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Examination of Diazaspiro Cores as Piperazine Bioisosteres in the Olaparib Framework Shows Reduced DNA Damage and Cytotoxicity

Sean W. Reilly^a, Laura N. Puentes^b, Khadija Wilson^b, Chia-Ju Hsieh^a, Chi-Chang Weng^a, Mehran Makvandi^{a,*}, and Robert H. Mach^{a,*}

^aDepartment of Radiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States

^bDepartment of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania, 421 Curie Blvd., Philadelphia, PA 19104, United States

Abstract

Development of poly(ADP-ribose) polymerase inhibitors (PARPi) continues to be an attractive area of research due to synthetic lethality in DNA repair deficient cancers, however, PARPi also have potential as therapeutics to prevent harmful inflammation. We investigated the pharmacological impact of incorporating spirodiamine motifs into the phthalazine architecture of FDA approved PARPi, olaparib. Synthesized analogues were screened for PARP-1 affinity, enzyme specificity, catalytic inhibition, DNA damage, and cytotoxicity. This work led to the identification of **10e** (12.6 ± 1.1 nM), which did not induce DNA damage at similar drug concentrations as olaparib. Interestingly, several worst in class compounds with low PARP-1 affinity, including **15b** ($4,397 \pm 1.1$ nM), induced DNA damage at micromolar concentrations, which can explain the cytotoxicity observed in vitro. This work provides further evidence that high affinity PARPi can be developed without DNA damaging properties offering potential new drugs for treating inflammatory related diseases.

TOC Graphic

* **Corresponding Author:** MM - Phone (215) 746-8755; makvandi@pennmedicine.upenn.edu, RHM - Phone (215) 746-8233; rmach@mail.med.upenn.edu.

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website Cytotoxicity data at 1 μM, 10 μM, and 100 μM concentrations, along with ¹H and ¹³C spectrum of compounds **10-17** (PDF) Molecular formula strings and some data (CSV)

Author Contributions

All authors have given approval to the final version of the manuscript.

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INTRODUCTION

Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear protein involved in various cellular processes including detection and repair of damaged DNA.^{1, 2} Among the 17 identified PARP enzymes,³ PARP-1 is the most widely investigated and pursued therapeutic target for cancer treatment due to the unique role of the protein in base excision repair (BER), however, new evidence suggests PARP-1 hyperactivation may also drive the pathogenesis associated with neuroinflammation and neurodegeneration.^{4–8}

Initial therapeutic strategies employing PARP inhibitors (PARPi) (Figure 1) were based on reports illustrating the synthetic lethality of cancers deficient in breast and ovarian cancer susceptibility genes (BRCA1/2) responsible for encoding proteins which orchestrate homologous recombination (HR) DNA repair.^{9, 10} Since then, applications for PARP inhibition research has expanded into other areas of research such as cardiovascular disease,¹¹ inflammation,¹² neurodegeneration,¹³ neuroimaging,^{14, 15} and even drug addiction.^{16–18} Therefore, understanding PARP inhibitor (PARPi) pharmacology through a medicinal chemistry approach may prove to be critical in translating these agents for uses beyond cancer therapy.^{7, 13, 17}

Currently, there are three FDA-approved PARPi including, olaparib, rucaparib, and niraparib for treatment of ovarian cancer with BRCA mutations or in platinum sensitive patients as maintenance therapy.¹⁹ Since then, PARPi development has quickly progressed with two additional PARPi, veliparib and talazoparib, currently under clinical evaluation for cancer therapy.²⁰ Interestingly, veliparib shows high affinity to the PARP-1 enzyme with good catalytic inhibitory properties, similar to the previously mentioned PARPi, but a non-linear reduced ability to induce DNA damage is observed when compared to other agents in this class.²¹ It has been postulated that PARPis induce DNA damage through trapping the PARP-1 enzyme on DNA, thereby creating non-removable lesions that are highly cytotoxic.^{22, 23} However, it was shown that DNA damage induced by PARP-1 trapping was positively associated with affinity to the catalytic subdomain of the PARP-1 enzyme for all agents except veliparib.²¹ All together, this evidence suggests high affinity PARPi, such as veliparib, can induce catalytic inhibition of PARP-1 without eliciting DNA damage, thereby making these agents suitable therapeutics for PARP-1 mediated disease states where DNA damage is not warranted.

Anticancer mechanisms of PARPi continue to be heavily investigated to further our understanding in cancer cell drug resistance to PARPi,^{4, 9, 10, 20, 24–28} with olaparib among the most extensively studied.²⁹ Herin, we examine the pharmacological consequences of replacing the piperazine core in olaparib with diazaspiro systems outlined in Figure 2, in hopes of developing a non-cytotoxic congener of the FDA-approved drug. Our previous work has shown these piperazine alternatives to be effective in alleviating off-target promiscuity and inducing unique protein-ligand interactions.³⁰ Here, we report the synthesis and the pharmacological profiling of **16b-17b**, utilizing in vitro and computational modeling. This work afforded best in class compound, **10e**, an olaparib congener with nanomolar PARP-1 affinity and poor DNA damaging properties, which can be explored as a potential therapeutic for inflammation or neurodegeneration applications where cytotoxicity

is not desired. In addition, we report several worst in class compounds that show micromolar affinity to PARP-1 and induce DNA damage in the concentration range of their affinity.

RESULTS AND DISCUSSION

Compound **9** was prepared following previously reported literature conditions.^{31, 32} Amide coupling of **9** with commercially available spiro analogues **1-8** (A, Scheme 1) afforded **10a-17a**, **10c**, and **10b-17b** in good to moderate yields. Compounds **10d** and **11c-17c** were accessed by Boc-deprotection and basification to obtain the free-amine analogues. Finally, olaparib analogues **10e** and **11d-17d** were synthesized using identical amide coupling conditions in step A, outlined in Scheme 1.

Compounds **10-17** were evaluated for PARP-1 affinity using our previously reported radioligand binding assay protocol with BRCA1 methylated ovarian cancer cells (OVCAR8) illustrated in Table 1.³³ With the exception of **13a** and **16a**, good to moderate PARP-1 inhibitor potencies were observed with methylene spiro-motifs **1a-8a** in the phthalazone architecture with IC₅₀ values ranging from 32.4–57.1 nM. Compared to **10a**, oxa-azaspiro analogue **10b** performed slightly better displaying a PARP-1 affinity value of 24.9 nM.

PARP-1 affinity was drastically reduced when examining ligand scaffolds containing diazaspiro cores with the boc-functional group. Compared to methylene congener **10a**, compound **10c** demonstrated a ~16-fold lower PARP-1 affinity (IC₅₀ = 551.6 nM). Amino core **6b** resulted in the largest reduction in enzyme affinity, in contrast to **15a**, resulting in a ~85-fold decrease in PARP-1 inhibition for **15b**, (IC₅₀ = 4,397 nM). Among the boc-containing analogues examined during this investigation, compound **17b** (IC₅₀ = 452.8 nM) was identified as the most potent inhibitor.

In comparison to the boc-protected analogues examined, increased PARP-1 IC₅₀ values were observed with free-amine analogues **10d**, **11c-17c**. Accordingly, PARP-1 affinity values improved over ~29 fold upon removing the boc-group from compound **13b**, to afford free amine compound **13c** (IC₅₀ = 109.5 nM). A slight increase in enzyme affinity was also observed with free-amine derivatives **11c**, **12c**, **15c**, and **17c**, in contrast to their respective boc-protected analogues.

Coupling the cyclopropanecarbonyl to the aforementioned free-amine intermediates improved the IC₅₀ profiles for most of the compounds developed in this study. PARPi **10e** highlighted this trend with an IC₅₀ value of 12.6 nM, suggesting spirocore **1** can act as a viable structural surrogate for the piperazine ring. Compound **17d** also displayed improved PARP-1 potency (IC₅₀ = 44.3 nM) when compared to the boc-protected (**17b**) and free-amine (**17c**) derivatives. However, **13d-15d** inhibited PARP-1 at higher concentrations, compared to the respective free-amine analogues of these agents.

Molecular docking studies were then performed with olaparib congeners, **10e**, **11d-17d**, to evaluate the predicted binding energies (Table 2) resulting from hydrogen bond interactions in the catalytic domain. This domain was previously illustrated by Salmas and co-workers through computational modeling in order to gain insight into binding interactions and

conformations of PARPi.³⁴ They reported hydrogen bond interactions between the ligand and residues Ser904, Gly863, and Arg878 afforded stability in the NAD⁺ binding pocket of the catalytic domain, while Ser904 and Gly863 dictated inhibitory potency of the compounds. Our computational modeling studies evaluated the interactions of these residues with **10e**, **11d-17d**, and revealed **17d** to have the lowest binding energy of -13.84 kcal/mol, compared to the -12.5 binding energy predicted with olaparib (Figure 3). For the most part, we found the lowest binding energies obtained throughout this modeling study to coincide with the in vitro PARP-1 IC₅₀ values of the selected compounds. These docking evaluations can be a useful strategy for discovering future PARPi in silico.

We then screened **10-17** with olaparib and AZD2461 controls for cell cytotoxicity at 10 μ M concentrations (Figure 3) using an OVCAR8 (BRCA1-methylated) cell line. Due to hypermethylation in the promoter region, these cells demonstrate DNA repair deficiencies,³⁵⁻³⁸ affording increased sensitivity to PARPi. Interestingly, compounds that exhibited high affinity to PARP-1 in this study were found to be non-cytotoxic. For example, **10e**, a structurally-similar analogue of olaparib with an IC₅₀ value of 12.6 nM, displayed reduced anti-proliferative activity (~80% cell survival) in OVCAR8 cells at 10 μ M. This trend was also observed with compounds **10a** (IC₅₀ = 33.9 nM), **12a** (IC₅₀ = 65.4 nM), and **17d** (IC₅₀ = 44.3 $\pm$ 1.2), both affording poor cytotoxic properties. In contrast, several compounds with μ M PARP-1 IC₅₀ values displayed anti-proliferative activity which rivaled those obtained with FDA approved olaparib and AZD2461. This trend was observed mostly with analogues containing boc-protected diazaspiro cores, with poor PARP-1 IC₅₀ values, such as **12b** (IC₅₀ = 3,118 nM), **14b** (IC₅₀ = 1,193 nM), and **15b** (IC₅₀ = 4,397 nM).

Next, we sought to determine if differential cytotoxicity profiles correlated with variations in PARP inhibition by measuring the biochemical product of PARP-1, poly(ADP-ribose) (PAR), after treatment with olaparib, **15b** or **10e**. Western blot analysis was performed to quantify PAR expression after OVCAR8 cells were treated with olaparib, **15b** or **10e** for 6 h. We found compounds **15b** and **10e** to inhibit PARP activity in a manner similar to olaparib, however, compared to the FDA approved drug, higher concentration levels of these two agents were required to completely block PAR synthesis (Figure 5a). In addition, γ -H2AX, a known biomarker for DNA double-strand breaks (DSBs)³⁹, was analyzed by cell microscopy (Figure 5b). This showed olaparib and **15b** induced DNA damage in a dose dependent manner, however **10e** was unable to induce DNA damage at concentrations as high as 10 μ M. These data provide strong evidence that **10e** shows high PARP-1 affinity, good catalytic inhibitory properties without DNA damaging properties.

To gain a better understanding of the anti-proliferative activity observed, we tested PARP selectivity of compounds **10-17** to determine if cell cytotoxicity is mediated by PARP-1, using cell viability assays with MEF WT, MEF *PARP-1* KO^{-/-}, and MEF *PARP-2* KO^{-/-} cell lines. This isogenic model takes into consideration the highly conserved nature of the PARP catalytic domain, and allows investigation into enzyme specificity of the compounds.⁴⁰ We found the EC₅₀ values obtained with this model (Table 3) to be generally consistent with the cytotoxicity data acquired with the OVCAR8 BRCA1-methylated cells. For example, no measurable cell death was recorded with **10e** in the MEF WT and *PARP-1* KO

^{-/-} assay, similar to the poor cytotoxic effects observed at 10 μ M in the OVCAR8 cell line. In addition, no cell death was observed with free amine intermediates **10d**, and **11c-17c**.

