Discovery of 2-{4-[(3S)-Piperidin-3-yl]phenyl}-2H-indazole-7-carboxamide (MK-4827): A Novel Oral Poly(ADP-ribose)polymerase (PARP) Inhibitor Efficacious in BRCA-1 and -2 Mutant Tumors

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We disclose the development of a novel series of 2-phenyl-2H-indazole-7-carboxamides as poly(ADP-ribose)polymerase (PARP) 1 and 2 inhibitors. This series was optimized to improve enzyme and cellular activity, and the resulting PARP inhibitors display antiproliferation activities against BRCA-1 and BRCA-2 deficient cancer cells, with high selectivity over BRCA proficient cells. Extrahepatic oxidation by CYP450 1A1 and 1A2 was identified as a metabolic concern, and strategies to improve pharmacokinetic properties are reported. These efforts culminated in the identification of 2-{4-[(3S)-piperidin-3-yl]phenyl}-2H-indazole-7-carboxamide 56 (MK-4827), which displays good pharmacokinetic properties and is currently in phase I clinical trials. This compound displays excellent PARP 1 and 2 inhibition with IC_{50} = 3.8 and 2.1 nM, respectively, and in a whole cell assay, it inhibited PARP activity with EC_{50} = 4 nM and inhibited proliferation of cancer cells with mutant BRCA-1 and BRCA-2 with CC_{50} in the 10–100 nM range. Compound 56 was well tolerated in vivo and demonstrated efficacy as a single agent in a xenograft model of BRCA-1 deficient cancer.

Introduction

Poly(ADP-ribose) polymerase (PARP)-1 is the founding member and most abundant member of poly(ADP-ribose)polymerizing proteins, a family of some 18 proteins. Several of the PARPs family of enzymes share a catalytic PARP homology domain and are characterized by the ability to catalyze the transfer of ADP-ribose units to proteins using nicotinamide adenine dinucleotide (NAD\(^+\)) as the substrate, resulting in the formation of long and branched poly(ADP-ribose) (PAR) chains. More recent characterization suggests that while PARPs 1–5 function as poly(ADP-ribosyl)ating proteins, some of the other family members may indeed be mono-ADP-riboseyltransferases and other members of the family may be catalytically inactive. PARP-1 and the closely related PARP-2 are nuclear proteins and possess DNA binding domains. These DNA binding domains serve to localize and rapidly bind these PARPs to the sites of DNA single- and double-strand breaks (SSB and DSB, respectively). With binding at sites of lesion, the catalytic activity of these enzymes is stimulated more than 500-fold, which results in the addition of PAR chains on several proteins associated with chromatin, including histones, p53, topoisomerases, and PARP itself, plus various DNA repair proteins. This results in chromatin relaxation and fast recruitment of DNA repair factors which access the DNA breaks and repair them by a process known as base excision repair (BER).

The knockout of PARP-1 significantly impairs repair of DNA damage following exposure to radiation or cytotoxic insult, with the residual PARP dependent repair activity being due to PARP-2, which contributes about 10% to nuclear PARP activity. Similarly, PARP 1 and 2 inhibition with small molecule inhibitors has been demonstrated to sensitize tumor cells to cytotoxic agents that induce DNA damage that would normally be repaired by BER, notably alkylating agents (like temozolomide and cyclophosphamide) and topoisomerase I poisons (irinotecan and topotecan). Sensitization of platinum, like cisplatin and carboplatin, has also been demonstrated, as has the use of PARP inhibitors as radiopotentiators, whereby the cellular damage caused by ionizing radiation is enhanced.

In addition, hyperactivation of PARP activity has been reported, caused by excessive DNA damage following oxidative insult upon ischemia reperfusion. The subsequent extensive PARylation of substrates results in depletion of cellular NAD\(^+\) pools and energy stores, leading to acute neuronal and myocardial cell death by necrosis. Indeed several classes of PARP inhibitors have already been developed and have demonstrated efficacy in animal models of these diseases. These PARP inhibitors have also shown applicability in models of inflammation, cardiovascular disease, and neurodegenerative disorders.

PARP-1 is also known to participate in a range of other cellular processes including regulation of apoptosis, cell...
division, transcriptional regulation, and differentiation, as well as chromosome stability.\textsuperscript{23} Despite PARP-1 being identified over 40 years ago, the biology of the other family members is still being elucidated.\textsuperscript{1,2} although PARP-4/vault PARP is known to be the catalytic component of the vault particles, which are ribonucleoprotein complexes that are involved in multidrug resistance of tumors. Tankyrase I and II are known to regulate telomere homeostasis and also to play key roles during mitotic segregation.

Recently, data have emerged suggesting that targeting more than one DNA repair pathway in tumor cells could induce “synthetic lethality.”\textsuperscript{17–19} Specifically, publications have appeared that described the selective killing of BRCA-1 or BRCA-2 deficient tumor cells by PARP inhibitors.\textsuperscript{17,18} In contrast, normal cells with an intact BRCA pathway when treated with a PARP inhibitor are viable and minimal cytotoxicity is seen. BRCA-1 and -2 are known tumor suppressors and are key components involved in the repair of DNA double strand breaks by the homologous recombination (HR) pathway, and mutations in these genes predispose individuals to hereditary breast and ovarian cancer and also prostate and pancreatic cancer.\textsuperscript{20} Every day cells are faced by an onslaught of DNA damage, through metabolic processes and/or exogenous damage, and in the presence of a PARP inhibitor this attack results in persistent DNA SSB. During replication, these SSBs cause stalled replication forks and subsequently develop into DSB. In BRCA-1 and -2 deficient cells, these lesions are not repaired by the alternative DSB repair pathway of homologous recombination, resulting in gross genomic instability, cell cycle arrest, and apoptosis. Cells that are deficient in BRCA-1 and -2 have been demonstrated to be acutely sensitive to killing by PARP inhibitors in vitro and in vivo.\textsuperscript{17,18} In a genetically engineered mouse model for BRCA-1 associated breast cancer treatment of tumor bearing mice with the PARP inhibitor 4-[3-(4-cyclopropanecarbonylpirazone-1-carbonyl)-4-fluorobenzyl]-1H-1,2,3-triazole (MK-4827) which is currently in human phase I clinical trials. This compound displays excellent potency against both the PARP-1 and PARP-2 enzymes with \( IC_{50} = 3.8 \) and 2.1 nM, respectively, and in a whole cell assay it inhibits PARP activity with \( EC_{50} = 4.0 \) nM and \( EC_{90} = 45 \) nM. This indazole-7-carboxamide inhibits proliferation of cancer cell lines with mutant BRCA-1 and BRCA-2 in vitro and is efficacious in xenograft models following oral dosing.

