hz, 1H), 6.59 – 6.51 (m, 2H), 5.16 (s, 2H), 3.62 (s, 2H), 2.92 (t, *J* = 7.8 Hz, 2H), 2.70 (t, *J* = 7.9 Hz, 2H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.8, 146.5, 141.7, 136.0, 129.4, 128.6, 128.2, 128.2, 118.5, 115.1, 113.2, 66.2, 35.8, 30.9; LC-MS (ESI) $R_t$ = 7.74 min; *m/z* calcd for $C_{16}H_{18}NO_2^+$: 256.1, found: 255.9.

*General method for the 4-oxo-β-lactam formation with malonyl dichloride using optimized conditions to form compounds **43** and **45***

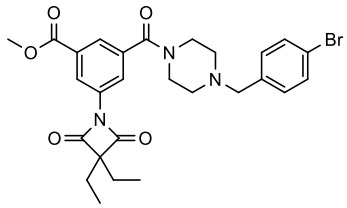
The corresponding aniline **75a** or **78** (1 equiv) and $Et_3N$ (2 equiv) were dissolved in DCM (0.16 M with respect to the limiting reagent) and subsequently added to a solution of malonyl dichloride **55** (1.5 equiv) in DCM (0.27 M with respect to the limiting reagent) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and then at rt for 16 h. After filtration of the mixture, the filtrate was concentrated in vacuo and purified by flash column chromatography (silica gel, EtOAc:cyclohexane, 2:1), affording 4-oxo-β-lactams **43** and **45** in pure form.

**(43) 1-(3-(4-(4-Bromobenzyl)piperazine-1-carbonyl)phenyl)-3,3-diethylazetidine-2,4-dione**



Orange oil; yield: 91% (364 mg, 0.730 mmol); $R_f$ = 0.31 (silica gel, EtOAc:cyclohexane, 2:1); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.94 – 7.89 (m, 2H), 7.49 – 7.43 (m, 3H), 7.32 – 7.27 (m, 1H), 7.25 – 7.19 (m, 2H), 3.81 (s, 2H), 3.51 (s, 2H), 3.45 (s, 2H), 2.55 (s, 2H), 2.42 (s, 2H), 1.87 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.0, 168.9, 137.1, 134.1, 131.6, 130.8, 129.7, 125.2, 121.2, 120.2, 117.7, 72.5, 62.1, 53.2, 52.7, 47.7, 42.2, 24.0, 9.3; LC-MS (ESI) $R_t$ = 7.28 min; *m/z* calcd for $C_{25}H_{29}BrN_3O_3^+$: 498.1, found: 498.2; HRMS (ESI): calcd for $C_{25}H_{28}BrN_3O_3$: 497.1314, found: (deconvoluted) 497.1317.

**(45) *tert*-Butyl (3-(4-(4-bromobenzyl)piperazine-1-carbonyl)-5-((*tert*-butyldimethylsilyl)oxy)phenyl)carbamate**

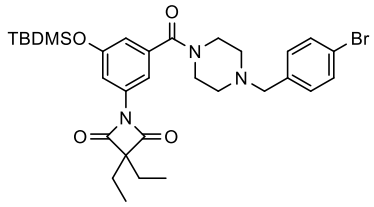


Yellowish solid; yield: 38% (145 mg, 0.261 mmol); $R_f$ = 0.37 (silica gel, EtOAc:cyclohexane, 2:1); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.52 – 8.49 (m, 1H), 8.11 – 8.08 (m, 1H), 7.95 – 7.92 (m, 1H), 7.47 – 7.42 (m, 2H), 7.25 – 7.20 (m, 2H), 3.94 (s, 3H), 3.83 (s, 2H), 3.55 (s, 2H), 3.46 (s, 2H), 2.59 (s, 2H), 2.47 (s, 2H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.06 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.8, 168.0, 165.4, 137.4, 134.3, 132.0, 131.7, 131.0, 126.1, 121.8, 121.0, 72.8, 61.9, 52.8, 47.5, 42.1, 24.1, 9.3; LC-MS (ESI) $R_t$ = 7.28 min; *m/z* calcd for $C_{27}H_{31}BrN_3O_5^+$: 556.1, found: 556.2; HRMS (ESI): calcd for $C_{27}H_{30}BrN_3O_5$: 555.1369, found: (deconvoluted) 555.1368.

*General method for the 4-oxo-β-lactam formation with malonyl dichloride leading to* compounds **46, 82a** and **82b**

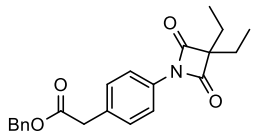
The corresponding aniline **75b, 81a or 81b** (1 equiv) was dissolved in DCM (1 M) and subsequently added to a solution of malonyl dichloride (1 equiv) in DCM (1 M). $Et_3N$ (2 equiv), also dissolved in DCM (2 M with respect to the limiting reagent) was added and the resulting mixture was heated to 48 °C and stirred at this temperature for 5 h. After filtration of the mixture, the filtrate was concentrated in vacuo and purified by flash column chromatography (silica gel, EtOAc:cyclohexane, 1:2 for **46**, 1:7 for **82a** and 1:12 → 1:7 for **82b**), affording 4-oxo-β-lactams **46, 82a** and **82b** in pure form.

**(46) 1-(3-(4-(4-Bromobenzyl)piperazine-1-carbonyl)-5-((*tert*-butyldimethylsilyl)oxy)phenyl)-3,3-diethylazetidine-2,4-dione**



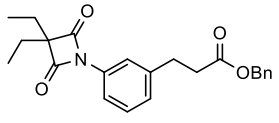
Yellowish solid; yield: 18% (73 mg, 0.12 mmol); $R_f$ = 0.30 (silica gel, EtOAc:cyclohexane, 1:2); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.46 (m, 3H), 7.45 – 7.42 (m, 1H), 7.35 – 7.26 (m, 2H), 6.77 – 6.72 (m, 1H), 4.03 – 3.81 (m, 2H), 3.75 – 3.47 (m, 4H), 2.87 – 2.42 (m, 4H), 1.86 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H), 0.99 (s, 9H), 0.24 (s, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.0, 168.8, 156.7, 137.6, 135.0, 131.9, 131.4, 116.9, 111.9, 110.3, 72.5, 25.7, 24.0, 18.3, 11.9, 9.3, -4.3; LC-MS (ESI) $R_t$ = 11.41 min; *m/z* calcd for $C_{31}H_{43}BrN_3O_4Si^+$: 628.2, found: 628.1; HRMS (ESI): calcd for $C_{25}H_{28}BrN_3O_4$ [M – TBDMS + $H^+$]: 513.1263, found: (deconvoluted) 513.1262.

**(82a) Benzyl 2-(4-(3,3-diethyl-2,4-dioxoazetidin-1-yl)phenyl)acetate**



White solid; yield: 15% (327 mg, 0.895 mmol); $R_f$ = 0.48 (silica gel, EtOAc:cyclohexane, 1:7); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.5 Hz, 2H), 7.39 – 7.28 (m, 7H), 5.14 (s, 2H), 3.67 (s, 2H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.6 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.1, 171.0, 135.7, 132.9, 132.5, 130.2, 128.6, 128.4, 128.3, 119.4, 72.2, 66.8, 40.9, 24.0, 9.3; LC-MS (ESI) $R_t$ = 11.45 min; *m/z* calcd for $C_{22}H_{24}NO_4^+$: 366.2, found: 366.0.

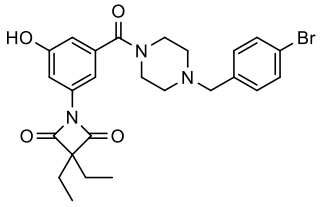
**(82b) Benzyl 3-(3-(3,3-diethyl-2,4-dioxoazetidin-1-yl)phenyl)propanoate**



White solid; yield 12% (283 mg, 0.746 mmol); $R_f$ = 0.36 (silica gel, EtOAc:cyclohexane, 1:12); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.66 (m, 2H), 7.41 – 7.27 (m, 6H), 7.10 (d, *J* = 8.7 Hz, 1H), 5.13 (s, 2H), 3.00 (t, *J* = 7.8 Hz, 2H), 2.71 (t, *J* = 7.8 Hz, 2H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.06 (t, *J* = 7.5 Hz, 6H);

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.5, 172.3, 142.0, 135.9, 134.1, 129.5, 128.6, 128.3, 126.8, 119.0, 117.3, 72.2, 66.5, 35.7, 30.9, 24.0, 9.3; LC-MS (ESI) $R_t$ = 11.73 min; *m/z* calcd for $C_{23}H_{26}NO_4^+$: 380.2, found: 380.1.

**(44) 1-(3-(4-(4-Bromobenzyl)piperazine-1-carbonyl)-5-hydroxyphenyl)-3,3-diethylazetidine-2,4-dione**

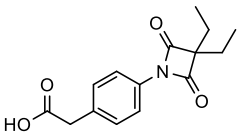


4-Oxo-β-lactam **46** (1 equiv, 61 mg, 0.097 mmol) was dissolved in THF (0.8 mL) and the mixture was cooled to 0 °C. A 1 M TBAF solution in THF (1 equiv, 0.107 mL) was added and the resulting mixture was stirred at rt for 2 h. The reaction was quenched with $H_2O$ and extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, EtOAc:cyclohexane, 3:1), affording 4-oxo-β-lactam **44** in pure form. White solid; yield: 68% (34 mg, 0.066 mmol); $R_f$ = 0.34 (silica gel, EtOAc:cyclohexane, 3:1); $^1$H NMR (400 MHz, $CD_3OD$) δ 7.51 – 7.44 (m, 2H), 7.40 – 7.36 (m, 1H), 7.33 – 7.30 (m, 1H), 7.30 – 7.25 (m, 2H), 6.74 – 6.71 (m, 1H), 3.76 (s, 2H), 3.57 (s, 2H), 3.48 (s, 2H), 2.57 (s, 2H), 2.48 (s, 2H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.04 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (101 MHz, $CD_3OD$) δ 173.2, 171.1, 159.9, 138.9, 137.6, 136.0, 132.6, 132.4, 122.3, 113.2, 109.3, 108.4, 73.5, 62.7, 24.7, 9.5; LC-MS (ESI) $R_t$ = 6.86 min; *m/z* calcd for $C_{25}H_{29}BrN_3O_4^+$: 514.1, found: 514.2; HRMS (ESI): calcd for $C_{25}H_{28}BrN_3O_4$: 513.1263, found: (deconvoluted) 513.1256.

*General method for the deprotection of benzylesters leading to the final products **47** and **48***

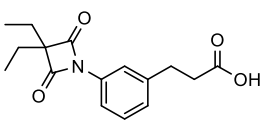
The corresponding benzyl-protected 4-oxo-β-lactam **82a** or **82b** (1 equiv) and Pd/C 10 wt% (3 mol%) were suspended in MeOH (0.12 M with respect to the limiting reagent) and the resulting mixture was degassed with $H_2$. The pressure of $H_2$ was kept constant with a balloon and the mixture was stirred at rt for 4 h. After filtration of the mixture over celite, the filtrate was concentrated in vacuo. Purification of the crude product by prep. HPLC afforded the final 4-oxo-β-lactams **47** and **48** in pure form.

**(47) 2-(4-(3,3-Diethyl-2,4-dioxoazetidin-1-yl)phenyl)acetic acid**



White solid (TFA salt); yield: 31% (76 mg, 0.28 mmol); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 3.65 (s, 2H), 1.84 (q, *J* = 7.5 Hz, 4H), 1.05 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.5, 172.2, 133.2, 131.9, 130.4, 119.5, 72.4, 40.7, 24.1, 9.3; LC-MS (ESI) $R_t$ = 8.78 min; *m/z* calcd for $C_{15}H_{18}NO_4^+$: 276.1, found: 275.9; HRMS (ESI): *m/z* calcd for $C_{15}H_{18}NO_4^+$: 276.1230, found: 276.1229.

**(48) 3-(3-(3,3-Diethyl-2,4-dioxoazetidin-1-yl)phenyl)propanoic acid**



White solid (TFA salt); yield: 47% (101 mg, 0.349 mmol); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.67 (m, 2H), 7.37 – 7.30 (m, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 2.99 (t, *J* = 7.9 Hz, 2H), 2.71 (t, *J* = 7.8 Hz, 2H), 1.85 (q, *J* = 7.5 Hz, 4H), 1.07 (t, *J* = 7.5 Hz, 6H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 178.5, 172.4, 141.8, 134.2, 129.6, 126.8, 119.1, 117.4, 72.3, 35.4, 30.6, 24.1, 9.4; LC-MS (ESI) $R_t$ = 9.12 min; *m/z* calcd for $C_{16}H_{20}NO_4^+$: 290.1, found: 289.8; HRMS (ESI): *m/z* calcd for $C_{16}H_{20}NO_4^+$: 290.1387, found: 290.1383.

# 3. X-Ray Crystallography

**Protein purification and crystallization**

Protein purification is described in ref. [2]: Briefly, human cDNA of DPP8 isoform 1 (UniProtKB Q6V1X1) was purchased from GeneArt and DPP9 isoform 2 from OriGene (UniProtKB Q86TI2-2). Either, DPP8 (1-898) or DPP9 (1-892) or the truncated version of DPP9 (20-892) proteins were expressed in *Spodoptera frugiperda* cells (Sf9) and purified using a His-Tag. Protein crystallization was performed by the hanging drop method. DPP8 crystals of space group $C222_1$ appeared after one day using 0.46 M Na-citrate pH 6.75 as precipitant and 10 mg/mL of protein in a 1:1 ratio. Co-crystallization with 1 mM of **6, 9, 43** and **45** rendered crystals in $P6_1$ space group, while apo crystals were soaked with **11** and **12** powder. DPP9 crystals were grown with 10 % PEG 8000, 30 % glycerol, 0.16 M Ca acetate and Na cacodylate pH 5.25 as precipitant solution and 20 mg/mL of protein in a 1:1 ratio. Crystals grew in P1 space group after one week, however none of the 4-oxo-β-lactam inhibitors was bound in these crystals. Futher improvement of DPP9 crystallization, using a truncated construct, rendered better diffracting crystal in P1 space group, grown in a condition containing PEG 2K MME buffered by Tris pH 7 at 293 K. Soaking of compound **13** was carried out using 1 mM of powder. The cocrystal structure shows two dimmers in the asymmetric unit.

**Structure solution and refinement**

X-ray diffraction data were collected at the SLS-X06SA beamline. Data sets were processed with XDS.[3] or with STARANISO.[4]. The apo DPP8 (6EOO) and apo DPP9 structures (6EOQ) were used for molecular replacement using Phaser.[5] The model was restrained refined keeping either 0.8% or 5% of free reflections to calculate Rfree factor using Refmac5.[6] Coordinates and structure factors were deposited in the Protein Data Bank[7] with the following access codes; 7OZ7 (DPP8-**6**), 7A3L (DPP8-**11**), 7A3J (DPP8-**12**), 7ZXS (DPP9-**13**), 7A3G (DPP8-**9**), 7AYQ (DPP8-**43**) and 7OR4 (DPP8-**45**). A summary of data collection and refinement statistics for each structure is provided in Supporting Table 2.

# SUPPORTING INFORMATION

**Table S3.1.** Crystallographic table. Data collection and refinement statistics.

| | DPP8-6 | DPP8-9 | DPP8-11 | DPP9-13 | DPP8-12 | DPP8-43 | DPP8-45 |
|---|---|---|---|---|---|---|---|
| *PDB code* | 7OZ7 | 7A3G | 7A3L | 7ZXS | 7A3J | 7AYQ | 7OR4 |
| *Data Collection* | | | | | | | |
| Wavelength (Å) | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Space Group | $P6_1$ | $P6_1$ | $C222_1$ | P1 | $C222_1$ | $P6_1$ | $P6_1$ |
| Resolution (Å) | 43.74 - 2.60 (2.66 - 2.60)[a] | 49.42 - 2.80 (2.87 - 2.80)[a] | 44.07 - 2.80 (2.87 - 2.80)[a] | 106.09 - 1.81 (1.96 – 1.81)[a] | 49.53 – 3.00 (3.08 – 3.00)[a] | 48.94 – 2.45 (2.51 – 2.45)[a] | 49.74 - 2.44 (2.50 – 2.44)[a] |
| *Cell dimensions* | | | | | | | |
| a, b, c (Å) | 149.42 149.42 269.77 | 153.59 153.59 270.22 | 164.03 252.77 261.23 | 88.35 106.18 121.13 | 164.37 252.95 260.86 | 149.50 149.50 269.33 | 149.44 149.44 269.38 |
| α, β, γ (°) | 90 90 120 | 90 90 120 | 90 90 90 | 65.2 70.4 75.9 | 90 90 90 | 90 90 120 | 90 90 120 |
| $R_{meas}$ (%) | 11.6 (172.0)[a] | 9.5 (160.6)[a] | 9.3 (137.3)[a] | 6.2 (58.7)[a] | 9.4 (118.7)[a] | 7.2 (153.2)[a] | 7.6 (130.9)[a] |
| $CC_{1/2}$ (%) | 99.9 (74.9)[a] | 99.9 (79.2)[a] | 99.9 (73.8)[a] | 99.80 (73.60)[a] | 99.9 (77.1)[a] | 100 (76.4)[a] | 99.9 (74.8)[a] |
| I/σ(I) | 16.20 (1.95)[a] | 19.17 (2.04)[a] | 18.90 (2.08)[a] | 5.4 (1.6)[a] | 16.43 (2.00)[a] | 23.16 (2.02)[a] | 22.96 (2.06)[a] |
| Completeness (%) | 100.0 (100.0)[a] | 100.0 (100.0)[a] | 100.0 (100.0)[a] | 91.5 (65.0)[a] | 100.0 (100.0)[a] | 100.0 (100.0)[a] | 100.0 (100.0)[a] |
| Multiplicity | 12.6 (12.8)[a] | 13.1 (13.6)[a] | 8.4 (8.6)[a] | 2.3 (2.4)[a] | 8.5 (8.4)[a] | 13.1 (12.2)[a] | 13.1 (12.2)[a] |
| Total observations | 1319446 (100619)[a] | 1165051 (88938)[a] | 1118675 (84941)[a] | 508196 (26515)[a] | 920839 (68196)[a] | 1639145 (112956)[a] | 1661730 (112989)[a] |
| Total unique observations | 104327 (7814)[a] | 88413 (6518)[a] | 132897 (9801)[a] | 220955 (11048)[a] | 108427 (7996)[a] | 124546 (9206)[a] | 126021 (9258)[a] |
| *Refinement* | | | | | | | |
| $R_{cryst}/R_{free}$ (%) | 22.23/25.95 | 20.90/24.13 | 21.27/24.45 | 17.5/20.3 | 19.8/23.22 | 19.25/22.77 | 21.39/25.34 |
| Number of reflections | 99110 (5217)[b] | 83992 (4421)[b] | 126252 (6645)[b] | 219248 (1707)[c] | 103005 (5422)[b] | 118318 (6228)[b] | 119719 (6301)[b] |
| R.m.s.d. bond lengths (Å) | 0.007 | 0.007 | 0.002 | 0.005 | 0.007 | 0.009 | 0.008 |
| R.m.s.d. bond angles (°) | 1.146 | 1.354 | 1.187 | 1.36 | 1.152 | 1.582 | 1.615 |
| Number of atoms | 13406 | 13651 | 20714 | 27406 | 20102 | 13419 | 13408 |
| Average B-factors (Å$^2$) | 69.81 | 89.57 | 74.38 | 34.66 | 101.42 | 65.65 | 64.44 |
| *Ramachandran plot* | | | | | | | |
| Preferred region (%) | 96.46 | 95.08 | 95.15 | 90.9 | 95.82 | 94.60 | 93.43 |
| Allowed region (%) | 3.28 | 4.70 | 4.14 | 8.9 | 3.65 | 5.02 | 6.00 |
| Outliers (%) | 0.20 | 0.80 | 0.72 | 0.1 | 0.53 | 0.38 | 0.56 |

a Values in parentheses correspond to the highest-resolution shell

b Values in parentheses correspond to free R-value test set (5%)

c Values in parentheses correspond to free R-value test set (0.8%)

# 4. Biochemical and biological Assays

**Biochemical inhibition assay**

75 ng of purified DPP8 or DPP9 was incubated with 0.16, 0.31, 0.63, 1.25, 2.50, or 5.00 µM of the tested inhibitors for 1 h at 24°C in 20 mM HEPES/KOH pH 7.3, 110 mM potassium acetate, 2 mM magnesium acetate, 1 mM EGTA, 0.02% Tween-20) supplemented with 1 mM DTT. The inhibitor-enzyme mixture was added to 500 µM H-Gly-Pro-7-amino-4-methylcoumarin (GP-AMC) substrate in a final reaction volume of 20 µL. Fluorescence was analyzed using an EnSpire microplate fluorimeter (Perkin-Elmer) with 360-nm (excitation) and 460-nm (emission) filters. Percentage inhibition was normalised to the corresponding mock-treated DPP9 controls. $K_i$ values were calculated assuming for tight inhibition using the Morrison equation:

Q=(Ki*(1+(S/Km))), Y=Vo*(1-((((Et+X+Q)-(((Et+X+Q)^2)-4*Et*X)^0.5))/(2*Et)))

with GraphPad Prism 8 version 8.4.3.

**Biological inhibition assays**

*Mice*

Pycard$^{-/-}$ (ref. [8]) and Casp1$^{-/-}$ (ref. [9]) mice on C57BL/6 background were housed under SOPF or SPF conditions at the Center for Experimental Models and Transgenic Services (CEMT, Freiburg, Germany), the Zentrum für Präklinische Forschung (ZPF, Munich, Germany), Charles River Laboratories (Calco, Italy), or the Center of Infection and Immunity (University of Lausanne, Epalinges, Switzerland) in accordance with local guidelines.

*BMDCs and BMDMs Preparation and Stimulation*

Cells were cultured at 37 °C, 5 % $CO_2$ in a humidified incubator. Murine bone marrow-derived dendritic cells (BMDCs) were differentiated from tibial and femoral bone marrow as previously described in ref. [10]. Recombinant murine GM-CSF was from Immunotools and was used at 20 ng ml$^{-1}$, respectively. After 6 - 8 days of differentiation, cells were plated in 96-well plates at a density of 0.08 - 0.1x106 cells per well, primed with 50 ng ml$^{-1}$ *E. coli* K12 ultra-pure LPS (InvivoGen) for 3 h and were afterwards treated with the inhibitors for 8 – 16 h. The DPP Inhibitors were used with a concentration of 1-100 µM. As positive controls, the DPP8/9 Inhibitors VbP and 1G244 were used at a concentration of 10 µM. The NLRP3 activator nigericin was used at 5 µM. All stimulations were performed in triplicates and cytokine production in cell-free supernatants was measured by ELISA.

*Immunodetection of Proteins*

For cytokine quantification of cell-free supernatants, ELISA kit for murine IL-1β (eBioscience) was used. ELISA data is depicted as mean ± SD of technical triplicates as previously described in ref. [10]. For immunoblot analysis, cell-free supernatant and cell lysate samples in SDS- and DTT-containing sample buffer were analyzed. Triplicates were pooled and proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes using standard techniques. The following primary antibodies were used: anti-Caspase-1 (p20) mAb (Casper-1, Adipogen), IL-1β/IL-1F2 pAb (AF-401-NA, R&D Systems), anti-GSDMD (EPR19828, Abcam), and GAPDH mAb (MAB5718, R&D Systems).

## SUPPORTING INFORMATION

*Cell Viability Assays*

Lytic cell death was determined by measuring LDH release from cell-free supernatants using a colorimetric assay (Promega, Takara) according to the manufacturer's protocol. Medium served as blank value and was subtracted from the sample values. Results were plotted as percentage of 100 % dead cells lysed with lysis buffer 45 min prior to collection of the cell supernatants. Data is depicted as mean ± SD of technical triplicates.

# 5. ABPP Experiments

**Cell Culture conditions of the ABPP experiments**

Cells were cultured at 37 °C under a humidified 5% carbon dioxide atmosphere. Culture media were supplemented with 10% fetal bovine serum (FBS), penicillin (50 IU/mL), streptomycin (50 µg/mL), and glutamine (2 mM), unless otherwise specified. U937 cells were cultured in tissue culture flasks using RPMI-1640. Cells were seeded at $2 \times 10^5$ viable cells/mL and cultured by addition of fresh media every 2 to 3 days, maintaining cell density below $2 \times 10^6$ viable cells/mL. HEK293T cells were cultured in tissue culture plates using DMEM. A subcultivation ratio of 1:3 to 1:5 was used for passaging cells every 2 to 3 days when the cells reached approximately 80% confluency.

For SILAC experiments, the culture medium for SILAC U937 cells was SILAC RPMI supplemented with 10% dialyzed FBS, penicillin, streptomycin, and glutamine (concentrations as above). "Light" medium was supplemented with L-arginine-HCl and L-lysine-HCl (each at 100 µg/mL); "Heavy" medium was supplemented with [$^{13}C_6$,$^{15}N_4$]L-arginine-HCl and [$^{13}C_6$,$^{15}N_2$]L-lysine-HCl (each at 100 µg/mL). SILAC U937 cells were generated by culturing cells in for a minimum of 5–8 passages. Aliquots were frozen in the corresponding SILAC medium supplemented with 10% DMSO and stored in liquid nitrogen where required. After thawing, cells were passaged a minimum of three times before being used in experiments.

**Serine Hydrolase overexpression in HEK293T cells**

Mammalian expression vectors containing the serine hydrolases DPP4, 7, 8, and 9 were available in the Cravatt lab. Plasmids were transformed into chemically competent E. coli; briefly, 16 µL of E. coli suspension was mixed with 2 µL of DNA solution. The mixture was incubated at 0 °C for 30 min, heat-shocked at 42 °C, and immediately returned to ice for 5 min. SOC media (600 µL) was added and the bacterial suspension was incubated at 37 °C for 1 h. An aliquot (10 µL) was inoculated onto an LB-carbenicillin plate and incubated at 37 °C overnight. The next day, a single antibiotic-resistant colony was picked and inoculated in 5 mL of LB media at 37 °C overnight, with shaking. The cells were centrifuged and DNA was extracted via miniprep (Zymo Research). The isolated DNA was quantified using a NanoDrop device (A260/280) and checked by sequencing before being used for transfection. HEK293T cells were seeded at $5 \times 10^5$ cells/mL and grown for 48 h. A transfection mixture was prepared for each well of a 6-well plate: 2 µg of plasmid DNA was gently diluted into 200 µL of serum-free DMEM and 6 µg of PEI MAX (1 mg/mL solution) was added. The mixture was mixed vigorously and incubated for 30 minutes, then added dropwise to the cells. The cells were grown for 24—48h before harvesting. Overexpression of the catalytically active form of the selected serine hydrolases was evaluated by ABPP using FP-Rhodamine labeling. Control cells were transfected with METTL7A, a non-serine hydrolase.

**Gel-based ABPP**

Whole cell lysates were normalized to 1 mg/mL of protein using a protein concentration assay (DC assay, BioRad). For labeling experiments, 1 µL of the tested probe (50X concentrated) or DMSO was incubated for 30 minutes with 50 µL of lysate at rt. For competitive experiments, 1 µL of competitor (50X concentrated) was added and incubated for 30 minutes with 50 µL of lysate at rt, followed by 1 µL of probe (50X concentrated) for an additional 30 minutes. The reaction was quenched by adding 4X gel loading buffer (17 µL). Proteins were resolved by SDS-PAGE (10% acrylamide gel, constant 300 V, approx 850–900 V·hr). In-gel fluorescence scanning was performed on a ChemiDoc MP imager (BioRad, excitation: Green Epi, emission: 605/50).

**ABPP-MudPIT-SILAC**

*Proteome treatment with ABP*

SILAC U937 whole cell lysates were normalized to a concentration of 1 mg/mL and 500 µL was used for each experiment. In the non-competitive experiment, ABP **10** was used at 10 µM concentration. Three replicate experiments were performed. "Light" samples were treated with DMSO, and "heavy" samples were treated with probe **10** for 30 min at rt.

In the competition experiment, "light" samples were pre-treated with DMSO and "heavy" samples with 4-Oxo-β-Lactam **8** for 30 min, at rt, then FP-Biotin (10 µM) was added to both samples for 30 min. Light and heavy samples were quenched and mixed by addition to pre-chilled (ice) methanol (2 mL).

*Preparation of samples for MudPIT analysis*

The subsequent steps for protein precipitation were performed using chilled buffers kept on ice. $CHCl_3$ (0.5 mL) and DPBS (1 mL) were added sequentially and the cloudy mixture was vortexed and centrifuged (5,000 ×g, 15 min, 4 ºC). The organic and aqueous layers were aspirated leaving a protein disc that had formed between the organic and aqueous phases. The protein disc was washed with cold 1:1 MeOH:$CHCl_3$ (3 × 1 mL) and then dispersed by sonication back into 4:1 MeOH:$CHCl_3$ (2.5 mL). A protein pellet was obtained by centrifugation (5000 ×g, 10 min, 4 °C) and the supernatant removed. The remaining pellet was resuspended in a freshly prepared solution of urea (500 µL, 6 M in DPBS); TCEP (50 µL, 100 mM in DBPS, basified with $K_2CO_3$) was added, and the suspension incubated with shaking (30 min, 37 °C) to redissolve proteins. Proteins were alkylated with iodoacetamide (70 µL, 400 mM in DPBS) for 30 min at rt, in the dark. SDS was added (10% in DPBS) and the sample diluted with DPBS (5 mL). Pre-washed streptavidin-beads (100 µL/sample, 0.25% SDS in DPBS) were added and the samples were rotated for 2.5 h. Beads were then pelleted by centrifugation (1,000 ×g, 2 min) and sequentially washed with 0.25% SDS (3 × 10 mL). Beads were transferred to a low-binding 1.7-mL tube, and further washed with DPBS (3 × 1 mL) and Millipore $H_2O$ (3 × 1 mL). Trypsin solution (20 µg sequencing-grade modified trypsin, [Promega], in 2 mL 2M urea, buffered with 100 mM TEAB) was added to each sample and on-bead digestion proceeded overnight with shaking (14 h at 37 °C). The mixture was filtered using a Bio-Spin column, eluting into a fresh low-binding tube and quenched with formic acid (16 µL). Samples were stored at –20 °C prior to MudPIT analysis.

*MudPIT analysis of tryptic peptides*

Peptides were analyzed by LC-MS/MS on a ThermoFinnigan LTQ mass spectrometer (Thermo Scientific) or on an LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific) according to previously described methods.[11] Peptides from on-bead tryptic digests were pressure loaded onto a 250 µm (inner diameter) fused silica capillary column packed with 4 cm $C_{18}$ resin (5 µm, Phenomenex). Peptides were then eluted onto a 100 µm (inner diameter) fused silica capillary column packed with 3 cm strong cation exchange (SCX) resin followed by 10 cm $C_{18}$ resin. Chromatographic separation of the peptide mixture was achieved using a 5-step multidimensional LC-MS (MudPIT) protocol consisting of 0%, 25%, 50%, 80% and 100% salt bumps of $NH_4OAc$ (500 mM) salt bumps followed by an increasing gradient of $CH_3CN$ (0.1% $HCO_2H$) in $H_2O$.[12]

## SUPPORTING INFORMATION

**Protein identification and quantification**

RAW files were directly uploaded to the Integrated Proteomics Pipeline (IP2) for analysis, where they were searched using ProLuCID against a human reverse-concatenated non-redundant (gene-centric) FASTA database, assembled from the Uniprot database. The precursor-ion mass tolerance was set to 50 ppm. Oxidation on methionine (+15.9949) was specified as a variable modification and iodoacetamide alkylation (+57.0215) on cysteine as a static modification. Matched MS2 spectra from ProLuCID searches were assembled by protein and filtered using DTASelect (version 2.0.47) which allowed only half-tryptic or fully-tryptic peptides for identification and quantification. Peptides were restricted to a specified false positive rate of 1%. SILAC ratios were quantified using CIMAGE software.[13] Briefly, the software extracts MS1 ion chromatograms (±20 ppm) from 'light' and 'heavy' target peptide masses (*m/z*) using a retention time window (±10 min) centered at the time the peptide ion was identified by MS/MS fragmentation; the ratio of light and heavy peptide peak areas are then calculated. To ensure the correct peak-pair is used for quantification, CIMAGE applies a co-elution correlation score filter ($R^2 \geq 0.8$) for heavy and light peptide peaks to exclude target peptides with poor co-elution profiles. Furthermore, an envelope correlation score filter is applied to ensure the experimentally observed high-resolution MS1 spectrum matches ($R^2 > 0.8$) the predicted isotopic distribution. Peptide SILAC ratios are capped at a maximum of 20 to mitigate the effects of the large dynamic range of protein levels; this is also the ratio assigned to "singletons"—peptides that exclusively had extracted ion signal for one of the isotopes but not the other.

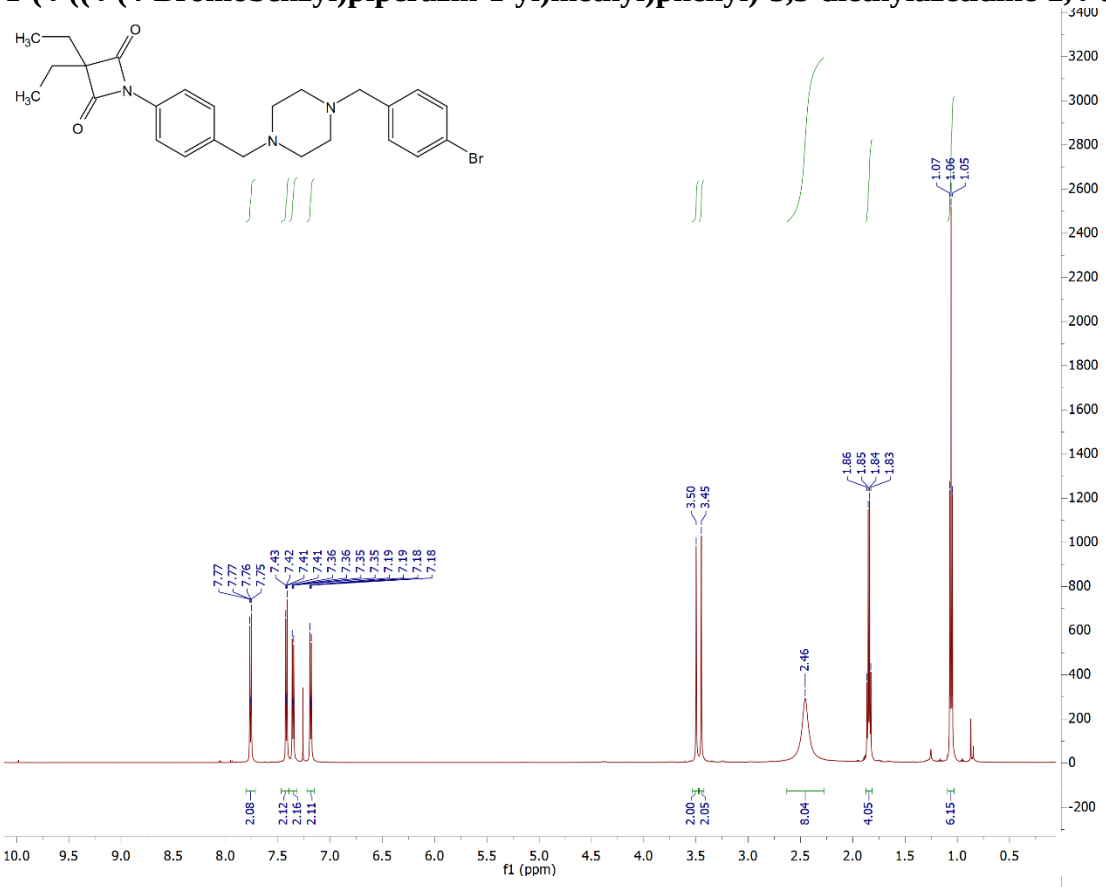
SILAC ratios for proteins were calculated from the peptide ratios generated by CIMAGE. Log2-transformed SILAC ratios were first analyzed on a per sample level. A SILAC ratio of ≥ 3.0 was chosen as a cutoff for defining enriched and competed targets. Peptides were grouped by sequence and the median value used for further analysis. In cases where a maximum ratio was observed along with ratios less than the cutoff, the maximum ratio was excluded from calculations, since the prior probability of outlier "singletons" is known to be high. Peptides were then grouped by protein and the median SILAC ratio was used for further calculations. Protein $\log_2$-SILAC ratios were grouped by experimental replicate, then the mean of 2 or 3 replicates was calculated and transformed back into base 10. Protein ratio values retained for further analysis were required to be quantified in at least 2 replicates and represented by at least 2 sequence-unique quantified peptides. Saturable targets were defined as proteins that had SILAC ratio of ≥ 3.0 in both enrichment and competition experiments.