Apart from **10c** and **11b**, boc-protected agents screened in the WT model displayed comparable anti-proliferative activity when compared to olaparib and/or AZD2461 (14.6 μ M and 22.1 μ M, respectively). Compound **16b** was identified as the most cytotoxic agent with an EC₅₀ values of 9.4 μ M. We also found compounds **15b** (EC₅₀ 17.2 μ M) and **17b** (EC₅₀ 16.9 μ M) to elicit cell death at similar concentrations to those of olaparib and AZD2461. Accordingly, from cell microscopy studies we know compound **15b** can induce DNA damage in the micromolar range, providing evidence as to why it would show toxicity at this concentration as well.

In contrast to the cytotoxic effects observed in the MEF WT model, MEF *PARP-1* KO^{-/-} cells were more resistant to olaparib, AZD2461, and **15b-17b**, illustrating a clear enzyme dependency of PARP-1. We find these data to be consistent with previous reports of Pommier and co-workers,²³ and Ashworth and co-workers,⁴¹ both describing PARP-1 as the mediator of olaparib toxicity.^{22, 23, 41} In comparison to the *PARP-1* KO^{-/-} model, cell cytotoxicity increased with olaparib, AZD2461, and **15b-17b** in the PARP-1 restored MEF *PARP-2* KO^{-/-} cell line, further validating a PARP-1 dependent mechanism. A dose response curve for these compounds of cell survival in the WT, PARP-1 and -2 knockdown cell lines is provided in Figure 5 to highlight this trend.

It was expected for the MEF cells to not be hypersensitive to PARP inhibition due to this isogenic model not having a *BRCA1* mutation which would lead to DNA repair deficiencies. This explains the comparable cytotoxicity of olaparib with **15b-17b**, despite the differences in PARP-1 affinity. To confirm these findings, we performed additional experiments in an isogenic cell model with a *BRCA1* mutation and restoration of a functional copy of *BRCA1* to answer whether synthetic lethality is dependent on PARP inhibitor affinity. As such, cytotoxic profiling of **10e**, **15b-17b**, and olaparib, were conducted in *BRCA1*-mutant/restored UWB1.289 isogenic cell lines. This cell line model allowed us to evaluate the effect of compounds reported in an isogenic background with a single gene perturbation; one cell line with a *BRCA1* mutation and the isogenic partner with a cDNA restored copy of *BRCA1* to correct the mutation. Due to the *BRCA1* deficiency of the cells, UWB1.289 is highly sensitive to PARPi. Compounds **15b-17b** were found to be cytotoxic in the both cell lines at micromolar concentrations compared and expressed EC₅₀ values ~20–40 times than that of olaparib (Table 4). Compound **10e**, however, showed evidence of cytotoxicity that was greater in *BRCA1* mutant cells vs. *BRCA1* restored cells. This cytotoxicity was observed at concentrations approximately 7-fold higher than olaparib, further supporting **10e** as having reduced DNA damaging properties and toxicity even in cells with *BRCA1* mutations.

Off-target interactions with other PARPs were then investigated with compounds **10e**, and **15b-17b** at 10 μ M concentrations (Figure 7). In comparison to olaparib, we found **10e** to be a highly specific PARP-1 inhibitor with minimal off-target activity even at 10 μ M concentrations. This reduction in off-target interactions could be attributed to the unique chemical space **10e** can populate in the highly conserved PARP catalytic domain due to spirodiamine **1**, that may otherwise be inaccessible with piperazine congener olaparib. In

combination with low cytotoxicity, **10e** exhibits high PARP-1 specificity and shows promise as a best in class compound that could be translated for treating inflammatory disease.

In comparison to **10e**, compounds **15b-17b** demonstrated an increase in enzymatic inhibition at most PARPs examined, most notably at the TNKS-2 protein. However, upon further investigation, we identified low to moderate TNKS-1/2 IC₅₀ values for **15b-17b** that were comparable to the olaparib (Table 5).

CONCLUSIONS

This study examined the pharmacological effects of replacing the piperazine core in the olaparib framework with diazaspiro systems. Molecular modeling of eight olaparib congeners developed in this study revealed favorable binding energies consistent to the PARP-1 IC₅₀ values obtain in vitro, indicating in silico studies can be a useful tool in developing future PARPi's. Western blot and cell microscopy analysis with olaparib, **10e** and **15b** (compounds with contrasting PARP-1 IC₅₀ profiles), revealed these selected agents inhibit PAR activity and but do not induce γ -H2AX foci equally, demonstrating **10e** does not cause DNA DSB's at micromolar concentrations. This is only the second PARPi, after veliparib, to be reported that shows high affinity to PARP-1, good catalytic inhibition, and reduced ability cause DNA damage. Furthermore, this suggest that high affinity does not equal good DNA damaging properties providing strong support for investigating PARPi pharmacology to delineate catalytic inhibition from DNA damage. A better understanding of this would allow PARPi to be translated for the treatment of diseases other than cancer, especially those that involve inflammation.

We found implementing diazaspiro systems into the olaparib framework did not alleviate the dependency of PARP-1 for cytotoxic effects. However, we identified three novel PARPi's (**15b-17b**) with greatly reduced PARP-1 affinity and unexpected cytotoxicity. However, this cytotoxicity was in agreement with the concentration range that these agents induced DNA damage. These data support the concept that DNA damage is dependent on PARP-1 affinity, but high affinity does not guarantee DNA damaging properties.

Off-target interactions with other PARPs were explored with olaparib congener **10e**, and analogues **15b-17b**. Compared to olaparib, **10e** demonstrated less enzymatic inhibition with most other PARPs, further highlighting **10e** as the best in class compound with a 12.6 nM PARP-1 IC₅₀ obtained, with reduced DNA damaging properties. It is known that HR deficient cells are highly sensitive to disruption of PAR signaling as well as DNA damage, and this was demonstrated with compound **10e** that showed greater cytotoxicity in *BRCA1* mutant vs. *BRCA1* cells with a functional copy of the gene restored.

Current work is underway in evaluating **10e** in neurodegenerative and anti-inflammatory models, due to the unique PARP-1 potency and non-cytotoxic nature of the compound. We will also continue to evaluate the anti-proliferative effects of **15b-17b** in PARPi resistant cell lines, and investigate if these compounds can induce selective toxicity to cancer cells when used with chemotherapeutic agents.

EXPERIMENTAL SECTION

General.

Chemical compounds **1–8** were purchased and used without further purification. Compound **9** was prepared following previously reported literature conditions.^{31, 32} NMR spectra were taken on a Bruker DMX 500 MHz. Compound structures and identity were confirmed by ¹H and ¹³C NMR, and mass spectroscopy. Compound purity greater than 95% was determined by LCMS analysis using a 2695 Alliance LCMS. All other commercial reagents were purchased and used without further purification. Purification of organic compounds were carried out on a Biotage Isolera One with a dual-wavelength UV-VIS detector. Chemical shifts (δ) in the NMR spectra (¹H and ¹³C) were referenced by assigning the residual solvent peaks.

General Synthetic Procedure for Compounds 10–17.

A one-pot mixture of azaspirocyclo (1.0 mmol), **9** (1.0 mmol), HOBt hydrate (1.0 mmol), EDC hydrochloride (1.0 mmol), and Et₃N (2.0 mmol) was stirred in 5 mL of THF at 60 °C for 12 h. A saturated NaHCO₃ (aq) solution (15 mL) was then added to the crude reaction mixture and stirred at room temp for 1 h. The reaction mixture was extracted with CH₂Cl₂ (3 × 20 mL) to afford the crude product. The residue was loaded onto a Biotage SNAP flash purification cartridge, eluting with 10% 7N NH₃ in MeOH solution/CH₂Cl₂ to give the target compounds **10a–17a**, **10c** and **10b–17b**. The appropriate Boc-protected compound (**10c**, and **11b–17b**) was dissolved in CH₂Cl₂ (2 mL), followed by dropwise addition of CF₃COOH (2 mL), and stirred at room temperature for 3 h. Volatiles were then removed under reduced pressure and the crude product was neutralized with a saturated NaHCO₃ (aq) solution (10 mL). The reaction mixture was extracted with CH₂Cl₂ (3 × 20 mL), and the organic layers were combined, dried, and concentrated to afford the free-amine intermediates (**10c–17c**) as white foams in near quantitative yields. Compounds were analyzed for purity using LCMS, ¹H and ¹³C NMR spectroscopy, and, if necessary, purified using a Biotage SNAP flash purification cartridge, eluting with 10% 7N NH₃ in MeOH solution/EtOAc.

4-(4-Fluoro-3-(2-azaspiro[3.3]heptane-2-carbonyl)benzyl)phthalazin-1(2H)-one (**10a**).

Following the general procedure, **10a** was purified by flash chromatography to afford the desired product as a white foam (0.108 g, 30%). ¹H NMR (500 MHz, CDCl₃) δ 11.38 (s, 1H), 8.48–8.46 (m, 1H), 7.77–7.70 (m, 3H), 7.49–7.47 (dd, J = 2.0, 6.2 Hz, 1H), 7.30–7.27 (m, 1H), 6.98 (t, J = 9.1 Hz, 1H), 4.28 (s, 2H), 4.10 (m, 2H), 3.95 (s, 2H), 2.21–2.15 (, 2H), 2.14–2.08 (m, 2H), 1.85–1.80 (m, 1H), 1.79–1.76 (m, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 166.1, 161.0, 156.9 (d, J_{C-F} = 249.6 Hz), 145.8, 134.1 (d, $J_{C-C-C-F}$ = 3.4 Hz), 133.7, 132.1 (d, $J_{C-C-C-F}$ = 8.3 Hz), 131.6, 130.2 (d, $J_{C-C-C-F}$ = 3.7 Hz), 129.7, 128.4, 127.2, 125.3, 122.7 (d, J_{C-C-F} = 17.1 Hz), 116.3 (d, J_{C-C-F} = 22.7 Hz), 63.4 (2×CH), 60.8, 38.1, 37.9, 33.1; LC-MS (ESI) m/z : (m/z): 378.15 [M+H].

4-(4-Fluoro-3-(2-oxa-6-azaspiro[3.3]heptane-6-carbonyl)benzyl)phthalazin-1(2H)-one (10b).

Following the general procedure, **10b** was purified by flash chromatography to afford the desired product as a white foam (0.2662 g, 70%). ¹H NMR (500 MHz, CDCl₃) δ 11.54 (s, 1H), 8.47–8.45 (m, 1H), 7.77–7.69 (m, 3H), 7.49–7.47 (m, 1H), 7.34–7.31 (m, 1H), 7.01 (t, *J* = 8.8 Hz, 1H), 4.81–4.79 (m, 2H), 4.74–4.72 (m, 2H), 4.30 (s, 2H), 4.27 (s, 2H), 4.20 (s, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 166.2, 161.0, 157.0 (d, *J*_{C–F} = 249.6 Hz), 145.6, 134.3 (d, *J*_{C–C–C–F} = 3.5 Hz), 133.7, 132.6 (d, *J*_{C–C–C–F} = 8.4 Hz), 131.6, 130.2 (d, *J*_{C–C–C–F} = 3.3 Hz), 129.7, 128.4, 127.2, 125.1, 122.0 (d, *J*_{C–C–F} = 16.6 Hz), 116.6 (d, *J*_{C–C–F} = 25.5 Hz), 77.4, 77.2, 76.9, 60.6 (2×CH), 58.0, 38.1, 37.7; LC-MS (ESI) *m/z*: 380.15 [M+H].

Tert-butyl 6-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)-2,6-diazaspiro[3.3]heptane-2-carboxylate (10c).

Following the general procedure, **10c** was purified by flash chromatography to afford the desired product as a white foam (0.324 g, 69%). ¹H NMR (500 MHz, CDCl₃) δ 12.00 (s, 1H), 8.41 (m, 1H), 7.65 (m, 3H), 7.46 (dd, *J* = 2.2, 4.1 Hz, 1H), 7.29 (m, 1H), 6.94 (t, *J* = 9.2 Hz, 1H), 4.23 (d, *J* = 7.2 Hz, 4H), 4.10 (s, 2H), 4.02 (d, *J* = 9.5 Hz, 2H), 3.97 (d, *J* = 9.4 Hz, 2H), 1.36 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 166.1, 161.1, 156.8 (d, *J*_{C–F} = 250.1 Hz), 155.8, 145.4, 134.3 (d, *J*_{C–C–C–F} = 3.6 Hz), 133.5, 132.5 (d, *J*_{C–C–C–F} = 8.2 Hz), 131.4, 130.1 (d, *J*_{C–C–C–F} = 3.5 Hz), 129.5, 128.2, 127.0, 125.0, 121.8 (d, *J*_{C–C–F} = 16.3 Hz), 116.4 (d, *J*_{C–C–F} = 21.8 Hz), 79.8, 61.0, 59.5, 58.6, 37.7, 32.5, 28.2; LC-MS (ESI) *m/z*: 479.09 [M+H], 379.08 [M-BOC+H].