Results and Discussion

Chemistry. Synthetic routes for the preparations of diverse [6,5]-bicyclic heterocyclic systems 8, 22, 27, and 30 bearing pendant primary carboxamide and phenyl groups are described in Scheme 1. Indazole PARP inhibitors were prepared from methyl 3-formyl-2-nitrobenzoate (5), itself available from oxidation of methyl 3-methyl-2-nitrobenzoate. Condensation with substituted anilines in refluxing EtOH gave azomethines 6a–j that could then be treated with NaN\(_3\) in DMF at 90°C, and initial substitution of the ortho-nitro group and subsequent cyclization gave the corresponding indazoles 7a–j as described by Kuvshinov.\textsuperscript{28} Amide formation using ammonia in MeOH in a sealed tube furnished the desired compounds 8–17. The 1,2,3-benzotriazole 22 was prepared from methyl 2,3-diaminobenzoate (19) by condensation with nitrosobenzene in AcOH to give 20, followed by oxidation cyclization using stoichiometric Cu(OAc)\(_2\) under an O\(_2\) atmosphere. Functional group interconversion then yielded 22. The synthesis of the pyrazolo[1,5-\(a\)]pyridine 27 started from ethyl 2-picolinate (23) which upon treatment with O-mesitylenesulfonylhydroxylamine gave the N-aminopyridinium salt 24. The key bicyclic 25 was then prepared by a \([3 + 2]\) cycloadition of the N-aminopyridinium salt with an alkyne ester.\textsuperscript{29} Hydrogenation of the benzyl ester 25, followed by decarboxylation in 48% HBr\(_2\) gave the required 2-phenylpyrazolo[1,5-\(a\)]pyridine-7-carboxamide (27) after amide formation. A similar synthetic strategy was employed for the formation of 2-phenyl[1,2,4]triazolo[1,5-\(a\)]pyridine-8-carboxamide (30), in which case the N-aminopyridinium salt was condensed with benzaldehyde to elaborate the key heterocyclic intermediate 29 that was subsequently transformed to 30.\textsuperscript{30} Substituted 2-arylindazole-7-carboxamides were prepared using the previously described route from the appropriately
substituted aniline, or alternatively methyl-2H-indazole-7-carboxylate (31) and indazole-7-carboxamide (34) can undergo microwave assisted S$_{N}$Ar substitution of p-fluorobenzaldehyde or fluoroacetophenone in DCM in the presence of K$_2$CO$_3$ (Scheme 2). The reductive amination using ZnCl$_2$ and NaBH$_3$(CN) together with the appropriate amines gave derivatives 37–48. The corresponding gem-dimethyl analogue 50 was prepared by conversion of methyl ketone 36 to the corresponding tertiary alcohol 49. This alcohol underwent a Ritter rearrangement to yield the corresponding formamide, which was in turn reduced to give Ritter rearrangement to yield the corresponding formamide 31.

Derivatives bearing the 4-[(3$S$)-piperidin-3-yl]phenyl moiety were prepared by one of two routes illustrated in Scheme 3. Racemic N-Boc-3-(4-aminophenyl)piperidine was condensed with methyl 3-formyl-2-nitrobenzoate (5) and cyclized using Na$_2$N$_3$ in DMF as described previously to yield 7i. Separation of 16 by chiral SFC gave the enantiomers 56 and 57. Alternatively, 4-piperidin-3-ylaniline (60), readily available from Suzuki coupling of 1-iodo-4-nitrobenzene and pyridine-3-boronic acid followed by PtO$_2$ hydrogenation, was resolved using L-tartaric acid to subsequently yield 7i. This alcohol underwent a Ritter rearrangement to yield the corresponding formamide, which was in turn reduced to give Ritter rearrangement to yield the corresponding formamide 31.

Given the interest in PARP-1 inhibition in a wide variety of therapeutic areas, a significant number of industrial and academic groups have worked on developing inhibitors. Most of these inhibitors developed to date bind to the nicotinamide binding site, where they compete with the natural substrate nicotinamide adenine dinucleotide (NAD$^+$). X-ray crystal structures and molecular modeling studies have indicated that the amide of nicotinamide makes three key hydrogen bonds to the hydroxyl group of Ser904 and the amide backbone of Gly863. Moreover, the pyridyl ring is engaged in π–π stacking with Tyr907. The majority of the inhibitors developed to date are close structural mimics of nicotinamide, and attempts to improve the affinity of these derivatives have been made by trying to lock the carboxamide group, which is usually free to rotate, into the desired anti-conformation. On the basis of this knowledge, four [6,5]-bicyclic heteroaromatic carboxamide derivatives 8, 22, 27, and 30 were designed, all of which incorporate a nitrogen atom within the heteroaromatic ring to lock the carboxamide group into a six-membered intramolecular hydrogen bond. All four novel scaffolds were demonstrated to be modest PARP inhibitors, with the indazole derivative 8 with IC$_{50} = 24$ nM displaying higher affinity than the benzotriazole 22, pyrazolo[1,5-α]pyridine 27, and the [1,2,4]triazolo[1,5-α]pyridine 30 in a PARP-1 SPA assay (Table 1). The indazole 8 was also demonstrated to inhibit PARP activity in cells, being able to inhibit the formation of PAR polymers following the induction of DNA damage with hydrogen peroxide. In HeLa cervical cancer cells 8 displayed EC$_{50} = 3.7\, \mu$M and EC$_{90} = 6.2\, \mu$M. The pharmacokinetics of 8 were also encouraging, displaying moderate stability in both rat and human microsomes (Cl$_{int} = 123$ and 138 (μL/min)/mgP, respectively), and in vivo in rats 8 displayed acceptable plasma clearance (Cl = 30 (mL/min)/kg), volume of distribution of 1.8 L/kg, and terminal half-life of 5.1 h. This indazole also showed acceptable oral bioavailability, $F = 41\%$. 

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**Scheme 1**

![Scheme 1](image)
On the basis of these results, a more detailed exploration of the indazole lead 8 was undertaken in order to optimize potency, as well as pharmaceutical properties. In an effort to improve the activity, substituents were introduced at the ortho, meta, and para positions of the pendent phenyl ring, as well as the insertion of a methylene spacer between the indazole core and the phenyl moiety. Substituents were well tolerated at the distal meta and para positions, resulting in an improvement in at least 5-fold in cellular activity, with 11 and 12 displaying EC50 = 450 and 720 nM in the PARylation assay, respectively. However, substitution adjacent to the indazole was detrimental, with 10 displaying a 5-fold loss of activity.

Scheme 2

Reagents: (a) p-FC6H4COR, K2CO3, DMF, microwave; (b) R'R'NH, NaBH3(CN), ZnCl2, MeOH, DCE; (c) NH3, MeOH, sealed tube, 60 °C; (d) MeMgBr, THF; (e) NaCN, H2SO4, DCM; (f) BH3, THF, THF; (g) MeOCOCH3, POCl3, KO'Bu, Et2O/THF, −78 °C to room temp, then HCl, Et2O; (h) formaldehyde, NaBH3(CN), then H2, Pd/C, MeOH; (i) ArNH2, EtOH, Δ; (j) NaN3, DMF, 90 °C; (k) TFA, DCM, H2O.