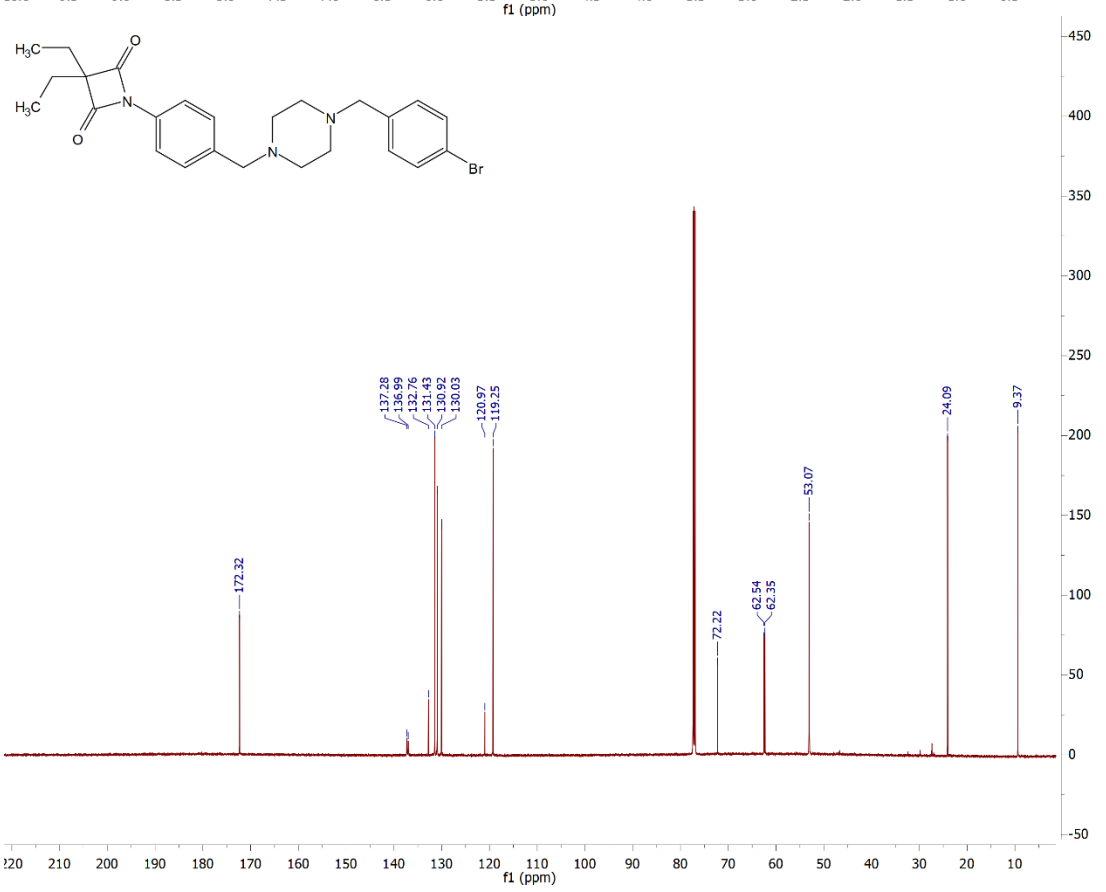
## 6. Cheminformatics

Ligand data for human DPP8 and DPP9 receptors was collected from ChEMBL v27. Only inhibitors with IC50, KD or Ki values ≤ 10 μM were kept for further analyses given that a high percentage of high micromolar inhibitors derive from colloidal aggregation.[14] The CATS2 descriptors[15] (MOE implementation) were calculated for all compounds in KNIME. The descriptor space was projected to the plane using the t-distributed neighborhood embedding (t-SNE) algorithm (learning rate= 600; iterations = 1000). The machine learning routine was implemented in Python 2.7 using the NumPy, Pandas and Scikit-learn libraries. Data was plotted with Matplotlib. The ECFP4-like Morgan fingerprints (2048 bits, radius 2) were calculated for compounds 6-9 and DPP8 inhibitor lists. The Tanimoto coefficient was calculated in KNIME.

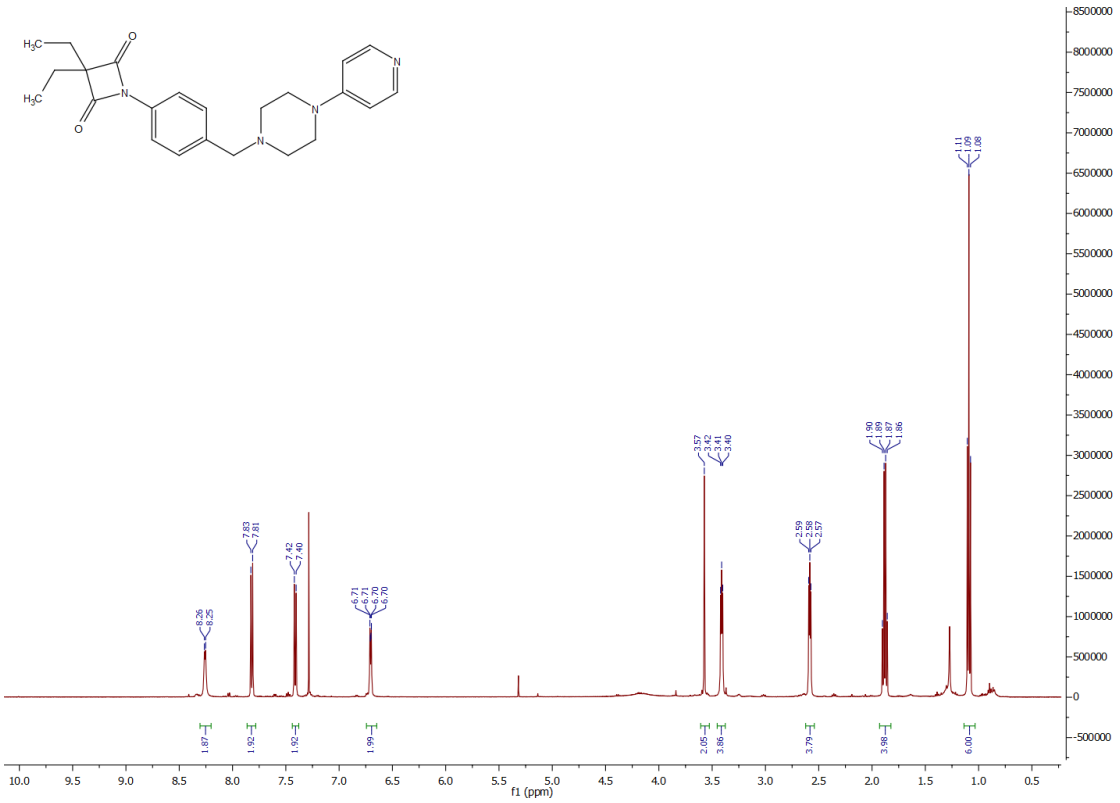
# 7. NMR Spectra of Tested Compounds

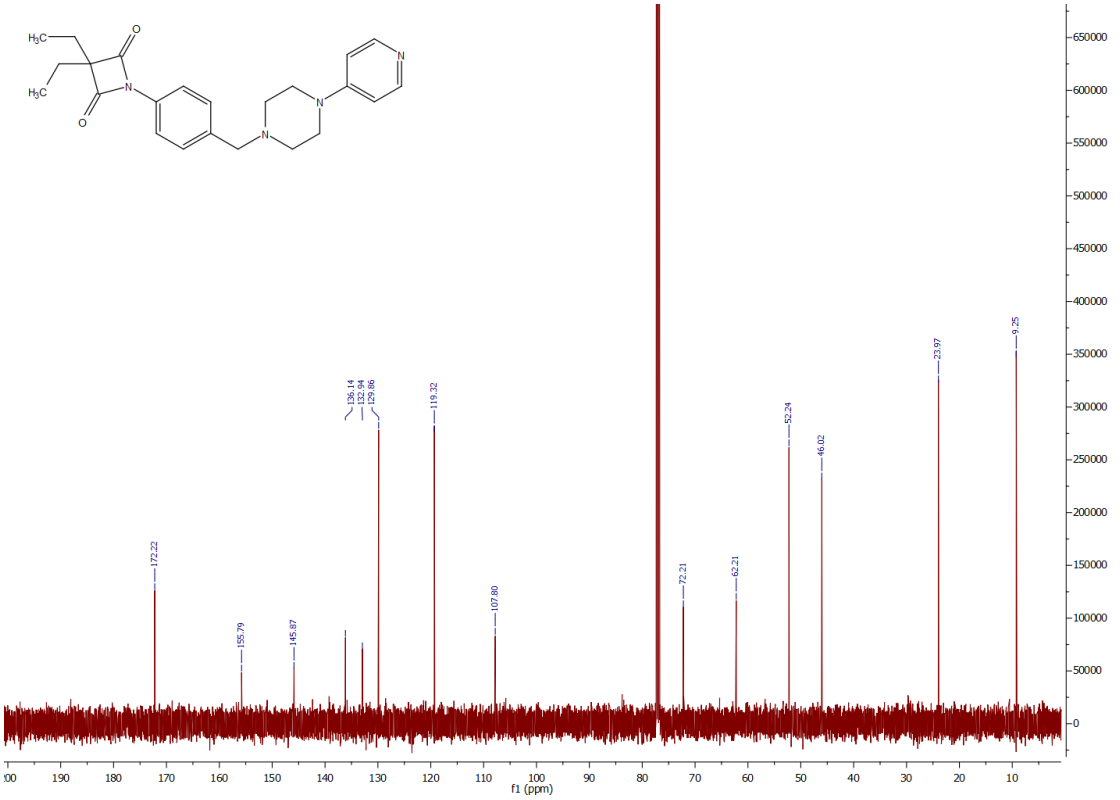
**1-(4-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (2)**



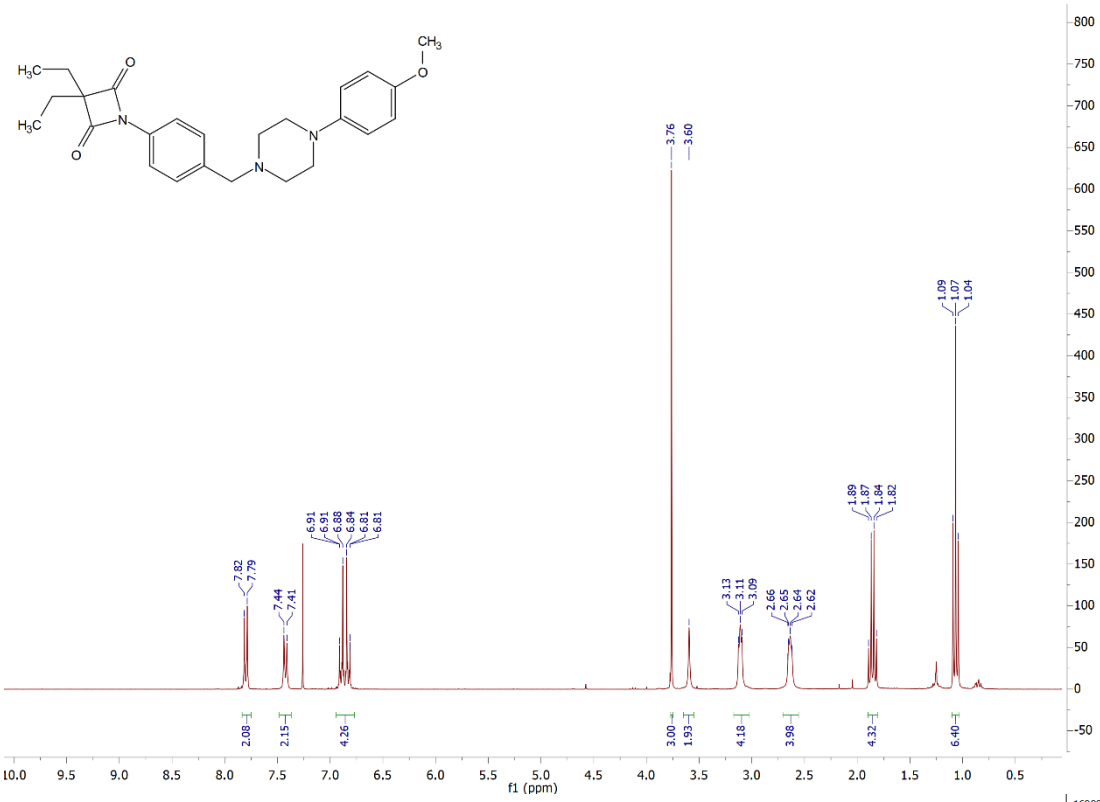



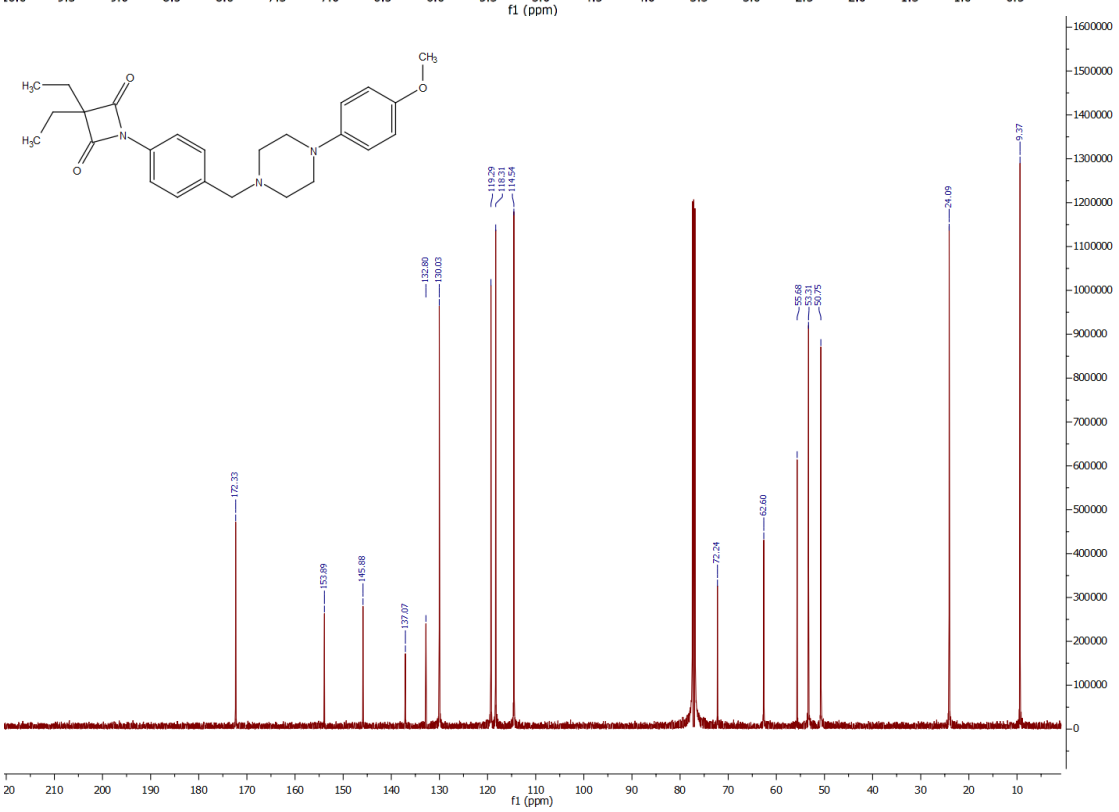
**3,3-Diethyl-1-(4-((4-(pyridin-4-yl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione (3)**



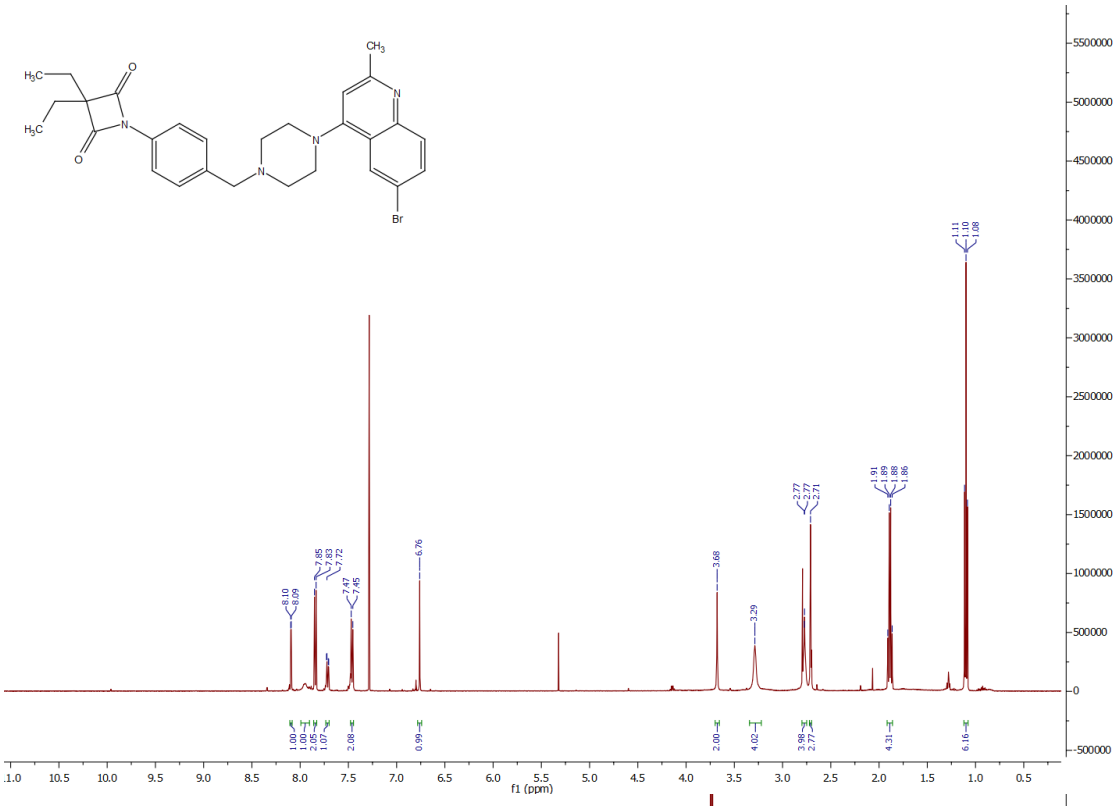
**3,3-Diethyl-1-(4-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione (4)**

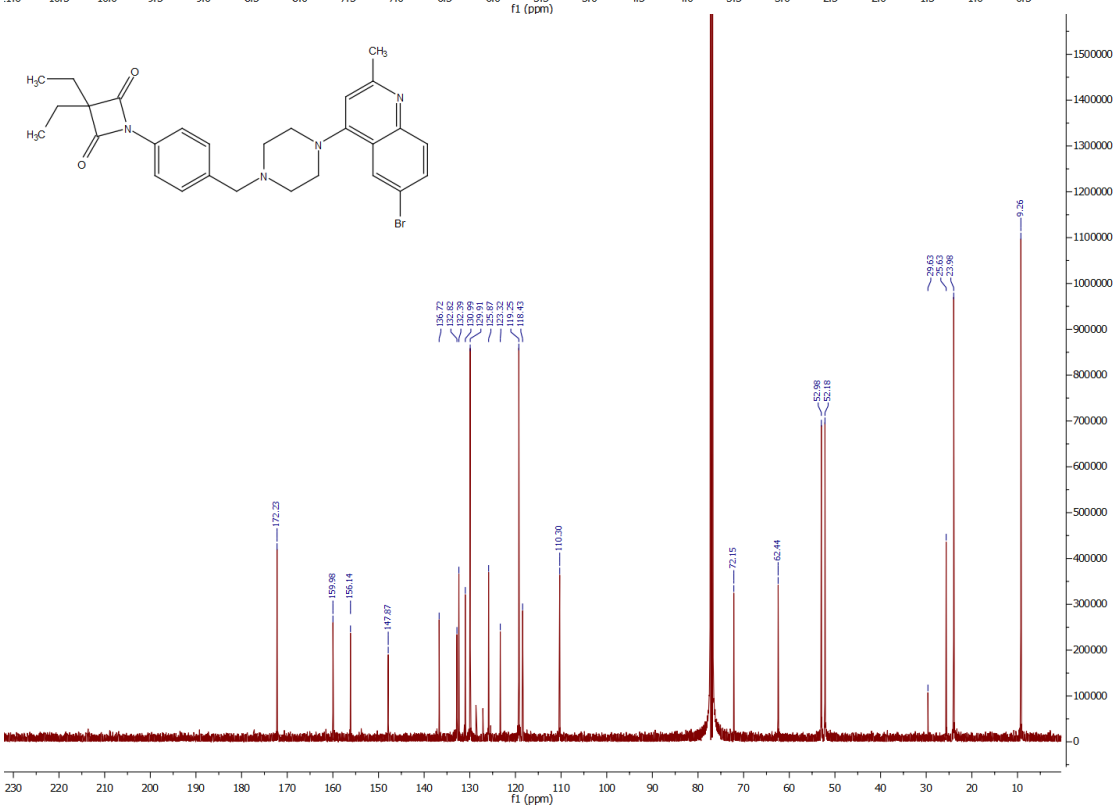


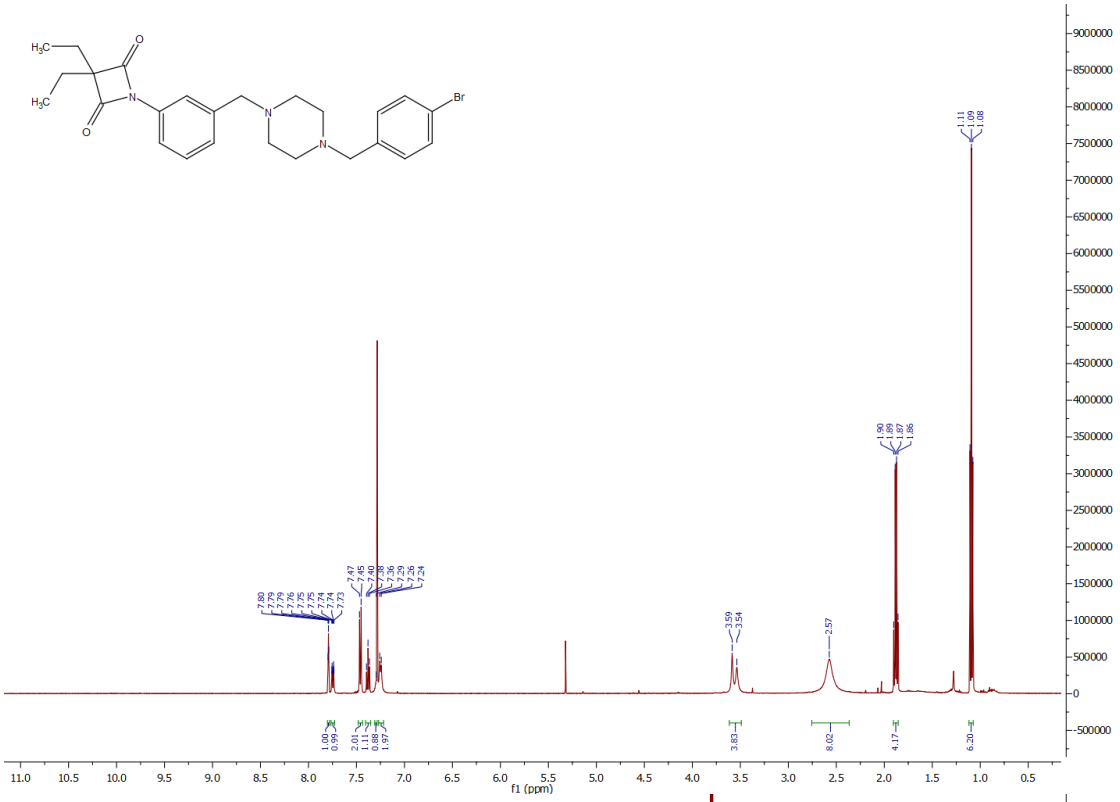
SUPPORTING INFORMATION

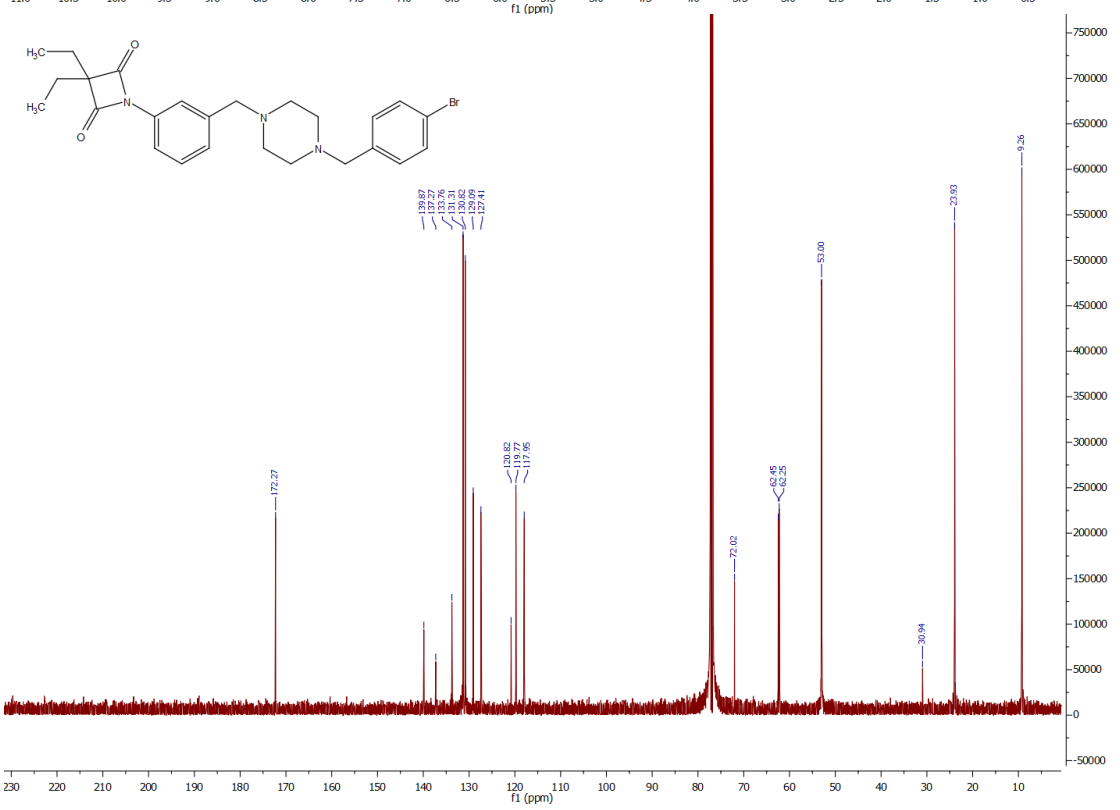
**1-(4-((4-(6-Bromo-2-methylquinolin-4-yl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (5)**



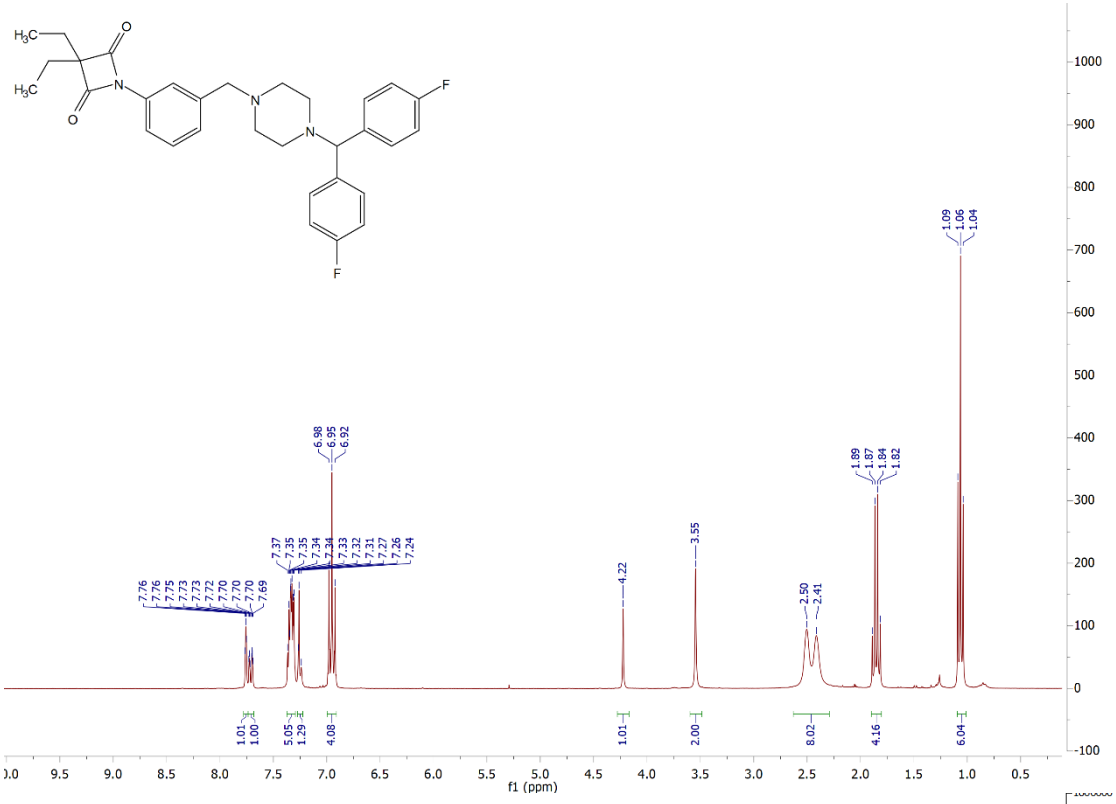



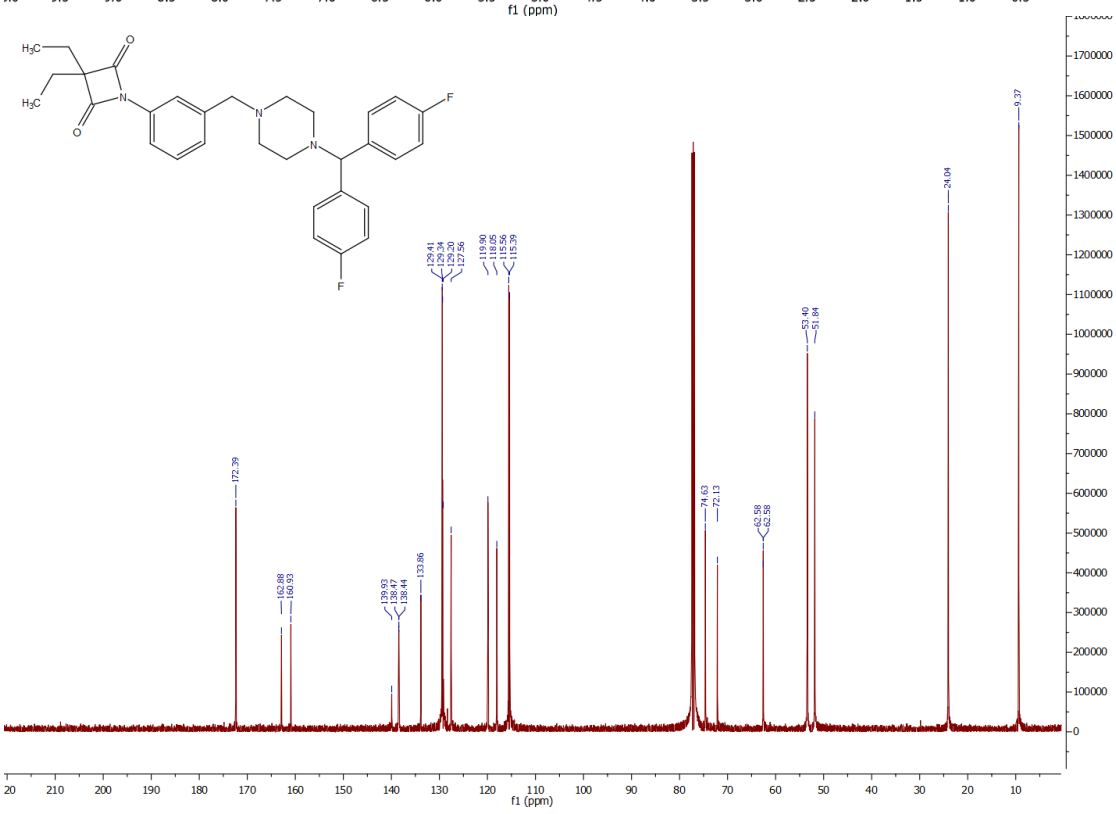
**1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (6)**



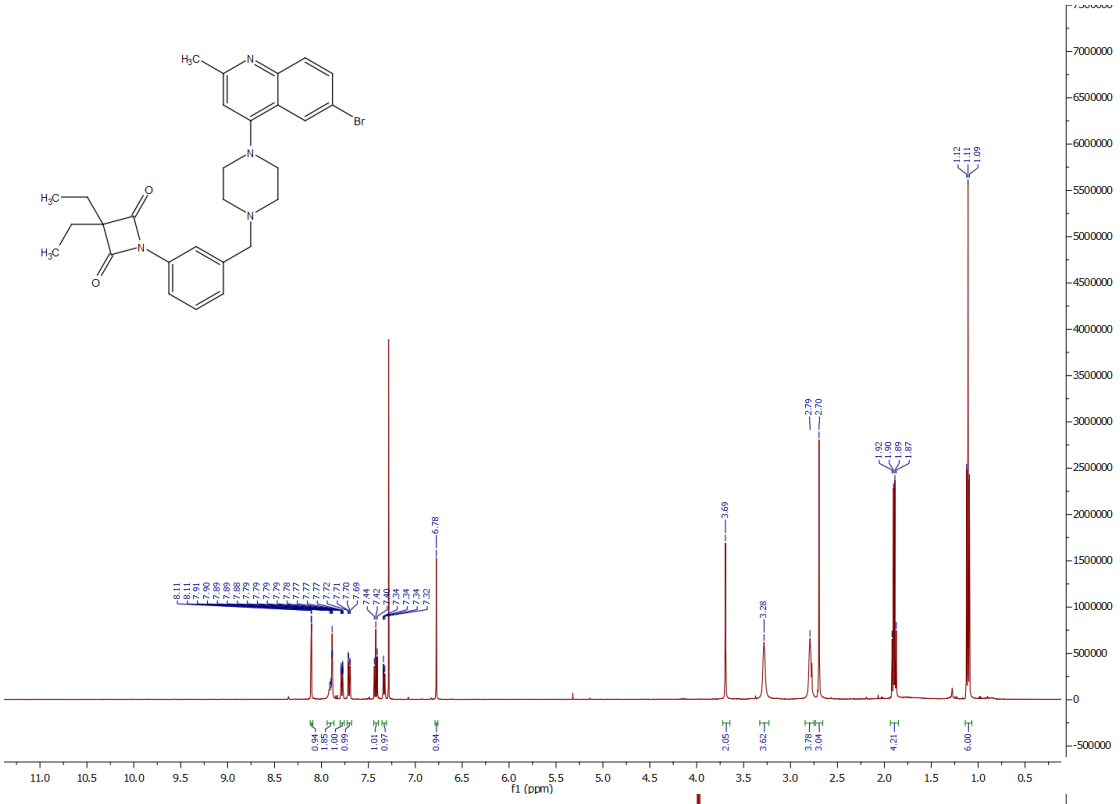



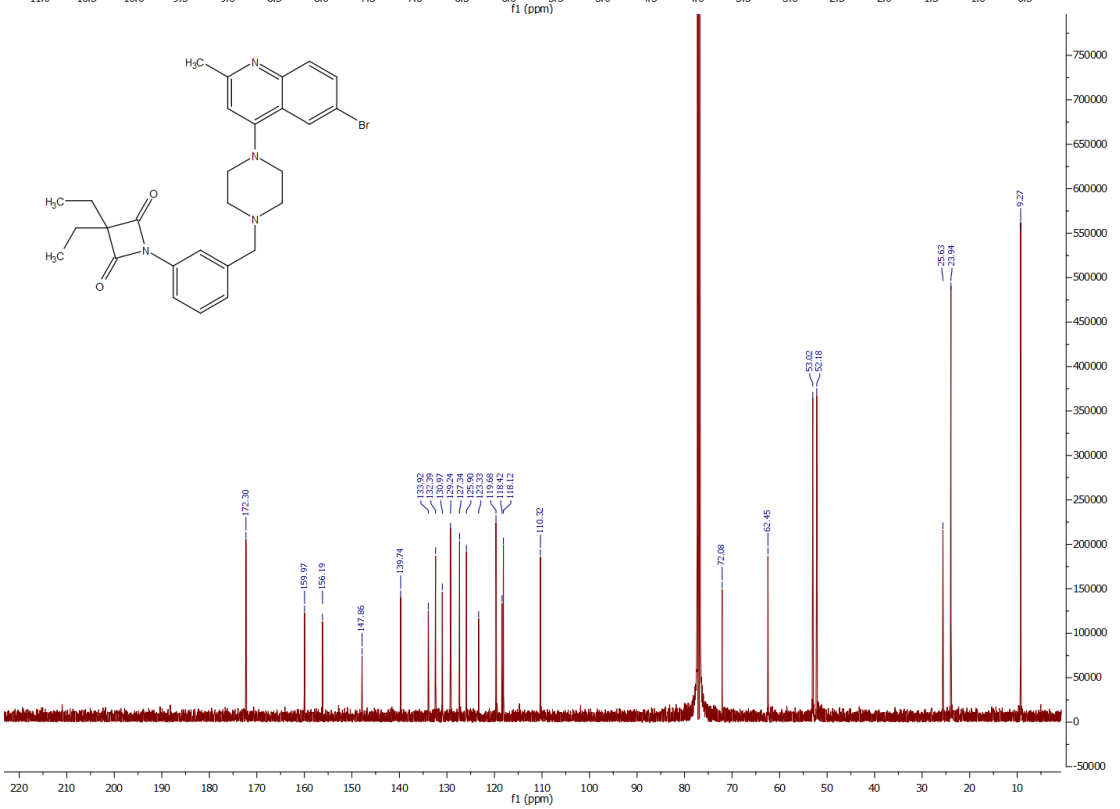
**1-(3-((4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (7)**