4-(4-Fluoro-3-(2,6-diazaspiro[3.3]heptane-2-carbonyl)benzyl)phthalazin-1(2H)-one (10d).

Following the general procedure, **10d** was afforded in near quantitative yield as a white foam. ¹H NMR (500 MHz, CDCl₃) δ 8.45–8.44 (m, 1H), 7.75–7.72 (m, 2H), 7.71–7.68 (m, 1H), 7.47 (dd, *J* = 2.2, 6.3 Hz, 1H), 7.48–7.28 (m, 1H), 6.98 (t, *J* = 9.0 Hz, 1H), 4.26 (s, 2H), 4.23 (s, 2H), 4.14 (s, 2H), 3.84 (d, *J* = 8.0 Hz, 2H), 3.75 (d, *J* = 8.0 Hz, 2H), 3.34–3.23 (m, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 166.1, 160.8, 158.9 (d, *J*_{C–F} = 250.0 Hz), 145.6, 134.2 (d, *J*_{C–C–C–F} = 3.6 Hz), 133.7, 132.5 (d, *J*_{C–C–C–F} = 8.4 Hz), 131.6, 130.1 (d, *J*_{C–C–C–F} = 3.6 Hz), 129.7, 128.4, 127.2, 125.1, 122.3 (d, *J*_{C–C–F} = 17.0 Hz), 116.4 (d, *J*_{C–C–F} = 22.9 Hz), 61.8, 61.7, 59.0, 57.4, 37.7; LC-MS (ESI) *m/z*: 479.09 [M+H], 379.21 [M+H].

4-(3-(6-(Cyclopropanecarbonyl)-2,6-diazaspiro[3.3]heptane-2-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one (10e).

Following the general procedure, **10e** was purified by flash chromatography to afford the desired product as a white foam (0.257 g, 82%). ¹H NMR (500 MHz, CDCl₃) δ 12.01 (s, 1H), 8.40–8.38 (m, 1H), 7.69–7.65 (m, 3H), 7.46–7.45 (m, 1H), 7.31–7.28 (m, 1H), 6.95 (t, *J* = 8.9 Hz, 1H), 4.38 (d, *J* = 8.9 Hz, 1H), 4.31 (d, *J* = 8.6 Hz, 1H), 4.26 (s, 2H), 4.23 (s, 2H), 4.16 (s, 2H), 4.12 (d, *J* = 10.5 Hz, 1H), 4.05 (d, *J* = 10.3 Hz, 1H), 1.30–1.28 (m, 1H), 0.88 (d, *J* = 3.0 Hz, 2H), 0.67 (d, *J* = 5.7 Hz, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 173.9, 166.0, 161.0, 156.9 (d, *J*_{C–F} = 250.7 Hz), 145.4, 134.3 (d, *J*_{C–C–C–F} = 3.4 Hz), 133.6, 132.7 (d, *J*_{C–C–C–F} = 8.3 Hz), 131.5, 130.2 (d, *J*_{C–C–C–F} = 3.6 Hz), 129.5, 128.3, 127.0,

125.0, 121.7 (d, J_{C-F} = 16.6 Hz), 116.6 (d, J_{C-F} = 23.0 Hz), 61.1, 60.0, 58.6, 58.1, 37.6, 32.6, 10.1, 7.5; LC-MS (ESI) m/z : (m/z): 447.04 [M+H].

4-(4-Fluoro-3-(1-azaspiro[3.3]heptane-1-carbonyl)benzyl)phthalazin-1(2H)-one (11a).

Following the general procedure, **11a** was purified by flash chromatography to afford the desired product as a white foam (0.189 g, 51%). (major rotamer reported) 1H NMR (500 MHz, $CDCl_3$) δ 12.00 (s, 1H), 8.41 (d, J = 7.40 Hz, 1H), 7.71–7.66 (m, 3H), 7.38 (dd, J = 1.7, 4.4 Hz, 1H), 7.30–7.28 (m, 1H), 6.95 (t, J = 8.9 Hz, 1H), 5.15 (d, J = 8.4 Hz, 1H), 4.79 (d, J = 9.9 Hz, 1H), 4.23 (s, 2H), 4.22–4.21 (m, 1H), 4.05 (d, J = 9.9 Hz, 1H), 3.89–3.84 (m, 2H), 2.48–2.45 (m, 2H), 1.38–1.35 (m, 1H), 0.90 (s, 2H), 0.67 (dd, J = 2.7, 2.6 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.7, 165.9, 161.1, 156.6 (d, J_{C-F} = 248.5 Hz), 145.6, 134.3 (d, $J_{C-C-C-F}$ = 3.50 Hz), 133.6, 132.3 (d, $J_{C-C-C-F}$ = 8.1 Hz), 131.5, 129.7, 129.6 (d, $J_{C-C-C-F}$ = 3.7 Hz), 128.3, 127.0, 125.1, 122.7 (d, J_{C-F} = 17.2 Hz), 116.3 (d, J_{C-F} = 22.2 Hz), 63.3, 59.8, 58.6, 47.5, 37.7, 28.7, 10.5, 7.4; LC-MS (ESI) m/z : 447.17 [M +H].

Tert-butyl 1-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)-1,6-diazaspiro[3.3]heptane-6-carboxylate (11b).

Following the general procedure, **11b** was purified by flash chromatography to afford the desired product as a white foam (0.219 g, 98%). (major rotamer reported) 1H NMR (500 MHz, $CDCl_3$) δ 12.00 (s, 1H), 8.42–8.40 (m, 1H), 7.70–7.65 (m, 3H), 7.38 (dd, J = 2.2, 4.0 Hz, 1H), 7.29–7.26 (m, 1H), 6.94 (t, J = 8.9 Hz, 1H), 4.79 (d, J = 9.1 Hz, 2H), 4.24 (s, 2H), 3.92 (d, J = 9.3 Hz, 2H), 3.83 (t, J = 7.4 Hz, 2H), 2.42 (t, J = 7.3 Hz, 2H), 1.38 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.8, 161.2, 156.6 (d, J_{C-F} = 249.2 Hz), 156.3, 145.7, 134.3 (d, $J_{C-C-C-F}$ = 2.9 Hz), 133.7, 132.2 (d, $J_{C-C-C-F}$ = 8.1 Hz), 131.5, 129.7, 129.6 (d, $J_{C-C-C-F}$ = 2.4 Hz), 128.3, 127.1, 125.1, 123.0 (d, J_{C-F} = 17.6 Hz), 116.5 (d, J_{C-F} = 22.4 Hz), 79.6, 63.5, 59.6, 47.4, 37.7, 28.5, 28.4; LC-MS (ESI) m/z : 501.12 [M +Na], 379.21 [M-BOC+H].

4-(4-Fluoro-3-(1,6-diazaspiro[3.3]heptane-1-carbonyl)benzyl)phthalazin-1(2H)-one (11c).

Following the general procedure, **11c** was afforded in near quantitative yield as a white foam. (major rotamer reported) 1H NMR (500 MHz, $CDCl_3$) δ 11.76 (s, 1H), 8.46–8.44 (m, 1H), 7.71–7.68 (m, 3H), 7.39–7.38 (m, 1H), 7.26–7.24 (m, 1H), 6.97–6.93 (m, 2H), 4.26 (s, 2H), 3.79 (t, J = 7.8 Hz, 2H), 3.17–3.11 (m, 2H), 2.31–2.28 (m, 2H), 2.07–2.03 (m, 2H), 1.86–1.82 (m, 1H), 1.70–1.63 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.7, 161.1, 156.5 (d, J_{C-F} = 248.6 Hz), 145.8, 134.0 (d, $J_{C-C-C-F}$ = 3.5 Hz), 133.6, 131.5, 131.4 (d, $J_{C-C-C-F}$ = 7.6 Hz), 129.6, 129.5 (d, $J_{C-C-C-F}$ = 3.8 Hz), 128.3, 127.1, 125.2, 124.1 (d, J_{C-F} = 18.2 Hz), 116.4 (d, J_{C-F} = 22.6 Hz), 68.6, 46.6, 44.2, 37.9, 36.0, 33.7, 30.9, 13.2; LC-MS (ESI) m/z : 501.12 [M+Na], 378.28 [M +H].

4-(3-(6-(Cyclopropanecarbonyl)-1,6-diazaspiro[3.3]heptane-1-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one (11d).

Following the general procedure, **11d** was purified by flash chromatography to afford the desired product as a white foam (0.180 g, 54%). (major rotamer reported) 1H NMR (500

MHz, CDCl₃) δ 12.00 (s, 1H), 8.41 (d, J = 7.40 Hz, 1H), 7.71–7.66 (m, 3H), 7.38 (dd, J = 1.7, 4.4 Hz, 1H), 7.30–7.28 (m, 1H), 6.95 (t, J = 8.9 Hz, 1H), 5.15 (d, J = 8.4 Hz, 1H), 4.79 (d, J = 9.9 Hz, 1H), 4.23 (s, 2H), 4.22–4.21 (m, 1H), 4.05 (d, J = 9.9 Hz, 1H), 3.89–3.84 (m, 2H), 2.48–2.45 (m, 2H), 1.38–1.35 (m, 1H), 0.90 (s, 2H), 0.67 (dd, J = 2.7, 2.6 Hz, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 173.7, 165.9, 161.1, 156.6 (d, $J_{\text{C-F}}$ = 248.5 Hz), 145.6, 134.3 (d, $J_{\text{C-C-C-F}}$ = 3.50 Hz), 133.6, 132.3 (d, $J_{\text{C-C-C-F}}$ = 8.1 Hz), 131.5, 129.7, 129.6 (d, $J_{\text{C-C-C-F}}$ = 3.7 Hz), 128.3, 127.0, 125.1, 122.7 (d, $J_{\text{C-C-F}}$ = 17.2 Hz), 116.3 (d, $J_{\text{C-C-F}}$ = 22.2 Hz), 63.3, 59.8, 58.6, 47.5, 37.7, 28.7, 10.5, 7.4; LC-MS (ESI) m/z : 447.17 [M + H].

4-(4-Fluoro-3-(2-azaspiro[3.5]nonane-2-carbonyl)benzyl)phthalazin-1(2H)-one (12a).

Following the general procedure, **12a** was purified by flash chromatography to afford the desired product as a white foam (0.185 g, 46%). ¹H NMR (500 MHz, CDCl₃) δ 11.84 (s, 1H), 8.46–8.45 (m, 1H), 7.72–7.69 (m, 3H), 7.49–7.47 (m, 1H), 7.29–7.25 (m, 1H), 6.96 (t, J = 8.7 Hz, 1H), 4.27 (s, 2H), 3.77 (s, 2H), 3.61 (s, 2H), 1.59 (bs, 4H), 1.41 (bs, 2H), 1.33 (bs, 4H); ¹³C NMR (125 MHz, CDCl₃) δ 166.5, 161.1, 156.9 (d, $J_{\text{C-F}}$ = 249.3 Hz), 145.7, 134.1 (d, $J_{\text{C-C-C-F}}$ = 3.4 Hz), 133.6, 132.0 (d, $J_{\text{C-C-C-F}}$ = 8.1 Hz), 131.5, 130.0 (d, $J_{\text{C-C-C-F}}$ = 3.6 Hz), 128.3, 127.1, 125.2, 122.7 (d, $J_{\text{C-C-F}}$ = 16.8 Hz), 116.4 (d, $J_{\text{C-C-F}}$ = 22.2 Hz), 61.5, 59.0, 37.8, 36.0, 35.7, 25.2, 23.0; LC-MS (ESI) m/z : 475.08 [M + H].

Tert-butyl 2-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)-2,7-diazaspiro[3.5]nonane-7-carboxylate (12b).