Scheme 3

Reagents: (a) ArNH2, EtOH, Δ; (b) NaN3, DMF, 90 °C; (c) NH3, MeOH, sealed tube, 60 °C; (d) HCl, dioxane; (e) SFC separation; (f) 3-pyridyl-B(OH)2, Pd(PPh3)4, Na2CO3, THF/H2O, Δ; (g) 50 psi of H2, PtO2, MeOH; (h) resolution L-tartaric acid, EtOH; (i) Boc2O, DCM; (j) 5, EtOH, Δ.
Table 1. In Vitro Activity of PARP Inhibitor Scaffolds

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*Values are the mean of at least four experiments (standard deviations were within 25% of the mean values). ND = not determined.

The benzyl derivative 9 resulted in a similar loss of enzymatic activity.

Knowing that substitution was tolerated at the remote positions, alternative groups were introduced in the hope of picking up additional binding interactions in the adenosine binding pocket to improve the affinity and cellular activity. Efforts concentrated on the addition of polar groups with a view to improve the solubility of these lipophilic compounds, given that the prototype 8 was only sparingly soluble in phosphate buffer, 26 μg/mL.

The addition of a dimethylaminomethyl group to give 37 resulted in a 6-fold improvement in enzymatic activity, IC_{50} = 3.7 nM, and further improved the cellular activity, inhibiting the formation of poly-ADP-ribose chains following DNA damage with EC_{50} = 110 nM and EC_{90} = 630 nM (Table 2). Given the submicromolar activity of this compound, its ability to inhibit the growth of cervical cancer HeLa cells where BRCA-1 had been silenced with a lentivirus expressing a short hairpin interfering RNA (shRNA) targeting BRCA-1 was tested. In parallel, the cytotoxicity against a matched pair cell line expressing an empty lentivector was also measured. In 7-day proliferation assays 37 displayed CC_{50} = 520 nM against the BRCA-1 deficient cells and 10-fold selectivity over the BRCA-1 proficient HeLa cells where CC_{50} = 5.6 μM, thereby confirming the previous literature reports that PARP inhibitors are capable of selectively killing BRCA-1 deficient cells.17,18 The addition of the basic amine also improved the physicochemical properties, with 37 displaying solubility in excess of 3 mg/mL in phosphate buffer.

Subsequent SAR studies confirmed these results with pyrrolidine 38 and piperidine 39 displaying similar PARP inhibitory activity in vitro and in cells, inhibiting the growth of BRCA-1 silenced HeLa cells with CC_{50} = 1.2 and 0.88 μM, respectively, and showing at least 5-fold selective cytotoxicity versus HeLa BRCA-1 wild-type cells. The less basic derivatives such as morpholine 40 displayed inferior PARP inhibition, with EC_{50} = 6.2 μM in the PARylation assay and CC_{50} = 5.3 μM against BRCA-1 silenced cells. Cellular activity could be improved by the incorporation of the N-methylpiperazine, and 41 displayed CC_{50} = 2.7 μM in the BRCA-1 deficient cells, despite this compound displaying weaker enzyme activity, IC_{50} = 31 nM. However, 41 showed only 3-fold selective cytotoxicity between BRCA-1 silenced and wild-type cells, suggesting some underlying toxicity. Similar micromolar activity was also seen with the trimethylhydrazine derivative 42, although with at least a 5-fold selectivity between BRCA-1 deficient and wild-type cells. Secondary amines were also tolerated, with the methylamine 43 displaying slightly improved activity compared to dimethylamino analogue 37 in cells, with CC_{50} = 460 nM and more than 10-fold selectivity for BRCA-1 deficient cells. In contrast, more lipophilic ethylamine 44, although displaying comparable enzymatic activity, was 3-fold less active in cells, and further substitution was detrimental, as the isopropylamine 47 lost both enzyme and cellular activity, with IC_{50} = 6.7 nM and CC_{50} = 2.0 μM in BRCA-1 deficient cells. Introduction of mono- and difluoro groups to the ethylamine, in 45 and 46, reduced the basicity of the amine and reduced cellular activity as seen with the morpholine 40. In general, there was good correlation between the extent of PARP inhibition in cells and the concentrations needed to inhibit growth of BRCA-1 silenced HeLa cells. In most cases EC_{50} for inhibition of PARylation correlated with the CC_{50} for inhibition of the proliferation of BRCA-1 silenced HeLa cells. This suggests that strong sustained PARP inhibition is required to inhibit growth in this context.

Exploration was also conducted in the isomeric series, where the methylaminomethyl group was introduced in the meta position of the phenyl ring, resulting in 54, and although this was tolerated, inhibiting PARP activity with IC_{50} = 5.3 nM, this derivative displayed only weak cellular activity, BRCA-1 deficient CC_{50} = 10 μM. A loss of activity was also observed in the para regioisomer if the amine was homologated to the corresponding phenethylamine, with 52 losing almost 2 orders of magnitude in activity, PARP-1 IC_{50} = 200 nM.

Encouraged by these results and the selective growth inhibition caused by these PARP inhibitors in BRCA-1 deficient cells, a number of compounds were profiled in microsomal stability studies and in vivo in rats (Table 3). The dimethylamino derivative 37 displayed relatively high turnover in rat liver microsomes (RLM), Cl_{int} = 177 (μL/min)/mg P, and in vivo high plasma clearance was observed in rats, in excess of hepatic blood flow. In contrast, the monomethylamine 43 showed improved stability in vitro (RLM Cl_{int} = 28 (μL/min)/mg P), yet in vivo rats similar high plasma clearance was measured, Cl = 220 (mL/min)/kg. The plasma clearance value in excess of rat liver blood flow suggested extrahepatic metabolism. Similar observations were seen with the piperidine 39 and the isopropyl 47 derivatives, all of which displayed good stability in rat liver microsomes but plasma clearances in excess of 100 (mL/min)/kg in vivo in rats. Plasma stability studies were conducted on a number of derivatives, and no metabolism was seen in rat or human plasma. Furthermore, blood/plasma partitioning experiments revealed B/P ratios in the range 1.2–1.6, thereby failing to explain the blood clearance in excess of hepatic blood flow.

A [3H]-radiolabeled version of 43 was prepared and administered iv to rats, and 80% of the radioactivity was recovered in the urine and bile within 24 h of dosing, being equally distributed between the two fluids (Figure 2). In urine, one significant metabolite was detected corresponding to the benzoic acid 63, probably arising from oxidation of the benzylic position to the corresponding aldehyde 35 followed by further oxidation to the carboxylic acid. In contrast, in...
Table 2. In Vitro Activity and Antiproliferation Activity of 2-Phenyl-2H-indazole-7-carboxamide PARP Inhibitors

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bile, both the carboxylic acid 63 and the corresponding glucuronide 64 were detected. In total the formation of 63 accounted for 50% of the radioactivity dosed and 60% of that recovered between 0 and 24 h.