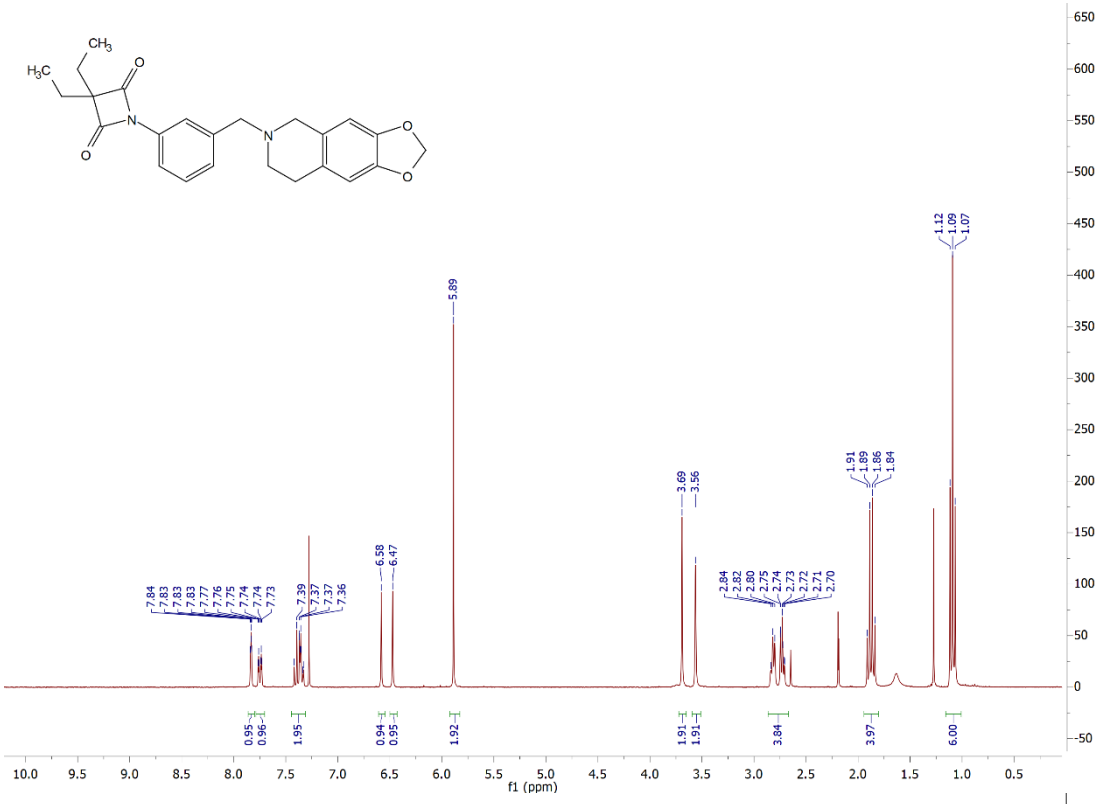



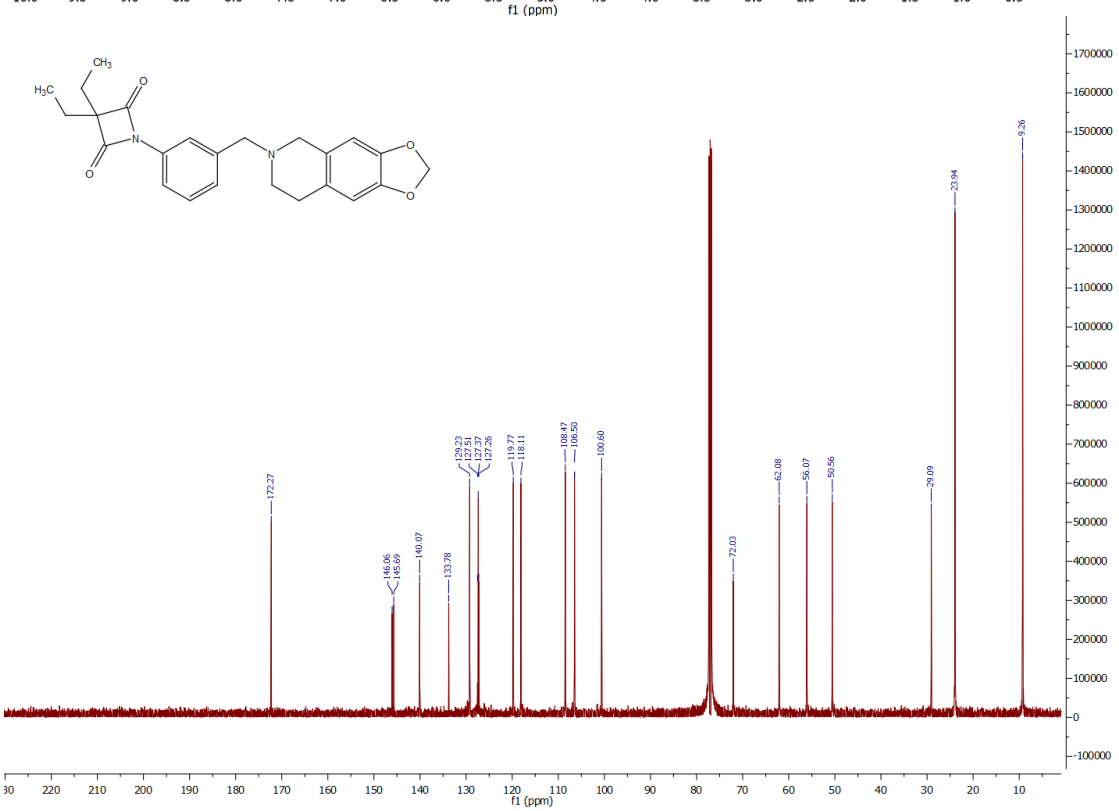
**1-(3-((4-(6-Bromo-2-methylquinolin-4-yl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (8)**



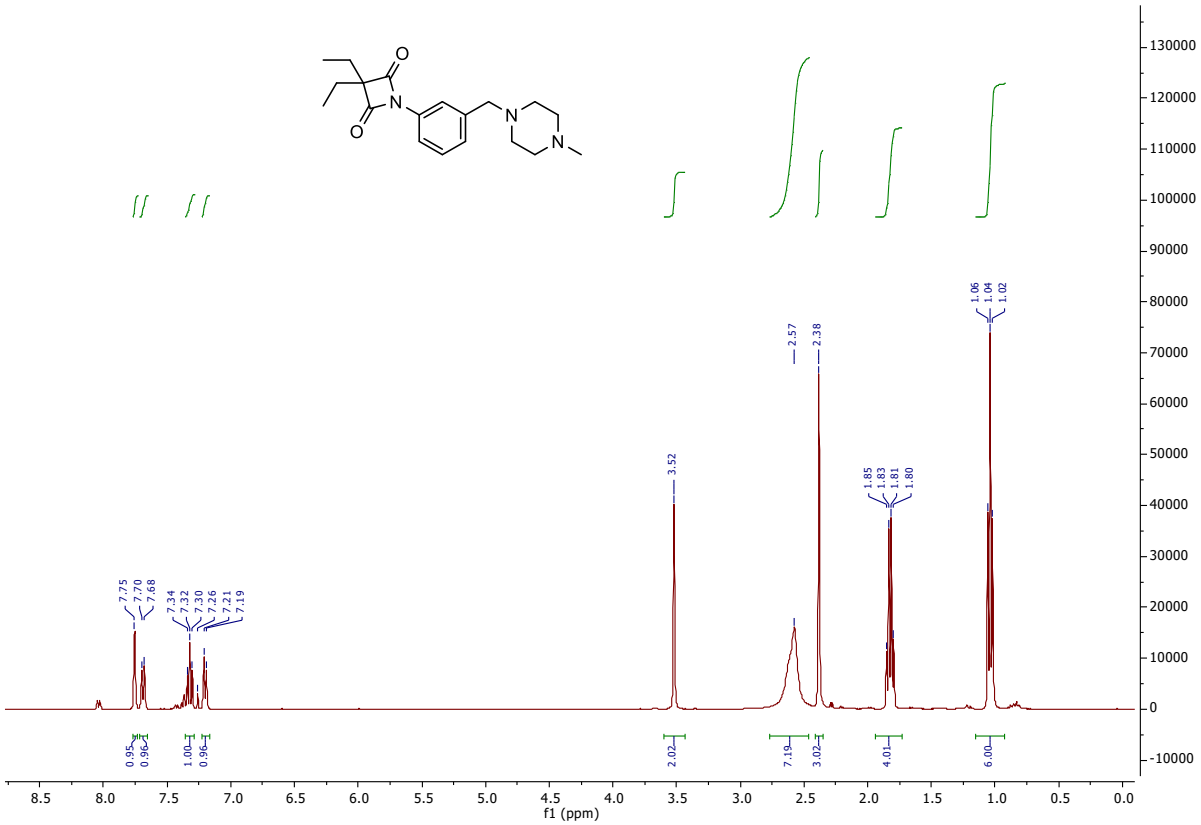



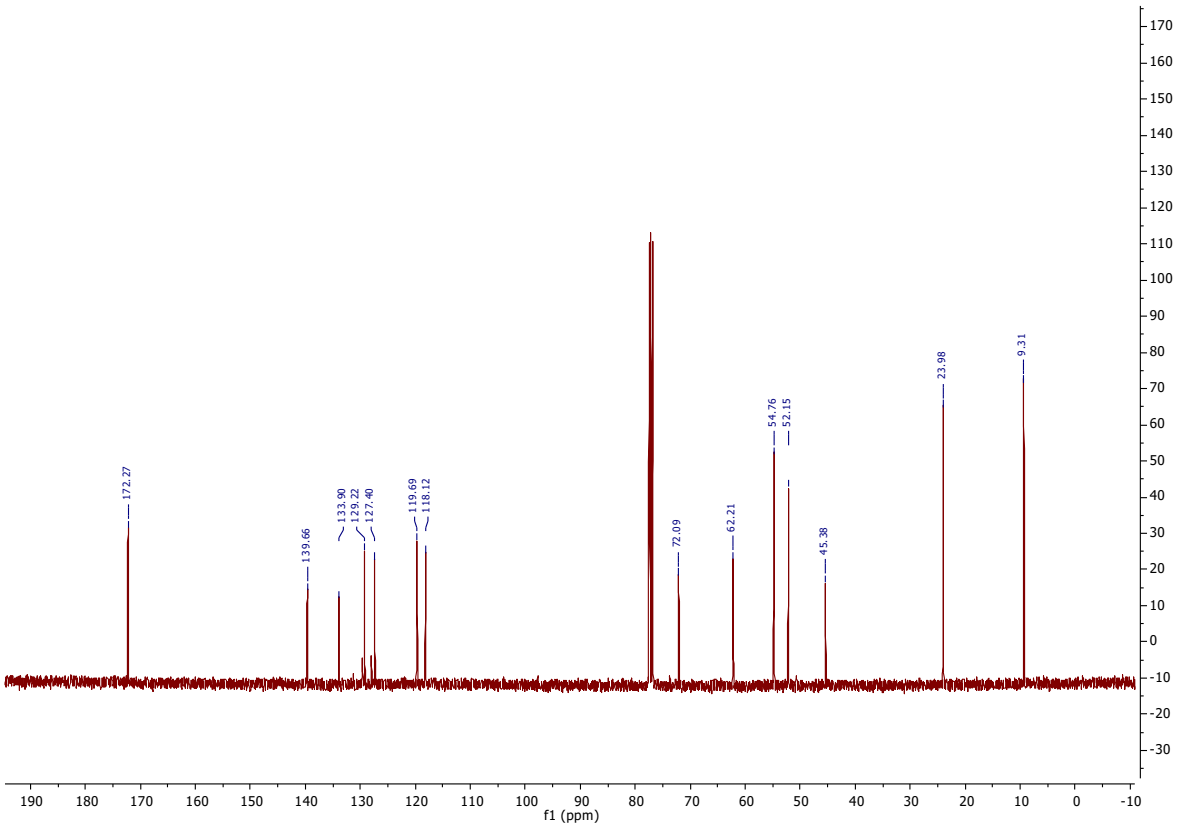
**1-(3-((7,8-Dihydro-[1,3]dioxolo[4,5-g]isoquinolin-6(5H)-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (9)**



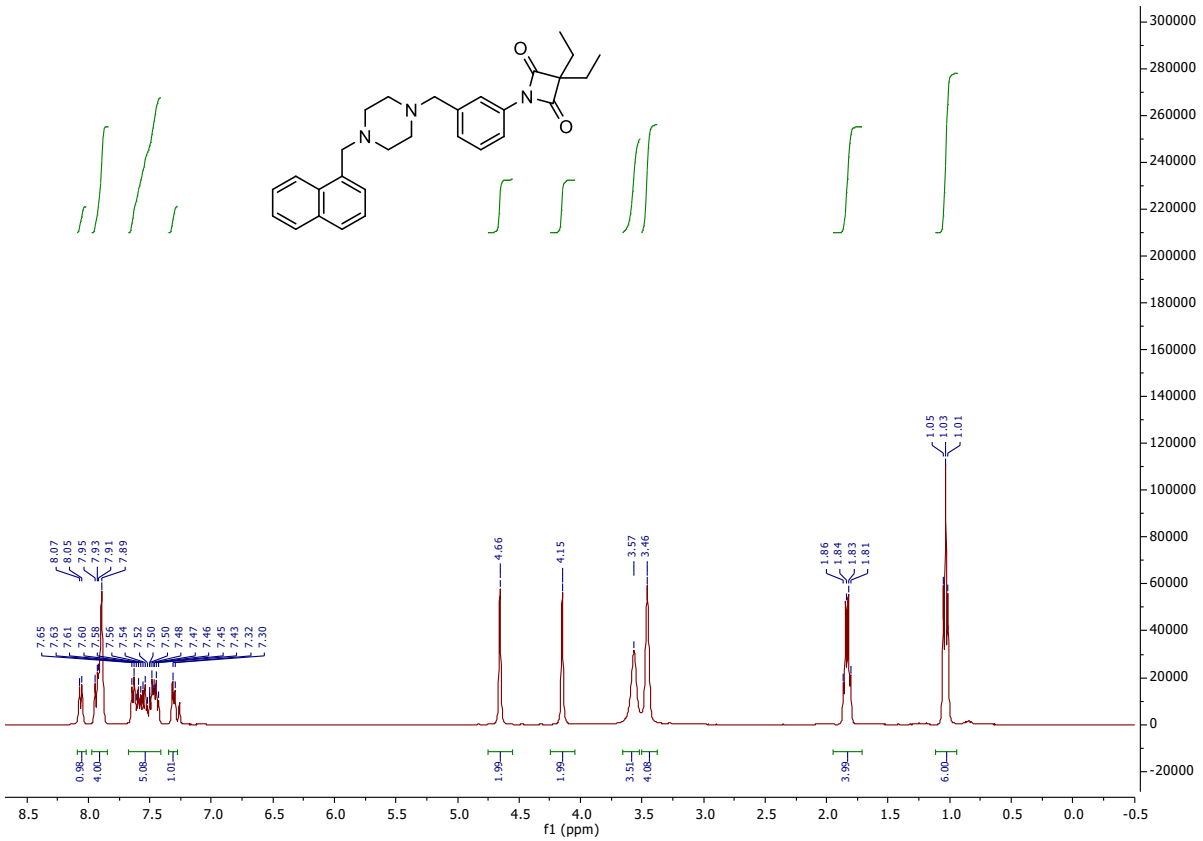



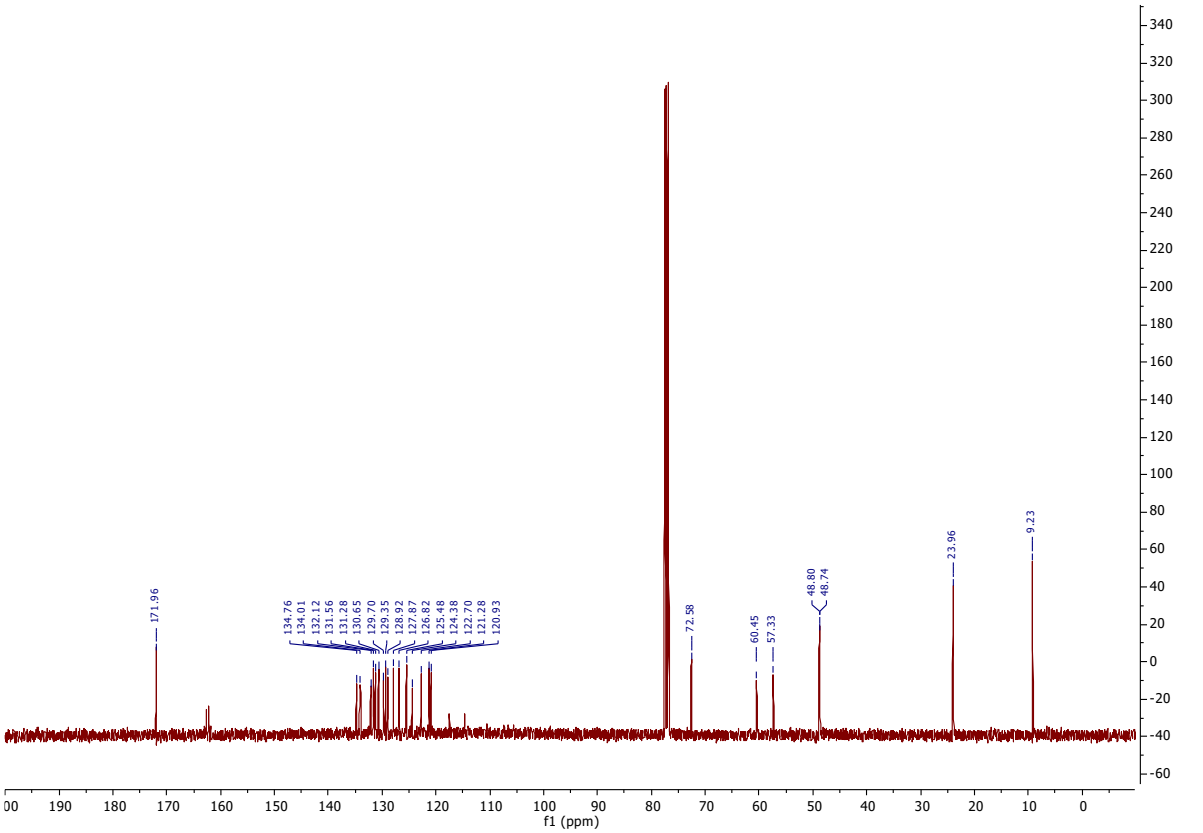
**3,3-Diethyl-1-(3-((4-methylpiperazin-1-yl)methyl)phenyl)azetidine-2,4-dione (11)**



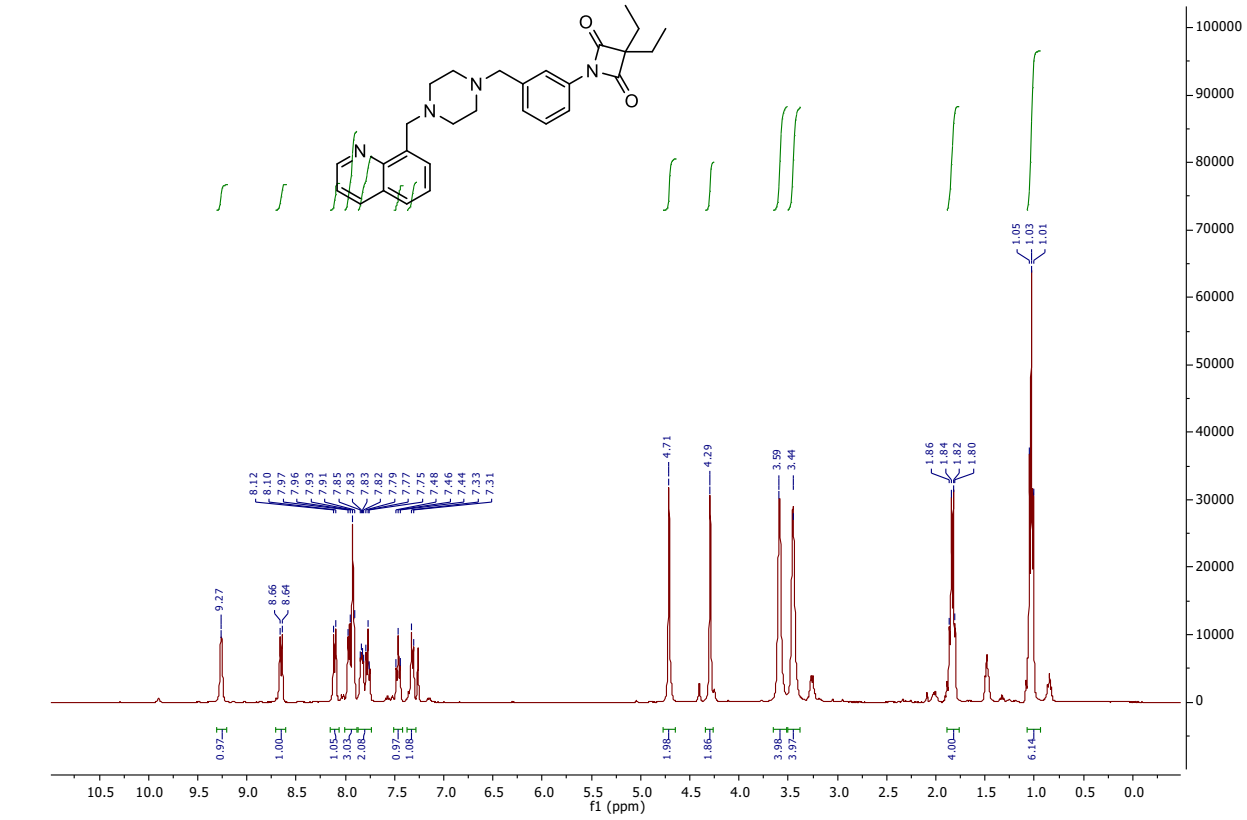



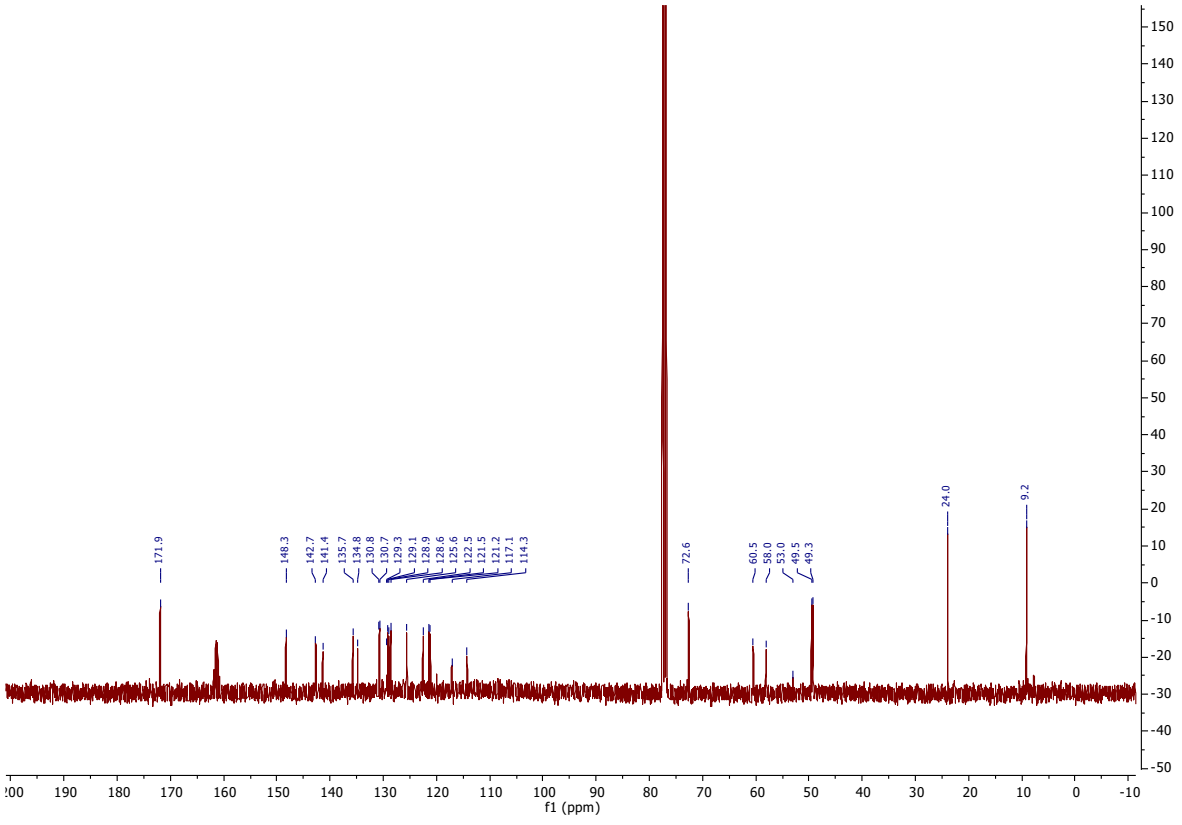
**3,3-Diethyl-1-(3-((4-(naphthalen-1-ylmethyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione (12)**



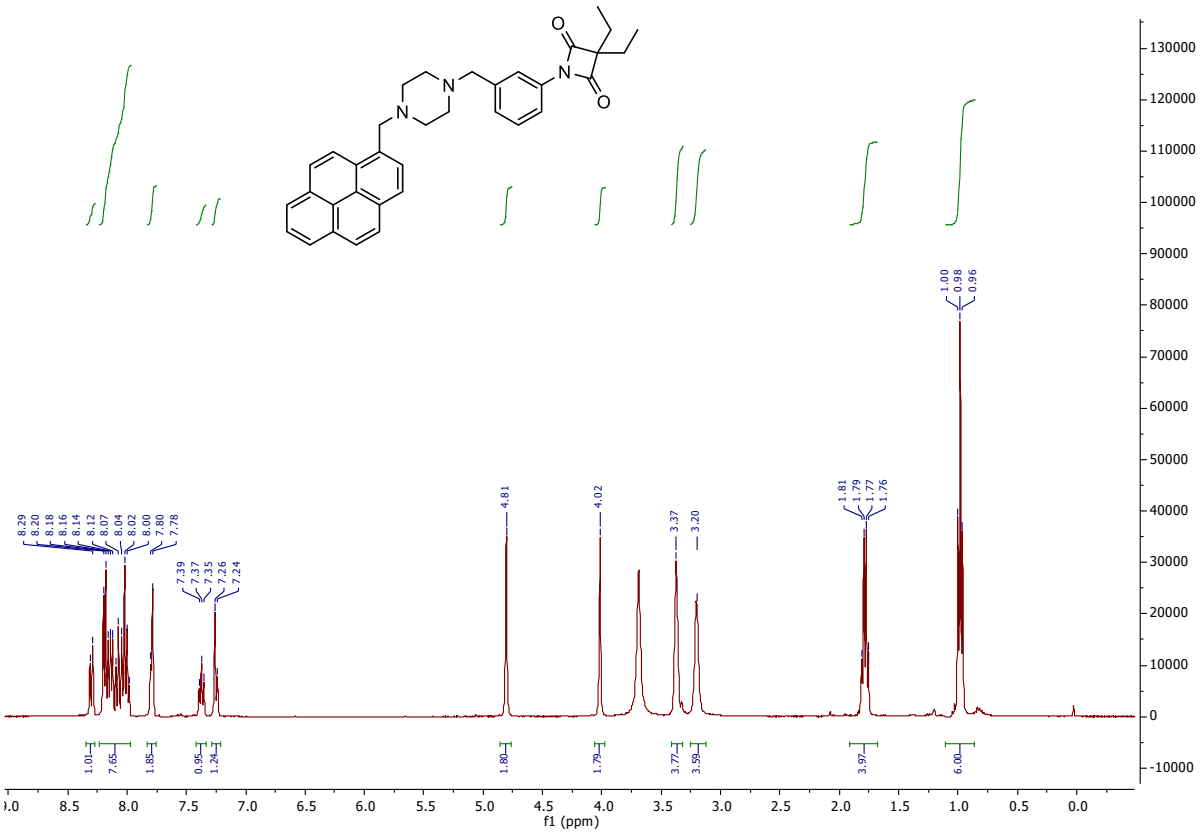



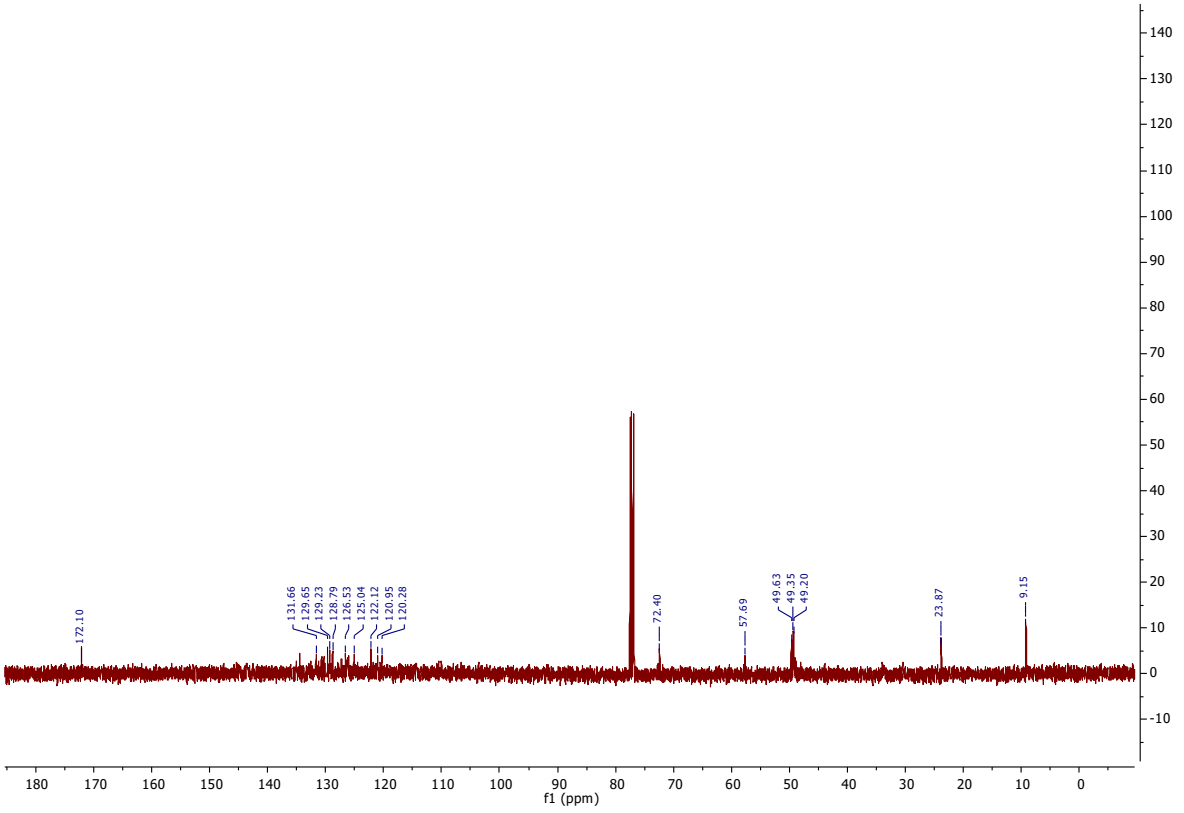
**3,3-Diethyl-1-(3-((4-(quinolin-8-ylmethyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione (13)**



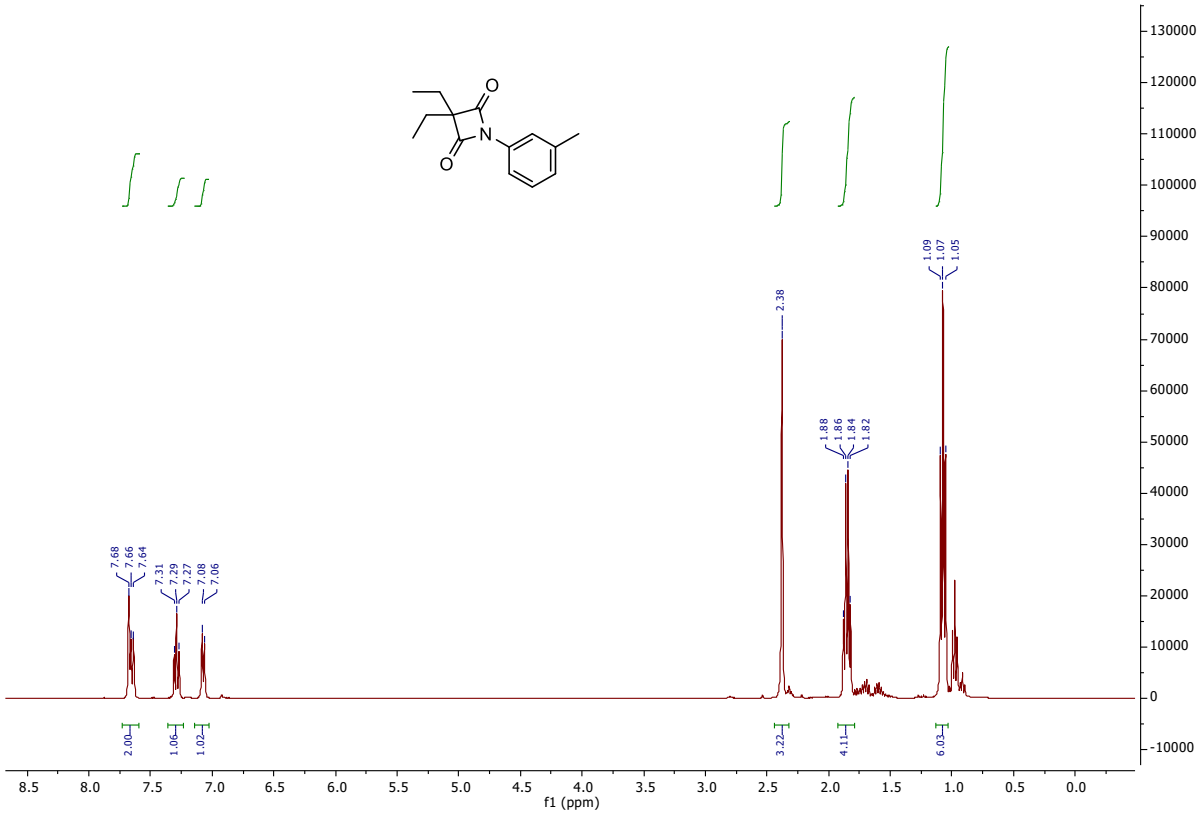



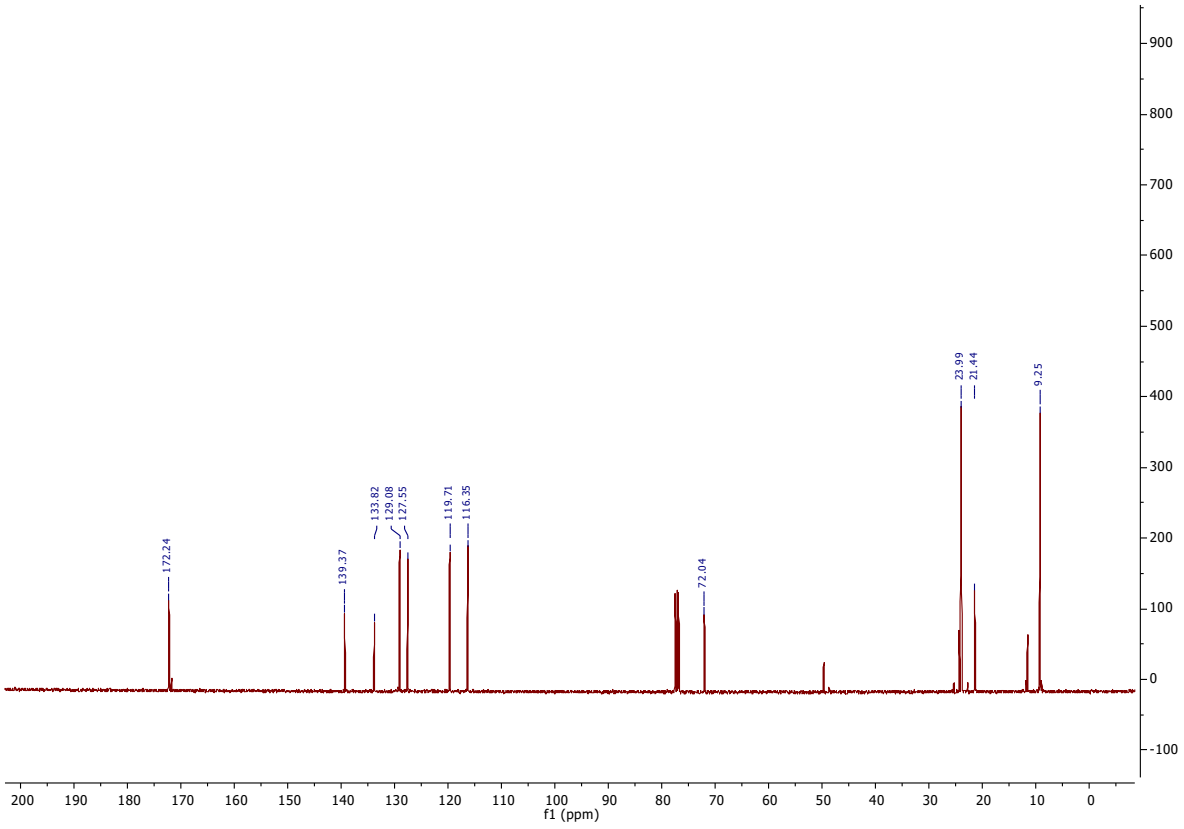
**3,3-Diethyl-1-(3-((4-(pyren-1-ylmethyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione (14)**