Following the general procedure, **12b** was purified by flash chromatography to afford the desired product as a white foam (0.350 g, 69%). ¹H NMR (500 MHz, CDCl₃) δ 12.07 (s, 1H), 8.41–8.39 (m, 1H), 7.66–7.65 (m, 3H), 7.46 (d, J = 5.5 Hz, 1H), 7.28–7.25 (m, 1H), 6.94 (t, J = 9.3 Hz), 4.23 (s, 2H), 3.82 (s, 2H), 3.68 (s, 2H), 3.29–3.25 (m, 4H), 1.67–1.62 (m, 4H), 1.07 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 166.4, 161.0, 156.7 (d, $J_{\text{C-F}}$ = 249.5 Hz), 154.6, 145.5, 134.1 (d, $J_{\text{C-C-C-F}}$ = 3.3 Hz), 133.5, 132.1 (d, $J_{\text{C-C-C-F}}$ = 8.4 Hz), 131.3, 129.9 (d, $J_{\text{C-C-C-F}}$ = 3.9 Hz), 129.5, 128.2, 126.9, 125.0, 122.2 (d, $J_{\text{C-C-F}}$ = 17.1 Hz), 116.3 (d, $J_{\text{C-C-F}}$ = 22.7 Hz), 79.6, 60.5, 57.9, 40.6, 37.6, 34.9, 34.0, 28.3; LC-MS (ESI) m/z : 407.12 [M-BOC+H].

4-(4-Fluoro-3-(2,7-diazaspiro[3.5]nonane-2-carbonyl)benzyl)phthalazin-1(2H)-one (12c).

Following the general procedure, **12c** was afforded in near quantitative yield as a white foam. ¹H NMR (500 MHz, CDCl₃) δ 8.44 (d, J = 6.5 Hz, 1H), 7.27–7.70 (m, 3H), 7.47–7.46 (m, 1H), 7.29–7.26 (m, 1H), 6.98 (t, J = 9.1 Hz, 1H), 4.26 (s, 2H), 3.84 (s, 2H), 3.69 (s, 2H), 2.80 (bs, 2H), 2.76 (bs, 2H), 1.73 (bs, 4H); ¹³C NMR (125 MHz, CDCl₃) δ 166.5, 160.9, 156.8 (d, $J_{\text{C-F}}$ = 249.9 Hz), 145.7, 134.2 (d, $J_{\text{C-C-C-F}}$ = 3.6 Hz), 133.7, 132.3 (d, $J_{\text{C-C-C-F}}$ = 8.1 Hz), 131.6, 130.0 (d, $J_{\text{C-C-C-F}}$ = 3.9 Hz), 129.7, 128.4, 127.2, 125.2, 122.5 (d, $J_{\text{C-C-F}}$ = 17.2 Hz), 116.5 (d, $J_{\text{C-C-F}}$ = 22.6 Hz), 61.2, 58.7, 43.2, 37.8, 35.9, 34.3; LC-MS (ESI) m/z : 407.25 [M+H].

4-(3-(7-(Cyclopropanecarbonyl)-2,7-diazaspiro[3.5]nonane-2-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one (12d).

Following the general procedure, **12d** was purified by flash chromatography to afford the desired product as a white foam (0.145 g, 68%). ¹H NMR (500 MHz, CDCl₃) δ 12.1 (s, 1H), 7.67–7.62 (m, 3H), 7.44 (dd, *J* = 1.9, 4.7 Hz, 1H), 7.27–7.24 (m, 1H), 6.94 (t, *J* = 9.3 Hz, 1H), 4.22 (s, 2H), 3.83 (s, 2H), 3.69 (s, 2H), 3.47 (bs, 4H), 1.68–1.63 (m, 5H), 0.87–0.85 (m, 2H), 0.65–0.64 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 171.9, 166.4, 161.0, 156.7 (d, *J*_{C–F} = 249.6 Hz), 145.4, 134.2 (d, *J*_{C–C–C–C–F} = 3.6 Hz), 133.5, 132.3 (d, *J*_{C–C–C–F} = 7.7 Hz), 131.4, 129.9 (d, *J*_{C–C–C–F} = 3.6 Hz), 128.2, 129.5, 126.9, 125.0, 122.1 (d, *J*_{C–C–F} = 17.3 Hz), 116.4 (d, *J*_{C–C–F} = 22.1 Hz), 60.4, 58.0, 42.5, 39.3, 37.6, 35.8, 34.3, 10.9, 7.3; LC-MS (ESI) *m/z*: 475.08 [M+H].

4-(4-Fluoro-3-(1-azaspiro[3.5]nonane-1-carbonyl)benzyl)phthalazin-1(2H)-one (13a).

Following the general procedure, **13a** was purified by flash chromatography to afford the desired product as a white foam (0.128 g, 32%). (major rotamer reported) ¹H NMR (500 MHz, CDCl₃) δ 11.52 (s, 1H), 8.47–8.45 (m, 1H), 7.74–7.70 (m, 3H), 7.37–7.36 (m, 1H), 7.25–7.22 (m, 1H), 6.97–6.94 (m, 1H), 4.26 (s, 2H), 3.80 (t, *J* = 8.0 Hz, 2H), 2.41–2.38 (m, 2H), 2.00 (t, *J* = 7.2 Hz, 2H), 1.89 (s, 1H), 1.87 (s, 1H), 1.74–1.71 (m, 2H), 1.33–1.30 (m, 1H), 1.23–1.21 (m, 2H), 1.08–1.00 (m, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 165.2, 160.0, 156.5 (d, *J*_{C–F} = 247.9 Hz), 145.9, 134.0 (d, *J*_{C–C–C–C–F} = 3.6 Hz), 133.7, 131.6, 131.3 (d, *J*_{C–C–C–F} = 8.2 Hz), 129.7, 129.3 (d, *J*_{C–C–C–F} = 3.7 Hz), 128.4, 127.2, 125.3, 124.4 (d, *J*_{C–C–F} = 19.3 Hz), 116.4 (d, *J*_{C–C–F} = 22.4 Hz), 71.2, 45.5 (2×CH), 43.2, 37.9, 36.7, 34.7, 27.8, 24.9, 22.7; LC-MS (ESI) *m/z*: 406.18 [M+H].

Tert-butyl 1-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)-1,7-diazaspiro[3.5]nonane-7-carboxylate (13b).

Following the general procedure, **13b** was purified by flash chromatography to afford the desired product as a white foam (0.409 g, 81%). (major rotamer reported) ¹H NMR (500 MHz, CDCl₃) δ 12.09 (s, 1H), 8.38–8.36 (m, 1H), 7.64–7.61 (m, 3H), 7.30 (dd, *J* = 2.2, 4.1 Hz, 1H), 7.22–7.19 (m, 1H), 6.89 (t, *J* = 8.9 Hz, 1H), 4.20 (s, 2H), 4.06 (bs, 2H), 3.97 (t, *J* = 7.3, 1H), 3.79 (t, *J* = 7.46 Hz, 2H), 2.65 (bs, 2H), 2.52 (dt, *J* = 4.2, 8.8 Hz, 2H), 2.01 (t, *J* = 7.9 Hz, 2H), 1.75 (d, *J* = 12.05 Hz, 2H), 1.37 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 165.7, 161.0, 156.2 (d, *J*_{C–F} = 248.1 Hz), 154.5, 145.5, 134.0 (d, *J*_{C–C–C–C–F} = 3.0 Hz), 133.4, 131.5 (d, *J*_{C–C–C–F} = 8.0 Hz), 129.4, 129.1 (d, *J*_{C–C–C–F} = 3.6 Hz), 128.1, 126.8, 125.6, 125.0, 123.5 (d, *J*_{C–C–F} = 18.2 Hz), 116.0 (d, *J*_{C–C–F} = 21.9 Hz), 79.4, 68.6, 45.4, 40.5, 37.6, 34.0, 28.3, 27.3; LC-MS (ESI) *m/z*: 529.04 [M+Na], 407.12 [M-BOC+H].

4-(4-Fluoro-3-(1,7-diazaspiro[3.5]nonane-1-carbonyl)benzyl)phthalazin-1(2H)-one (13c).

Following the general procedure, **13c** was afforded in near quantitative yield as a white foam. (major rotamer reported). ¹H NMR (500 MHz, CDCl₃) δ 8.44–8.42 (m, 1H), 7.75–7.66 (m, 3H), 7.26–7.18 (m, 2H), 7.05–6.93 (m, 1H), 4.24 (s, 2H), 4.05–4.02 (m, 1H), 3.84–3.81 (m, 1H), 3.14–3.11 (m, 1H), 3.02–2.97 (m, 1H), 2.88–2.86 (m, 1H), 2.63–2.51 (m, 2H), 2.13–2.05 (m, 2H), 1.87–1.79 (m, 1H), 1.74–1.72 (m, 1H), 1.61–1.56 (m, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 165.8, 160.9, 156.4 (d, *J*_{C–F} = 246.0 Hz), 145.6, 134.0 (d,

$J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.0$ Hz), 133.6, 131.5 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 8.0$ Hz), 129.8, 129.3 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 4.1$ Hz), 128.4, 127.1, 125.2, 124.2, 123.9 (d, $J_{\text{C}-\text{C}-\text{F}} = 18.1$ Hz), 116.2 (d, $J_{\text{C}-\text{C}-\text{F}} = 20.8$ Hz), 71.0, 45.4, 43.5, 42.8, 42.7, 36.4, 28.8; LC-MS (ESI) m/z : 407.12 [M+H].

4-(3-(7-(Cyclopropanecarbonyl)-1,7-diazaspiro[3.5]nonane-1-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one (13d).

Following the general procedure, **13d** was purified by flash chromatography to afford the desired product as a white foam (0.074 g, 53%). (major rotamer reported) ^1H NMR (500 MHz, CDCl_3) δ 11.54 (s, 1H), 8.46 (m, 1H), 7.74–7.68 (m, 3H), 7.36 (dd, $J = 2.2, 4.1$ Hz, 1H), 7.27–7.25 (m, 1H), 6.98–6.95 (m, 1H), 4.25 (s, 2H), 4.13 (m, 2H), 3.88 (t, $J = 8.0$ Hz, 2H), 3.10 (bs, 1H), 2.69–2.54 (m, 3H), 2.13 (t, $J = 7.7$ Hz, 2H), 1.81 (m, 2H), 1.76–1.70 (m, 1H), 0.97–0.94 (m, 2H), 0.73–0.69 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 171.9, 165.5, 160.1, 156.4 (d, $J_{\text{C}-\text{F}} = 247.2$ Hz), 145.7, 134.1 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.5$ Hz), 133.7, 131.6 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 8.5$ Hz), 129.6, 129.3 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.6$ Hz), 128.3, 127.1, 125.2, 124.3, 123.8 (d, $J_{\text{C}-\text{C}-\text{F}} = 18.3$ Hz), 116.4 (d, $J_{\text{C}-\text{C}-\text{F}} = 22.1$ Hz), 68.8, 45.5, 42.3, 37.8, 36.5, 34.2, 27.4, 11.1, 7.4; LC-MS (ESI) m/z : 475.08 [M+H], 496.98 [M+Na].

4-(4-Fluoro-3-(6-azaspiro[3.4]octane-6-carbonyl)benzyl)phthalazin-1(2H)-one (14a).

Following the general procedure, **14a** was purified by flash chromatography to afford the desired product as a white foam (0.263 g, 54%). (mixture of two rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 12.30 (s, 1H), 8.40–8.37 (m, 1H), 7.64–7.62 (m, 3H), 7.32–7.29 (m, 1H), 7.25–7.19 (m, 1H), 6.95–6.88 (m, 1H), 4.23–4.22 (m, 2H), 3.55–3.51 (m, 2H), 3.19–3.17 (t, $J = 6.8$ Hz, 1H), 3.09 (s, 1H), 1.99–1.95 (m, 1H), 1.86–1.74 (m, 7H); ^{13}C NMR (125 MHz, CDCl_3) δ 164.9, 164.8, 161.1, 156.0 (d, $J_{\text{C}-\text{F}} = 247.8$ Hz), 145.6, 134.1 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.4$ Hz), 134.0 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.4$ Hz), 133.4, 133.3, 131.3, 131.1 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 8.1$ Hz), 131.0 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 8.1$ Hz), 129.4, 128.7 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.6$ Hz), 128.6 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.6$ Hz), 128.1, 128.0, 126.9, 125.5 (d, $J_{\text{C}-\text{C}-\text{F}} = 17.8$ Hz), 125.4 (d, $J_{\text{C}-\text{C}-\text{F}} = 17.8$ Hz), 125.1, 116.1 (d, $J_{\text{C}-\text{C}-\text{F}} = 21.9$ Hz), 116.0 (d, $J_{\text{C}-\text{C}-\text{F}} = 21.9$ Hz), 58.7, 57.0, 46.3, 44.7, 44.5, 43.7, 37.7, 37.6, 36.2, 31.1, 30.5, 15.9, 15.7; LC-MS (ESI) m/z : 392.16 [M+H].