Knowing the identity of the primary route of metabolism, CYP phenotyping was undertaken with recombinant CYP450 isoforms, which revealed that CYPs 1A1 and 1A2 are primarily responsible for the metabolism of 43 (Figure 3a). CYP1A1 is expressed at only very low levels in the liver and is predominantly an extrahepatic CYP, although it can be induced via the aryl hydrocarbon receptor.\(^{34,35}\) CYP1A2 is expressed mainly in the liver but is also expressed extraheptically in rats.\(^{34,36}\) Incubations of 43 with CYPs 1A1 and 1A2 revealed that formation of 63 correlated with disappearance of 43, and only minor levels of the aldehyde 35 were seen in vitro. Knowing that these CYP450 isoforms are expressed extrahepatically, stability studies were undertaken with microsomes isolated from different organs (Figure 3b), which revealed that 43 was degraded by lung and kidney microsomes in the presence of NADPH, in addition to liver microsomes, with intrinsic clearance of 22, 22, and 28 (μL/min)/mgP. In contrast, 43 was stable in heart microsomes and showed only modest metabolism in intestinal microsomes Cl\(_{\text{int}}\) = 5 (μL/min)/mgP.

With this information analogues were screened using recombinant CYP1A1; 43, the dimethylamino 37, the piperidine 39, and the isopropyl 47 derivatives were all degraded rapidly, with Cl\(_{\text{int}}\) = 6, 8.7, 3.8, and 0.8 (μL/min)/mgP. This is in good agreement with the high clearances seen in vivo in rats. On the other hand, the N-methylpiperazine derivative 41, which showed modest stability in RLM, showed improved stability in the presence of CYP1A1 Cl\(_{\text{int}}\) < 0.1 (μL/min)/mgP, and when this compound was dosed iv to rats, it displayed low plasma clearance Cl = 8 (mL/min)/kg, with long terminal half-life \(t_{1/2} = 9.7\) h.

A strategy to improve the PK properties was to block the methylene group through steric crowding; therefore, the corresponding mono- and gem-dimethyl derivatives, 48 and 50, were prepared. Both compounds maintained PARP inhibitory activity with IC\(_{50} = 3.7\) and 16 nM, respectively, and were capable of blocking PARP activity in cells as revealed by EC\(_{90} = 690\) and 900 nM. This level of PARP inhibition resulted in the inhibition of proliferation of BRCA-1 deficient HeLa cells with CC\(_{50} = 3.1\) and 0.92 μM, respectively, albeit with only a modest selectivity over wild type cells. Both derivatives showed good stability in rat liver microsomes as seen previously with 43, with 48 and 50 having intrinsic clearances of 22 and 18 (μL/min)/mgP, respectively. The introduction of the steric crowding on the benzylic methylene served to protect these derivatives from oxidation by CYP1A1 as demonstrated by improved stability compared to 43 against this recombinant CYP, with Cl\(_{\text{int}}\) = 2.2 and 0.4 (μL/min)/mgP for 48 and 50, respectively. Moreover, this improved stability was recapitulated in vivo where the addition of a single methyl group reduced the rat

### Table 3. In Vitro and in Vivo Pharmacokinetic Parameters for 2-Phenyl-2H-indazole-7-carboxamide PARP Inhibitors

<table>
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<th>compd</th>
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<th>PARylation EC(_{50}) (nM)</th>
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<td>470</td>
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\(^{a}\)Values are the mean of at least four experiments (standard deviations were within 25% of the mean values).
plasma clearance to 58 (mL/min)/kg, while the gem-dimethyl group reduced plasma clearance still further to 30 (mL/min)/kg.

Despite the improvement in PK properties, the activity of these derivatives was still suboptimal, and accordingly attempts were undertaken to increase the activity by restricting the rotational freedom of the amines, either by closing the methylamine back onto the phenyl ring as in 13 and 14 (Table 2) or through cyclization onto the benzylic methylene, as in 15–17. Locking the amine into a tetrahydroisoquinoline system like 13 or 14 resulted in a modest improvement in enzymatic activity, and both analogues displayed around a 2-fold improvement in activity, with EC90 = 310 and 140 nM in the PARylation assay and antiproliferative activities of 130 and 270 nM, respectively, in BRCA-1 silenced cells. Both analogues showed excellent selectivity for the BRCA-1 silenced cells, with 16- and 20-fold higher concentrations required to cause antiproliferative effects in wild-type cells. Despite the encouraging profile, 13 was still characterized by high plasma clearance in rats, with Cl = 87 (mL/min)/kg, reflecting the moderate stability in both rat microsomes and CYP1A1, Clint = 52 and 0.7 (μL/min)/mgP, respectively. In contrast, the isomer 14 showed slightly better microsomal stability with Clint = 36 (μL/min)/mgP, and lower plasma clearance in vivo where Cl = 30 (mL/min)/kg. This compound also displayed modest oral bioavailability, F = 22%.

Unfortunately cellular activity was still suboptimal and efforts focused on the other cyclization strategy (Tables 2 and 3). Accordingly, pyrrolidine 15 and piperidine derivatives 16 and 17 were prepared. These three analogues bore the basic amine at increasing distances from the aromatic

Figure 2. Metabolite profiles were obtained by radiochromatography of urine and bile following dosing of [3H]43 (300 μCi/kg). Separation was by RP-HPLC, using a Acc Act RP C18 150 mm × 2.1 mm column (Mac Mod Analytical Inc., Chadds Ford, PA) coupled to a Packard 515TR radiodetector (Perkin-Elmer Life and Analytical Sciences, Boston, MA) equipped with a 500 μL liquid scintillation flow cell.

Figure 3. (a) Stability of 43 in the presence of rat rCYP isoforms. (b) Metabolic stability of 43 in liver, lung, kidney, intestine microsomes and heart homogenate in the presence of NADPH.
ring. The first two analogues displayed similar enzyme activities, although the piperidine 16 was characterized by 2-fold improvement in cellular activity, with EC$_{50} = 220$ nM in the PARylation assay. This analogue was the first compound from this series to display double digit antiproliferation activity, CC$_{50} = 72$ nM, in BRCA-1 deficient cells and displayed > 25-fold selectivity for BRCA-1 silenced cells compared to their wild type counterparts. In contrast, the 4-piperidinyl isomer 17 displayed around a 3-fold weaker enzyme activity, and this was reflected in weaker cellular activity with CC$_{50} = 410$ nM in BRCA-1 deficient cells. Unsurprisingly, the pyrrolidine 15 with the amine in a benzylic position was a substrate of CYP1A1 (Cl$_{int} = 2.5$ ($\mu$L/min)/mgP), but the 3-piperidinyl 16 derivative bearing a phenethylamine showed good stability both in rat liver microsomes and in recombinant CYP1A1, Cl$_{int} = 11$ and 0.3 ($\mu$L/min)/mgP, respectively. When dosed to rats, 16 displayed moderate plasma clearance Cl = 47 (mL/min)/kg and excellent oral bioavailability, $F = 74\%$. The 4-piperidinyl derivative 17 showed similar in vitro properties, but given the weaker cellular activity with BRCA-1, CC$_{50} = 410$ nM.