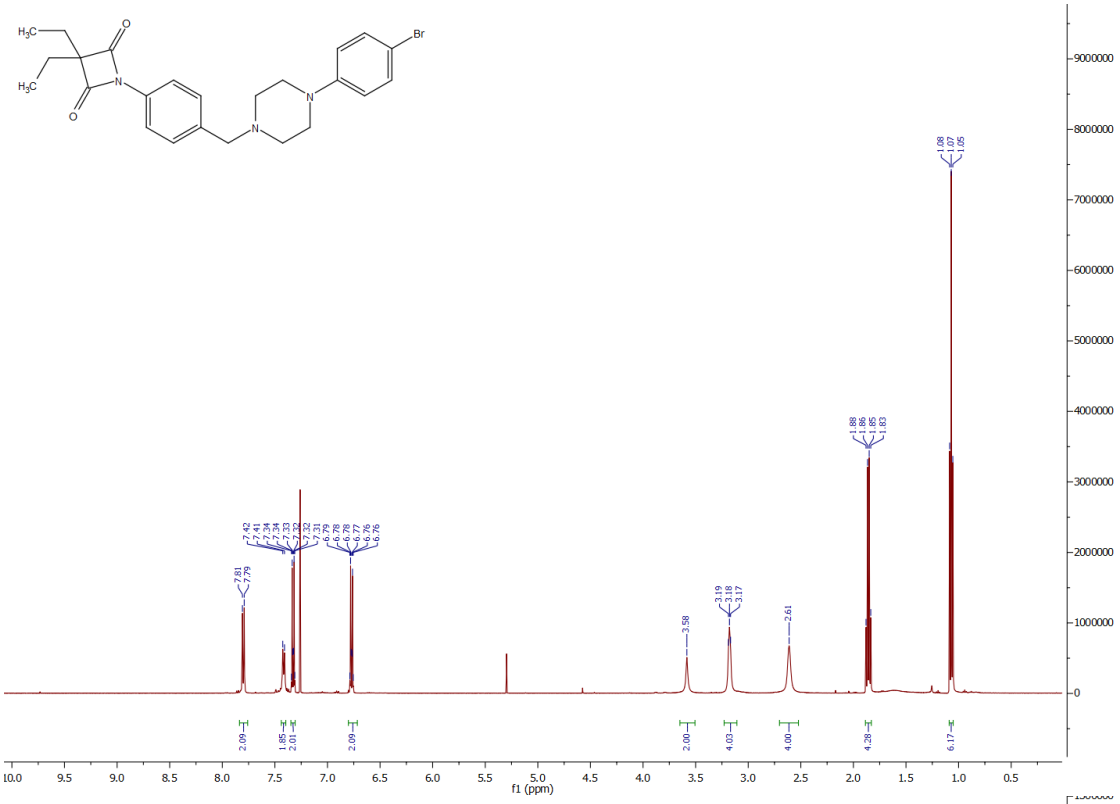



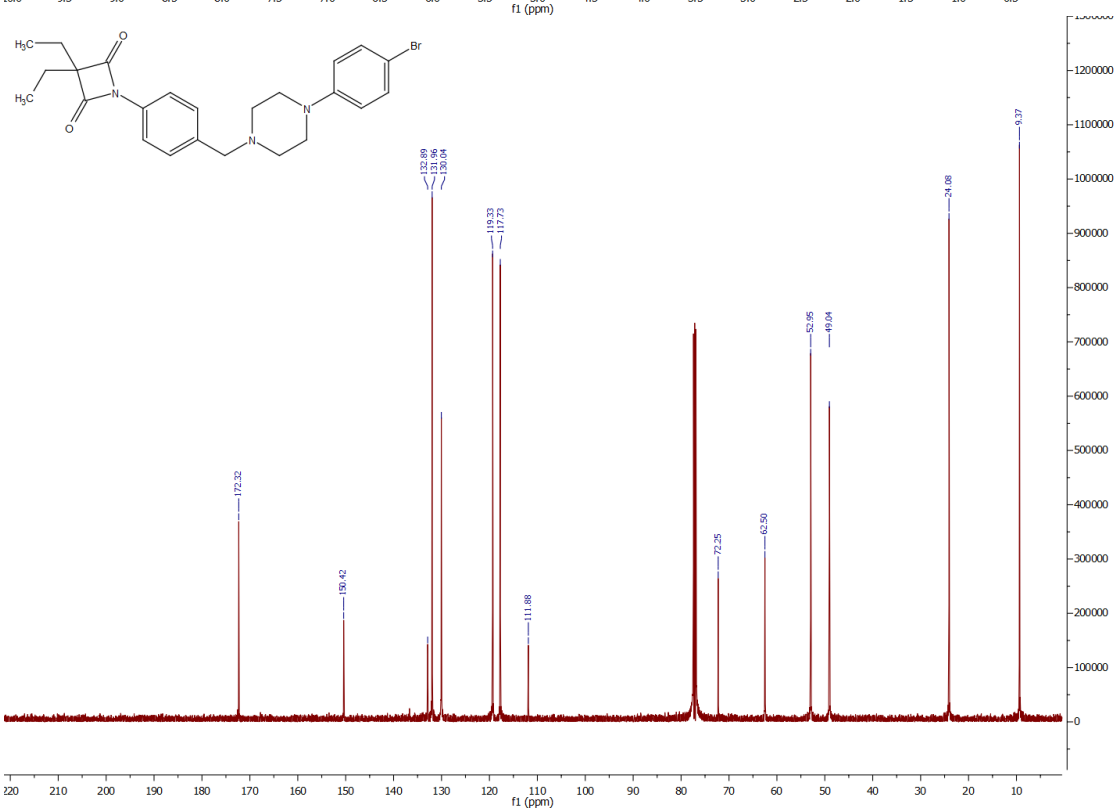
**3,3-Diethyl-1-(*m*-tolyl)azetidine-2,4-dione (17)**



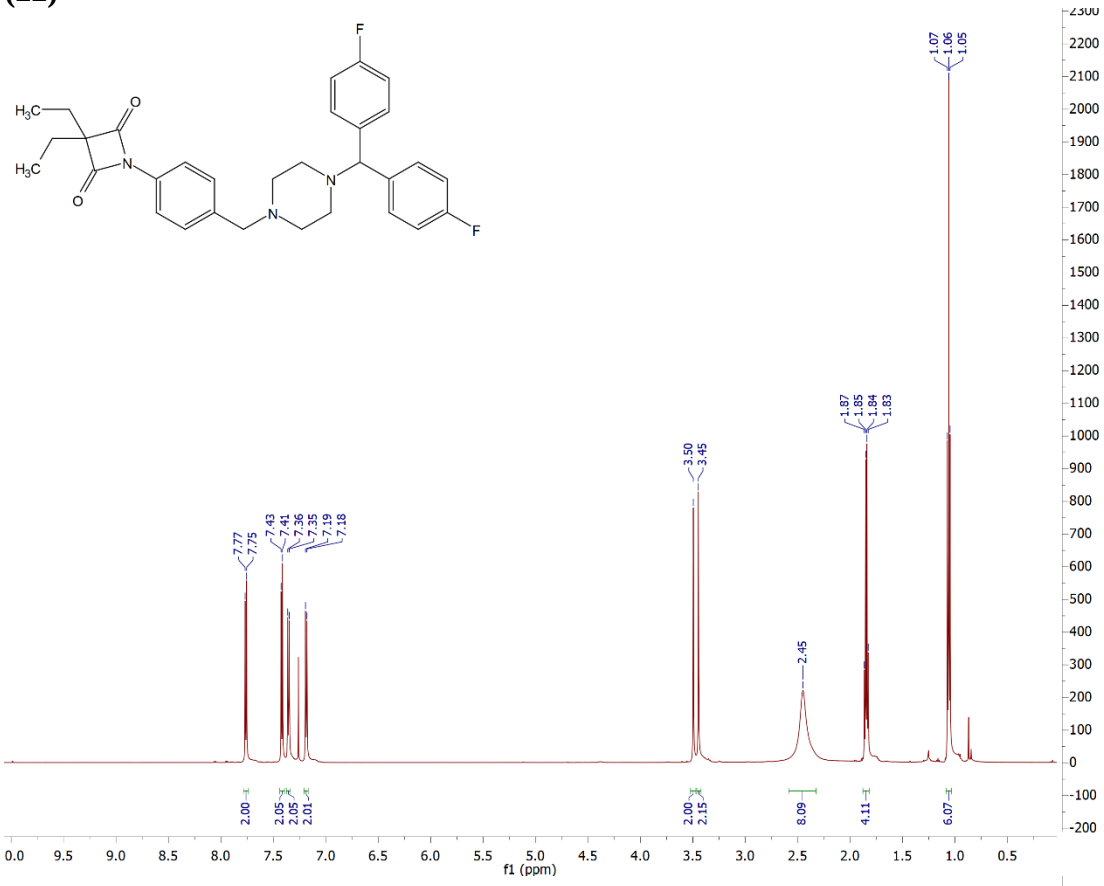



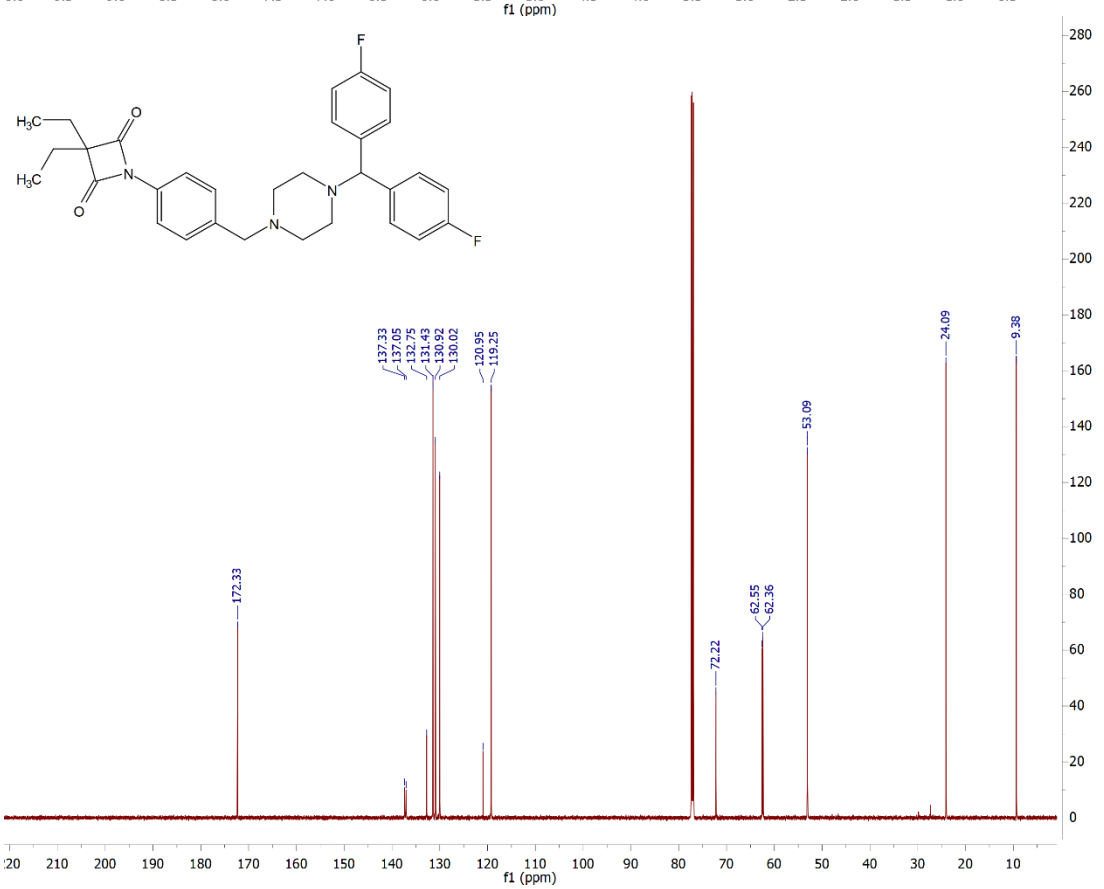
**1-(4-((4-(4-Bromophenyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (21)**



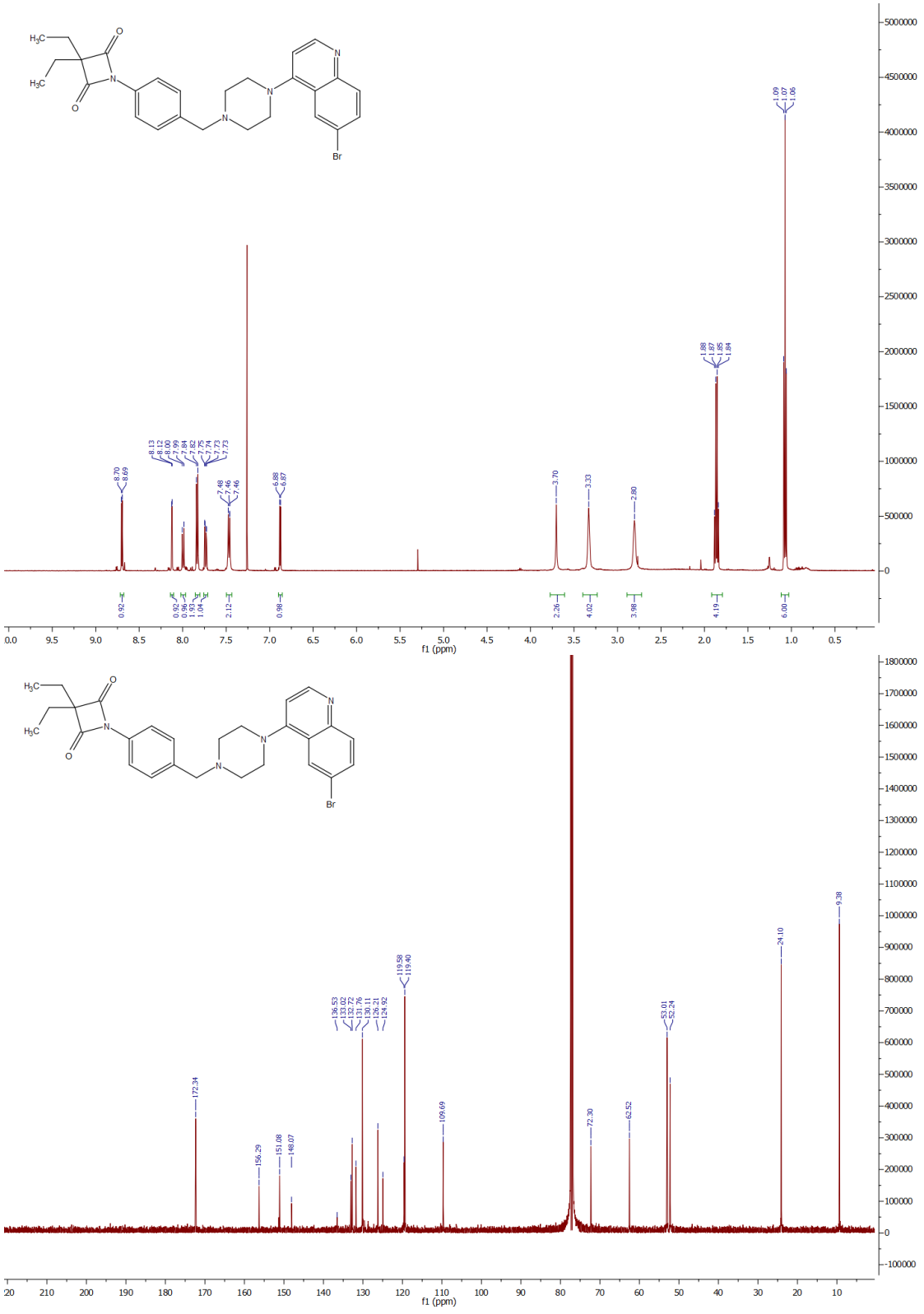



**1-(4-((4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (22)**

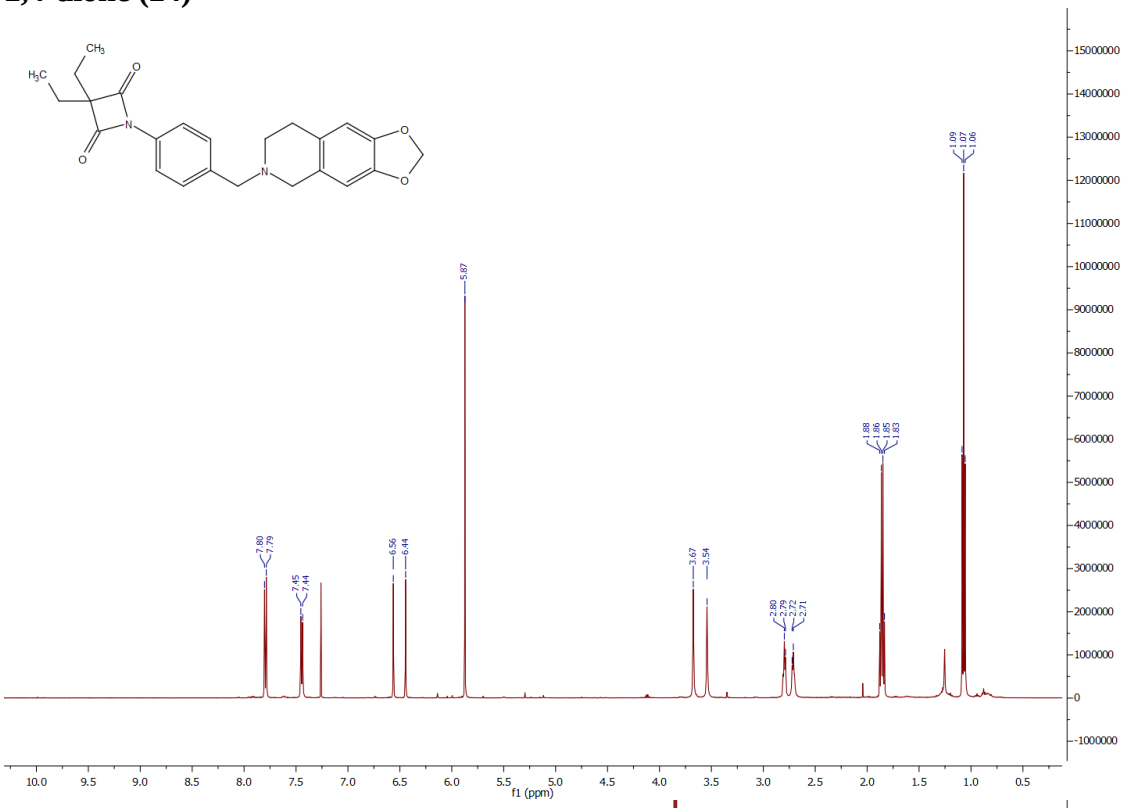


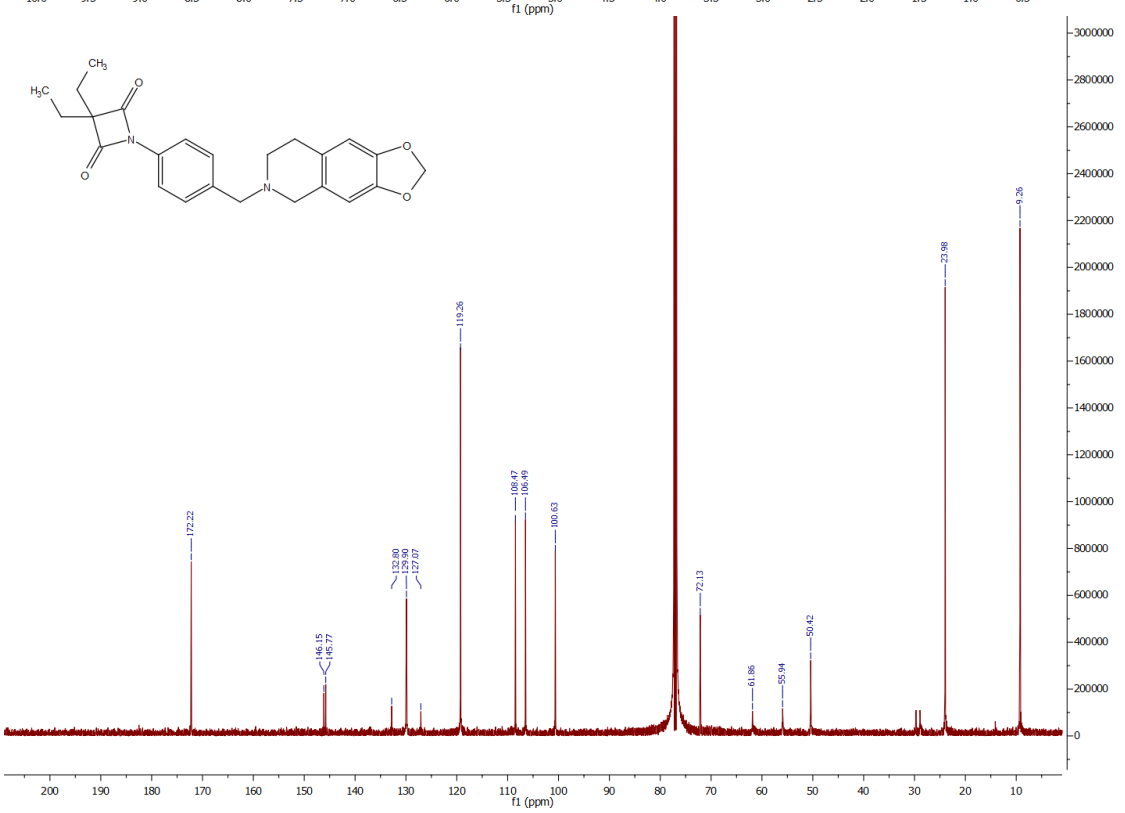



**1-(4-((4-(6-Bromoquinolin-4-yl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (23)**

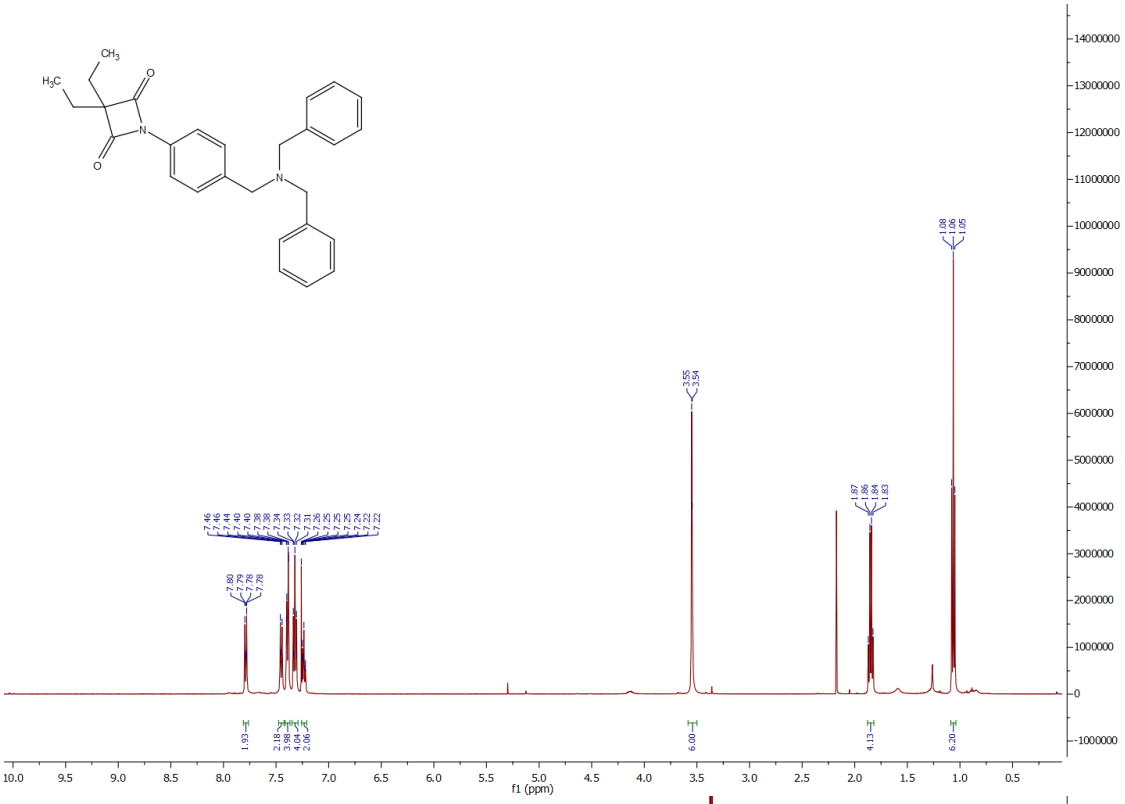


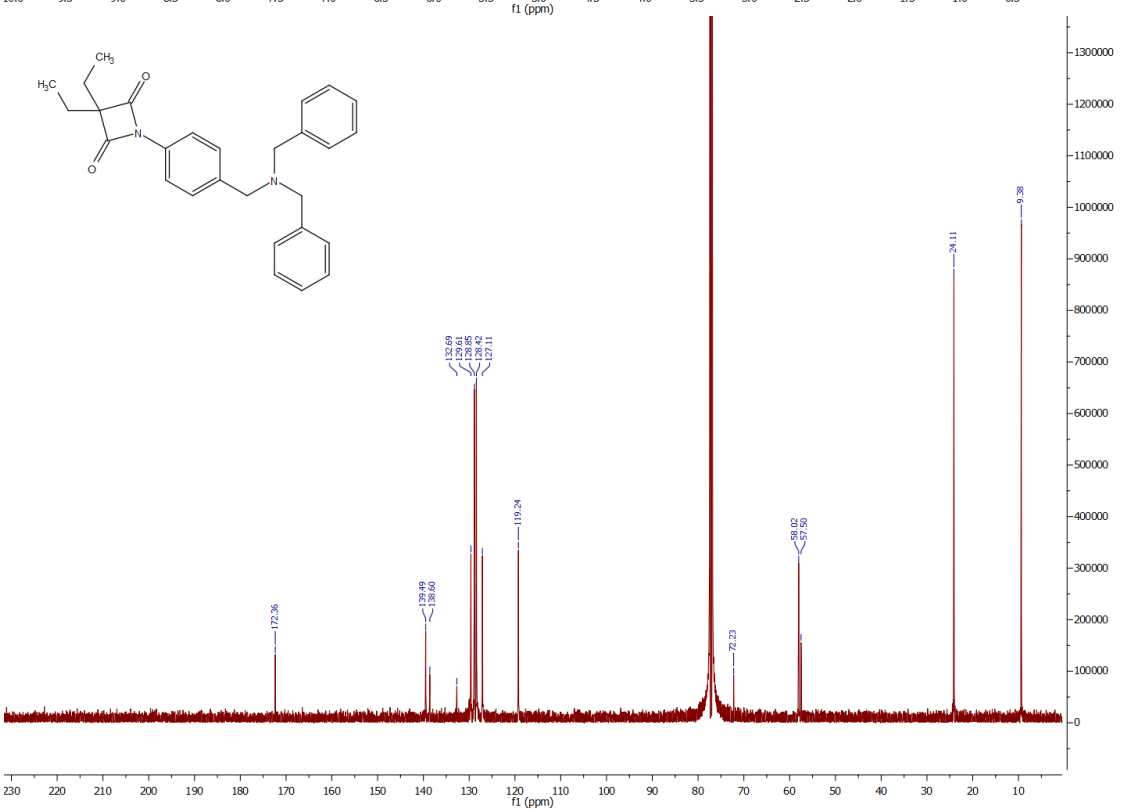
**1-(4-((7,8-Dihydro-[1,3]dioxolo[4,5-g]isoquinolin-6(5H)-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (24)**



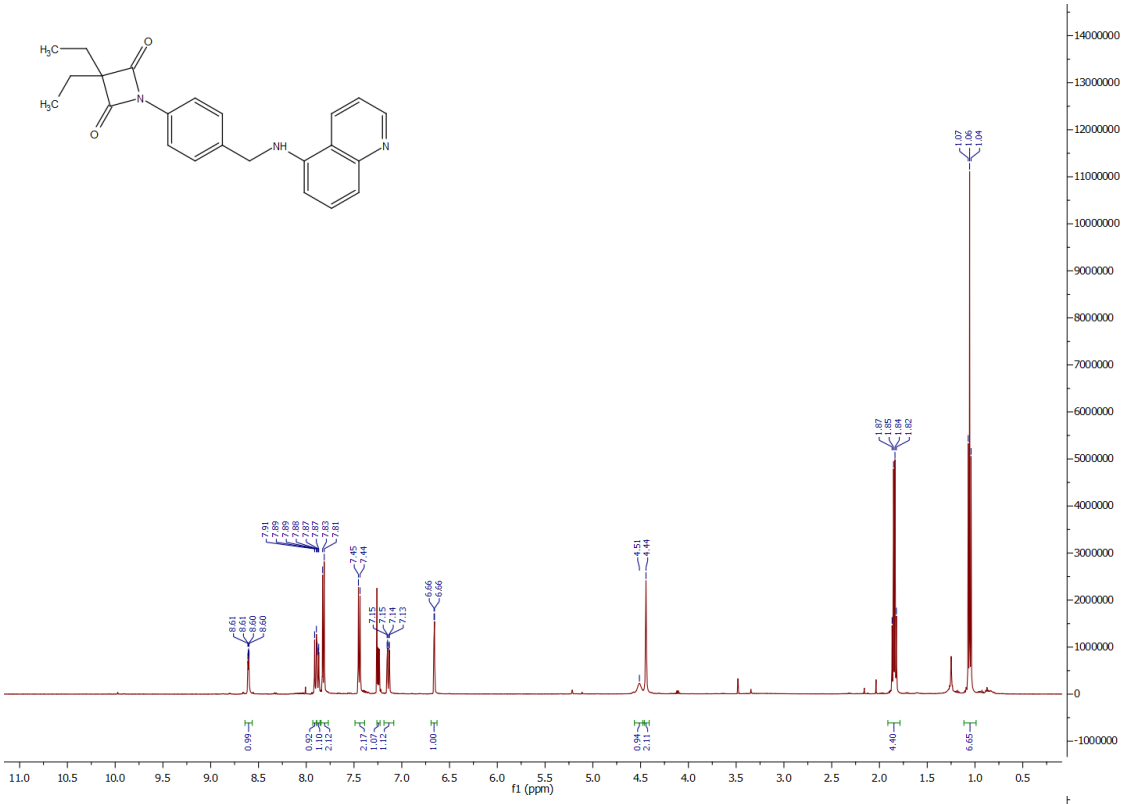



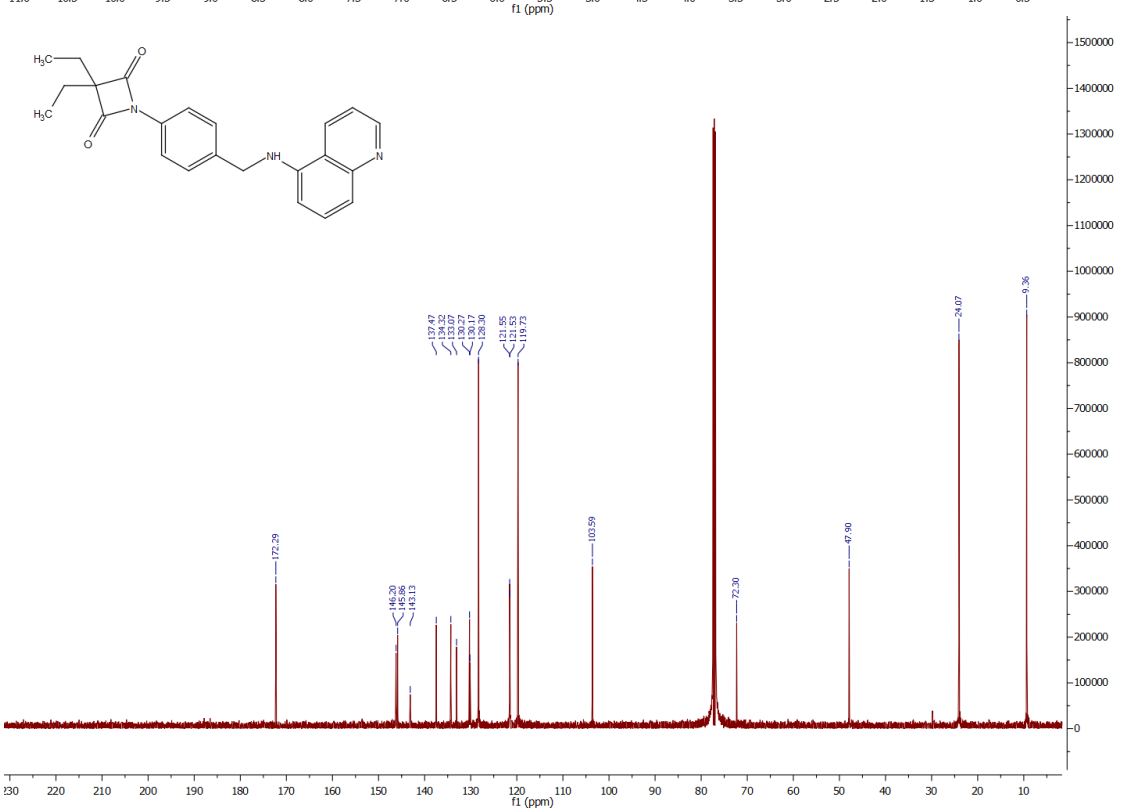
**1-(4-((Dibenzylamino)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (25)**



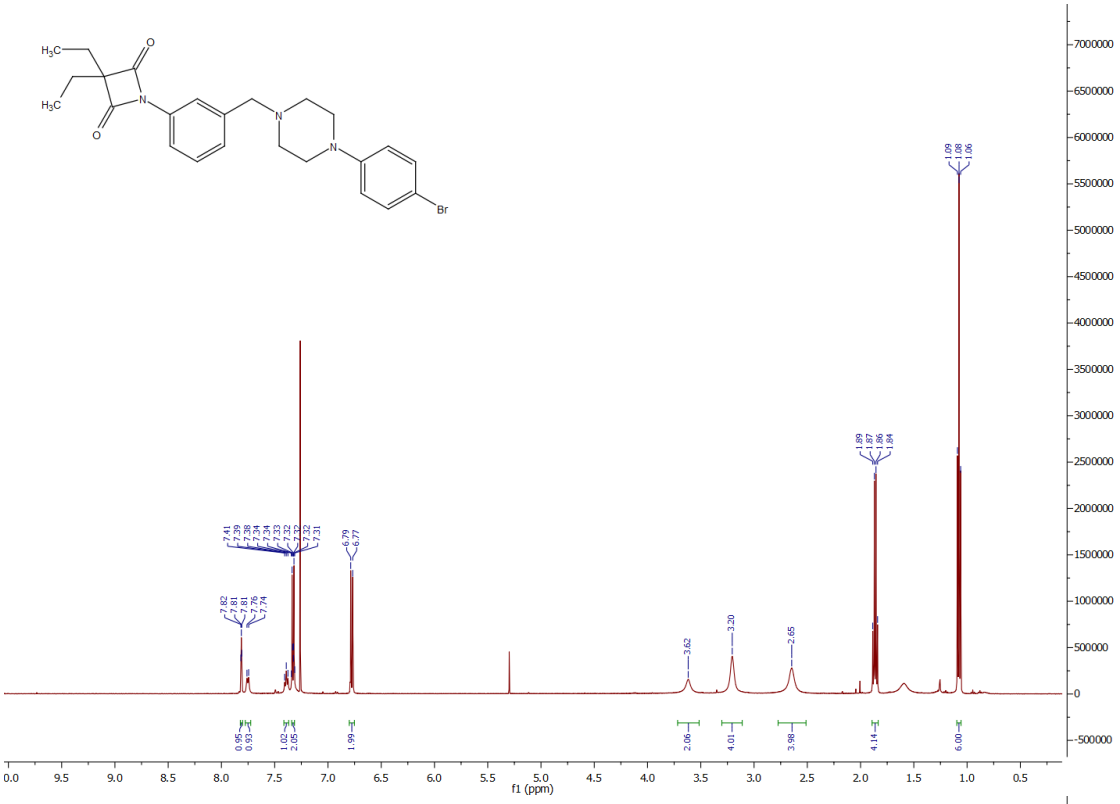



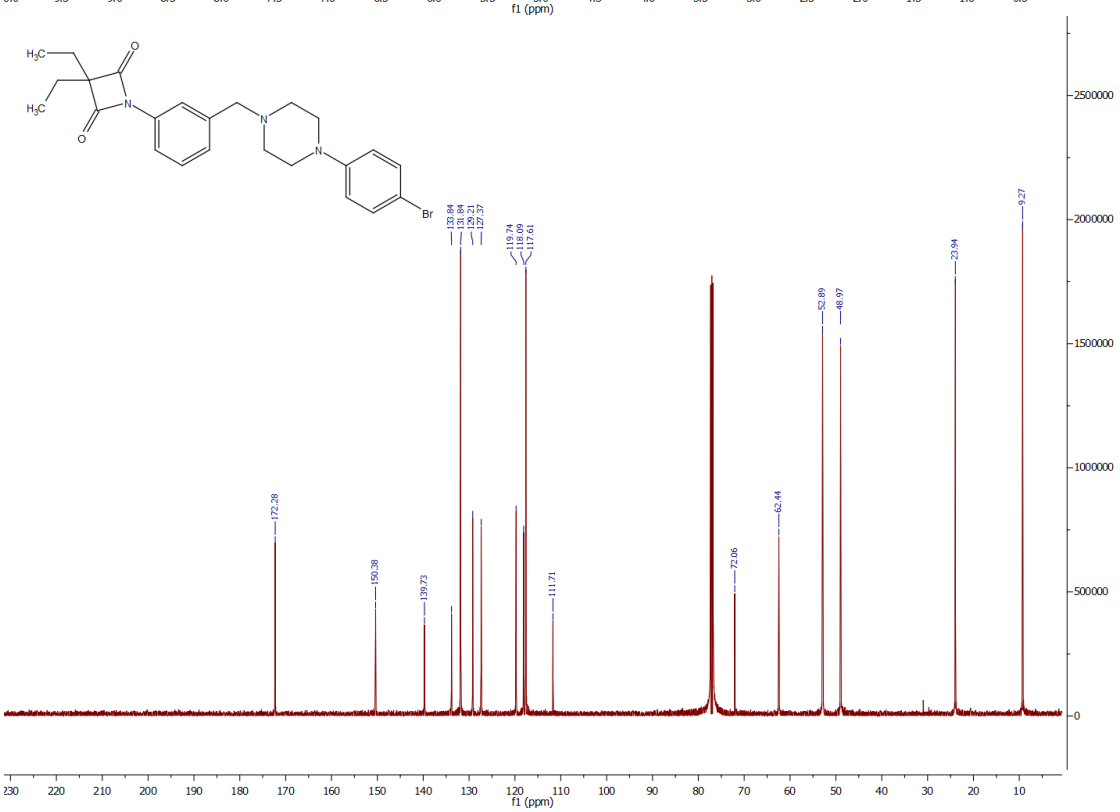
## 3,3-Diethyl-1-(4-((quinolin-5-ylamino)methyl)phenyl)azetidine-2,4-dione (26)