Tert-butyl 2-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)-2,6-diazaspiro[3.4]octane-6-carboxylate (14b).

Following the general procedure, **14b** was purified by flash chromatography to afford the desired product as a white foam (0.334 g, 72%). (mixture of two rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 12.08–12.06 (m, 1H), 8.39–8.37 (m, 1H), 7.68–7.62 (m, 3H), 7.31–7.29 (m, 1H), 7.26–7.22 (m, 1H), 6.96–6.90 (m, 1H), 4.22–4.21 (m, 2H), 3.88–3.87 (m, 2H), 3.77–3.73 (m, 2H), 3.70–3.66 (m, 2H), 3.60 (t, $J = 6.94$ Hz, 1H), 3.34–3.33 (m, 2H), 3.25 (t, $J = 6.90$ Hz, 1H), 2.08 (t, $J = 7.2$ Hz, 1H), 2.00 (t, $J = 6.7$ Hz, 1H), 1.36–1.33 (m, 9H); ^{13}C NMR (125 MHz, CDCl_3) δ 165.1, 165.0, 161.1 (2 $\times$ CH), 156.2 (d, $J_{\text{C}-\text{F}} = 247.6$ Hz), 156.1 (d, $J_{\text{C}-\text{F}} = 247.6$ Hz), 145.5 (2 $\times$ CH), 134.4 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.4$ Hz), 134.3 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.4$ Hz), 133.5, 131.6 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 7.9$ Hz), 131.4 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 7.9$ Hz), 129.5, 128.9, 127.0, 125.1 (d, $J_{\text{C}-\text{F}} = 17.3$ Hz), 125.0 (d, $J_{\text{C}-\text{F}} =$

17.2 Hz), 116.2 (d, $J_{\text{C}-\text{C}-\text{F}} = 21.9$ Hz), 116.2 (d, $J_{\text{C}-\text{C}-\text{F}} = 21.9$ Hz), 79.8, 79.7, 57.9, 56.8, 55.1, 50.4, 46.3, 44.6, 39.7, 38.7, 37.7, 36.3, 34.6, 28.3 (2 $\times$ CH); LC-MS (ESI) m/z : 515.01 [M+Na], 393.10 [M-BOC+H].

4-(4-Fluoro-3-(2,6-diazaspiro[3.4]octane-6-carbonyl)benzyl)phthalazin-1(2H)-one (14c).

Following the general procedure, **14c** was afforded in near quantitative yield as a white foam. (mixture of two rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 8.44 (d, $J = 7.0$ Hz, 1H), 7.76–7.68 (m, 3H), 7.36–7.32 (m, 1H), 7.26–7.24 (m, 1H), 7.01–6.96 (m, 1H), 4.26–4.25 (m, 2H), 3.72 (s, 1H), 3.62–3.59 (m, 3H), 3.51 (s, 2H), 3.41 (s, 1H), 3.27 (t, $J = 6.8$ Hz, 1H), 2.13–2.08 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 165.3, 165.0, 160.1, 156.4 (d, $J_{\text{C}-\text{F}} = 247.6$ Hz), 156.3 (d, $J_{\text{C}-\text{F}} = 246.7$ Hz), 145.6, 145.2, 134.3 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.4$ Hz), 134.7 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.7$ Hz), 131.6, 131.5 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 7.4$ Hz), 131.4 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 7.8$ Hz), 129.8, 129.7 (2 $\times$ CH), 129.6, 129.0, (2 $\times$ CH), 128.4, 127.2, 125.1 (d, $J_{\text{C}-\text{F}} = 17.3$ Hz), 125.2 (d, $J_{\text{C}-\text{F}} = 18.1$ Hz), 124.9, 116.2 (d, $J_{\text{C}-\text{C}-\text{F}} = 21.9$ Hz), 116.2 (d, $J_{\text{C}-\text{C}-\text{F}} = 21.9$ Hz), 57.4, 56.5, 55.8, 55.5, 46.5, 45.1, 44.5, 43.9, 37.8, 37.5, 36.4, 34.6; LC-MS (ESI) m/z : 393.23 [M+H].

4-(3-(6-(Cyclopropanecarbonyl)-2,6-diazaspiro[3.4]octane-2-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one (14d).

Following the general procedure, **14d** was purified by flash chromatography to afford the desired product as a white foam (0.119 g, 88%). (mixture of two rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 11.99 (m, 1H), 8.40–8.38 (m, 1H), 7.70–7.65 (m, 3H), 7.32–7.30 (m, 1H), 7.27–7.25 (m, 1H), 6.99–6.94 (m, 1H), 4.24–4.22 (m, 2H), 4.12–4.07 (m, 1H), 4.01–3.96 (m, 1H), 3.90–3.71 (m, 3H), 3.67–3.62 (m, 1H), 3.40 (s, 1H), 3.36 (s, 1H), 3.31 (t, $J = 6.71$ Hz, 1H), 2.15 (m, 1H), 2.09 (t, $J = 6.8$ Hz, 1H), 1.37–1.27 (m, 1H), 0.92–0.90 (m, 1H), 0.89–0.87 (m, 1H), 0.70–0.67 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 174.2 (2 $\times$ CH), 165.2, 165.0, 161.1 (2 $\times$ CH), 156.2 (d, $J_{\text{C}-\text{F}} = 249.1$ Hz), 156.1 (d, $J_{\text{C}-\text{F}} = 249.1$ Hz), 145.6, 145.5, 134.5 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.5$ Hz), 134.4 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.5$ Hz), 133.63, 133.60, 131.7 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 8.9$ Hz), 131.5 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 8.9$ Hz), 129.57, 129.0 (2 $\times$ C), 128.3 (2 $\times$ CH), 127.1, 125.1, 125.0 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 2.5$ Hz), 124.9 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 17.4$ Hz), 124.8 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 17.4$ Hz), 116.3 (d, $J_{\text{C}-\text{C}-\text{F}} = 21.8$ Hz), 116.2 (d, $J_{\text{C}-\text{C}-\text{F}} = 21.8$ Hz), 59.4, 58.8, 57.1, 56.9, 56.4, 55.2, 46.4, 44.6, 39.9, 38.9, 37.7, 36.4, 34.8, 10.1 (2 $\times$ CH), 7.5; LC-MS (ESI) m/z : 461.06 [M+H].

4-(4-Fluoro-3-(2-azaspiro[4.4]nonane-2-carbonyl)benzyl)phthalazin-1(2H)-one (15a).

Following the general procedure, **15a** was purified by flash chromatography to afford the desired product as a white foam (0.292 g, 72%). (mixture of rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 11.85 (s, 1H), 8.46–8.44 (m, 1H), 7.72–7.68 (m, 3H), 7.36–7.32 (m, 1H), 7.27–7.23 (m, 1H), 6.97 (dt, $J = 1.94, 7.0$ Hz, 1H), 4.27 (s, 2H), 3.63 (t, $J = 7.3$ Hz, 1H), 3.29 (t, $J = 6.9$ Hz, 1H), 3.02 (s, 1H), 1.79 (t, $J = 7.1$ Hz, 1H), 1.73 (t, $J = 7.1$ Hz, 1H), 1.65–1.63 (m, 2H), 1.61–1.59 (m, 2H), 1.51–1.41 (m, 5H); ^{13}C NMR (125 MHz, CDCl_3) δ 165.2, 165.0, 161.1, 156.2 (d, $J_{\text{C}-\text{F}} = 247.3$ Hz), 156.1 (d, $J_{\text{C}-\text{F}} = 247.3$ Hz), 145.7, 134.2 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.2$ Hz), 133.6, 133.5, 131.6 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 8.6$ Hz), 131.5, 131.1 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 7.9$ Hz), 129.6, 128.8 (t, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.7$ Hz), 128.3, 127.1, 125.7 (d,

$J_{\text{C}-\text{C}-\text{F}} = 18.2 \text{ Hz}$), 125.6 (d, $J_{\text{C}-\text{C}-\text{F}} = 18.4 \text{ Hz}$), 116.2 (d, $J_{\text{C}-\text{C}-\text{F}} = 22.0 \text{ Hz}$), 116.1 (d, $J_{\text{C}-\text{C}-\text{F}} = 22.0 \text{ Hz}$), 59.0 (2×CH), 57.2, 49.7, 48.5, 47.3 (2×CH), 45.5, 37.9, 37.8, 37.5, 36.7, 36.5, 36.1, 24.8, 24.6; LC-MS (ESI) m/z 406.18 [M+H].

Tert-butyl 7-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)-2,7-diazaspiro[4.4]nonane-2-carboxylate (15b).

Following the general procedure, **15b** was purified by flash chromatography to afford the desired product as a white foam (0.411 g, 79%). (mixture of two rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 12.19 (s, 1H), 8.32–8.31 (m, 1H), 7.59–7.57 (m, 3H), 7.29–7.27 (m, 1H), 7.19 (t, $J = 8.42 \text{ Hz}$, 1H), 4.17 (s, 2H), 3.61–3.57 (s, 1H), 3.49–3.42 (m, 1H), 3.34–3.26 (m, 3H), 3.19–3.04 (m, 3H), 1.79–1.72 (m, 3H), 1.67–1.65 (m, 1H), 1.33–1.31 (m, 9H); ^{13}C NMR (125 MHz, CDCl_3) δ 165.1, 164.9, 161.0 (2×C), 156.1 (d, $J_{\text{C}-\text{F}} = 247.2 \text{ Hz}$), 156.0 (d, $J_{\text{C}-\text{F}} = 248.2 \text{ Hz}$), 154.2 (2×C), 145.5, 145.4, 134.3 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.3 \text{ Hz}$), 134.2 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.3 \text{ Hz}$), 133.5 (2×C), 131.6 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 8.3 \text{ Hz}$), 129.3, 128.9 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.7 \text{ Hz}$), 128.0, 126.7, 125.3, 124.9 (d, $J_{\text{C}-\text{C}-\text{F}} = 17.7 \text{ Hz}$), 116.1 (d, $J_{\text{C}-\text{C}-\text{F}} = 22.2 \text{ Hz}$), 116.0 (d, $J_{\text{C}-\text{C}-\text{F}} = 22.3 \text{ Hz}$), 79.2, 79.1, 56.2, 54.7, 54.5, 54.2, 54.1, 50.0, 48.7, 47.8, 47.4, 44.8, 44.5, 44.4, 37.4, 34.8, 34.7, 34.3, 34.2, 33.5, 33.4, 28.4, 28.2; LC-MS (ESI) m/z 407.12 [M-BOC+H].

4-(4-Fluoro-3-(2,7-diazaspiro[4.4]nonane-2-carbonyl)benzyl)phthalazin-1(2H)-one (15c).