Given the encouraging properties of 16, the two enantiomers were separated and profiled, and although only marginal differences in activity between 56 and 57 were seen on the enzyme (PARP-1 IC$_{50} = 3.2$ and 2.4 nM, respectively), the S-enantiomer 56 proved to be around an order of magnitude more active in cells with EC$_{50} = 4$ nM and EC$_{90} = 45$ nM in the PARylation assay, compared to EC$_{50} = 30$ nM and EC$_{90} = 280$ nM for the enantiomer 57. Similarly, the S-enantiomer 56 displayed improved antiproliferation effects in the BRCA-1 silenced cells with CC$_{50} = 33$ nM, compared to 470 nM for the R-enantiomer 57. As seen previously for the racemate, excellent selectivity was seen between BRCA-1 silenced and wild-type cells with 56 displaying a 23-fold window. The absolute stereochemistry of the piperidinone was determined by Mosher’s amide analysis (see Supporting Information), as well as through comparison to a synthetic intermediate prepared by nitration and reduction of (S)-3-phenylpiperidine prepared by enantioselectively as described by Bosch. The pharmacokinetics of the S-enantiomer 56 both in vitro and in vivo in rats were slightly inferior to those of the 57, but nevertheless 56 was characterized by acceptable pharmacokinetics in rats with plasma clearance of 28 (mL/min)/kg, very high volume of distribution (V$_{dss} = 6.9$ L/kg), long terminal half-life ($t_{1/2} = 3.4$ h), and excellent bioavailability, $F = 65\%$.

Given the interest in 56, it was profiled against several other PARP family members in a trichloroacetic acid precipitation assay looking at the incorporation of [3H]NAD into the growing PAR polymers (Table 4). It was demonstrated to be a potent and selective PARP-1 and PARP-2 inhibitor with IC$_{50} = 3.8$ and 2.1 nM, respectively. Furthermore, it displayed at least a 100-fold selectivity over PARP-3, V-PARP, and tankyrase-1, with IC$_{50} = 1300$, 330, and

<table>
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<th>Table 4. Inhibitory Activity of 56 on Individual PARP Isoform*</th>
<th>IC$_{50}$ (nM)</th>
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<td>1300</td>
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<tr>
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<td>TANK-1</td>
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*Values are the mean of at least four experiments (standard deviations were within 25% of the mean values).

<table>
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<th>Table 5. Antiproliferation Activity of 56*</th>
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<th>tumor type</th>
<th>mutation</th>
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<td>human mammary epithelial</td>
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</table>

*Values are the mean of at least four experiments (standard deviations were within 25% of the mean values).

Figure 4. Activity of 56 in MDA-MB-436 (BRCA-1 mutant) xenograft bearing mice when dosed orally at 100 mg/kg q.d. or 50 mg/kg b.i.d. for 33 days. For each group, $n = 7$. 

![Activity of 56 in MDA-MB-436 (BRCA-1 mutant) xenograft bearing mice when dosed orally at 100 mg/kg q.d. or 50 mg/kg b.i.d. for 33 days. For each group, $n = 7$.](image-url)
570 nM, respectively. As well as inhibiting the growth of HeLa cell lacking BRCA-1 because of silencing by RNA interference, this derivative is able to inhibit the proliferation of cancer cell lines carrying natural BRCA-1 or BRCA-2 mutations (Table 5). In MDA-MB-436 human mammary gland adenocarcinoma cells carrying BRCA-1 mutations, S6 displayed CC\textsubscript{50} = 18 nM, while in CAPAN-1 human pancreatic adenocarcinoma cells, which are BRCA-2 mutant, S6 displayed CC\textsubscript{50} = 90 nM. The latter result substantiates the evidence that potent PARP inhibitors are capable of killing BRCA-2 deficient cells.\textsuperscript{38} In contrast, normal human prostate and mammary epithelial cells are resistant to S6, displaying antiproliferative effects in the micromolar range, thereby demonstrating the very high selective cytotoxicity from these PARP inhibitors in BRCA-1 and -2 mutant cancer cells compared to surrounding tissue.

The in vivo efficacy of the compound was demonstrated preclinically in a BRCA-1 mutant MDA-MB-436 xenograft model (Figure 4), and 2 × 10\textsuperscript{6} cells were injected subcutaneously in the right flank of 6-week-old nude CD1 female mice. When tumors reached an average volume of 150 mm\textsuperscript{3}, mice were randomized to form homogeneous groups and treated with S6, dosing orally at either 100 mg/kg q.d. or 50 mg/kg b.i.d. Tumor regression was observed with both inhibitor, displaying more than 100-fold selectivity over the experiment.

The PARP-1 SPA Assay. Enzyme assay was conducted in buffer containing 25 mM Tris, pH 8.0, 1 mM DTT, 1 mM spermine, 50 mM KCl, 0.01% Nonidet P-40, and 1 mM MgCl\textsubscript{2}. PARP reactions contained 0.1 \mu Ci [\textsuperscript{3}H]NAD\textsuperscript{+}, 100 nM bovine NAD\textsuperscript{+}, 150 nM biotinylated NAD\textsuperscript{+}, 1 \mu g/mL activated calf thymus, and 1–5 nM PARP-1. Auto-reactions utilizing SPA bead-based detection were carried out in 50 \mu L volumes in white 96-well plates.

Compounds were prepared in 11-point serial dilution in 96-well plate, 5 \mu L/well in 5% DMSO/H\textsubscript{2}O (10× concentrated). Reactions were initiated by adding first 35 \mu L of PARP-1 enzyme in buffer and incubating for 5 min at room temperature and then 10 \mu L of NAD\textsuperscript{+} and DNA substrate mixture. After 3 h at room temperature, these reactions were terminated by the addition of 50 \mu L of streptavidin-SPA beads (2.5 mg/mL in 200 mM EDTA, pH 8). After 5 min, they were counted using a TopCount microplate scintillation counter. IC\textsubscript{50} data was determined from inhibition curves at various substrate concentrations.