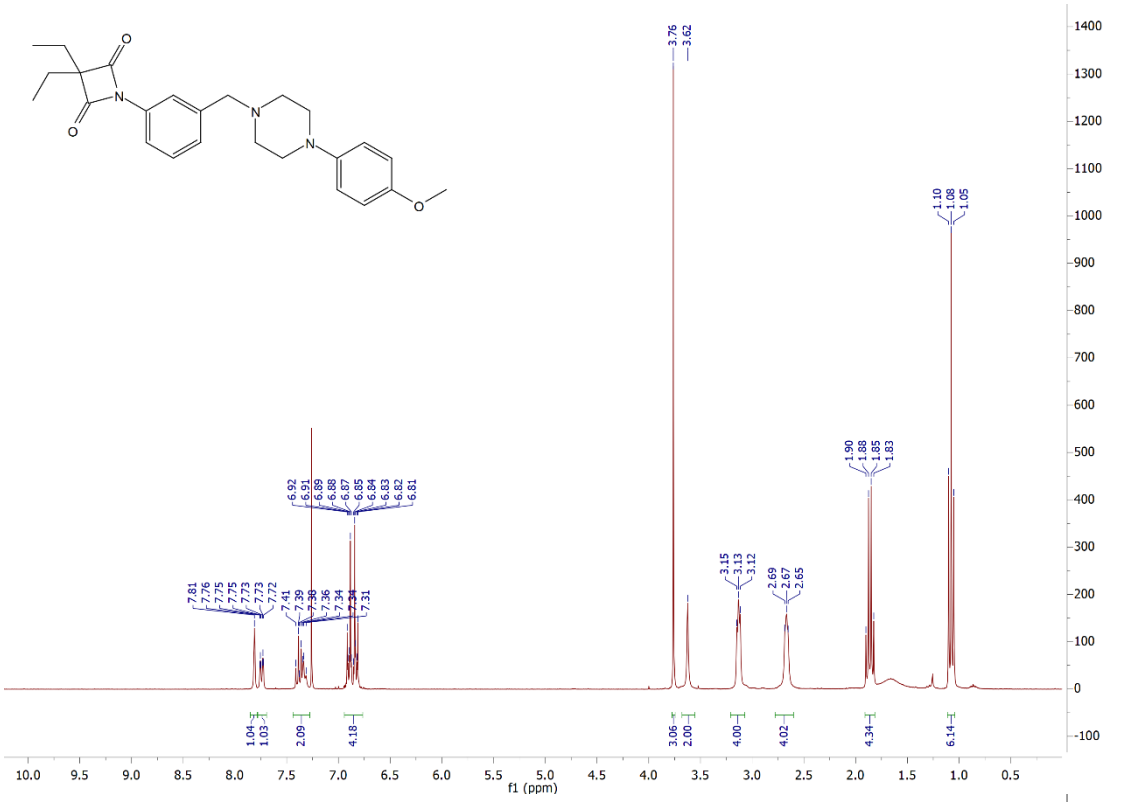



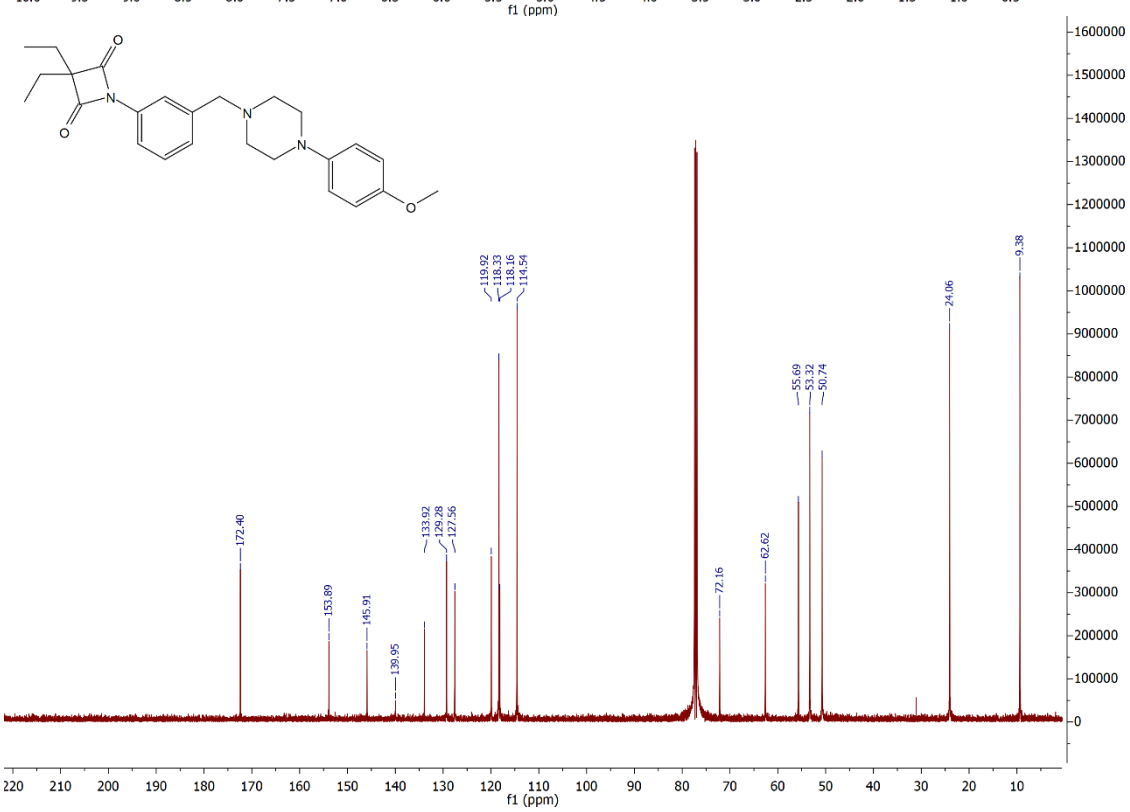
**1-(3-((4-(4-Bromophenyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (27)**



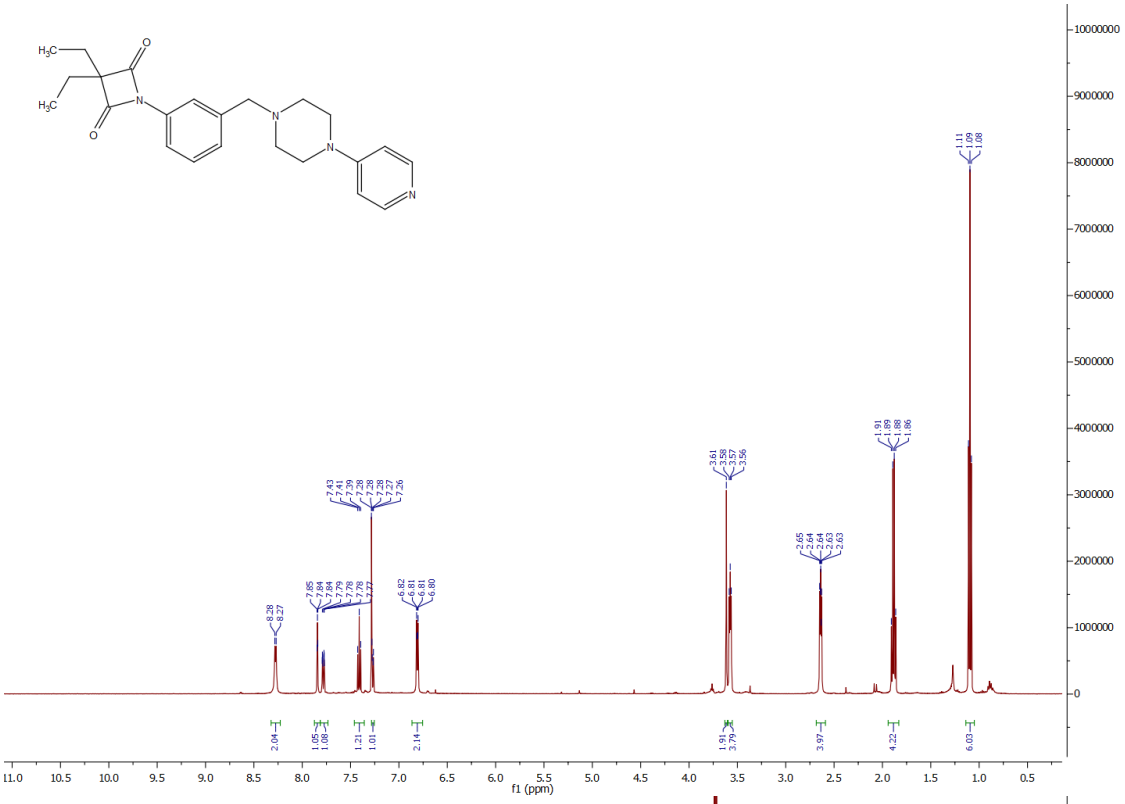



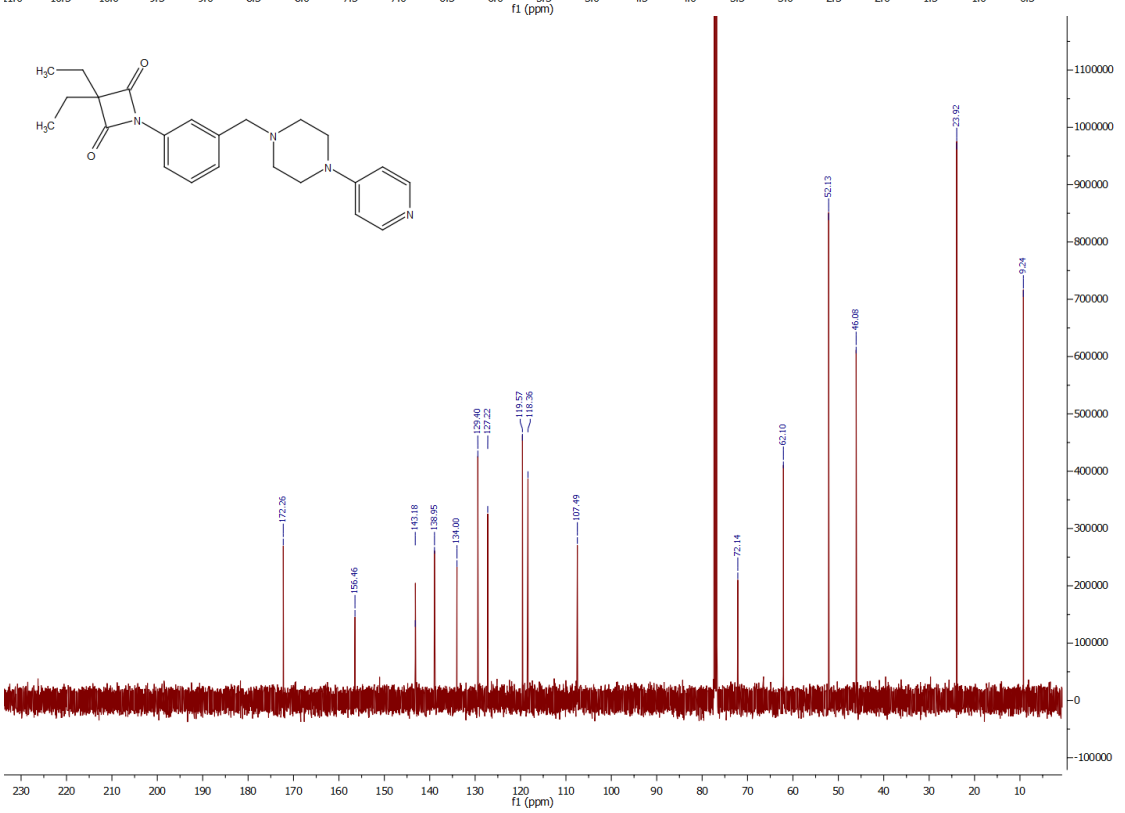
**3,3-Diethyl-1-(3-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione (28)**



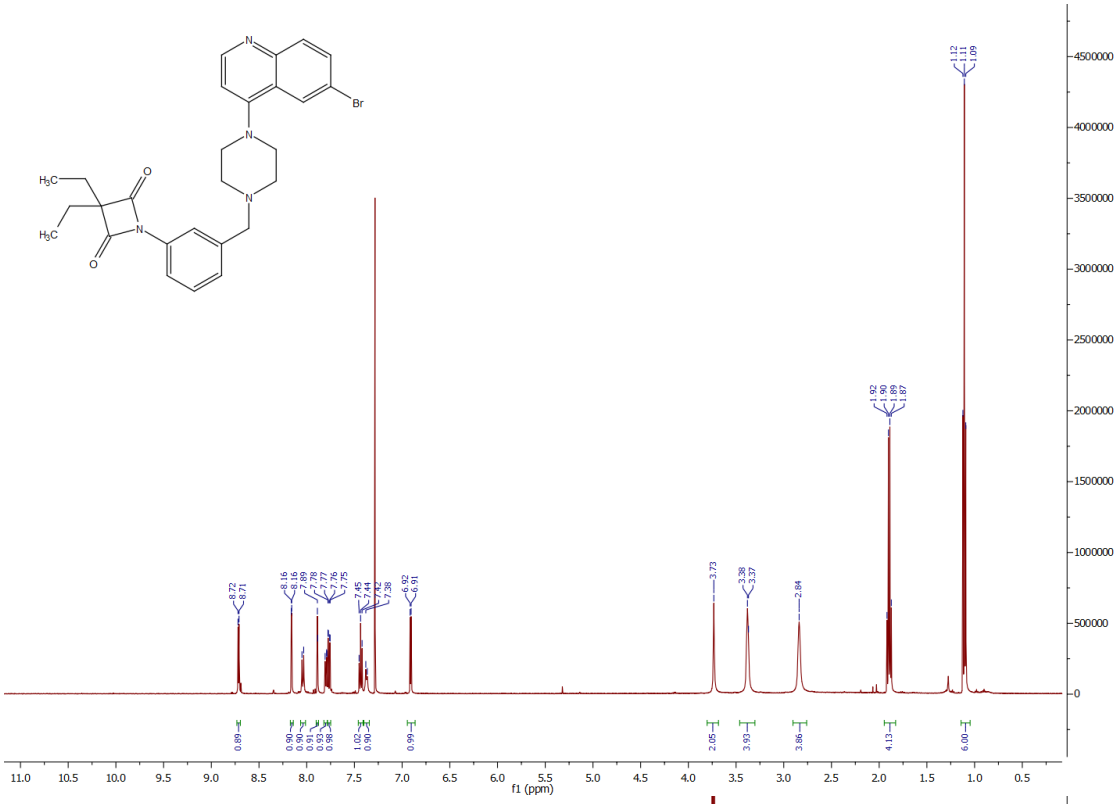
**3,3-Diethyl-1-(3-((4-(pyridin-4-yl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione (29)**

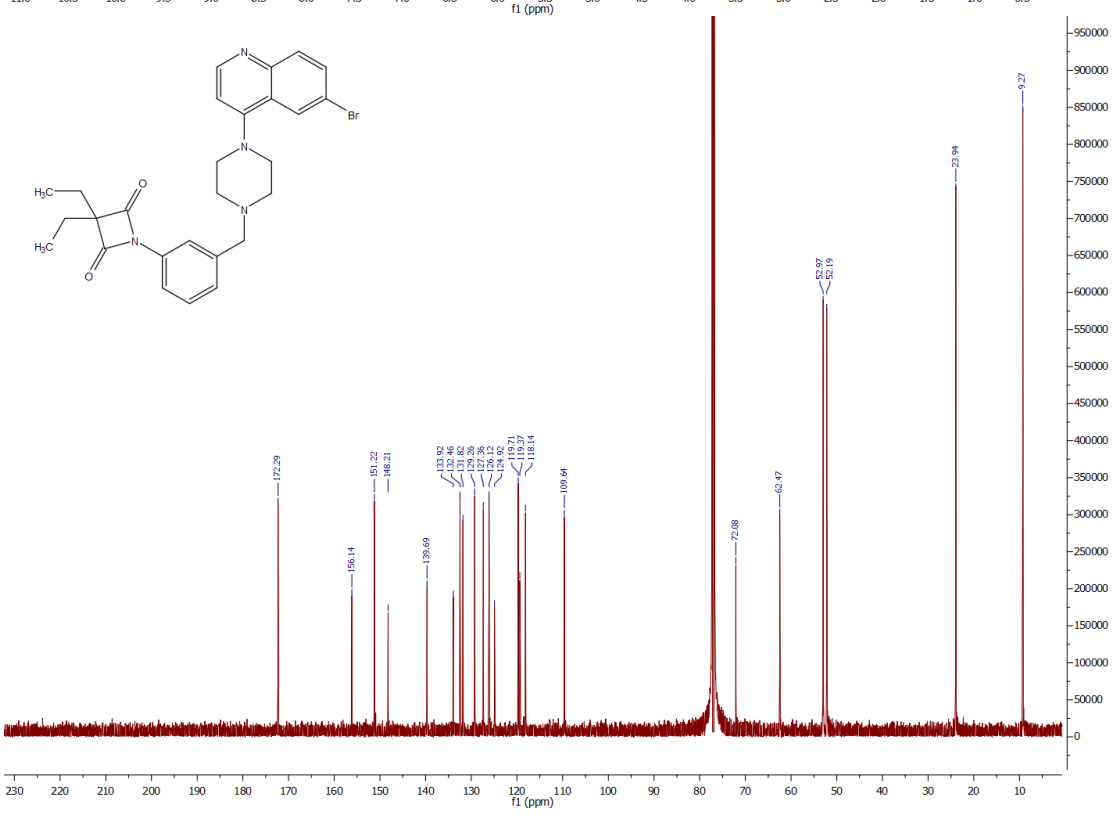


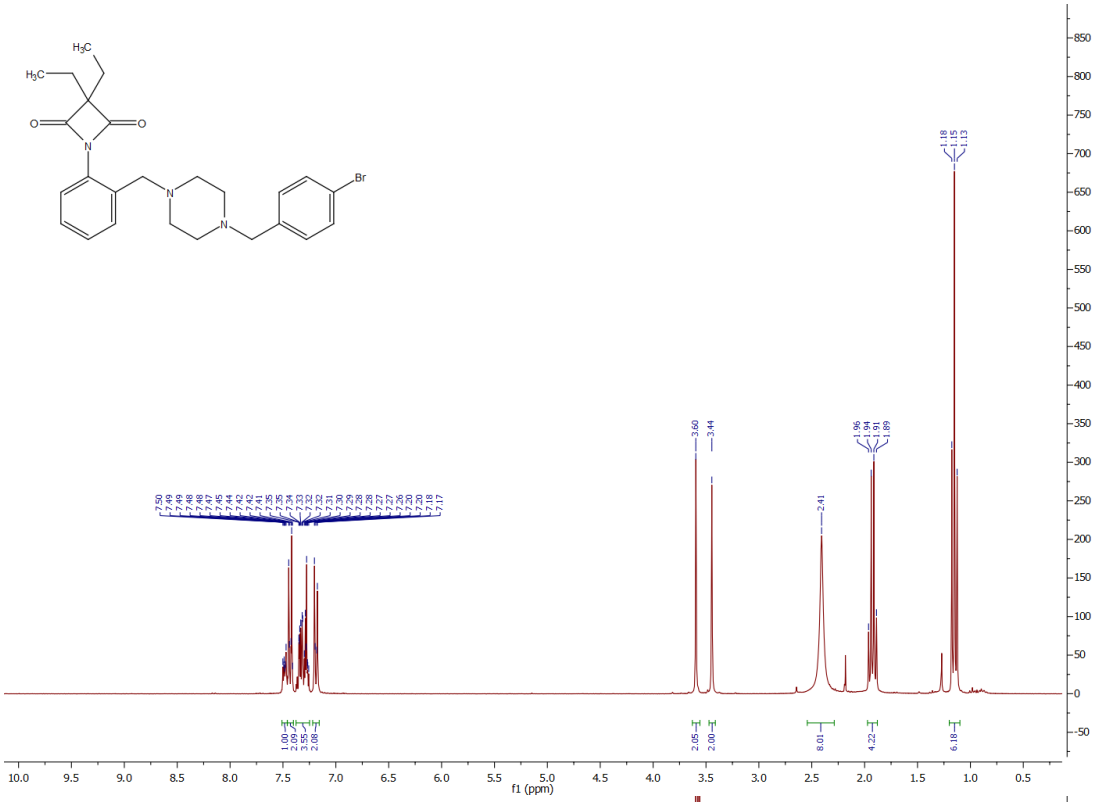
SUPPORTING INFORMATION

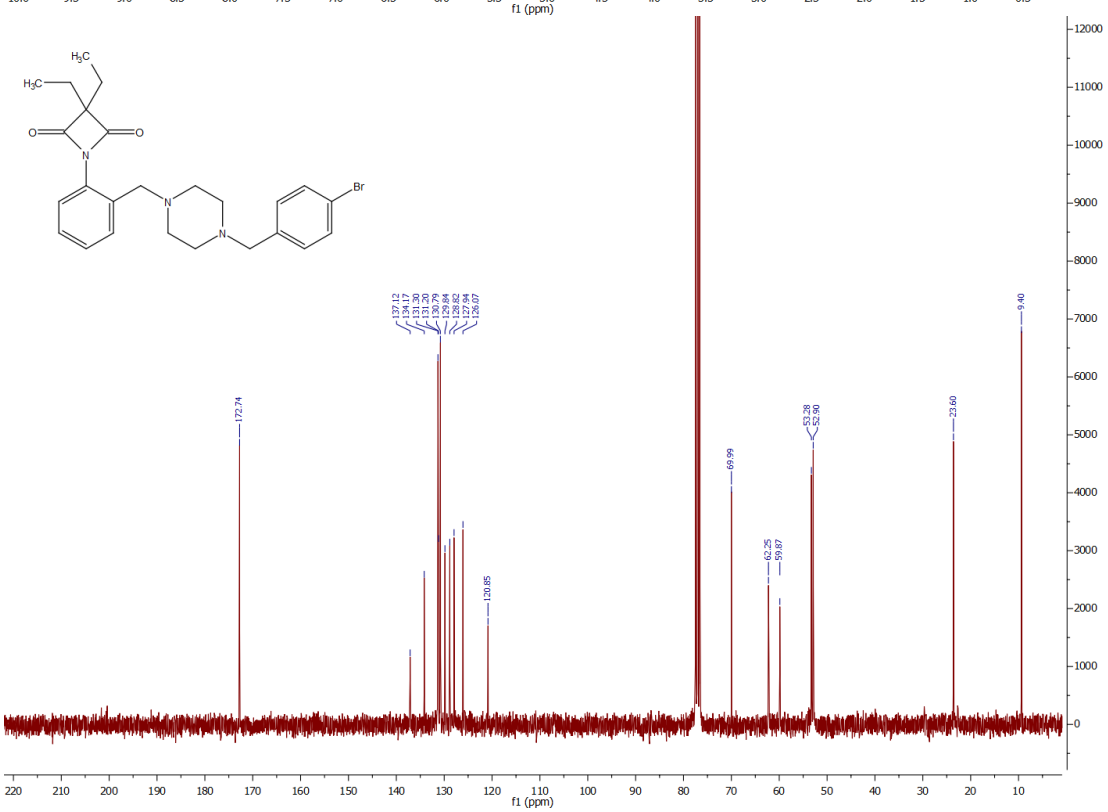
**1-(3-((4-(6-Bromoquinolin-4-yl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (30)**



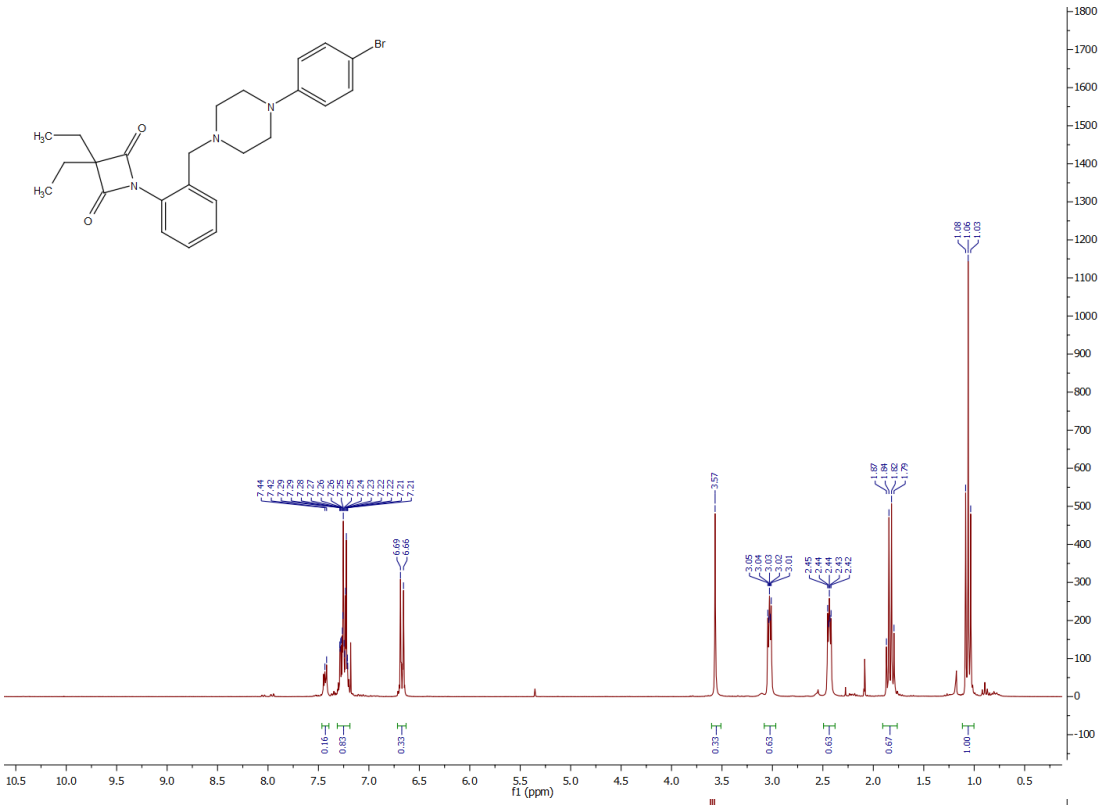



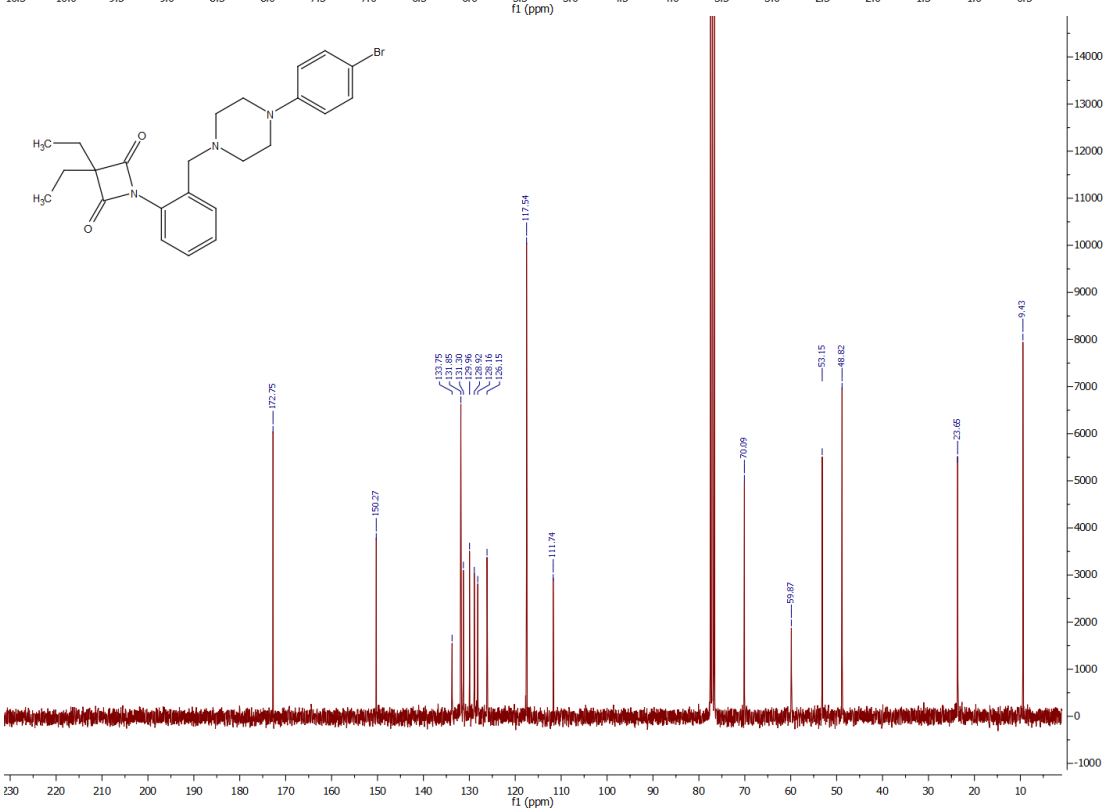
**1-(2-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (31)**



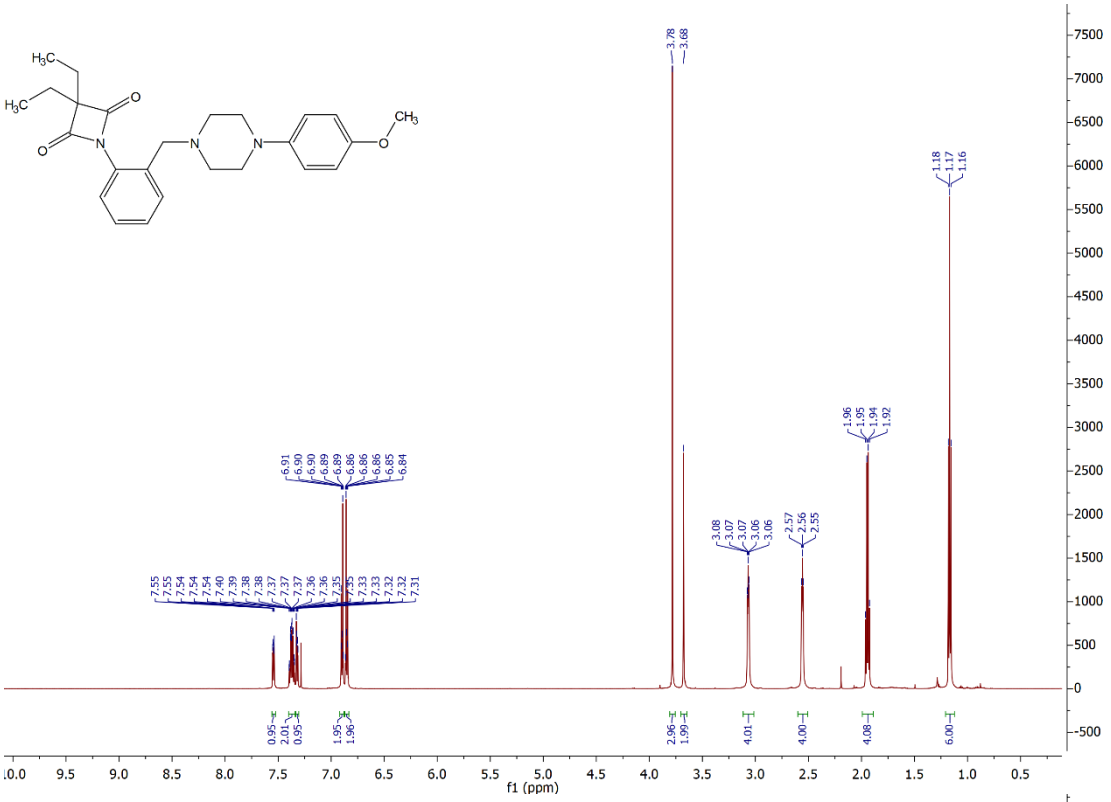



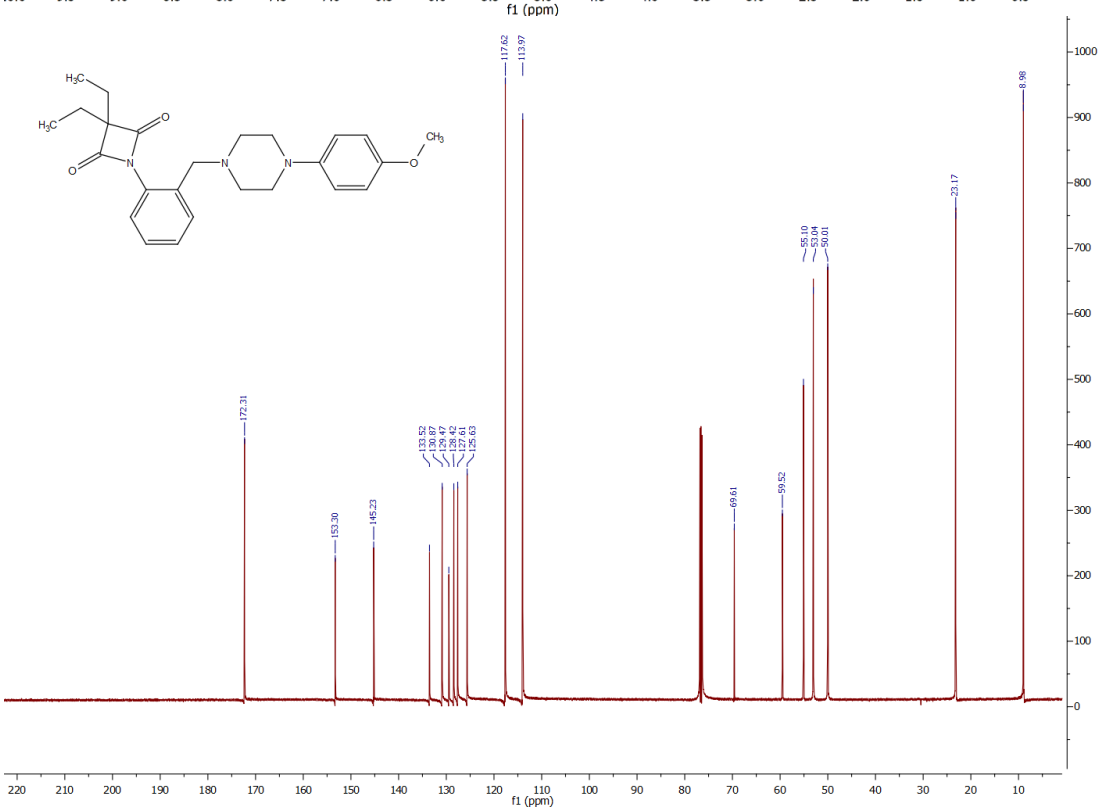
**1-(2-((4-(4-Bromophenyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (32)**