Following the general procedure, **15c** was afforded in near quantitative yield as a white foam. (mixture of two rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 8.45 (d, $J = 7.8 \text{ Hz}$, 1H), 7.74–7.68 (m, 3H), 7.35–7.34 (m, 1H), 7.78–7.22 (m, 1H), 7.01–6.96 (m, 1H), 4.26–4.25 (m, 2H), 3.71–3.62 (m, 1H), 3.58–3.51 (m, 1H), 3.36–3.30 (m, 1H), 3.18 (q, $J = 10.4, 12.0 \text{ Hz}$, 1H), 3.06–2.96 (m, 2H), 2.94–2.84 (m, 1H), 2.80–2.78 (m, 1H), 1.93 (t, $J = 7.3 \text{ Hz}$, 1H), 1.87–1.82 (m, 1H), 1.76–1.64 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 165.2, 165.1, 161.9, 156.3 (d, $J_{\text{C}-\text{F}} = 246.4 \text{ Hz}$), 145.6, 145.4, 134.2 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.5 \text{ Hz}$), 133.9 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.5 \text{ Hz}$), 133.5 (2×C), 131.5 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.6 \text{ Hz}$), 131.3 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 8.3 \text{ Hz}$), 129.7 (2×C), 129.4 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.8 \text{ Hz}$), 128.9 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.8 \text{ Hz}$), 128.4, 127.1, 125.6 (d, $J_{\text{C}-\text{C}-\text{F}} = 18.2 \text{ Hz}$), 125.0 (d, $J_{\text{C}-\text{C}-\text{F}} = 23.2 \text{ Hz}$), 116.4 (d, $J_{\text{C}-\text{C}-\text{F}} = 21.9 \text{ Hz}$), 116.3 (d, $J_{\text{C}-\text{C}-\text{F}} = 21.8 \text{ Hz}$), 58.3, 56.8, 56.3, 56.1, 49.7, 48.6, 47.2, 46.4, 46.3, 44.4, 37.9, 37.6, 37.0, 36.4, 36.1, 35.2; LC-MS (ESI) m/z 407.25 [M+H].

4-(3-(7-(Cyclopropanecarbonyl)-2,7-diazaspiro[4.4]nonane-2-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one (15d).

Following the general procedure, **15d** was purified by flash chromatography to afford the desired product as a white foam (0.056 g, 41%). (mixture of rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 11.89–11.83 (m, 1H), 8.42–8.41 (m, 1H), 7.70–7.67 (m, 3H), 7.35–7.34 (m, 1H), 7.30–7.26 (m, 1H), 6.99–6.95 (m, 1H), 4.25 (s, 2H), 3.73–3.67 (m, 2H), 3.59–3.48 (m, 3H), 3.42–3.35 (m, 2H), 3.18 (s, 1H), 2.20–1.77 (m, 4H), 1.57–1.48 (m, 1H), 0.96–0.89 (m, 2H), 0.74–0.69 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 172.6, 172.5, 172.4, 165.2, 165.3, 165.2, 165.1, 161.0 (3×C), 156.3 (d, $J_{\text{C}-\text{F}} = 247.8 \text{ Hz}$), 156.2 (d, $J_{\text{C}-\text{F}} = 247.3 \text{ Hz}$), 145.6, 145.5 (2×C), 134.3 (d, $J_{\text{C}-\text{C}-\text{C}-\text{F}} = 3.6 \text{ Hz}$), 134.3 (d,

$J_{\text{C-C-C-F}} = 3.7$ Hz), 133.6, 131.6 (d, $J_{\text{C-C-C-F}} = 8.6$ Hz), 131.5 (d, $J_{\text{C-C-C-F}} = 8.6$ Hz), 129.5, 128.2 (d, $J_{\text{C-C-C-F}} = 3.8$ Hz), 128.8 (d, $J_{\text{C-C-C-F}} = 3.8$ Hz), 128.3, 127.0, 125.2 (d, $J_{\text{C-C-F}} = 17.2$ Hz), 125.1 (d, $J_{\text{C-C-F}} = 17.2$ Hz), 116.4 (d, $J_{\text{C-C-F}} = 22.1$ Hz), 116.2 (d, $J_{\text{C-C-F}} = 22.1$ Hz), 55.4, 55.3, 55.6, 55.0, 54.6, 54.1, 49.4, 48.0, 47.5, 46.7, 46.2, 45.6, 45.4, 45.1, 45.0, 44.9, 37.7, 37.6, 35.2, 34.8, 34.7, 33.8, 33.7, 33.5, 32.9, 12.5, 12.2, 12.1, 7.7, 7.6, 7.5; LC-MS (ESI) m/z : 475.08 [M+H].

4-(4-Fluoro-3-(2-azaspiro[4.5]decane-2-carbonyl)benzyl)phthalazin-1(2H)-one (16a).

Following the general procedure, **16a** was purified by flash chromatography to afford the desired product as a white foam (0.208 g, 50%). (mixture of two rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 11.33 (s, 1H), 8.48–8.45 (m, 1H), 7.76–7.69 (m, 3H), 7.36–7.29 (m, 1H), 7.28–7.23 (m, 1H), 7.02–6.97 (m, 1H), 4.28–4.27 (m, 2H), 3.64 (t, $J = 7.3$ Hz, 1H), 3.43 (s, 1H), 3.30 (t, $J = 7.1$ Hz, 1H), 3.01 (s, 1H), 1.75 (t, $J = 7.4$ Hz, 1H), 1.68 (t, $J = 7.1$ Hz, 1H), 1.51–1.32 (m, 9H), 1.26–1.23 (m, 1H); ^{13}C NMR (125 MHz, CDCl_3) δ 165.2, 165.1, 160.9, 156.3 (d, $J_{\text{C-F}} = 247.7$ Hz), 156.2 (d, $J_{\text{C-F}} = 247.7$ Hz), 145.8, 134.2 (d, $J_{\text{C-C-C-F}} = 3.4$ Hz), 134.1 (d, $J_{\text{C-C-C-F}} = 3.4$ Hz), 133.7 (2 $\times$ CH), 131.6, 131.2 (d, $J_{\text{C-C-C-F}} = 8.0$ Hz), 129.7, 128.9 (d, $J_{\text{C-C-C-F}} = 4.3$ Hz), 128.8 (d, $J_{\text{C-C-C-F}} = 3.9$ Hz), 129.7, 128.9 (d, $J_{\text{C-C-C-F}} = 8.2$ Hz), 129.6, 128.8 (d, $J_{\text{C-C-C-F}} = 3.2$ Hz), 128.7 (d, $J_{\text{C-C-C-F}} = 3.2$ Hz), 128.4 (2 $\times$ CH), 127.2, 125.9 (d, $J_{\text{C-C-F}} = 18.5$ Hz), 125.8 (d, $J_{\text{C-C-F}} = 18.5$ Hz), 125.3, 116.5 (d, $J_{\text{C-C-F}} = 22.0$ Hz), 116.4 (d, $J_{\text{C-C-F}} = 22.1$ Hz), 58.2, 56.3, 46.2 (2 $\times$ CH), 44.4, 42.6, 41.2, 38.0, 37.9, 37.1, 35.6, 35.4, 35.2, 34.8, 26.2, 26.1, 23.4, 23.3; LC-MS (ESI) m/z : 420.20 [M+H].

Tert-butyl 2-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)-2,8-diazaspiro[4.5]decane-8-carboxylate (16b).

Following the general procedure, **16b** was purified by flash chromatography to afford the desired product as a white foam (0.427 g, 79%). (mixture of two rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 12.09 (s, 1H), 8.39–8.38 (m, 1H), 7.64–7.63 (m, 3H), 7.31–7.21 (m, 2H), 6.95–6.90 (m, 1H), 4.22–4.21 (m, 2H), 3.61 (t, $J = 7.2$ Hz, 1H), 3.49–3.44 (m, 2H), 3.32–3.26 (m, 2H), 3.24–3.19 (m, 1H), 3.17–3.14 (m, 1H), 3.02 (s, 1H), 1.75 (t, $J = 7.5$ Hz, 1H), 1.68 (t, $J = 6.8$ Hz, 1H), 1.52–1.40 (m, 4H), 1.38 (s, 4H), 1.36 (s, 5H); ^{13}C NMR (125 MHz, CDCl_3) δ 165.2, 165.0, 161.1, 161.0, 156.0 (d, $J_{\text{C-F}} = 247.7$ Hz), 155.9 (d, $J_{\text{C-F}} = 247.7$ Hz), 154.6, 154.5, 145.5, 145.4, 134.3 (d, $J_{\text{C-C-C-F}} = 3.6$ Hz), 134.2 (d, $J_{\text{C-C-C-F}} = 3.6$ Hz), 133.4, 133.3, 131.3, 131.2 (d, $J_{\text{C-C-C-F}} = 8.5$ Hz), 131.1 (d, $J_{\text{C-C-C-F}} = 8.5$ Hz), 129.4, 128.7 (d, $J_{\text{C-C-C-F}} = 3.9$ Hz), 128.6 (d, $J_{\text{C-C-C-F}} = 3.9$ Hz), 128.2, 128.1, 126.9, 125.4 (d, $J_{\text{C-C-F}} = 18.5$ Hz), 125.3 (d, $J_{\text{C-C-F}} = 18.5$ Hz), 125.0, 116.2 (d, $J_{\text{C-C-F}} = 21.9$ Hz), 116.0 (d, $J_{\text{C-C-F}} = 21.9$ Hz), 79.5, 79.4, 57.1, 55.0, 45.7, 45.6, 44.0, 40.9, 39.6, 37.7, 37.6, 36.1, 34.4, 34.1, 33.7, 28.3 (2 $\times$ C); LC-MS (ESI) m/z : 421.14 [M-BOC+H].

4-(4-Fluoro-3-(2,8-diazaspiro[4.5]decane-2-carbonyl)benzyl)phthalazin-1(2H)-one (16c).

Following the general procedure, **16c** was afforded in near quantitative yield as a white foam. (mixture of two rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 8.42–8.40 (m, 1H), 7.68–7.66 (m, 3H), 7.31–7.23 (m, 2H), 6.98–6.93 (m, 1H), 4.24–4.23 (m, 2H), 3.62 (t, $J =$

7.0), 3.46 (s, 1H), 3.28 (t, J = 7.0 Hz, 1H), 3.04 (s, 1H), 2.88–2.85 (m, 1H), 2.80–2.76 (m, 2H), 2.63–2.59 (m, 1H), 1.77 (t, J = 7.4 Hz, 1H), 1.70 (t, J = 7.0 Hz, 1H), 1.56–1.50 (m, 2H), 1.48–1.43 (m, 1H), 1.41–1.36 (m, 1H); ^{13}C NMR (125 MHz, CDCl_3) δ 165.2, 165.1, 161.0, 156.2 (d, $J_{\text{C-F}}$ = 247.2 Hz), 155.1 (d, $J_{\text{C-F}}$ = 247.2 Hz), 145.5, 134.2, 133.5 (2 $\times$ C), 131.4, 131.2 (d, $J_{\text{C-C-C-F}}$ = 3.5 Hz), 131.1 (d, $J_{\text{C-C-C-F}}$ = 3.4 Hz), 129.6, 128.8 (d, $J_{\text{C-C-C-F}}$ = 8.8 Hz), 128.7 (d, $J_{\text{C-C-C-F}}$ = 8.8 Hz), 128.3, 127.0, 125.7, 125.6 (d, $J_{\text{C-C-F}}$ = 18.3 Hz), 125.1, 116.2 (d, $J_{\text{C-C-F}}$ = 21.9 Hz), 116.1 (d, $J_{\text{C-C-F}}$ = 21.9 Hz), 57.8, 55.7, 45.7, 44.1, 43.7, 43.4, 41.2, 39.9, 37.8, 37.0, 35.7, 35.0; LC-MS (ESI) m/z : 421.13 [M+H].

4-(3-(8-(Cyclopropanecarbonyl)-2,8-diazaspiro[4.5]decane-2-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one (16d).