The PARP Isoform TCA Assays. The enzymatic reaction was conducted in the presence of 25 mM Tris-HCl pH 8.0, 1 mM MgCl\textsubscript{2}, 50 mM KCl, 1 mM spermine, 0.01% Nonidet P-40, and 1 mM DTT. PARP reactions contained 0.1 \mu Ci [\textsuperscript{3}H]NAD (200,000 DPM), 1.5 \mu M NAD\textsuperscript{+}, 1 \mu g/mL activated calf thymus, and 0.2–1 nM human PARP-1 enzyme. Assays were carried out in 50 \mu L volumes in white 96-well polypropylene microplate.

A 96-well plate was prepared with serial dilutions over 10 points over a 0.1–50 nM concentration range 5% DMSO/H\textsubscript{2}O, 5 \mu L. Reactions were initiated by adding first 35 \mu L of PARP-1 enzyme in buffer and incubating for 5 min at room temperature, then 10 \mu L of NAD\textsuperscript{+} and DNA substrate mixture. After 2 h incubation at room temperature, the reaction was stopped by the addition of TCA (50 \mu L/well, 20% in 20 mM NaPPi solution) and incubated for 10 min over ice. The resulting precipitate was filtered on a Unifilter GF/B microplate and washed four times with 2.5% TCA. After addition of 50 \mu L/well of scintillation liquid the amount of radioactivity incorporated into the PAR polymers was determined using a TopCount microplate scintillation counter. IC\textsubscript{50} data were determined from inhibition curves at various substrate concentrations. The protocols for the other PARP family members are very similar with subtle changes as described in the Supporting Information.

PARylation Assay. HeLa cells were seeded into a 96-well Viewplate black microplate at an initial concentration of 10,000 cells/well in culture medium (100 \mu L of DMEM containing 10% FCS, 0.1 mg/mL penicillin–streptomycin, and 2 mM L-glutamine). The plates were incubated for 4 h at 37 °C under 5% CO\textsubscript{2} atmosphere, and then compounds were added with serial dilutions over nine points over a 0.3–100 nM concentration range in 5% DMSO/H\textsubscript{2}O, 10 \mu L. Reactions were initiated by adding first 35 \mu L of PARP-1 enzyme in buffer and incubating for 5 min at room temperature, then 10 \mu L of NAD\textsuperscript{+} and DNA substrate mixture. After 2 h incubation at room temperature, the reaction was stopped by the addition of TCA (50 \mu L/well, 20% in 20 mM NaPPi solution) and incubated for 10 min over ice. The resulting precipitate was filtered on a Unifilter GF/B microplate and washed four times with 2.5% TCA. After addition of 50 \mu L/well of scintillation liquid the amount of radioactivity incorporated into the PAR polymers was determined using a TopCount microplate scintillation counter. IC\textsubscript{50} data were determined from inhibition curves at various substrate concentrations.

Conclusions

A novel series of 2-phenyl-2H-indazole-7-carboxamide PARP inhibitors has been developed. Introduction of a p-alkylaminomethyl group improved cellular activity but resulted in detrimental PK properties as a result of oxidation by CYP1A enzymes. Subsequent optimization culminated in the identification of S6 which is a potent and selective PARP 1 inhibitor, displaying antiproliferative effects in the micromolar range, thereby demonstrating the very high selective cytotoxicity from these PARP inhibitors in BRCA-1 and -2 mutant cancer cells compared to surrounding tissue.

Further characterization of the preclinical profile of S6 will be described elsewhere in due course, and S6 is currently undergoing clinical evaluation in a phase 1 clinical trial in cancer patients.\textsuperscript{39}

Experimental Section

General Experimental Details. Solvents and reagents were obtained from commercial suppliers and were used without further purification. HPLC—MS and UPLC—MS analyses were performed on either a Waters Alliance 2795 apparatus or an Acquity UPLC. Nuclear magnetic resonance spectra were obtained on Bruker AMX spectrometers and are referenced in ppm relative to TMS. High resolving power accurate mass measurement electrospray (ES) and atmospheric pressure chemical ionization (APCI) mass spectral data were acquired by use of a Bruker Daltonics 7T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS). All final compounds displayed ≥95% purity as determined by analytical RP-HPLC on an Acquity Waters UPLC using three different methods.
with Ex, HQ 620_60X, Em. HQ 700_75M, exposure time of 300 ms. The % PAR-positive cells was calculated by measuring the ratio between the numbers of PAR-positive nuclei over the total number of Draq5-labeled nuclei. The IC50 was determined on the basis of the residual enzyme activity in the presence of increasing PARP1 concentration.

**Proliferation Assay in BRCA-1 Silenced and Wild Type HeLa Cells.** HeLa BRCA1-silenced cells were generated by transducing HeLa cells at an MOI of 100 with a lentivirus containing an H1-derived expression cassette for a shRNA against BRCA-1 and an expression cassette for GFP (GFP under the control of EF1-a promoter). Silencing of BRCA1 was more than 80% as assessed by Taqman analysis. Control BRCA wild type HeLa cells were generated by transducing them with a lentivirus expressing GFP only.

Proliferation assays were conducted in 96-well black viewplates, and 300 cells/well (250 cell/well for BRCA-1 wt) in culture medium, 190 μL/well (DMEM containing 10% FCS, 0.1 mg/mL penicillin–streptomycin, and 2 mM l-glutamine), were plated and incubated for 4 h at 37 °C under 5% CO2 atmosphere. Inhibitors were then added with serial dilutions, 10 μL/well to obtain the desired final compound concentration in 0.5% DMSO. The cells were then incubated for 7 days at 37 °C in 5% CO2 after which time viability was assessed. Briefly, with CellTiter-Blue (Promega) solution prediluted 1:10 in medium, 100 μL/well was added and the cells left for 45 min at 37 °C under 5% CO2 and then a further 15 min at room temperature in the dark. The number of living cells was determined by reading the plate at fluorimeter, excitation at 550 nm and emission at 560 nm. Cell growth was expressed as the percentage growth with respect to vehicle treated cells. The concentration required to inhibit cell growth by 50% (CC50) was determined. The protocols for the other cell lines are very similar and are described in the Supporting Information.

**Xenograft Model.** The MDA-MB-436 human breast cancer cells (ATCC) were grown in RPMI 1640 medium with l-glutamine supplemented with 10% FCS, penicillin (100 U/mL), and streptomycin (100 μg/mL) in standard adherent culture conditions at 37 °C and 5% CO2. For establishment of xenograft tumors, cells were harvested from subconfluent cultures using EDTA/trypsin, washed in serum-free medium, and injected (2 × 10^6) cells subcutaneously in the right flank of 6-week-old nude CD1 female mice in 100 μL total volume of 1:1 mix of cell suspension in serum-free media and RGF-Matrigel. When tumors reached an average volume of 150 mm³, mice were randomized to form homogeneous groups and treatment started, dosing orally. Mice were dosed orally in water (10 mL/kg) with 100 mg/kg q.d. or 50 mg/kg b.i.d. for 33 days, with tumor growth and body weight measurements done at least once a week.