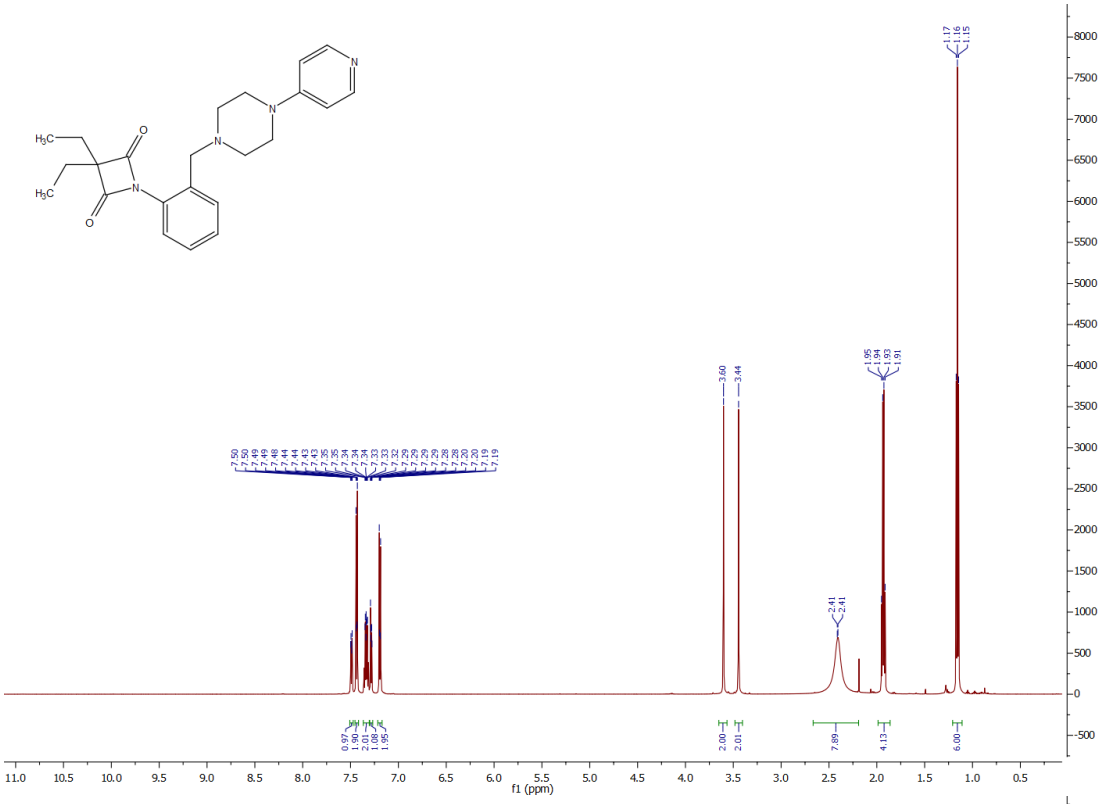



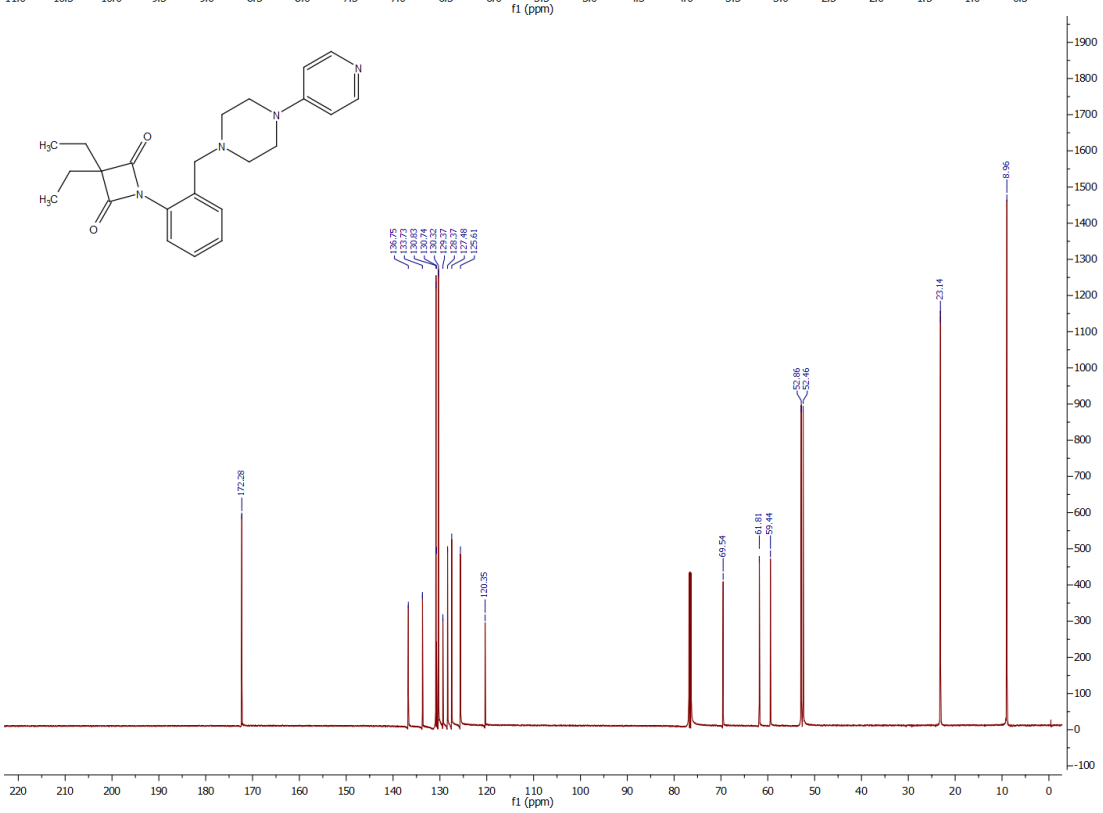
**3,3-Diethyl-1-(2-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione (33)**



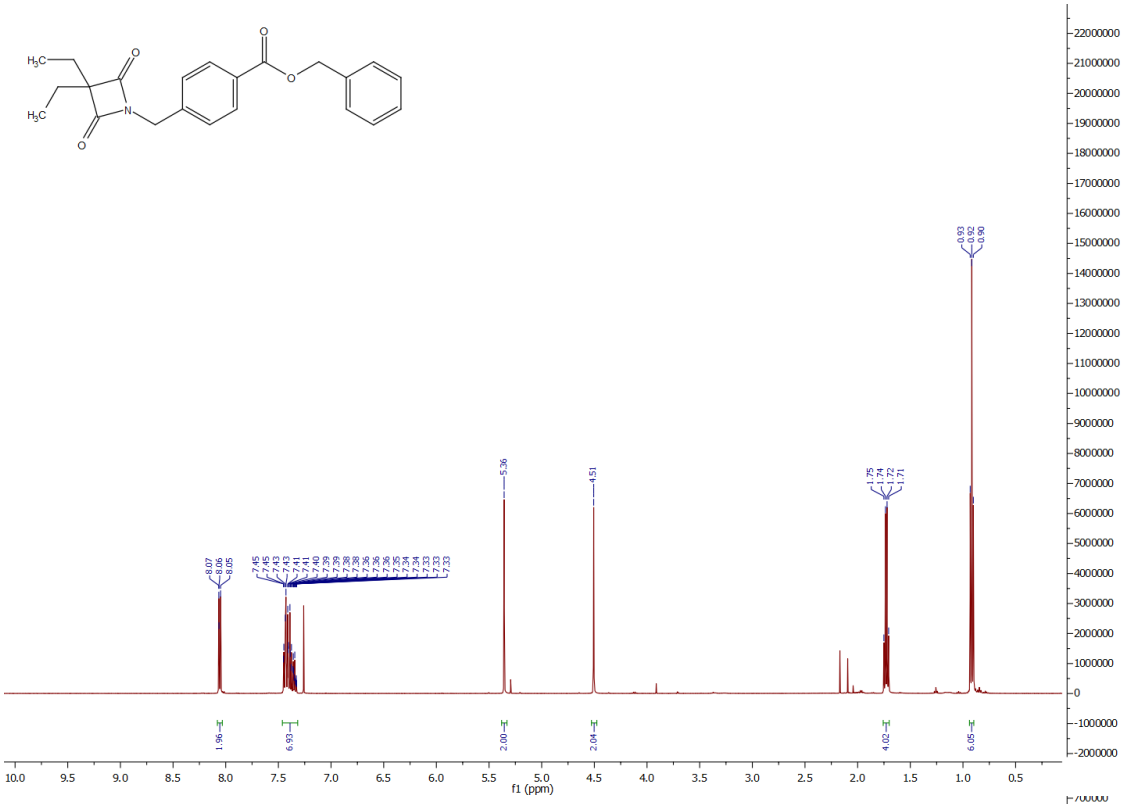



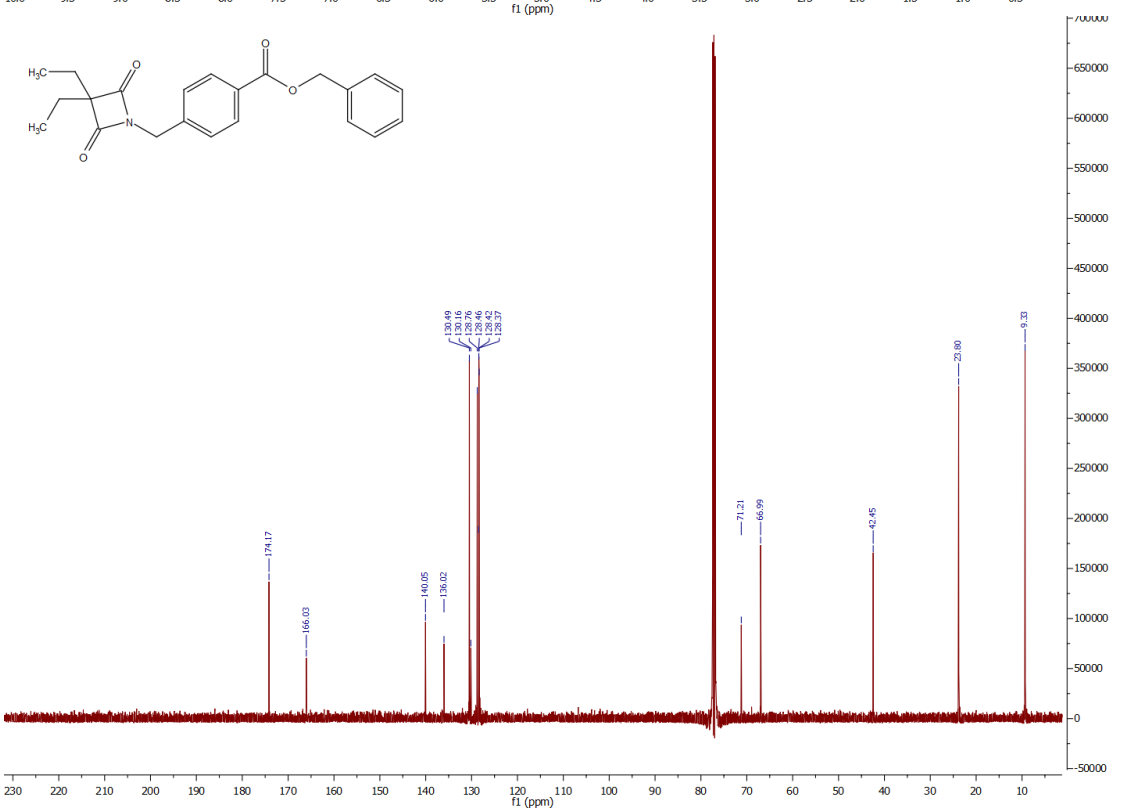
**3,3-Diethyl-1-(2-((4-(pyridin-4-yl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione (34)**



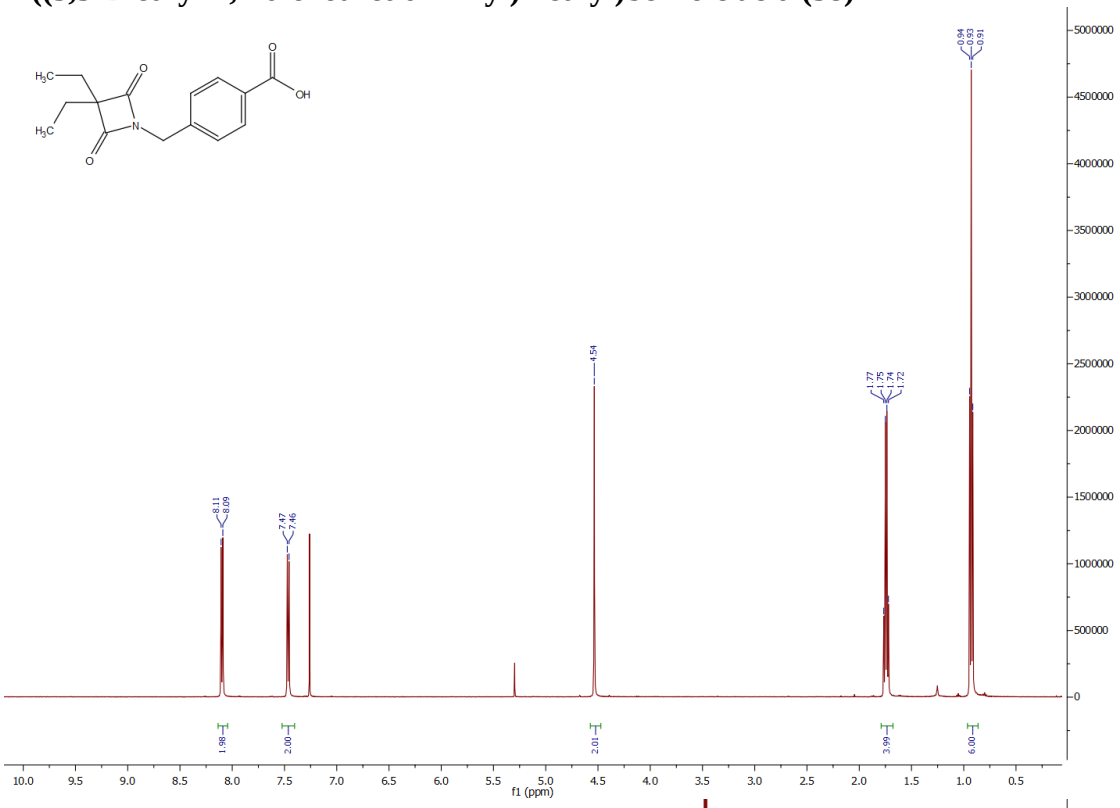



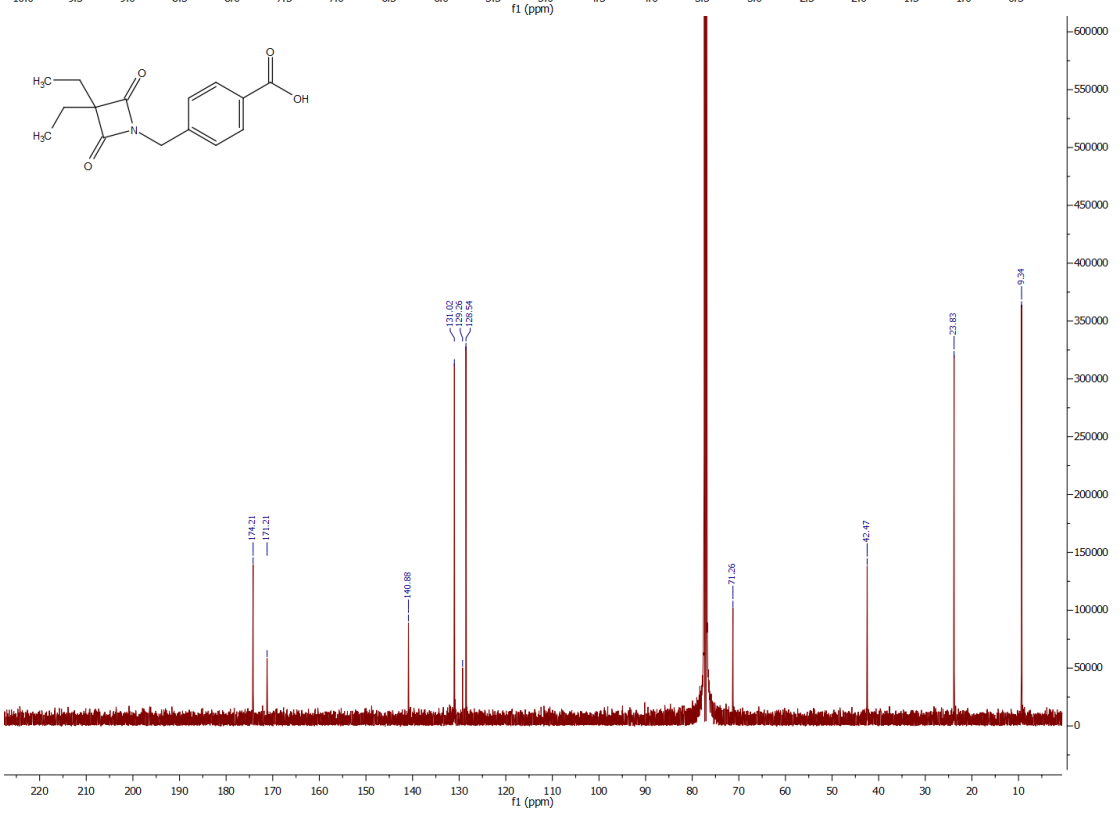
**Benzyl 4-((3,3-diethyl-2,4-dioxoazetidin-1-yl)methyl)benzoate (37)**



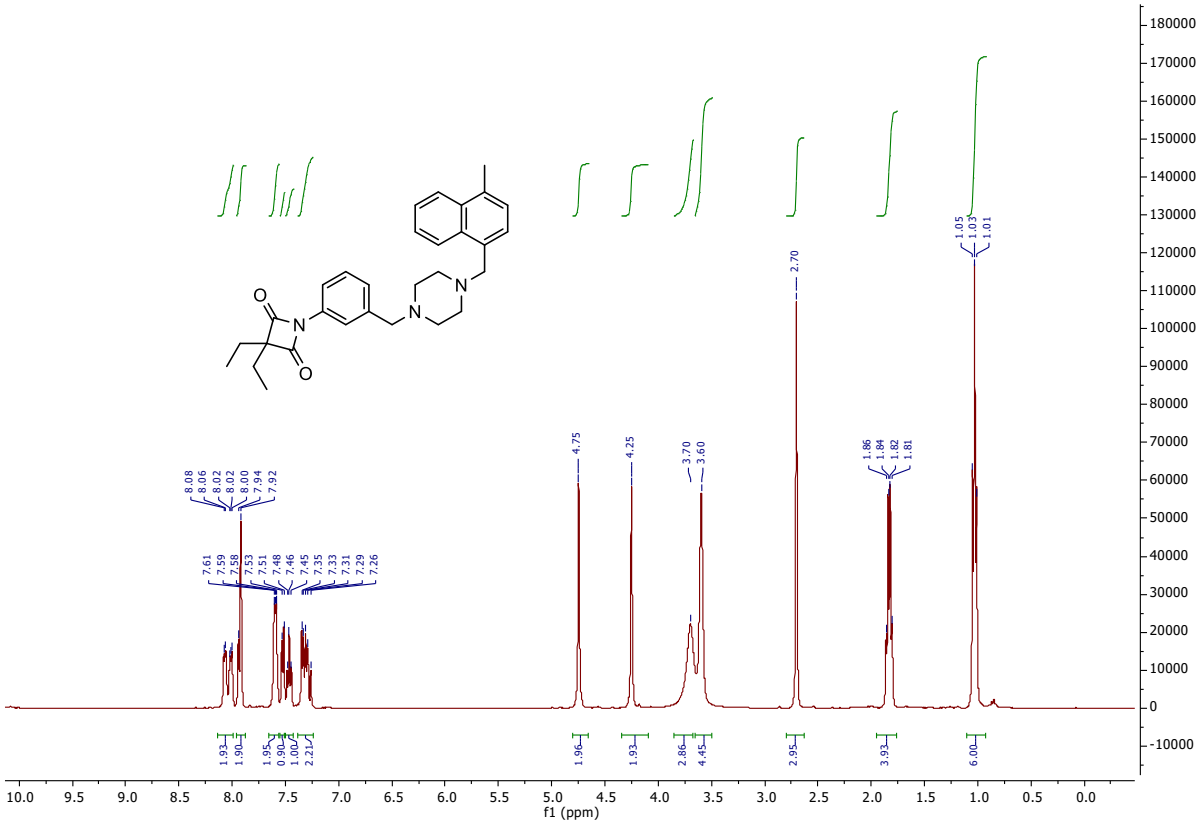



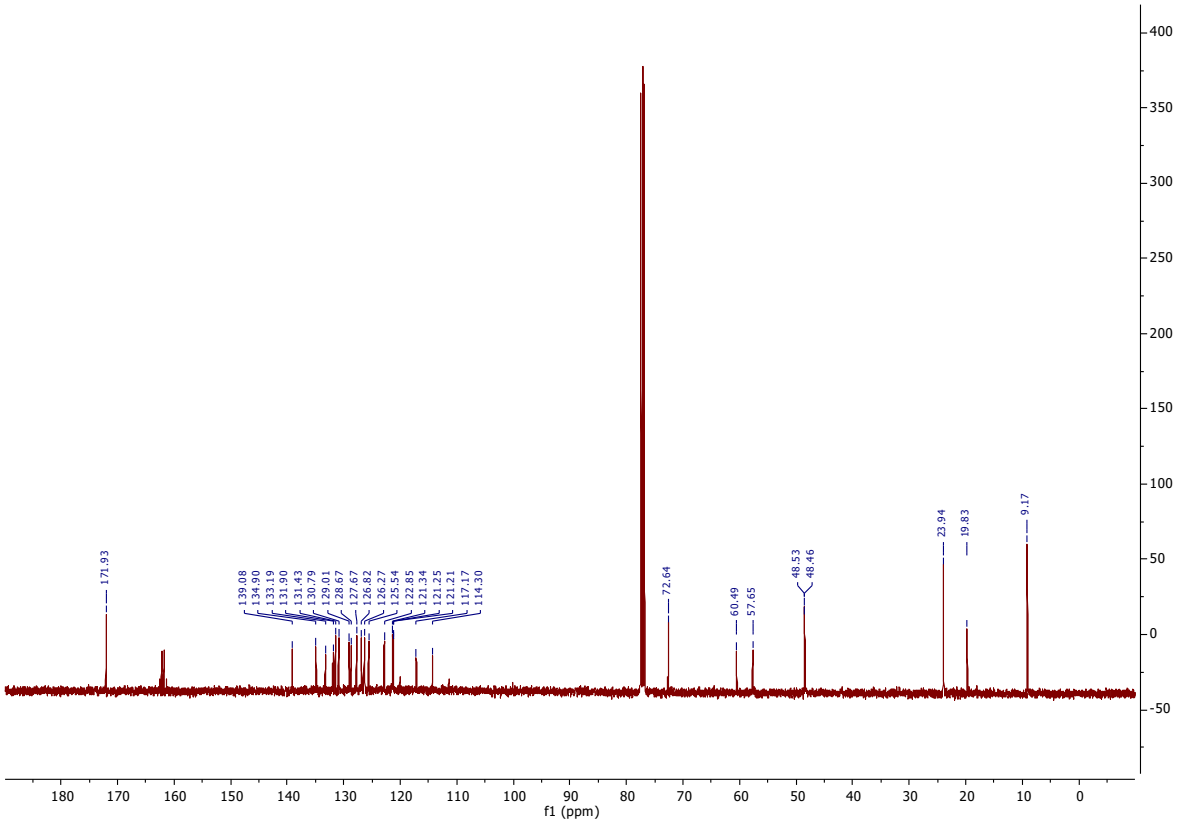
**4-((3,3-Diethyl-2,4-dioxoazetidin-1-yl)methyl)benzoic acid (38)**



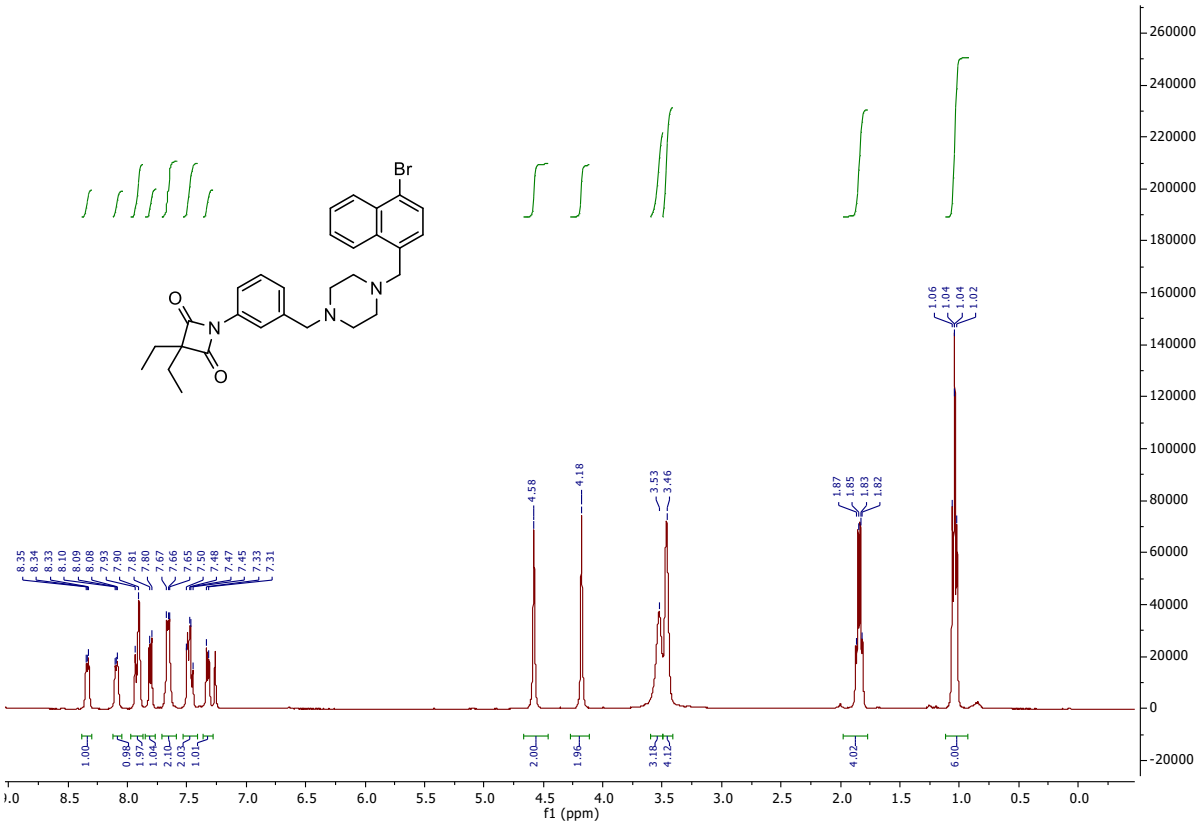



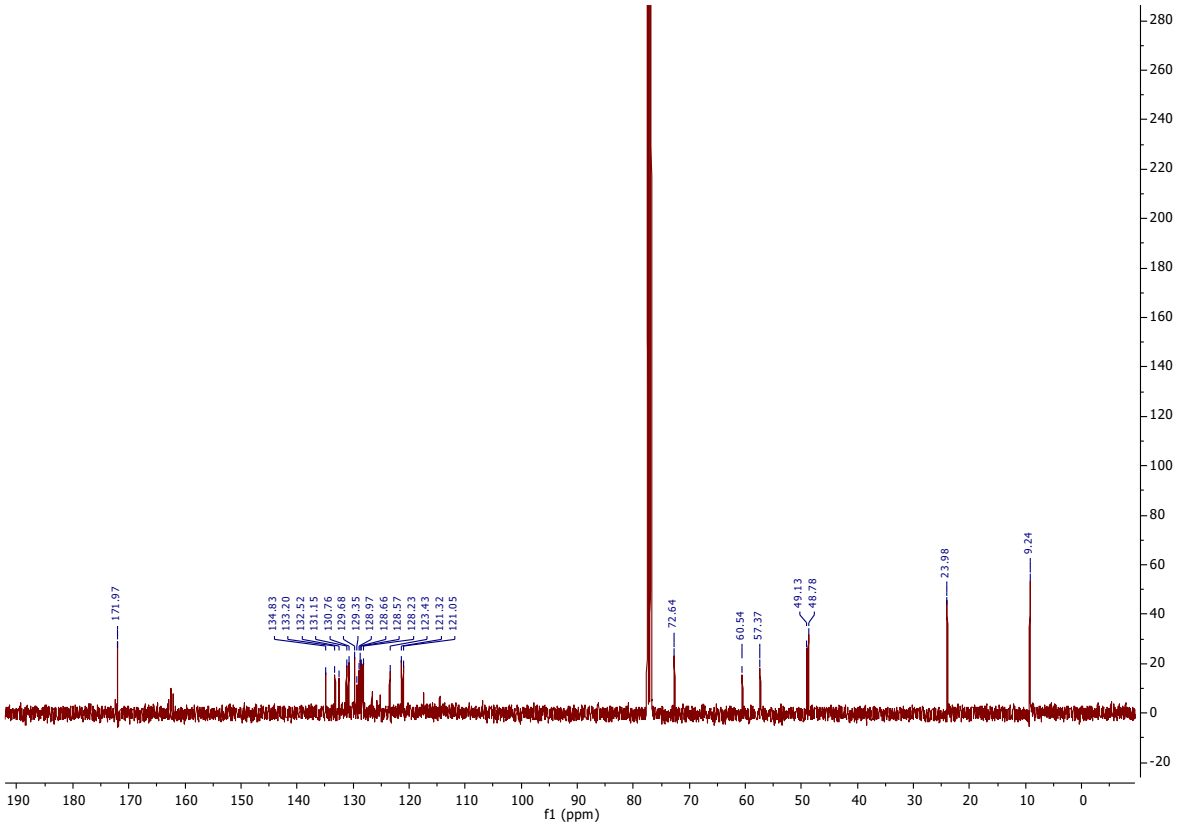
**3,3-Diethyl-1-(3-((4-((5-methylnaphthalen-1-yl)methyl)piperazin-1-yl)methyl)phenyl)azetidine-2,4-dione (39)**



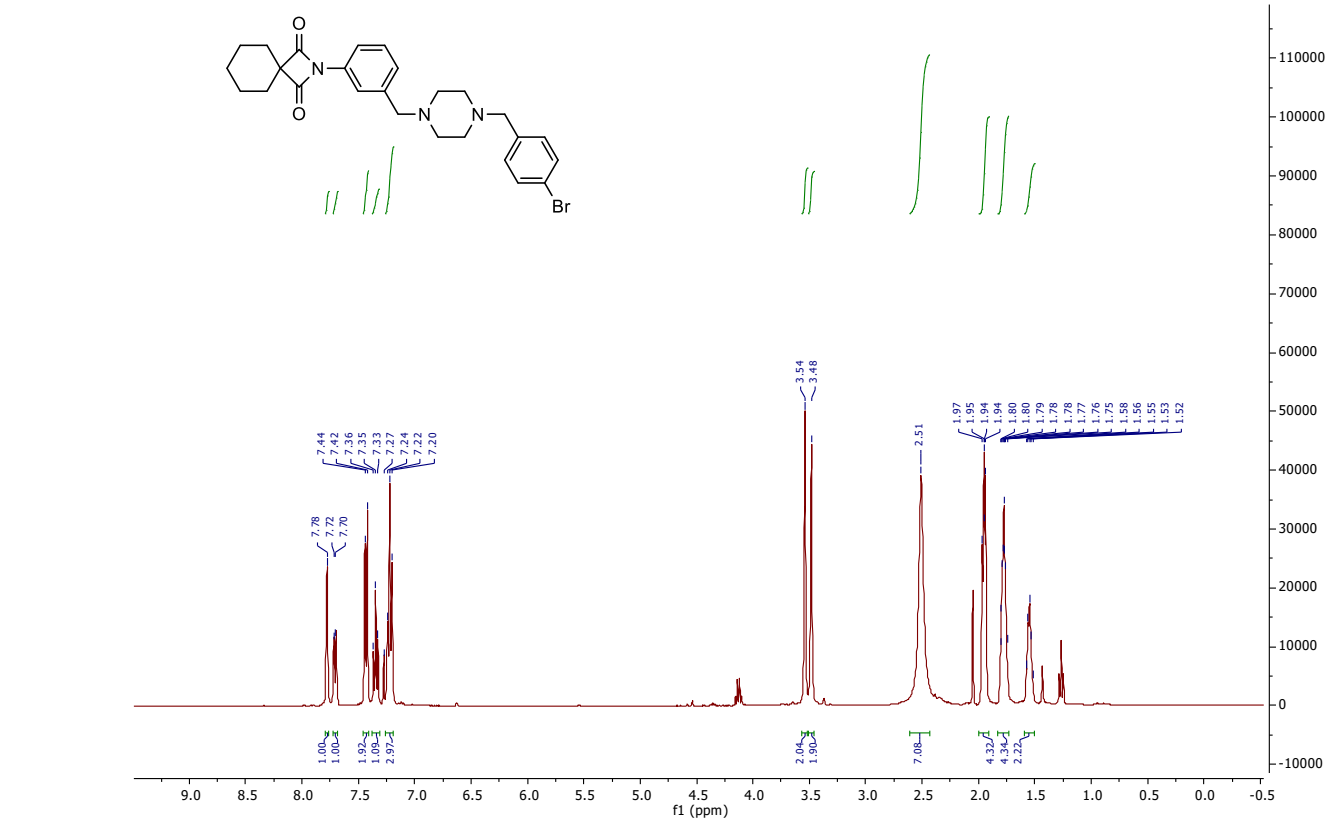



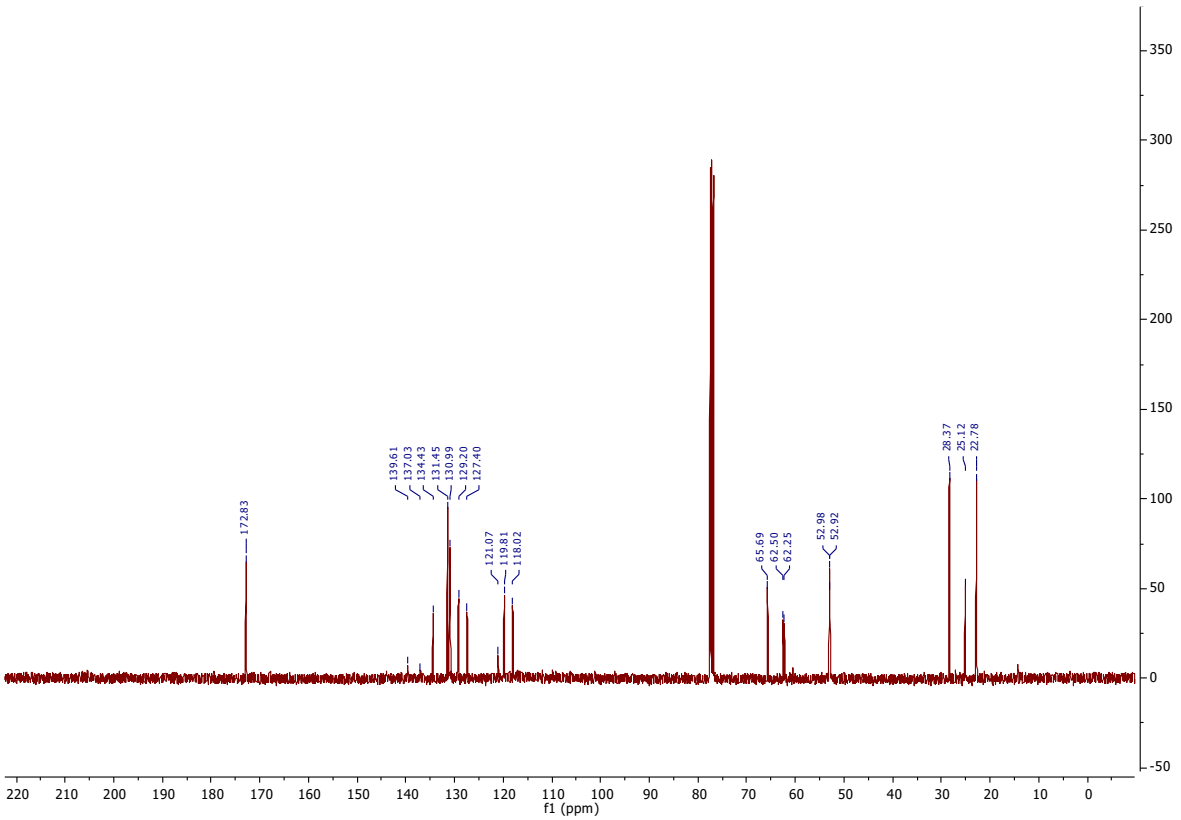
**1-(3-((4-((4-Bromonaphthalen-1-yl)methyl)piperazin-1-yl)methyl)phenyl)-3,3-diethylazetidine-2,4-dione (40)**