Following the general procedure, **16d** was purified by flash chromatography to afford the desired product as a white foam (0.158 g, 93%). (mixture of two rotamers reported) ^1H NMR (500 MHz, CDCl_3) δ 11.97–11.96 (m, 1H), 8.41–8.39 (m, 1H), 7.69–7.65 (m, 3H), 7.32–7.31 (m, 1H), 7.28–7.25 (m, 1H), 6.98–6.95 (m, 1H), 4.24 (s, 2H), 3.76 (bs, 1H), 3.65 (t, J = 7.21 Hz, 1H), 3.50 (m, 2H), 3.40 (bs, 2H), 3.33 (t, J = 6.8 Hz, 1H), 3.07 (s, 1H), 1.81 (m, 1H), 1.74 (t, J = 7.03 Hz, 1H), 1.70–1.63 (m, 1H), 1.54–1.42 (4H), 0.92–0.89 (2H), 0.67–0.66 (2H); ^{13}C NMR (125 MHz, CDCl_3) δ 171.9, 165.4, 165.1, 161.1, 161.0, 156.2 (d, $J_{\text{C-F}}$ = 247.7 Hz), 156.1 (d, $J_{\text{C-F}}$ = 247.7 Hz), 145.6 (2 $\times$ CH), 134.4 (d, $J_{\text{C-C-C-F}}$ = 3.1 Hz), 134.3 (d, $J_{\text{C-C-C-F}}$ = 3.1 Hz), 133.6 (2 $\times$ CH), 131.5 (d, $J_{\text{C-C-C-F}}$ = 8.3 Hz), 131.4 (d, $J_{\text{C-C-C-F}}$ = 8.2 Hz), 129.6, 128.8 (d, $J_{\text{C-C-C-F}}$ = 3.2 Hz), 128.7 (d, $J_{\text{C-C-C-F}}$ = 3.2 Hz), 128.3 (2 $\times$ CH), 127.1, 125.5 (d, $J_{\text{C-C-F}}$ = 17.5 Hz), 125.4 (d, $J_{\text{C-C-F}}$ = 17.3 Hz), 125.1, 116.3 (d, $J_{\text{C-C-F}}$ = 22.1 Hz), 116.2 (d, $J_{\text{C-C-F}}$ = 22.1 Hz), 57.4, 55.1, 45.8 (2 $\times$ C), 44.2, 43.1, 41.3, 40.1, 39.8, 37.8, 36.5, 35.4, 35.2, 34.3, 34.5, 11.1, 11.0, 7.4; LC-MS (ESI) m/z : 489.10 [M+H].

4-(4-Fluoro-3-(3-azaspiro[5.5]undecane-3-carbonyl)benzyl)phthalazin-1(2H)-one (17a).

Following the general procedure, **17a** was purified by flash chromatography to afford the desired product as a white foam (0.238 g, 55%). ^1H NMR (500 MHz, CDCl_3) δ 12.29 (s, 1H), 8.38–8.36 (m, 1H), 7.63–7.61 (m, 3H), 7.25–7.24 (m, 1H), 7.21–7.18 (m, 1H), 6.90–6.87 (t, J = 8.8 Hz, 1H), 4.20 (s, 2H), 3.62–3.60 (m, 2H), 3.10 (bs, 2H), 1.41–1.39 (m, 2H), 1.29–1.27 (m, 12 H); ^{13}C NMR (125 MHz, CDCl_3) δ 164.7, 161.2, 156.0 (d, $J_{\text{C-F}}$ = 247.2 Hz), 145.7, 134.2 (d, $J_{\text{C-C-C-F}}$ = 3.6 Hz), 133.5, 131.3, 130.9 (d, $J_{\text{C-C-C-F}}$ = 7.8 Hz), 129.5, 128.8 (d, $J_{\text{C-C-C-F}}$ = 3.7 Hz), 128.2, 125.1, 124.8 (d, $J_{\text{C-C-F}}$ = 18.6 Hz), 116.0 (d, $J_{\text{C-C-F}}$ = 21.8 Hz), 43.1, 37.8, 37.7, 36.4, 36.0, 35.7, 31.4, 26.6, 21.3; LC-MS (ESI) m/z : 434.09 [M+H].

Tert-butyl 9-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)-3,9-diazaspiro[5.5]undecane-3-carboxylate (17b).

Following the general procedure, **17b** was purified by flash chromatography to afford the desired product as a white foam (0.215 g, 40%). ^1H NMR (500 MHz, CDCl_3) δ 12.01 (s, 1H), 8.43–8.41 (m, 1H), 7.59–7.57 (m, 3H), 7.28–7.26 (m, 1H), 7.19 (bs, 1H), 6.87 (t, J = 8.3 Hz, 1H), 4.16 (s, 2H), 7.70–7.67 (m, 3H), 7.27–7.26 (m, 1H), 7.25–7.23 (m, 1H), 6.94 (t,

$J = 8.6$ Hz, 1H), 4.24 (s, 2H), 3.68 (s, 2H), 3.34–3.31 (m, 4H), 3.19 (s, 2H), 1.51 (t, $J = 5.2$ Hz, 2H), 1.44 (m, 4H), 1.39 (s, 9H), 1.35 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 164.9, 161.1, 158.1 (d, $J_{\text{C-F}} = 247.6$ Hz), 154.9, 145.7, 134.4 (d, $J_{\text{C-C-C-F}} = 3.4$ Hz), 133.6, 131.5, 131.1 (d, $J_{\text{C-C-C-F}} = 8.0$ Hz), 129.6, 128.9 (d, $J_{\text{C-C-C-F}} = 4.3$ Hz), 128.3, 127.1, 124.7 (d, $J_{\text{C-C-F}} = 18.3$ Hz), 116.2 (d, $J_{\text{C-C-F}} = 21.9$ Hz), 79.5, 42.9, 39.3, 37.8, 37.3, 35.8, 34.8, 30.4, 28.5; LC-MS (ESI) m/z 557.09 [M+Na], 435.15 [M-BOC+H].

4-(4-Fluoro-3-(3,9-diazaspiro[5.5]undecane-3-carbonyl)benzyl)phthalazin-1(2H)-one (17c).

Following the general procedure, **17c** was afforded in near quantitative yield as a white foam. ^1H NMR (500 MHz, CDCl_3) δ 8.44–8.42 (m, 1H), 7.71–7.66 (m, 3H), 7.27–7.22 (m, 2H), 6.96 (t, $J = 8.8$ Hz, 1H), 4.25 (s, 2H), 3.69 (t, $J = 5.4$ Hz, 2H), 3.18 (bs, 2H), 2.81–2.77 (m, 4H), 1.55–1.53 (m, 2H), 1.47–1.44 (m, 4H), 1.37 (bs, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 164.9, 161.0, 158.1 (d, $J_{\text{C-F}} = 246.9$ Hz), 145.6, 134.3 (d, $J_{\text{C-C-C-F}} = 3.1$ Hz), 133.6, 131.5, 131.0 (d, $J_{\text{C-C-C-F}} = 7.8$ Hz), 129.6, 128.9 (d, $J_{\text{C-C-C-F}} = 3.7$ Hz), 128.4, 127.1, 125.1, 124.8 (d, $J_{\text{C-C-F}} = 18.4$ Hz), 116.0 (d, $J_{\text{C-C-F}} = 22.0$ Hz), 42.9, 41.8, 37.8, 37.6, 36.5, 35.6, 30.6; LC-MS (ESI) m/z 435.15 [M+H].

4-(3-(9-(Cyclopropanecarbonyl)-3,9-diazaspiro[5.5]undecane-3-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one (17d).

Following the general procedure, **17d** was purified by flash chromatography to afford the desired product as a white foam (0.055 g, 60%). ^1H NMR (500 MHz, CDCl_3) δ 11.84 (s, 1H), 8.44–8.43 (m, 1H), 7.72–7.68 (m, 3H), 7.28–7.25 (m, 2H), 6.97 (t, $J = 8.5$ Hz, 1H), 4.26 (s, 2H), 3.76 (s, 1H), 3.65 (s, 1H), 3.58–3.55 (m, 4H), 3.21 (s, 2H), 1.72–1.66 (m, 1H), 1.57–1.40 (m, 8H), 0.93 (s, 2H), 0.71–0.69 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 172.0, 164.9, 161.1, 156.2 (d, $J_{\text{C-F}} = 248.1$ Hz), 145.7, 134.4 (d, $J_{\text{C-C-C-F}} = 3.7$ Hz), 133.7, 131.6, 131.2 (d, $J_{\text{C-F}} = 8.1$ Hz), 129.7, 128.9 (d, $J_{\text{C-C-C-F}} = 3.6$ Hz), 128.3, 127.1, 125.2, 124.5 (d, $J_{\text{C-F}} = 18.3$ Hz), 116.1 (d, $J_{\text{C-F}} = 22.0$ Hz), 43.0, 41.4, 37.8, 37.7, 36.3, 36.0, 34.9, 34.5, 30.8, 11.1, 7.4; LC-MS (ESI) m/z 503.13 [M+H], 525.30 [M+Na].

Cell Culture.

Cells were cultured using standard techniques at 37 °C with 5% CO_2 and 15% O_2 . In this work OVCAR8 ovarian cancer cell lines were used for radioligand binding to characterize PARP-1 affinity and to assess in vitro cytotoxicity of compounds synthesized in this work. Genetically engineered PARP-1 and PARP-2 double knockout mouse embryonic fibroblast (MEF) were used for cell viability studies with wildtype control. To evaluate the impact of BRCA1 mutations we used isogenic cell lines UWB1.289 that has a deleterious BRCA1 mutation and UWB1.289-BRCA1 restored which had a functional BRCA1 gene inserted. All cells were cultured in RPMI 1640 with 10% FBS and 1% penicillin/streptomycin, except for UWB1.289 which was cultured in a 1:1 solution of MEGM with bullet kit: RPMI 1640 with 10% FBS and 1% penicillin/stretomycin.

PARP-1 Radioligand Binding in Live OVCAR8 Cells.

The affinity of each compound for the PARP-1 enzyme was evaluated using a radioligand binding method previously reported by our group.³³ Briefly, OVCAR8 ovarian cancer cells

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were seeded in 96-well Stripwell plates at a density of 40,000 cells/well 24 hrs prior to the study. On the day of study, compounds were diluted in RPMI to concentrations of 100 μ M - 0.064 nM. Next, [125 I]KX1, a known PARP-1 specific radioligand, was added to the plate followed by each compound dilution. Reactions were allowed to equilibrate for 1 hr and were then washed with 200 μ L of PBS. Wells were separated and counted on an automatic Wizard gamma counter (Perkin Elmer, Waltham MA). Dose response curves were produced to calculate 50% maximum inhibition values (IC₅₀) using non-linear fit sigmoidal dose response curves in GraphPad 7.0 (Prism, La Jolla CA). Experiments were repeated three times with adjusting dose concentrations to increase accuracy of IC₅₀ values.

Computational Docking Experiments.

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In silico molecular docking was performed by using AUTODOCK 4.2⁴² plugin on Pymol (pymol.org). X-ray structure of catalytic domain of PARP1 (PDB ID: 4ZZZ, Resolution 1.90Å) was retrieved from RCSB Protein Data Bank (www.rcsb.org). Preparation of the protein prior to docking studies involved removal of waters and other hetero atoms, followed by adding polar hydrogens. Chain A was selected for further structure minimization and protein-ligand docking target. The structure was then minimized with Swiss-PDBViewer⁴³ using CHARMM energy minimization.⁴⁴ Compound **10e**, **11d-17d**, and olaparib were drawn on ChemDraw Profession 15.1 (PerkinElmer Informatics, Inc.), then imported to Chem3D Ultra 15.1 (PerkinElmer Informatics, Inc.) to minimize individual structures by MMFF94 force field. Blind docking was performed on olaparib in order to determine the binding pocket within PARP-1. A grid box of 54 $\times$ 54 $\times$ 54 Å was applied to the chain A of PARP-1 structure, which was large enough to encompass the entire chain. The Lamarckian Genetic Algorithm⁴⁵ was used for ligand conformation search and was run 1,000 times, generating 1,000 possible protein-ligand docking solutions. A new grid box of 22.5 $\times$ 22.5 $\times$ 22.5 Å was then determined around the main binding pocket, predicted by olaparib-PARP1 complex, for further individual compound to PARP-1 docking. Compounds ran 500 times to generated 500 possible protein-ligand docking solutions. The protein-ligand complex with the lowest free binding energy as well as the highest cluster number was reported.

Cell Viability Assays.

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Cell viability assays were carried out using six cell lines including OVCAR8, UWB1.289, UWB1.289-BRCA1, MEF *WT*, MEF *PARP-1 KO*^{-/-}, and MEF *PARP-2 KO*^{-/-} using previously reported methods.³³ Briefly, cells were seeded in black wall clear bottom 96-well plates at concentrations of 1,000 cells/well. After 24 h, cells were then treated with concentrations from 100–0.016 μ M. Cells were incubated with compounds for 4–7 days and then assayed for cell viability using the luminescent based assay, CellTiter Glo (Promega, Waltham MA). Plates were read on an Enspire multimode plate reader (Perkin Elmer). Data was normalized to percent survival at each concentration evaluated by dividing the luminescent signal in treated wells vs. the average of vehicle controls. Experiments were repeated three times and all compounds were assayed during each experiment.

Western Analysis.