**General Methods for the Synthesis of Indazole PARP Inhibitors. Preparation of Imines 6a–k (Method A).** A mixture of methyl 3-formyl-2-nitrobenzoic acid (5) (1.0 equiv) and the corresponding aniline (1.0 equiv) in EtOH (0.2 M) was stirred at room temperature for 2 h until TLC revealed completion of the reaction. Evaporation of the solvent gave a white solid which was used in the next step without further purification.

**Preparation of Methyl 2H-Indazole-7-carboxylate Esters 7a–k (Method B).** A mixture of the imine (6a–k) (1.0 equiv) and NaN₃ (1.0 equiv) in dry DMF (0.3 M) was stirred at 90 °C overnight under N₂ atmosphere. The crude was concentrated under reduced pressure and the residue purified by flash column chromatography on silica.

**Preparation of 2H-Indazole-7-carboxamides 8–17 (Method C).** A solution of the corresponding carboxylic ester (7a–k) in NH₃ (7 N solution in MeOH, 200 equiv) in a sealed tube was heated to 60 °C for 36 h. After the mixture was cooled, solvent was evaporated under reduced pressure and the residue purified by flash column chromatography.

**Preparation of Methyl 2-(4-Formylphenyl)-2H-indazole-7-carboxylate 32 (Method D).** To a solution of methyl 2H-indazole-7-carboxylate (31) (1.0 equiv) in DMF (0.8 M) were added K₂CO₃ (1.1 equiv) and 4-fluorobenzaldehyde (1.3 equiv), and the reaction mixture was heated under microwave conditions at 200 °C for 10 min. The reaction mixture was cooled to room temperature and diluted with EtOAc. The organic phase was washed with brine and dried (Na₂SO₄). Evaporation of the solvent gave (32) which was purified by flash column chromatography.

**Preparation of Methyl 2-[4-[Alkylamino(methyl)phenyl]-2H-indazole-7-carboxylate 33a–k (Method E).** Methyl 2-(4-formylphenyl)-2H-indazole-7-carboxylate (32) (1.0 equiv) was suspended in MeOH (0.1 M), and the amine (8 equiv) was added. To this solution was added a solution of NaBH₄(CN) (1.1 equiv) and ZnCl₂ (0.5 equiv) in MeOH (0.5 mL). The pH was adjusted to 6 with 1.25 M HCl in MeOH, and the mixture stirred at room temperature for 3 h. Then 6 N HCl (0.1 mL) was added and the solvent was reduced in vacuo. Saturated aqueous NaHCO₃ solution was added, the product was extracted with DCM and dried (Na₂SO₄), and the solvents were reduced under reduced pressure.

**Methyl 3-Formyl-2-nitrobenzoate (5).** To a suspension of 3-methyl-2-nitrobenzoic acid (13.5 g, 74 mmol) in MeOH (200 mL) at 0 °C was added dropwise AcCl (15.9 mL, 224 mmol). The reaction mixture was stirred for 20 h at reflux. The solvent was reduced under reduced pressure, and the residue was dissolved in EtOAc, washed several times with saturated aqueous NaHCO₃ solution and brine, and dried (Na₂SO₄). Evaporation of the solvent gave methyl 3-methyl-2-nitrobenzoate (4) (12.4 g, 85%) as a white solid which was used in the next step without further purification.

**1H NMR (400 MHz, CDCl₃, 300 K) δ 7.86 (1H, d, J = 7.5 Hz), 7.53–7.42 (2H, m), 3.89 (3H, s), 2.36 (3H, s). MS (ES) C₉H₉NO₂ requires 195, found 194 (M + Na)⁺.

A mixture of methyl 3-methyl-2-nitrobenzoate (12.4 g, 63.3 mmol), (BzO₂)(0.92 g, 3.8 mmol), and NBS (13.5 g, 74.7 mmol) in CCl₄ (320 mL) was heated at reflux under N₂ atmosphere for 12 h. The mixture was cooled to room temperature, diluted with DCM, concentrated under reduced pressure while dry loading onto silica gel. The residue was purified by flash column chromatography on silica gel using 10% EtOAc/petroleum ether to yield methyl 3-(methylmethyl)2-nitrobenzoate (6.94 g, 40%) as a white solid. **1H NMR (400 MHz, CDCl₃, 300 K) δ 7.93 (1H, d, J = 7.7 Hz), 7.72 (1H, d, J = 7.7 Hz), 7.57 (1H, t, J = 7.7 Hz), 4.43 (2H, s), 3.88 (3H, s). MS (ES) C₁₁H₁₃BrNO₄ requires 273/275, found 242/244 (M – OMe)⁺, 227/229 (M – NO₂)⁺.

To a mixture of methyl 3-(methylmethyl)2-nitrobenzoate (6.94 g, 25.3 mmol) and 4 A molecular sieves (35 g) in MeCN (125 mL) at room temperature was added N-methylmorpholine N-oxide (5.93 g, 50.6 mmol), and the reaction mixture was stirred for 1.5 h under N₂ atmosphere. Then the mixture was diluted with EtOAc and filtered and the filtrate was washed with H₂O. 1 N HCl, brine, and dried (Na₂SO₄). Evaporation of the solvent gave methyl 3-formyl-2-nitrobenzoate (5) as a white solid (3.92 g, 74%) which was used in the next step without further purification.

**1H NMR (400 MHz, CDCl₃, 300 K) δ 9.96 (1H, s), 8.26 (1H, d, J = 7.9 Hz), 8.18 (1H, d, J = 7.9 Hz), 7.77 (1H, t, J = 7.9 Hz), 3.93 (3H, s). MS (ES) C₉H₇NO₂ requires 209, found 208 (M + H)⁻.

**Methyl 2-Nitro-3-[(phenylimino)methyl]benzoate (6a).** 6a was prepared according to general method A, using 5 (0.4 g, 1.9 mmol) and aniline (0.18 g, 2.0 mmol) to afford methyl 2-nitro-3-[(phenylimino)methyl]benzoate (6a) which was used in the next step without further purification.

**1H NMR (400 MHz, CDCl₃, 300 K) δ 8.51 (1H, d, J = 7.3 Hz), 8.41 (1H, s), 8.11 (1H, d, J = 7.3 Hz), 7.67 (1H, t, J = 7.8 Hz), 7.43 (2H, t, J = 7.8 Hz), 7.31 (1H, t, J = 7.3 Hz), 7.16 (2H, d, J = 7.8 Hz), 3.94 (3H, s).