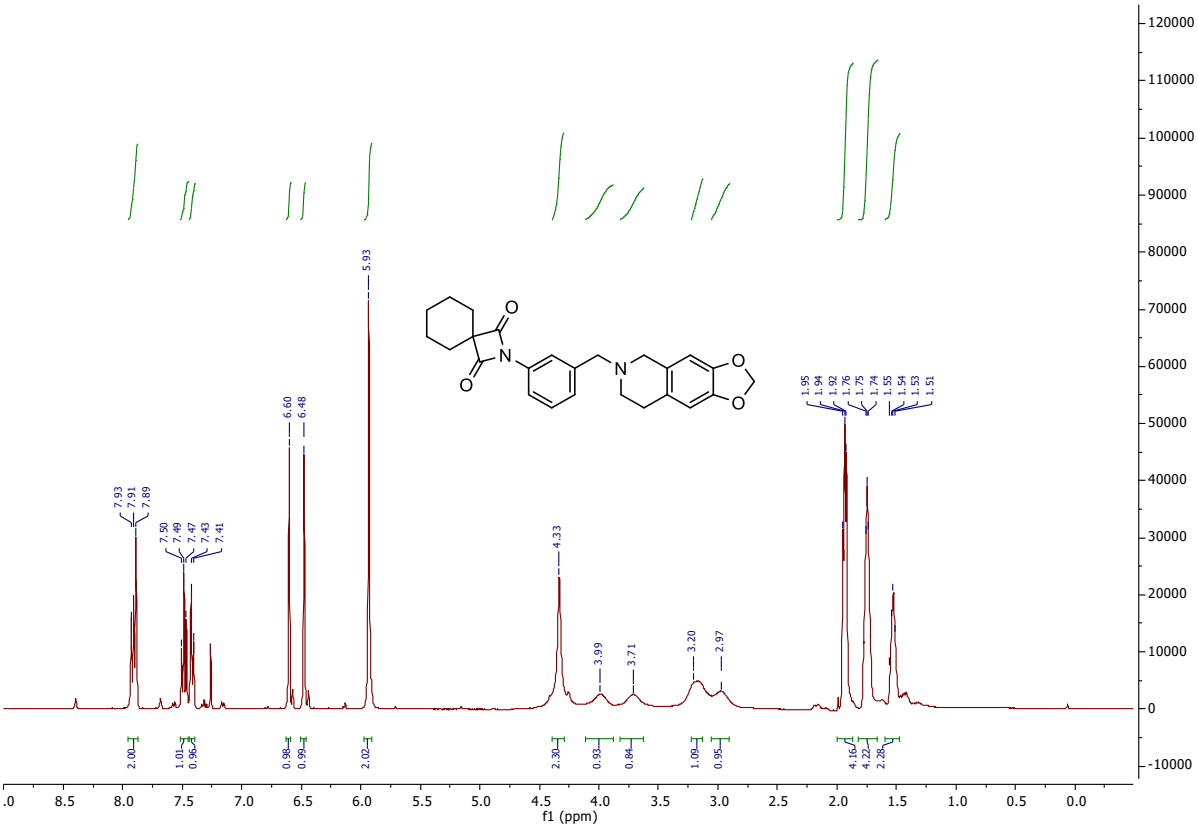



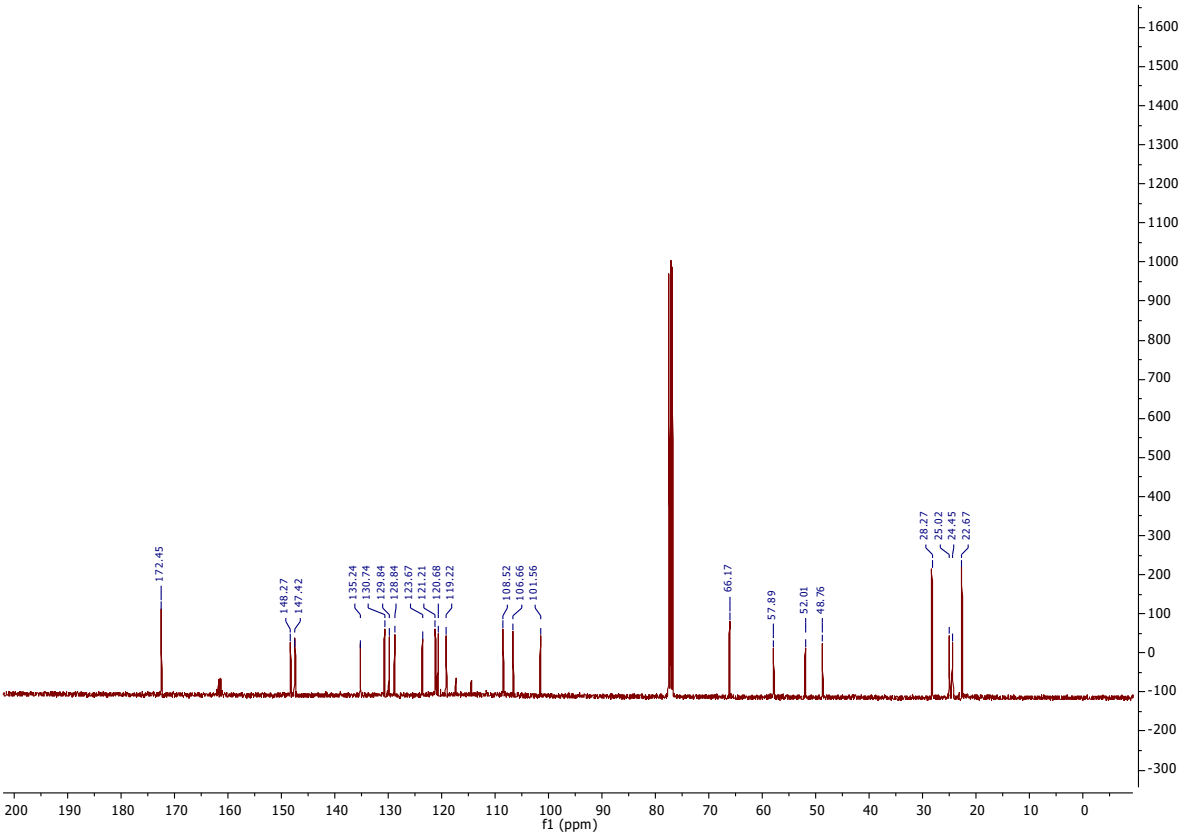
**2-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-2-azaspiro[3.5]nonane-1,3-dione (41)**



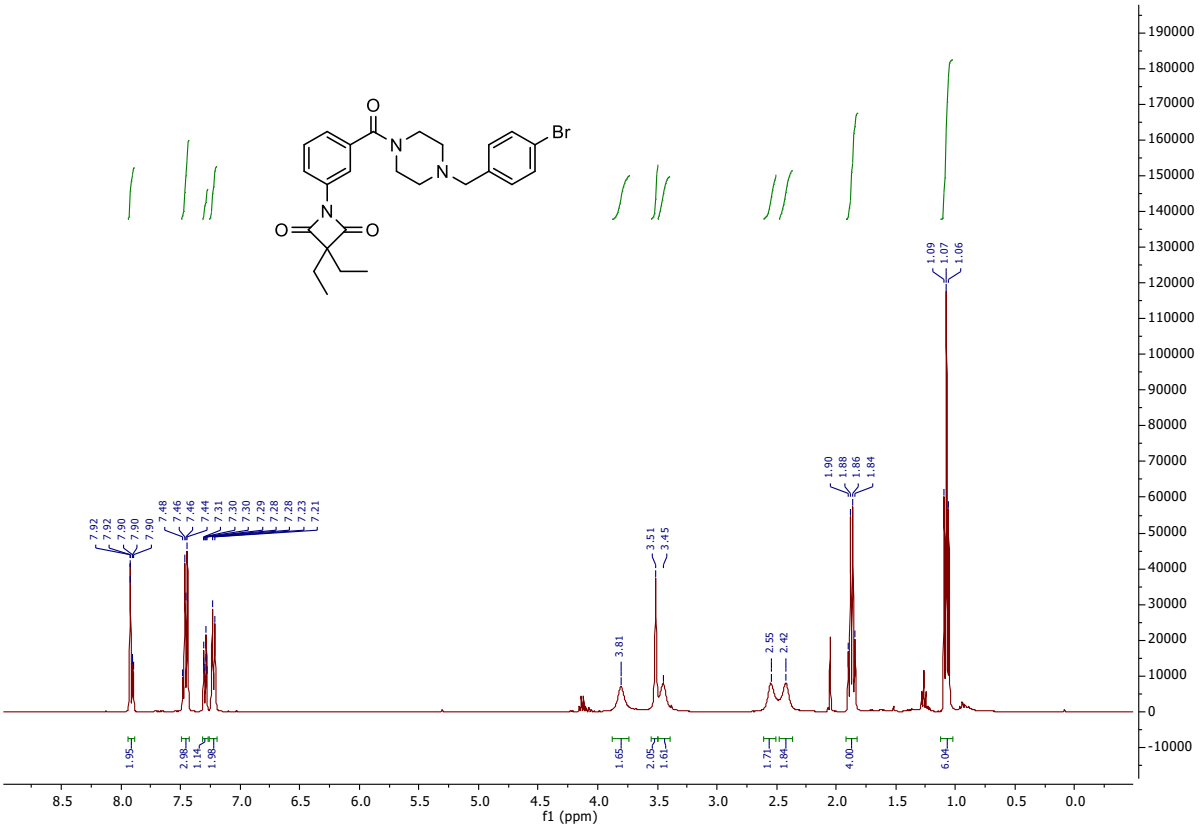



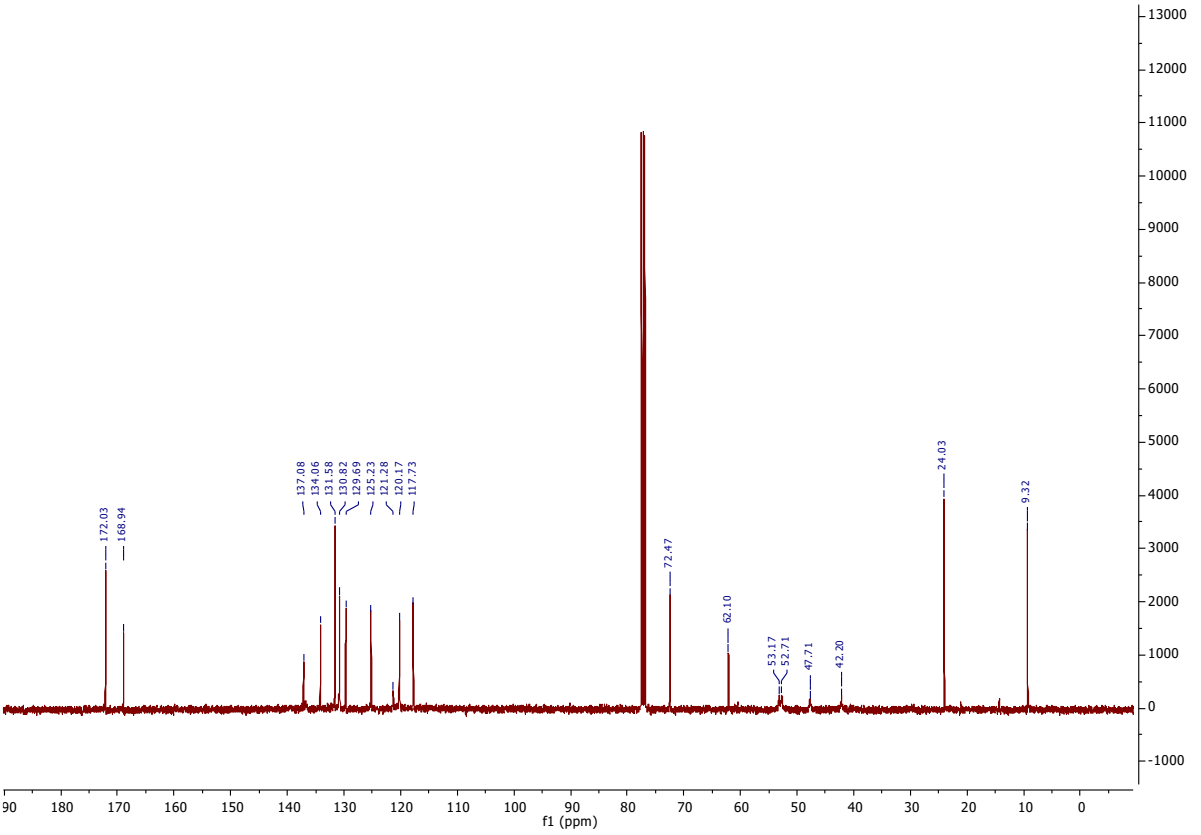
**2-(3-((7,8-Dihydro-[1,3]dioxolo[4,5-g]isoquinolin-6(5H)-yl)methyl)phenyl)-2-azaspiro[3.5]nonane-1,3-dione (42)**



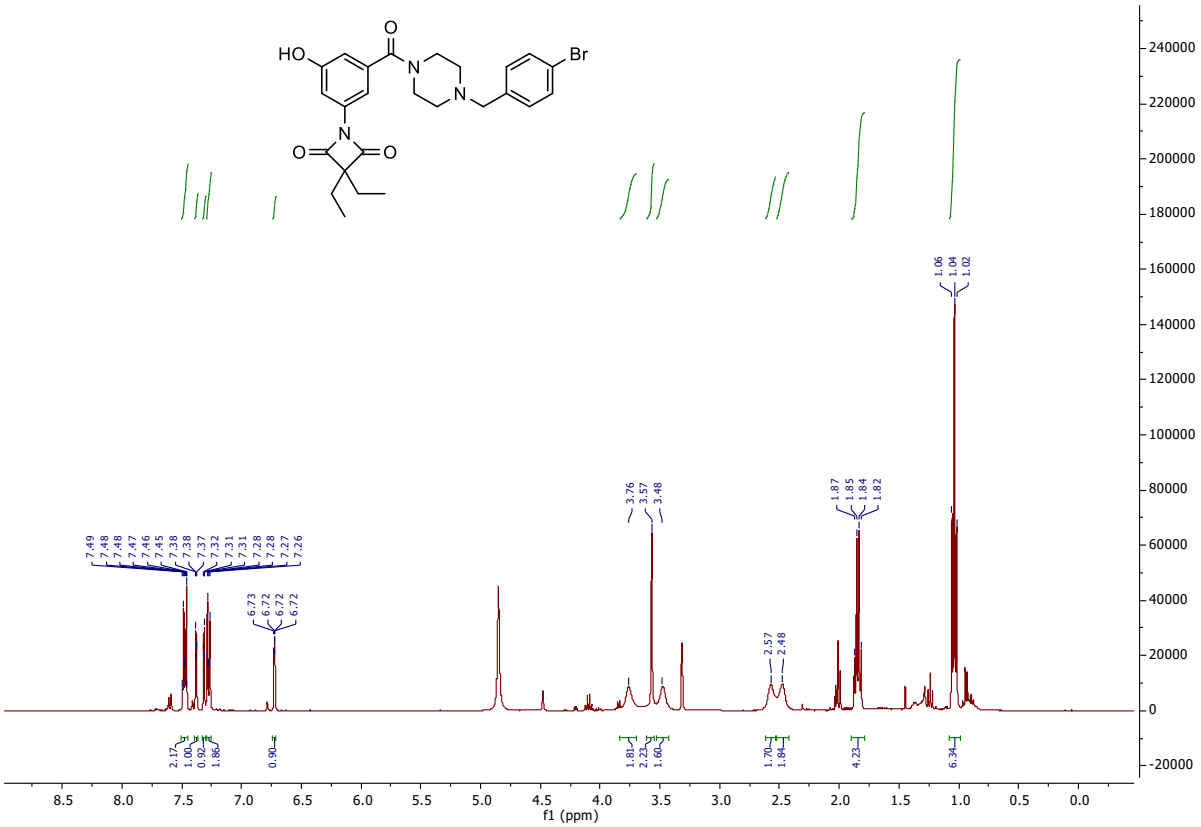



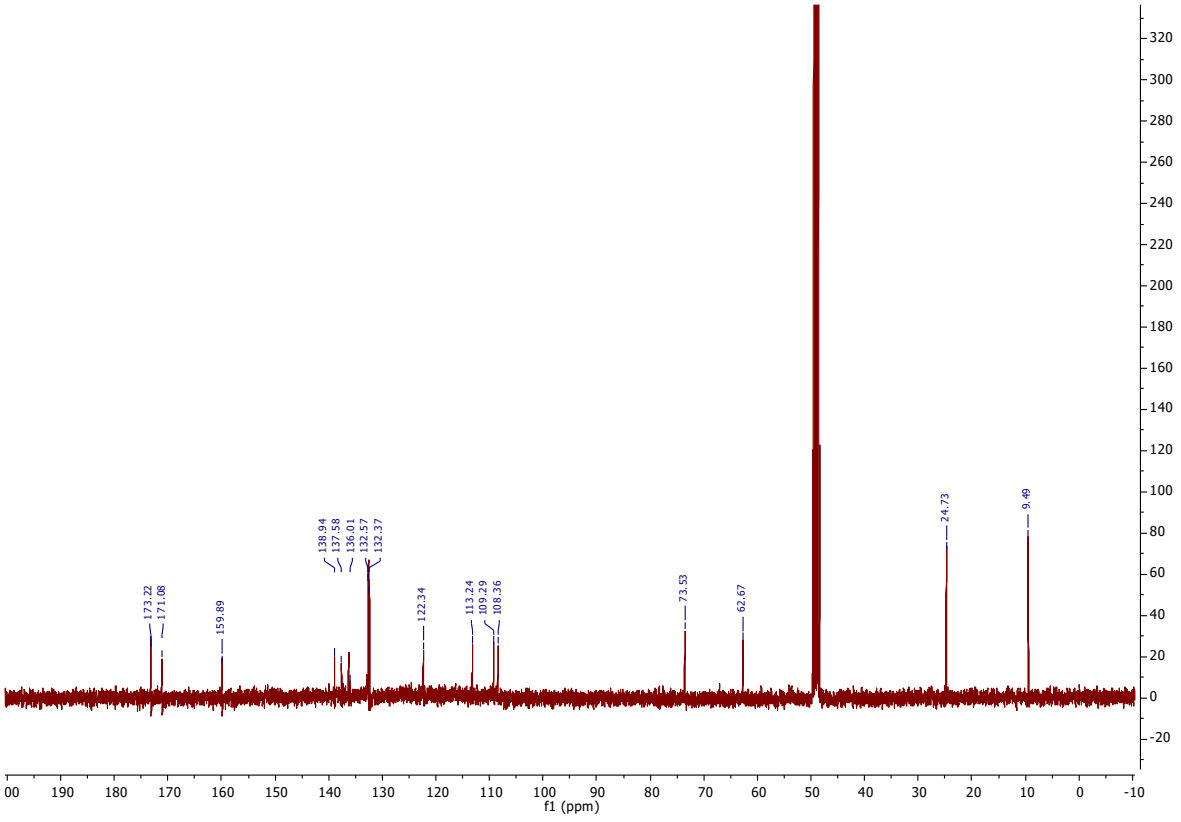
**1-(3-(4-(4-Bromobenzyl)piperazine-1-carbonyl)phenyl)-3,3-diethylazetidine-2,4-dione (43)**



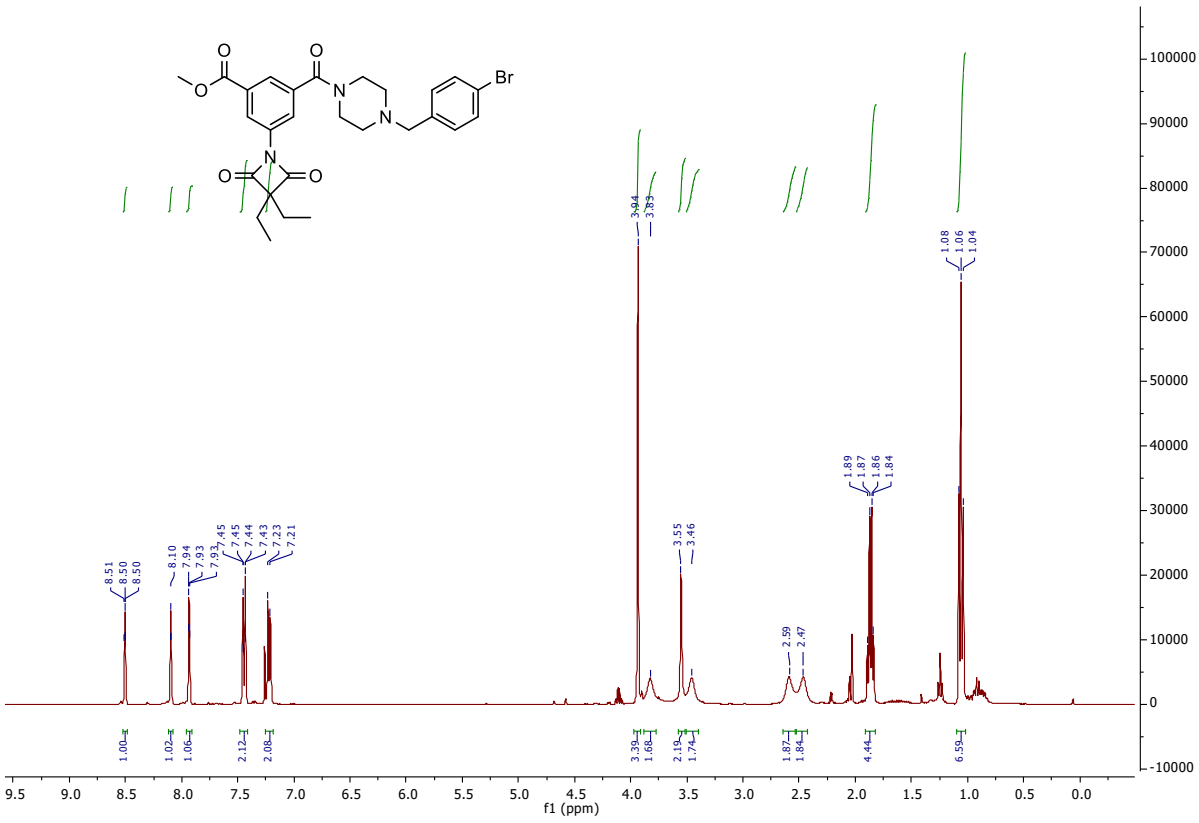



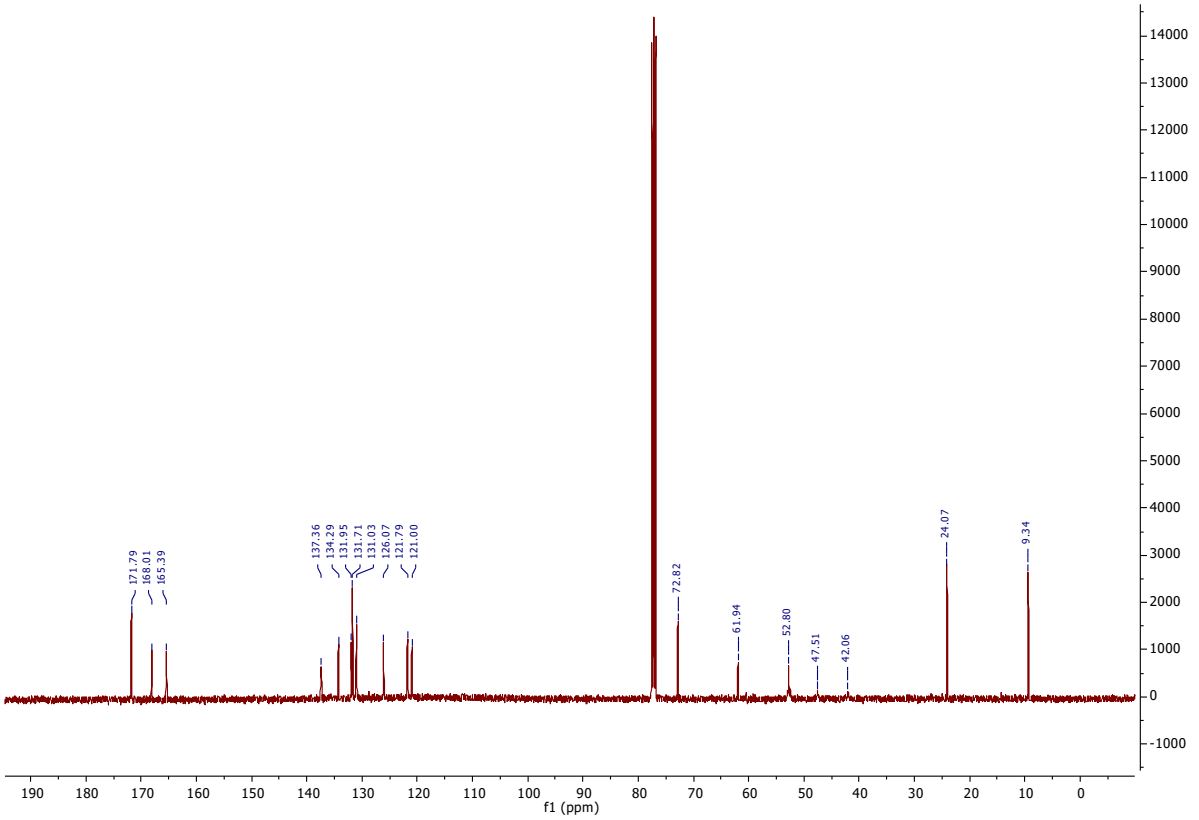
**1-(3-(4-(4-Bromobenzyl)piperazine-1-carbonyl)-5-hydroxyphenyl)-3,3-diethylazetidine-2,4-dione (44)**



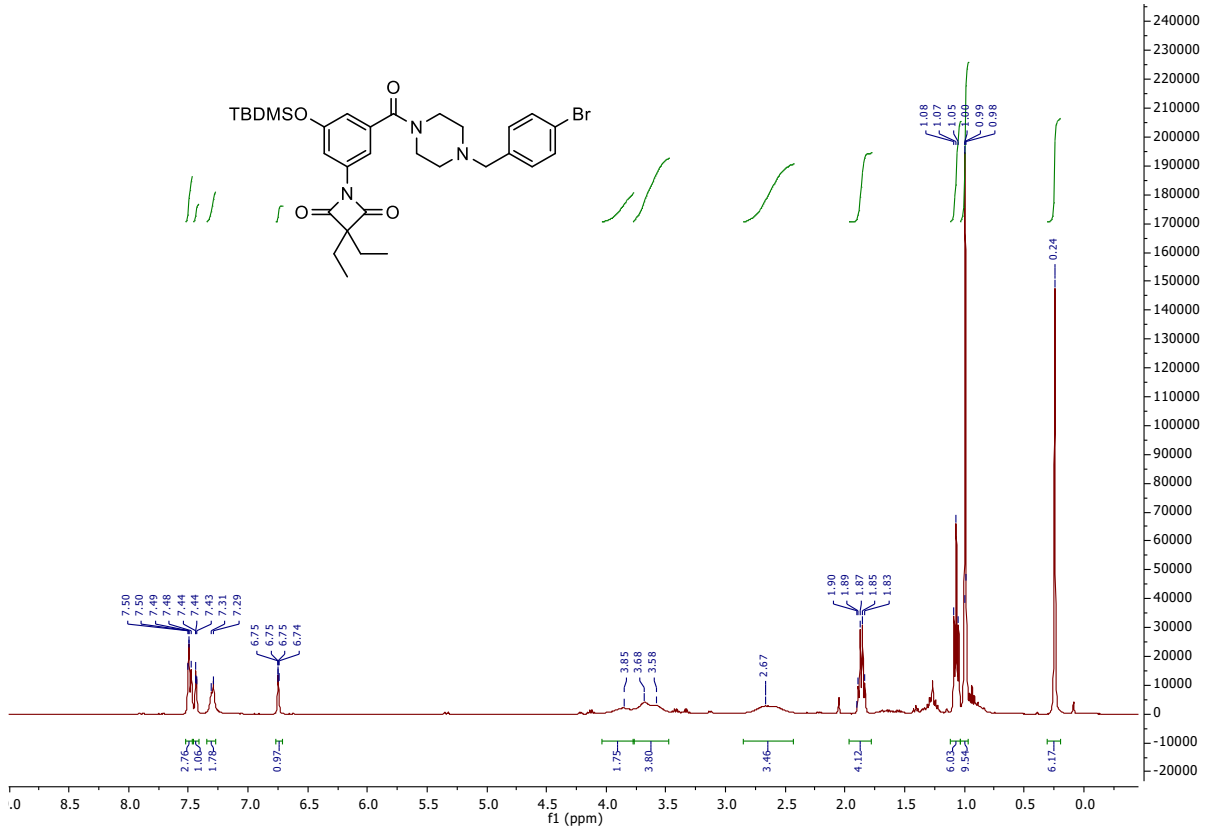



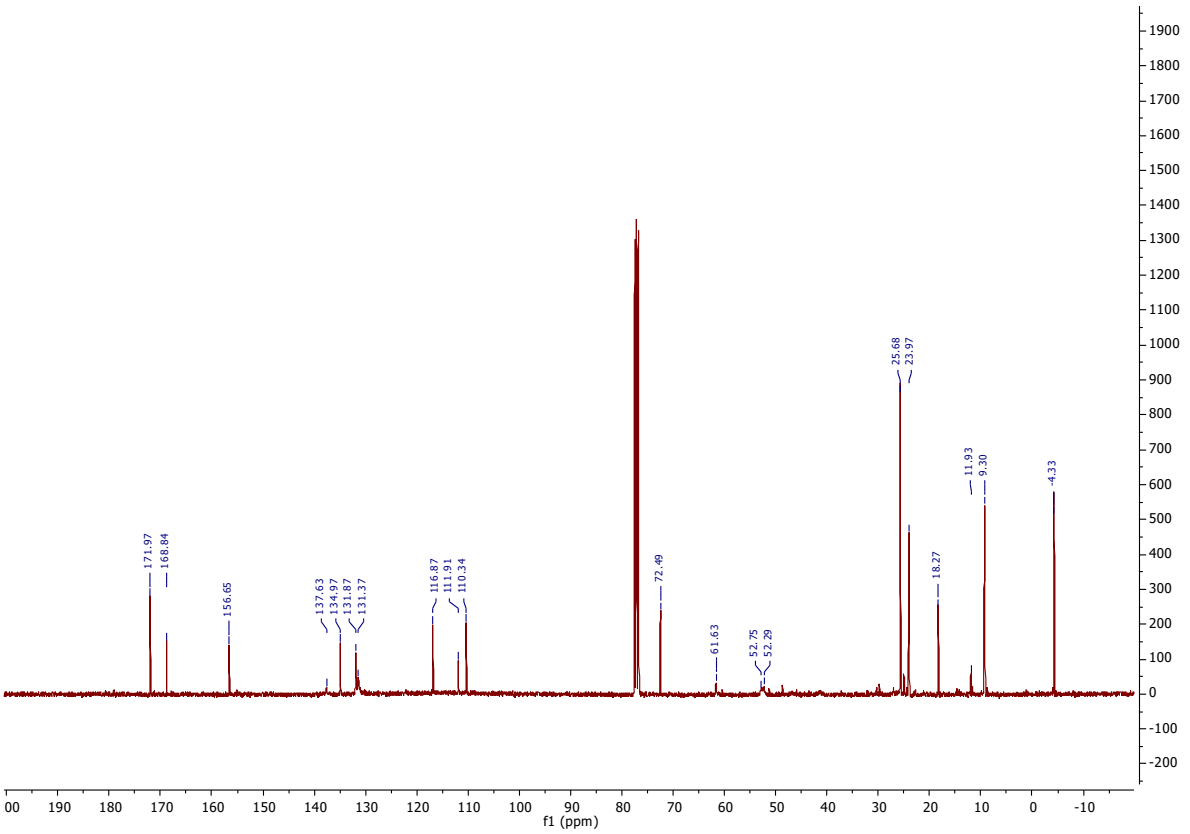
**_tert_-Butyl (3-(4-(4-bromobenzyl)piperazine-1-carbonyl)-5-((_tert_-butyldimethylsilyl)oxy)phenyl)carbamate (45)**



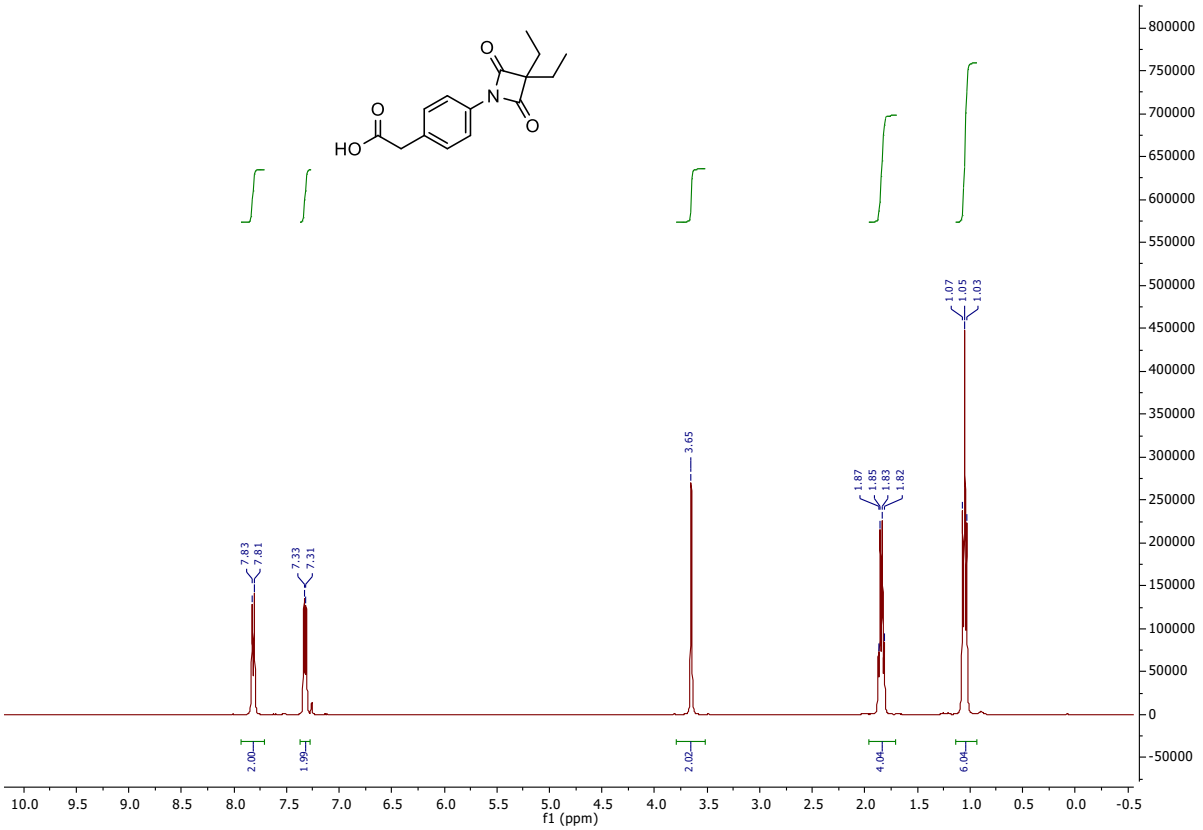



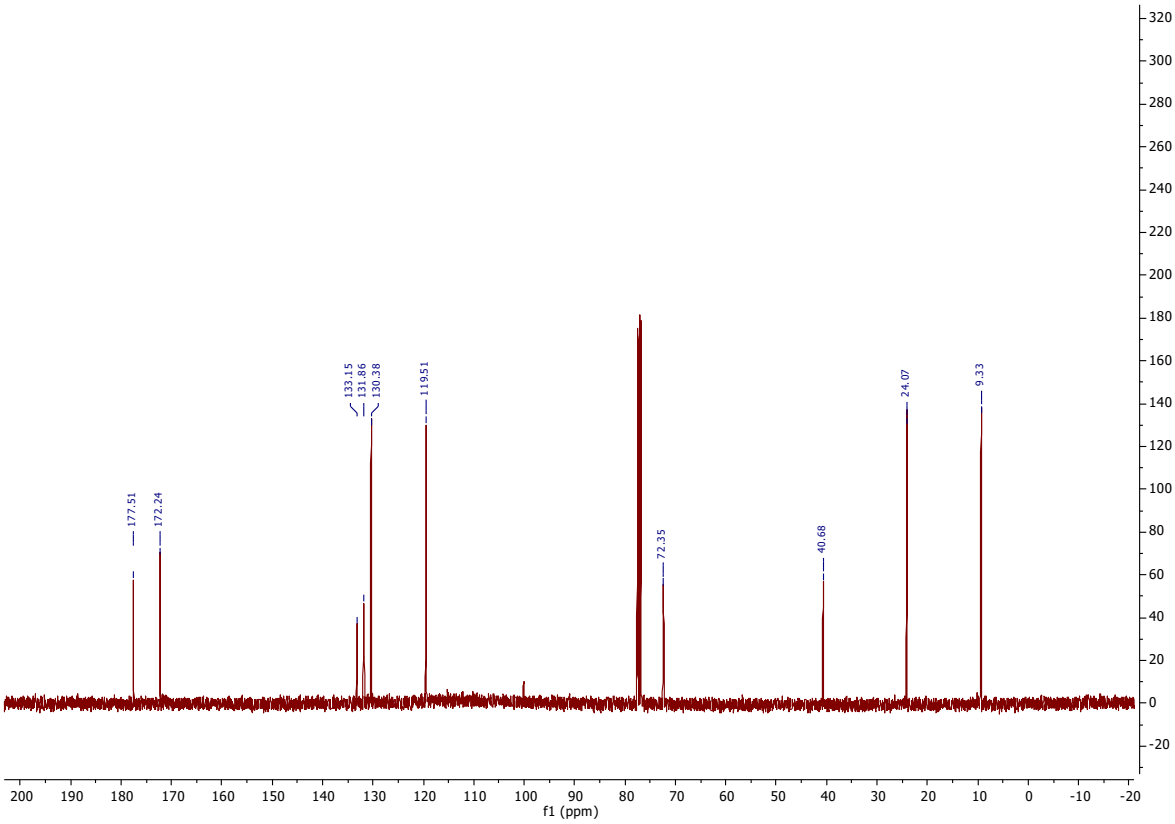
**1-(3-(4-(4-Bromobenzyl)piperazine-1-carbonyl)-5-((*tert*-butyldimethylsilyl)oxy)phenyl)-3,3-diethylazetidine-2,4-dione (46)**