PARPi treated cells were lysed in RIPA buffer at 4°C for 1 hour and then sonicated. Samples were centrifuged at 15 g for 20 minutes at 4° C. The supernatant was collected and protein was quantified using BioRad DC protein quantification assay. All samples were then diluted to a final concentration of 2 µg/µL and 1X laemmli buffer. Gel electrophoresis was performed using BioRad TGX pre-packed gels at 100 V for 1 hr. Gels were transferred to a PDVF membrane using BioRad turbo transfer at 1.3 A for 7 minutes. Membranes were blocked in Odyssey blocking buffer (LiCOR) for 1 hr. Membranes were incubated overnight at 4°C with either Anti-PAR (Trevigen) or anti-γ-H2AX (EMD Millipore) and anti-Histone H3 (Histone H3 Antibody #9715, Cell Signaling Technologies) at 1:1000 dilution.

Next, membranes were washed 4 times in PBS w/ 0.2% tween 20 and incubated with fluorescent secondary antibodies (IRDye, LiCOR) for 1 hr in Odyssey Blocking buffer, 0.2% tween 20 and 0.1% SDS. Uniform regions of interest were applied to each lane to calculate the total fluorescent intensity, which was representative of total target protein. Notice, PAR bands are visualized at multiple molecular weights and is characteristic of PAR due to its natural occurrence as a polymer that can range from 25–200 repeating sub-units. For the purpose of this study, molecular weights between 250kDa – 113kDa that resulted in fluorescent signal were attributed to PAR. Histone H3 was used as a loading control to calculate final relative protein expression for each lysate. Experimental data represent the average of 3 independent experiments.

Cell Microscopy.

We performed immunofluorescent cell microscopy studies to analyze DNA damage measured by γH2AX foci formation. OVCAR8 cells were plated on cover slips in 24 well-plates 24 hrs prior to treatment with 0.01, 0.1, 1, or 10 µM olaparib, 10e, 15b, or DMSO controls. Treatment with PARPi lasted for 24 hrs and at that time media and drug was removed, cells were fixed with 500 µL/well of 4% paraformaldehyde in PBS for 10 minutes. Following fixation cells were washed three times with PBS then permeabilized using 500µL/well triton-x. Cover slips with cells were then removed from the 24-well plate and were placed on a flat parafiled surface with the cells exposed to air. Primary for antibody anti-phospho-histoneH2A.X (EMD Millipore) was diluted 1:5000 in a 0.2% tween 20 PBS (0.2% PBST) solution and 30 µL was added to cover slips. Primary antibody was incubated at 37 °C for 1 hr at which time they were returned to the 24-well plate and washed 3 times with 0.2% PBST. Next secondary antibody (anti-mouse alexafluor488, thermofisher) was diluted 1:100 in 0.2% PBST and added following the same steps and incubation time used for the primary antibody. Next, cover slips were returned to 24-well plate and washed with 0.2% PBST 3 times, removed from plate, and mounted on slides using DAPI mounting medium.

PARPs Enzyme Inhibition Assay.

Data was produced by BPS Bioscience. In general, all assays were done by following the BPS PARP or TNKS assay kit protocols. The enzymatic reactions were conducted in duplicate at room temperature for 1 hour in a 96 well plate coated with histone substrate. 50 µL of reaction buffer (Tris·HCl, pH 8.0) contains NAD⁺, biotinylated NAD⁺, activated DNA, a PARP enzyme and the test compound. After enzymatic reactions, 50µl of Streptavidin-

horseradish peroxidase was added to each well and the plate was incubated at room temperature for an additional 30 min. 100 μ L of developer reagents were added to wells and luminescence was measured using a BioTek SynergyTM 2 microplate reader.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

DSBs	double strand breaks
EDC HCl	ethylcarbodiimide hydrochloride
HOBt	1-hydroxybenzotriazole
HR	homologous recombination
MEF	mouse embryonic fibroblast
PAR	Poly (ADP-ribose)
SSBs	single strand breaks
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran

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Scheme 1.

Reagents and conditions: (A) spiro compound (**1-8**), HOBt hydrate, EDC HCl, TEA, THF, 60 °C, 12 h; (b) TFA, CH₂Cl₂, rt, 3 h; (c) Free-amine compound (**10d**, **11c-17c**), cyclopropane carboxylic acid, HOBt hydrate, EDC HCl, TEA, THF, 60 °C, 12 h.

olaparib, AZD2281

AZD2461

niraparib, MK4827

talazoparib, BMN673

rucaparib, AG014699

veliparib, ABT888

Figure 1.
Examples of PARP-1 inhibitors.

Figure 2.
Linear and angular spirocycles examined.

Figure 3.
Olaparib (green) and **17d** (red) ligand docking pose illustrating active site residue interactions within the PARP-1 complex (PDB ID: 4ZZZ)

Figure 4.
Cytotoxicity screens for compounds carried out in OVCAR8 cells at 10 μM concentrations.

Figure 5. Evaluation of PARP-1 catalytic inhibition and DNA damage induced by PARPi. **(A)** Western blot analysis of PAR and Histone H3 expression at increasing concentrations of olaparib, **10e**, and **15b**. **(B)** Immunofluorescent microscopy experiments analyzing γ H2AX foci, as a marker of DNA damage at various concentrations of olaparib, **10e**, and **15b**.

Figure 6.

Dose response curves showing the relative potency of compounds **15b-17b** vs. olaparib and AZD2461 in MEF *WT* (A), *PARP-1* KO^{-/-} (B), and *PARP-2* KO^{-/-} (C) cells.

Figure 7.

Heat map illustrating the enzymatic inhibition of each PARP protein at a 10 μ M concentration of selected compounds.

Table 1.

PARP-1 IC₅₀ Values for 10–17^a

Compound		PARP-1IC ₅₀ ^b (nM)	Compound		PARP-1IC ₅₀ ^b (nM)
olaparib		6.0 ± 1.2	AZD2461		1.7 ± 1.0
10a , R = CH ₂	33.9 ± 1.5		14a , R = CH ₂	32.4 ± 1.1
	10b , R = O	24.9 ± 1.2		14b , R = NBoc	1,193 ± 1.0
	10c , R = NBoc	551.6 ± 1.0		14c , R = NH	1,237 ± 2.6
	10d , R = NH	2,395 ± NA		15d , R =	2,535 ± 1.0
	10e , R =	12.6 ± 1.1
11a , R = CH ₂	36.5 ± 1.2		15a , R = CH ₂	51.4 ± 1.1
	11b , R = NBoc	877 ± 1.0		15b , R = NBoc	4,397 ± 1.1
	11d , R =	526.1 ± 2.0		15d , R =	1,118 ± 1.7
		129.7 ± 1.0			2,586 ± 1.0
12a , R = CH ₂	65.4 ± 1.5		16a , R = CH ₂	141.4 ± 1.3
	12b , R = NBoc	3,118 ± 1.0		16b , R = NBoc	1,105 ± 1.0
	12c , R = NH	1,419 ± 2.3		16c , R = NH	3,881 ± 9.9
	12d , R =	100.1 ± 1.2		16d , R =	224.9 ± 1.0
13a , R = CH ₂	284 ± 1.2		17a , R = CH ₂	57.1 ± 1.2
	13b , R = NBoc	2,969 ± 1.1		17b , R = NBoc	452.8 ± 1.0
	13c , R = NH	109.5 ± 1.0		17c , R = NH	233.7 ± 1.0
	13d , R =	430.9 ± 1.1		17d , R =	44.3 ± 1.2

^aIC₅₀ (nM) ± (SEM) values represent inhibition of PARP-1 enzymatic activity using our previously reported radioligand binding methodology (ref. 33).

^bDose response curves were produced to calculate 50% maximum inhibition values (IC₅₀) where n=3.

Table 2.In Silico Binding Energies of Olaparib, 10e, and 11d-17d^a

Compound	Average Binding Energy (kcal/mol)±SD	Experimental PARP-1 IC ₅₀ ^b (nM)±SD
17d	−13.84±0.0155	44.3±1.2
olaparib	−12.5±0.2175	6.0±1.2
10e	−12.63±0.0149	12.6±1.1
13d	−12.53±0.0092	430.9±1.1
12d	−12.43±0.0323	100.1±1.2
16d	−12.03±0.059	224.9±1.0
11d	−11.87±0.0914	129.7±1.0
14d	−11.96±0.0429	2,535±1.0
15d	−11.89±0.0116	2,586±1.0

^a Average binding energies calculated using PyMol modeling software compared to experimental PARP-1 IC₅₀ obtained.

Table 3.EC₅₀ Values for 10–17 in *PARP-1* KO^{-/-} and *PARP-2* KO^{-/-} Cell Lines^a

Compound	WT	<i>PARP-1</i> ^{-/-}	<i>PARP-2</i> ^{-/-}	Compound	WT	<i>PARP-1</i> ^{-/-}	<i>PARP-2</i> ^{-/-}
	EC ₅₀ (μM)				EC ₅₀ (μM)		
olaparib	14.6 ± 1.2	49.6 ± NA	8.5 ± 1.0	AZD2461	22.1 ± 1.4	49.0 ± NA	14.9 ± 1.0
10a	48.3 ± NA	—	49.0 ± 1.1	14a	45.2 ± NA	—	54.7 ± 1.1
10b	52.2 ± NA	—	53.3 ± 1.1	14b	29.2 ± 1.8	—	31.8 ± 1.1
10c	31.1 ± 1.4	—	24.7 ± 1.1	14c	—	—	—
10d	—	—	—	14d	—	—	61.8 ± NA
10e	—	—	59.8 ± NA				
11a	48.0 ± NA	—	58.3 ± 1.2	15a	31.7 ± 1.6	—	55.5 ± 1.0
11b	42.9 ± NA	—	35.8 ± 1.5	15b	17.2 ± 1.5	46.4 ± NA	23.4 ± 1.0
11c	—	—	—	15c	—	—	511.6 ± 1.0
11d	53.2 ± NA	—	46.7 ± NA	15d	—	—	70.9 ± NA
12a	25.6 ± 1.3	48.3 ± NA	39.9 ± 1.1	16a	42.7 ± NA	51.6 ± NA	46.8 ± 1.1
12b	23.4 ± 1.2	49.2 ± NA	41.5 ± 1.1	16b	9.4 ± NA	45.9 ± 1.9	31.6 ± 1.1
12c	—	—	68.5 ± NA	16c	—	—	—
12d	49.3 ± NA	—	57.2 ± 1.1	16d	41.6 ± 1.1	—	61.0 ± 1.0
13a	32.6 ± 1.3	—	78.8 ± 1.3	17a	41.3 ± NA	—	41.3 ± 1.0
13b	23.3 ± 1.4	—	33.2 ± 1.0	17b	16.9 ± 1.5	43.4 ± 1.4	19.2 ± 1.1
13c	—	—	105.9 ± 1.1	17c	—	—	59.7 ± NA
13d	47.5 ± NA	—	44.4 ± 1.2	17d	24.8 ± 1.1	49.7 ± NA	21.6 ± 1.0

^aEC₅₀ (μM) ± (SEM) data for compounds in mouse embryonic fibroblasts PARP-1 and PARP-2 KO^{-/-} cells. “—” = effective concentration for 50% reduction in viability was not reached.

Table 4.EC₅₀ Values for 15b-17b in UWB1.289 Cell Lines^a

Compound	UWB1.289EC ₅₀ (μM)	UWB1.289+BRC EC ₅₀ (μM)
olaparib	0.2 ± 1.2	4.8 ± 1.3
10e	1.5 ± 0.8	12.1 ± 2.6
15b	4.2 ± 1.1	7.4 ± 1.1
16b	8.4 ± 1.1	6.2 ± 1.2
17b	2.7 ± 1.1	4.0 ± 1.2

^aEC₅₀ (μM) ± (SEM) data for compounds in BRCA1-null and BRCA1-restored UWB1.289 cell lines.

Table 5.TNKS-1 and TNKS-2 IC₅₀ Values for 15b-17b^a

	TNKS-1 IC ₅₀ (μM)	TNKS-2 IC ₅₀ (μM)
olaparib	1.8 ± 1.0	1.2 ± 1.3
15b	1.0 ± 1.0	0.576 ± 1.1
16b	3.0 ± NA	0.953 ± 1.0
17b	5.7 ± 1.1	2.8 ± 1.2

^aIC₅₀ (μM) ± (SEM) data for TNKS-1 and TNKS-2.