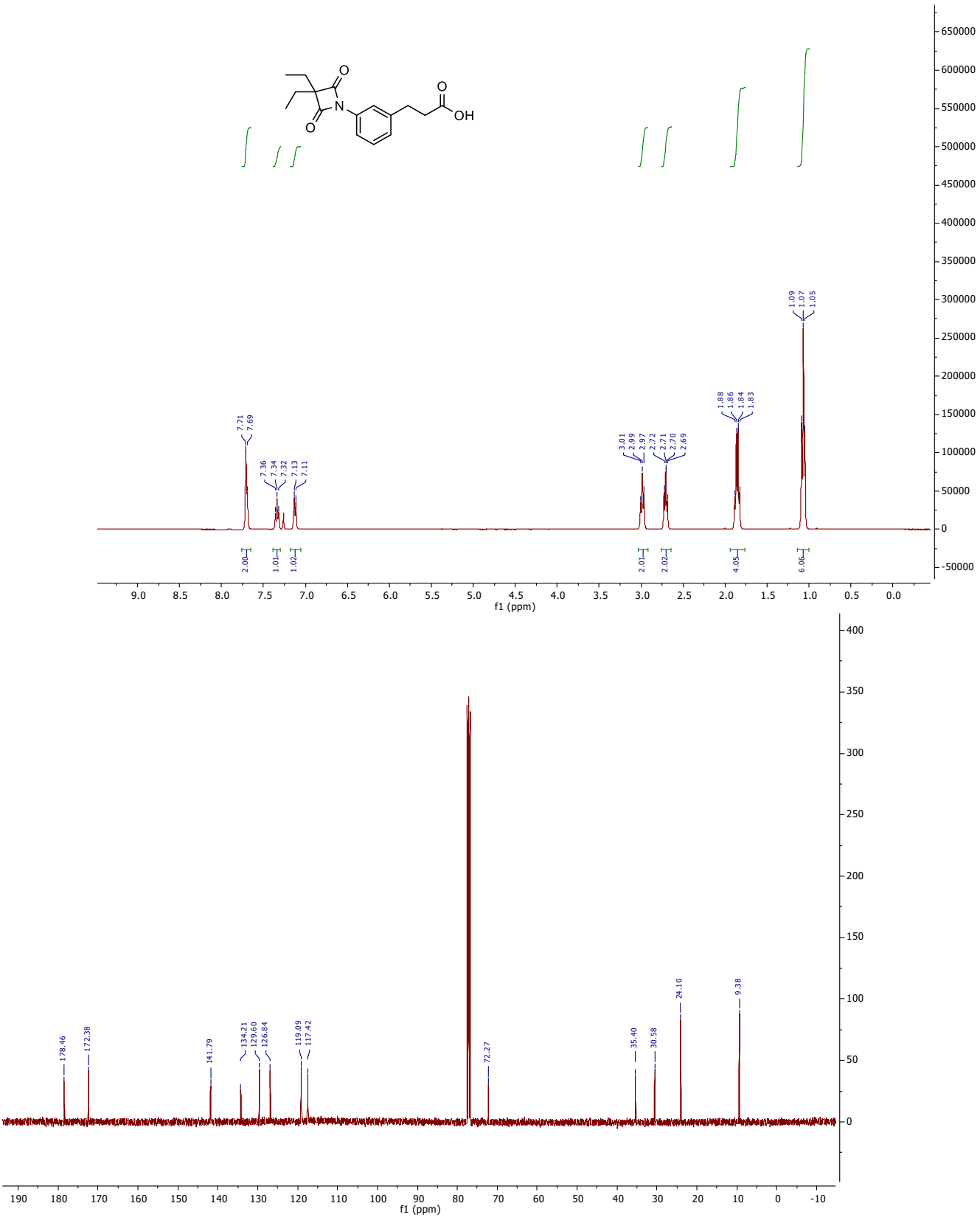



## 2-(4-(3,3-Diethyl-2,4-dioxoazetidin-1-yl)phenyl)acetic acid (47)

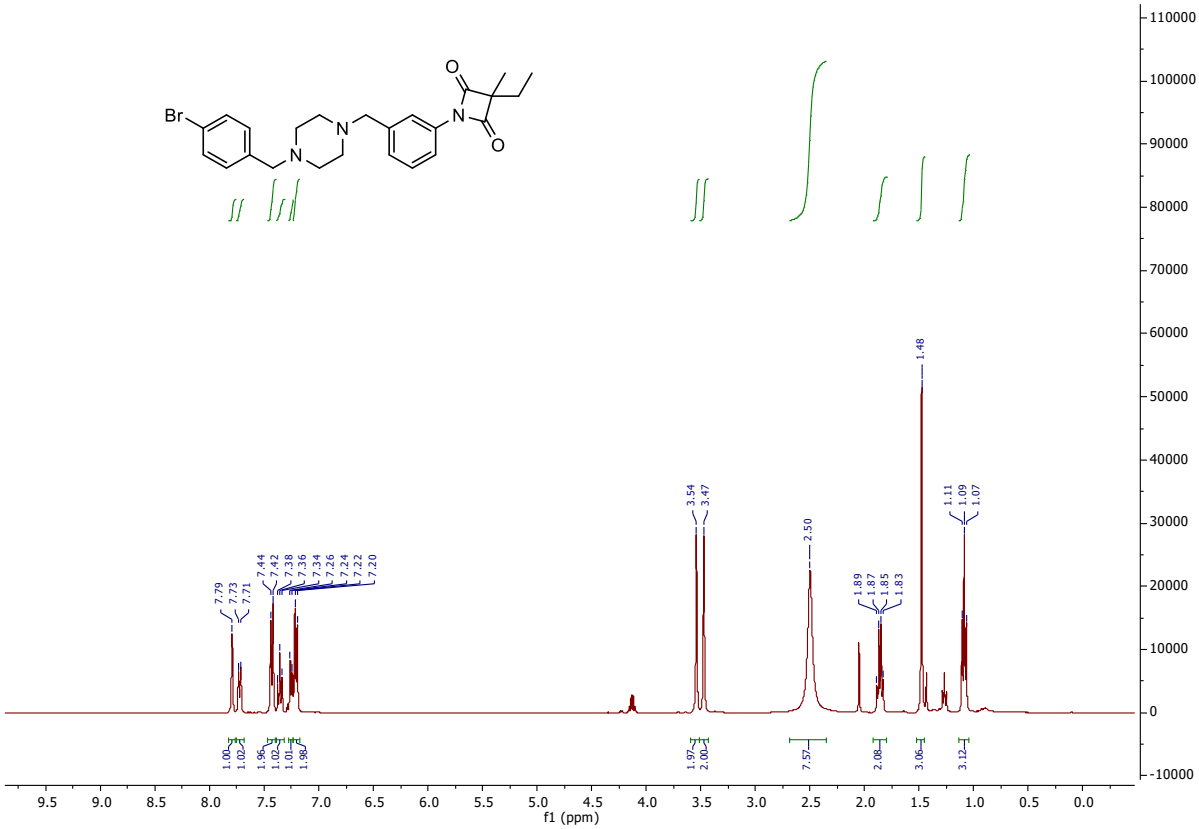


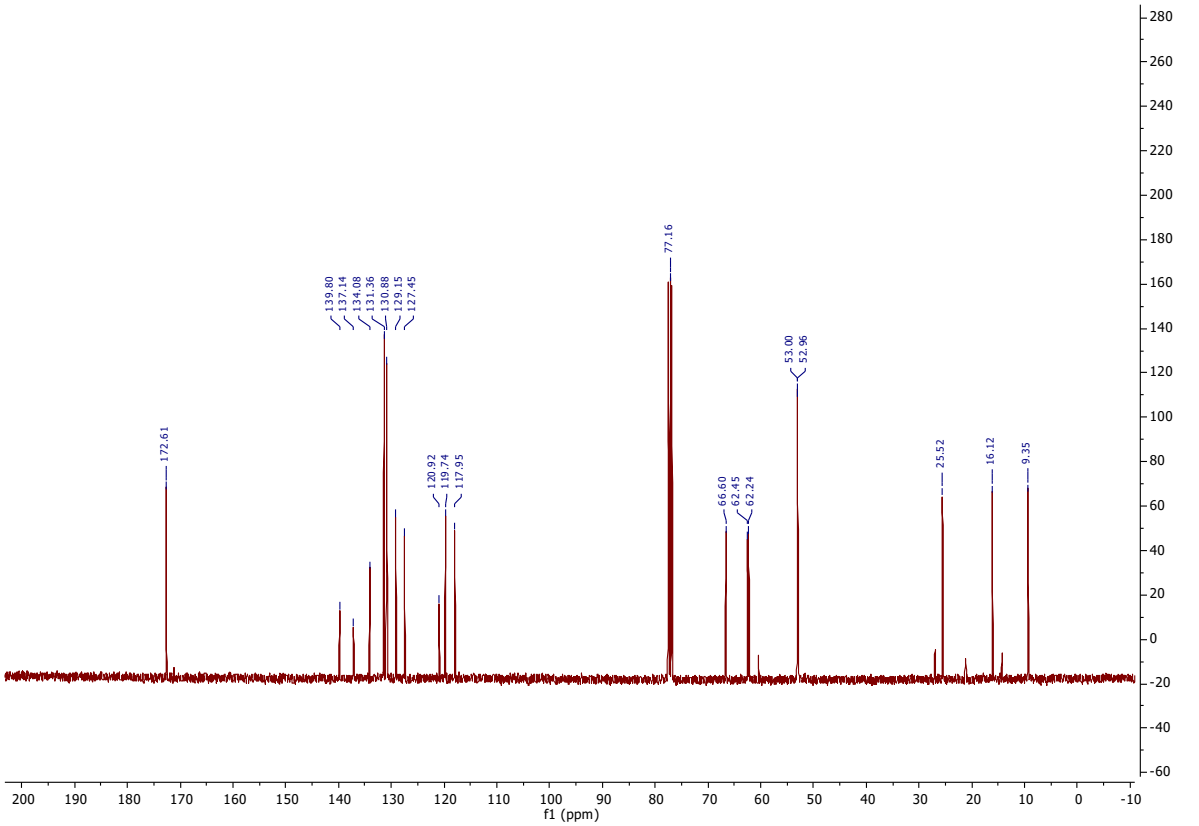



**3-(3-(3,3-Diethyl-2,4-dioxoazetidin-1-yl)phenyl)propanoic acid (48)**

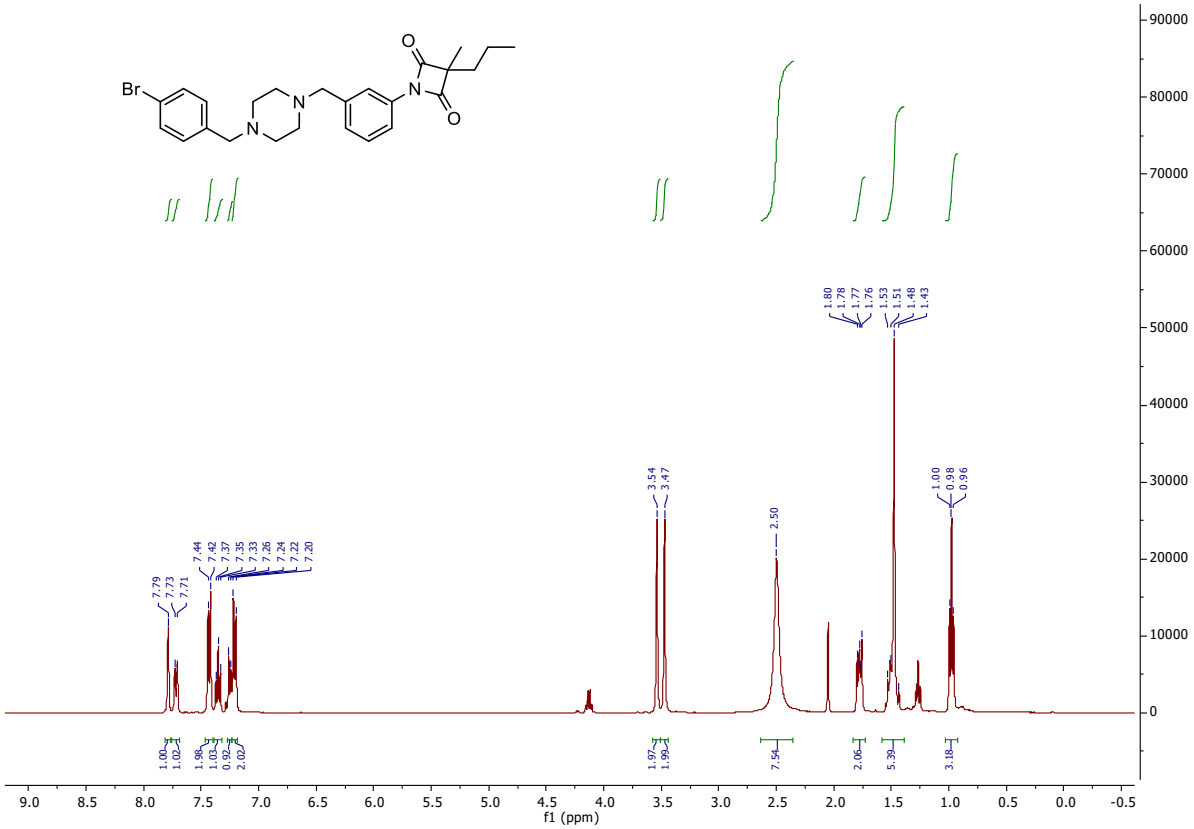


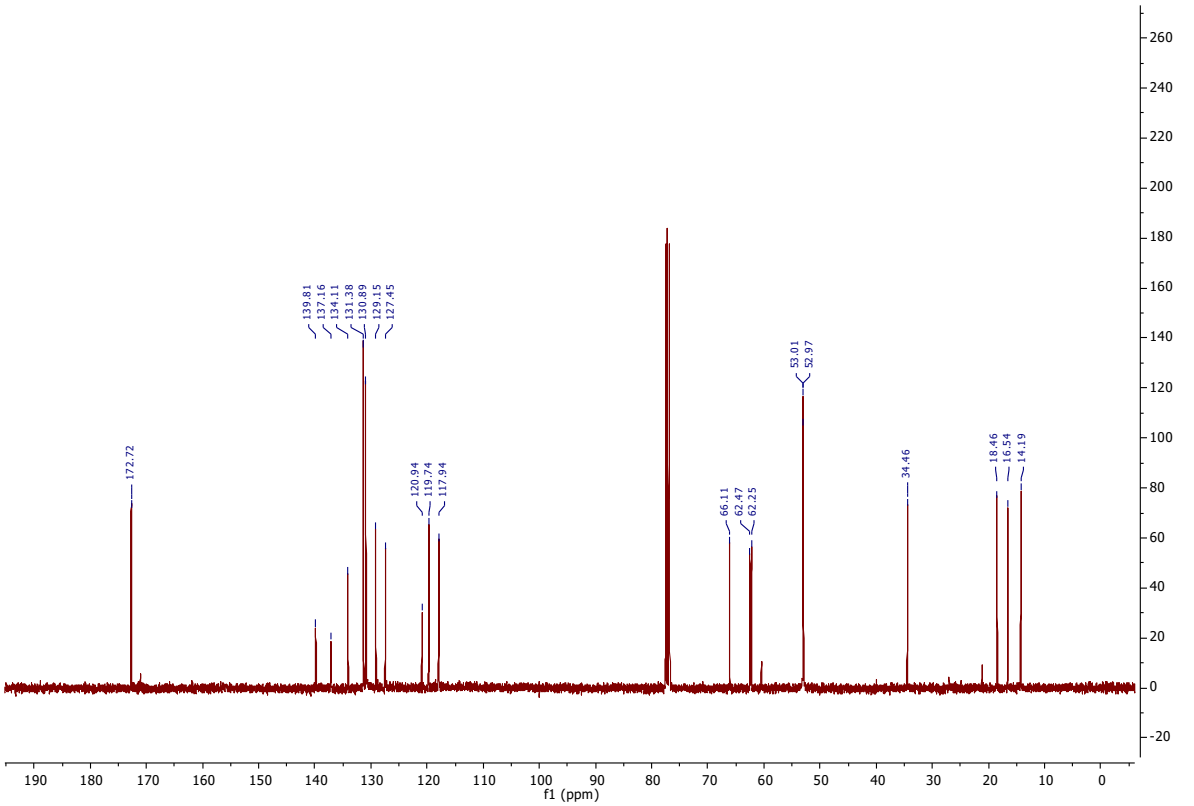
**1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3-ethyl-3-methylazetidine-2,4-dione (49)**



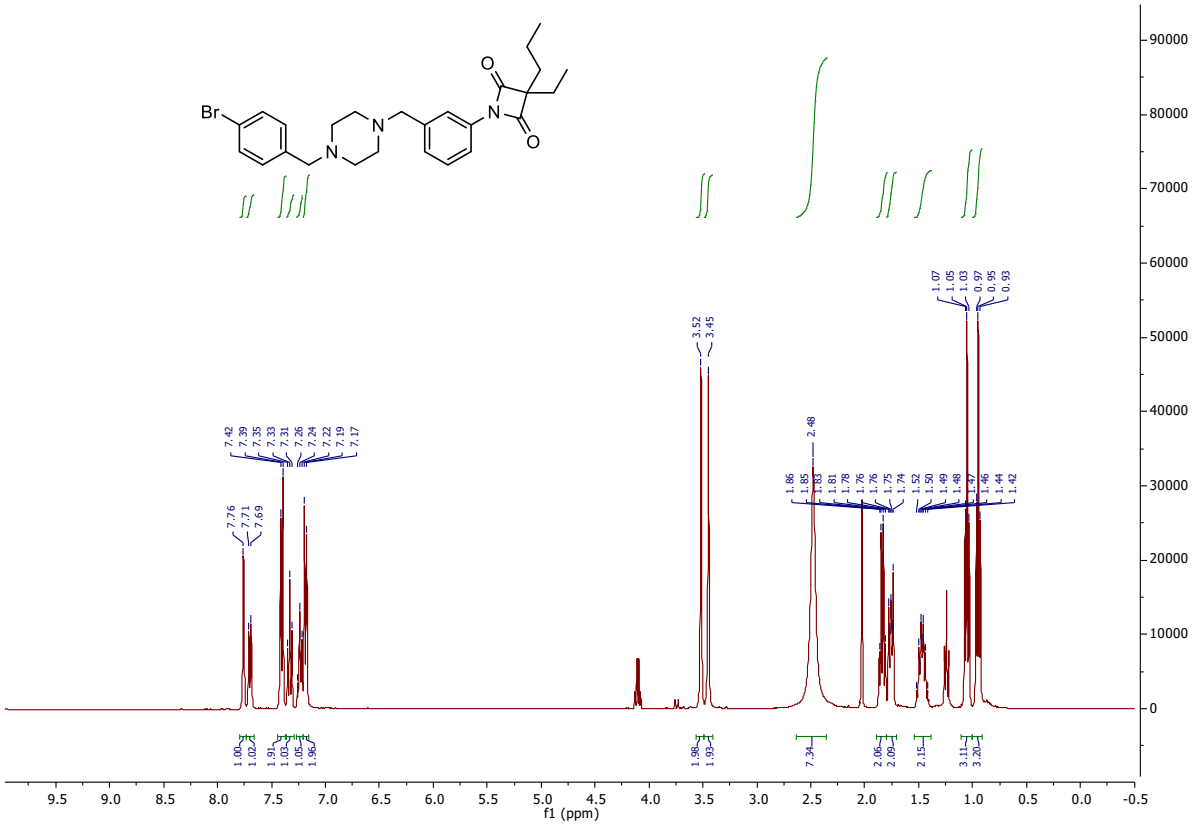



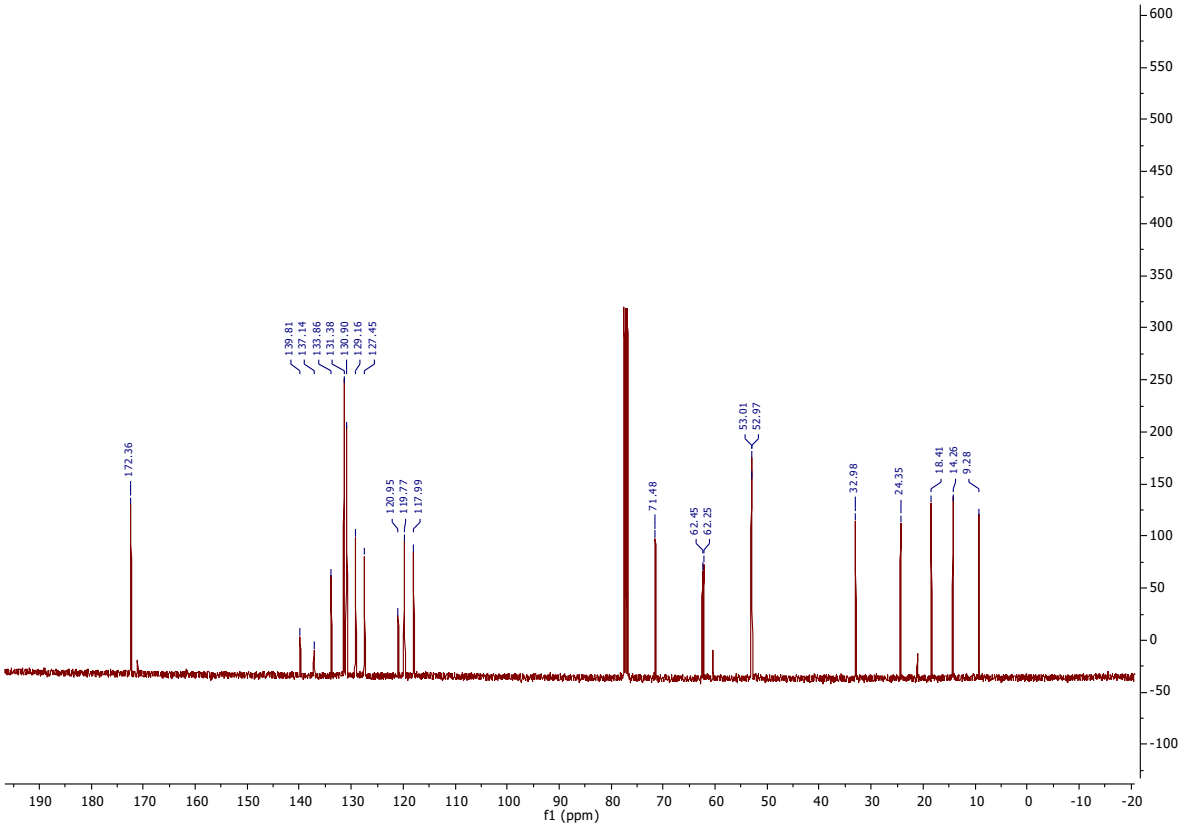
**1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3-methyl-3-propylazetidine-2,4-dione (50)**



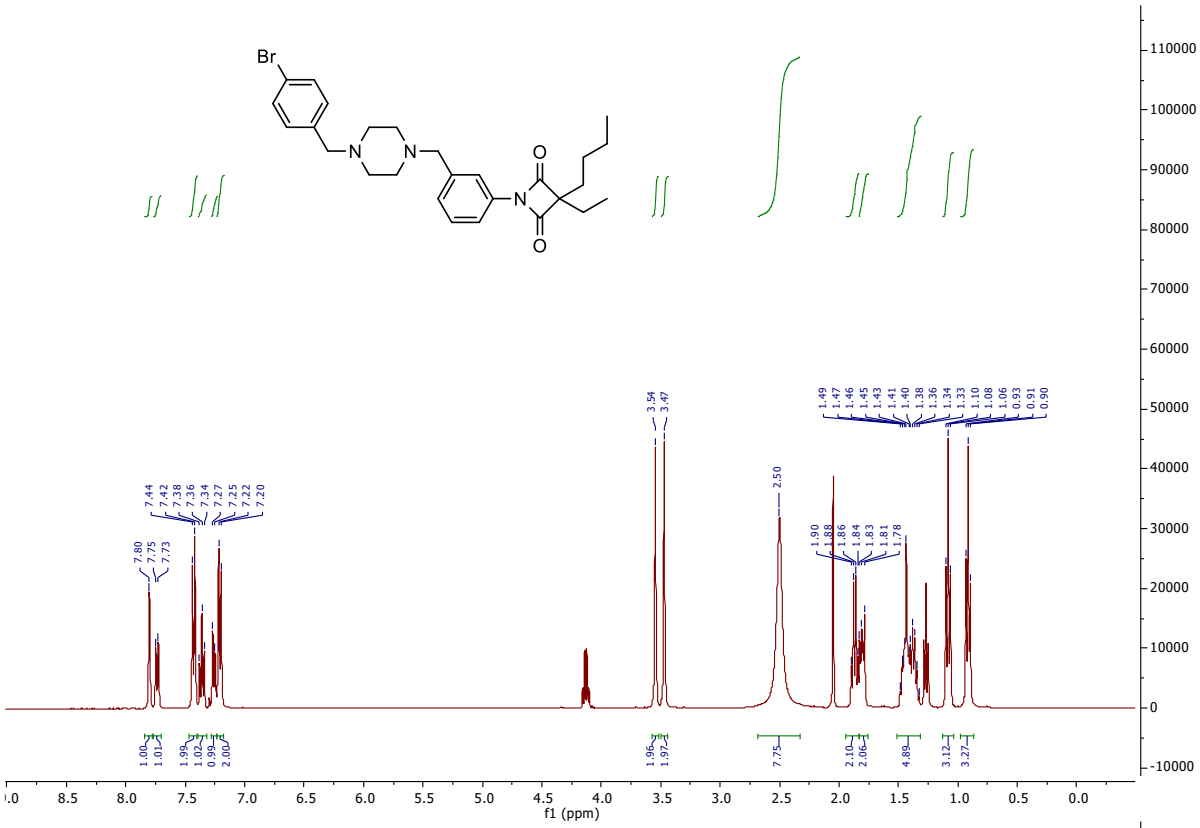



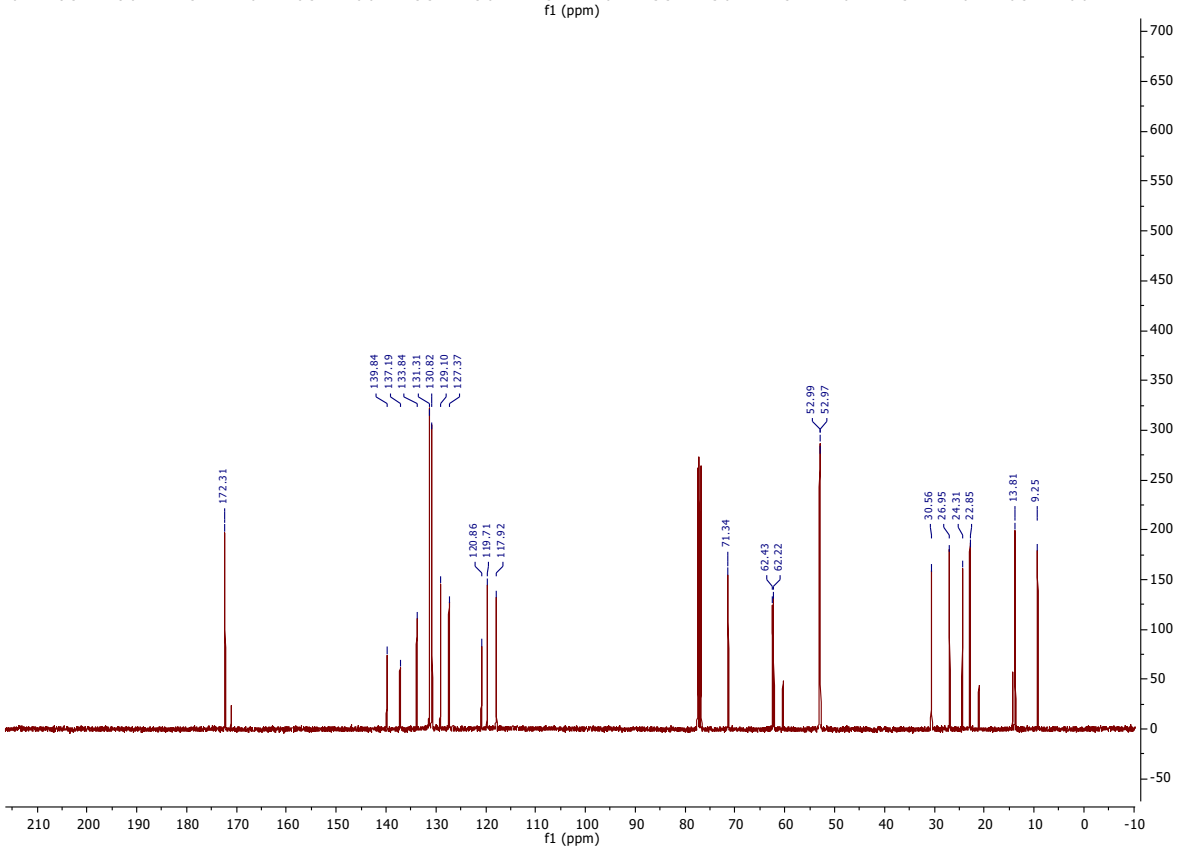
**1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3-ethyl-3-propylazetidine-2,4-dione (51)**



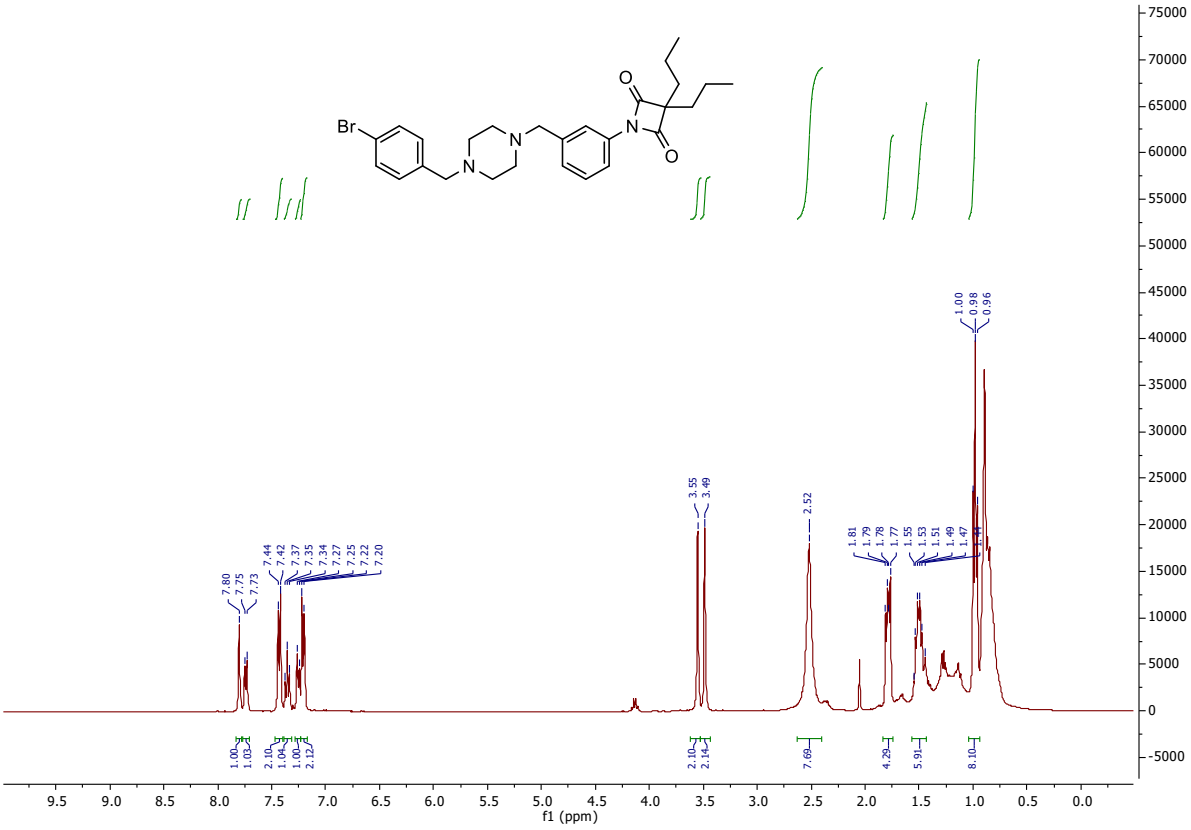



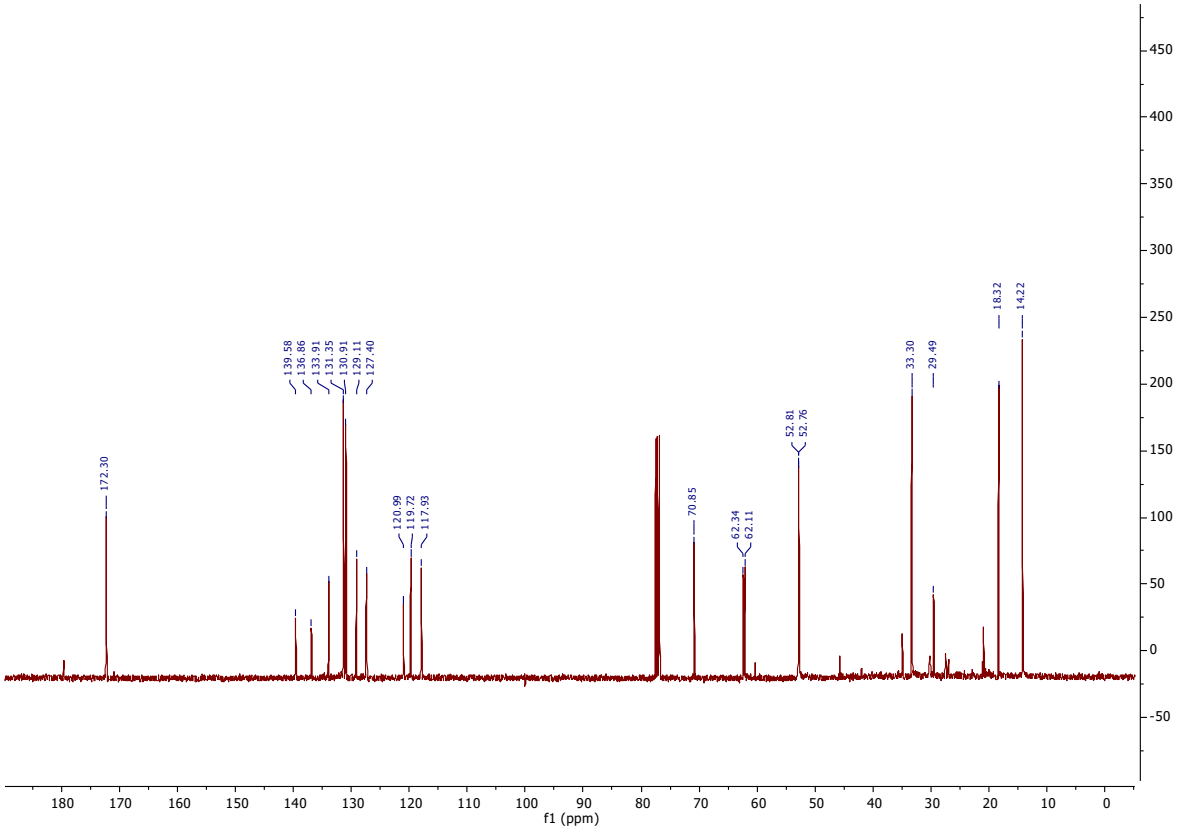
**1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3-butyl-3-ethylazetidine-2,4-dione (52)**



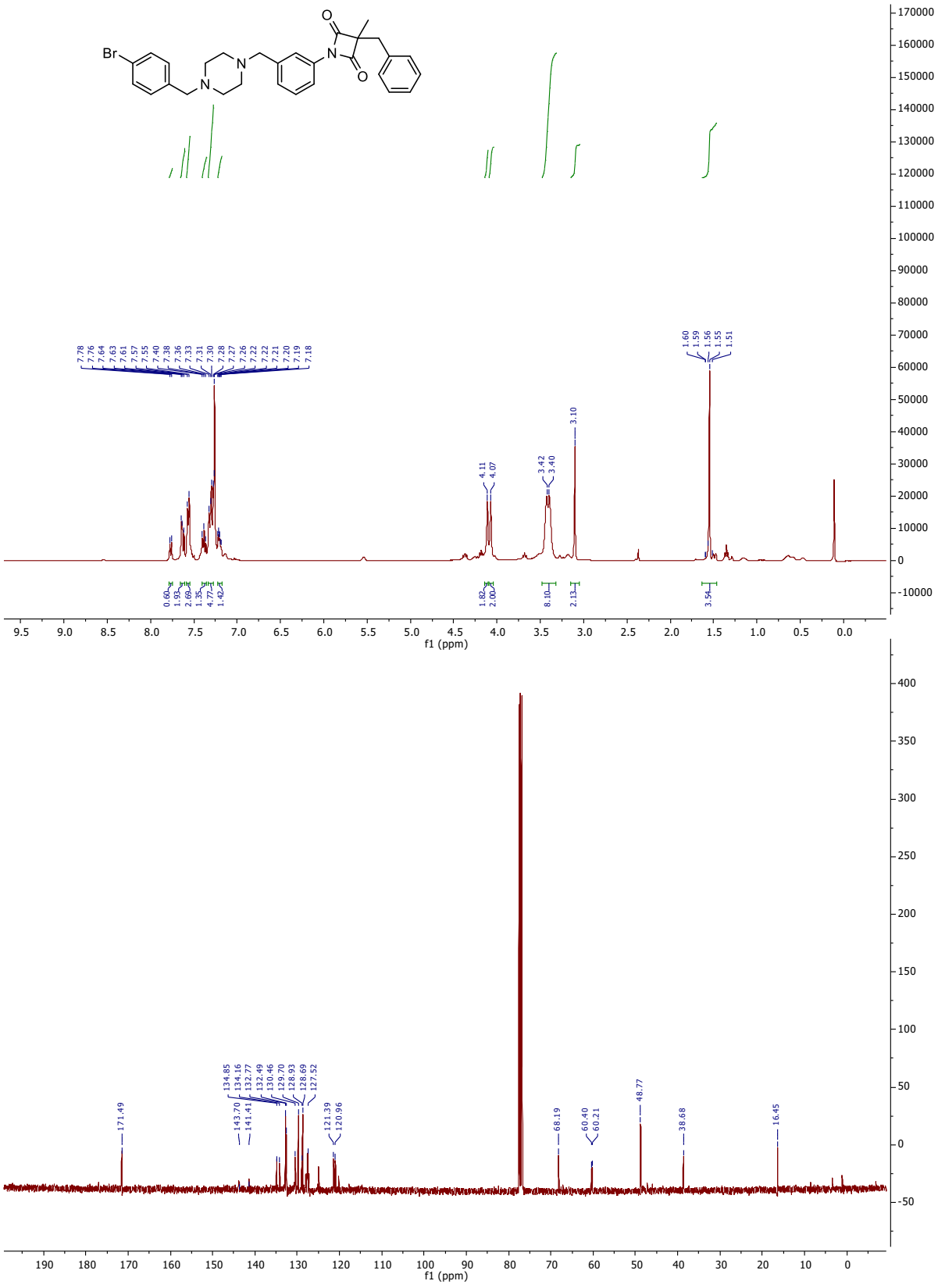



**1-(3-((4-(4-Bromobenzyl)piperazin-1-yl)methyl)phenyl)-3,3-dipropylazetidine-2,4-dione (53)**



**3-Benzyl-1-(3-((4-(4-bromobenzyl)piperazin-1-yl)methyl)phenyl)-3-methylazetidine-2,4-dione (54)**



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