**Methyl 2-Pheny1-2H-indazole-7-carboxylate (7a).** 7a was prepared according to general method B using 6a (0.54 g, 1.9 mmol)
to give, after purification by flash column chromatography on silica using a gradient of 10–40% EtOAc/petroleum ether, ether 2-phenyl-2H-indazole-7-carboxylate (7a) (0.18 g, 37% yield as a brown oil. 1H NMR (400 MHz, CDCl3, 300 K) δ 8.50 (1H, s), 8.12 (1H, d, J = 7.0 Hz), 7.96–7.90 (3H, m), 7.49 (2H, t, J = 7.6 Hz), 7.38 (1H, t, J = 7.4 Hz), 7.15 (1H, t, J = 7.4 Hz), 4.03 (3H, s). MS (ES) C14H11N3O2 requires 252, found 252 (M + H+)·

2-Phenyl-2H-indazole-7-carboxamide (8). 8 was prepared according to general method C using 7a (0.18 g, 0.7 mmol) to afford, after purification by flash column chromatography on silica using a gradient of 30–50% EtOAc/petroleum ether, 8 (47 mg, 28%) as a white solid. 1H NMR (400 MHz, DMSO, 300 K) δ 9.33 (1H, s), 8.56 (1H, br s), 8.16 (2H, d, J = 7.9 Hz), 8.08–8.00 (2H, m), 7.88 (1H, br s), 7.63 (2H, t, J = 7.7 Hz), 7.50 (1H, t, J = 7.4 Hz), 7.27 (1H, t, J = 7.9 Hz). HRMS (ESI) m/z calefd for C14H11N3O2 requires 253, found 276 (M + H+)·

Methyl 2,3-Diaminobenzoate (9). A mixture of 2-amino-3-nitrobenzoic acid methyl ester (8) (6 g, 30 mmol) and Pd/C (0.6 g, 10% w/w) in MeOH (100 mL) was stirred for 3 days at room temperature under H2 atmosphere (1 atm). The mixture was filtered through Celite, and then the solvent was evaporated under reduced pressure. The red solid of crude methyl 2,3-diaminobenzoate (10) (4.88 g, 96%) was used in the next step without further purification. 1H NMR (300 MHz, CDCl3, 300 K) δ 7.46 (1H, d, J = 8.1 and 1.4 Hz), 6.84 (1H, d, J = 7.5 Hz), 6.59 (1H, dd, J = 8.1 and 7.5 Hz), 3.87 (3H, s). MS (ES) C9H11N2O2 requires 166, found 167 (M + H+).

Methyl 3-Amino-2-phenylbenzoate (11). A mixture of methyl 2,3-diaminobenzoate (9) (400 mg, 2.4 mmol) and nitrosobenzene (284 mg, 2.6 mmol) in AcOH (25 mL) was stirred for 3 h at room temperature. Evaporation of the solvent gave methyl 3-amino-2-phenylbenzoate (11) (1.07 g, 5.0 mmol) at room temperature. The inorganics were removed by filtration, and the filtrate was concentrated under reduced pressure. The crude residue was dissolved in toluene (50 mL) and heated at reflux for 4 h and then concentrated under reduced pressure and purified by column chromatography on silica eluting with 20% EtOAc/petroleum ether to yield the titled compound (34 mg, 34%) as a white solid.

1-Amino-2-(ethoxycarbonyl)pyridinium 2,4,6-Trimethylbenzenesulfonate (24). Ethyl 2-picolinate (23) (500 mg, 3.3 mmol) in DCM (10 mL) was stirred with O-mesitylenesulfonylhydroxylamine (1.07 g, 5.0 mmol) at room temperature. After 15 min a further portion of O-mesitylenesulfonylhydroxylamine (1.07 g, 5.0 mmol) was added and stirred continuously for 2 h. The resulting solution was diluted with EtO (100 mL) and the resulting precipitate removed by filtration and dried under reduced pressure to yield the desired compound (1.15 g, 95%). 1H NMR (300 MHz, DMSO-d6, 300 K) δ 8.91 (1H, d, J = 5.5 Hz), 8.48 (1H, d, J = 5.5 Hz), 8.36 (1H, t, J = 5.5 Hz), 8.16 (1H, t, J = 5.5 Hz), 6.71 (2H, s), 4.48 (2H, q, J = 7.2 Hz), 2.52 (6H, s), 2.29 (3H, s), 1.39 (3H, q, J = 7.2 Hz).

Benzyl 7-Carbamoyl-2-phenylpyrazolo[1,5-a]pyridine-3-carboxylate (25). 1-Amino-(2-ethoxycarbonyl)pyridinium 2,4,6-trimethylbenzenesulfonate (24) (1.15 g, 3.1 mmol), benzyl 3-phenylpropiolate (0.74 g, 3.1 mmol), and K2CO3 (434 mg, 3.1 mmol) in THF (30 mL) were stirred at room temperature for 18 h. The inorganics were removed by filtration, and the filtrate was concentrated under reduced pressure. The crude residue was dissolved in toluene (50 mL) and heated at reflux for 4 h and then concentrated under reduced pressure and purified by column chromatography on silica eluting with 20% EtOAc/petroleum ether to yield the desired material (25) (184 mg, 15%). 1H NMR (300 MHz, DMSO-d6, 300 K) δ 8.36 (1H, d, J = 5.5 Hz), 8.25 (1H, d, J = 7.1 Hz), 7.83 (1H, m), 7.58 (1H, d, J = 6.9 Hz), 7.78–7.63 (4H, m), 7.59 (1H, d, J = 5.5 Hz), 7.48–7.27 (7H, m), 5.32 (2H, s), 4.47 (2H, q, J = 7.2 Hz), 1.19 (3H, t, J = 7.2 Hz). MS (ES) C24H20N2O4 requires 400 found 401 (M + H+)·

Ethyl 2-Phenylpyrazolo[1,5-a]pyridine-7-carboxylate (26). The substrate 25 (184 mg, 0.46 mmol) was taken up in EtOH (20 mL) and EtOAc (20 mL) and hydrogenated in the presence of 10% Pd/C (75 mg) under an H2 atmosphere for 4 h. The catalyst was filtered off and the solvent concentrated under reduced pressure to yield 2-phenylpyrazolo[1,5-a]pyridine-3-carboxylic acid (130 mg, 91%). MS (ES) C14H11N4O2 requires 239 found 240 (M + H+)·

2-Phenylpyrazolo[1,5-a]pyridine-7-carboxylate (27). Ethyl 2-phenylpyrazolo[1,5-a]pyridine-7-carboxylate (26) was converted to 2-phenylpyrazolo[1,5-a]pyridine-7-carboxamide according to general method C and then purified by column chromatography on silica eluting with 50% EtOAc/petroleum ether to yield the titled compound (34 mg, 34%) as a white solid.

1-Amino-(2-ethoxycarbonyl)pyridinium 2,4,6-trimethylbenzenesulfonate (24) (1.15 g, 3.1 mmol), benzyl 3-phenylpropiolate (0.74 g, 3.1 mmol), and K2CO3 (434 mg, 3.1 mmol) in THF (30 mL) were stirred at room temperature for 18 h. The inorganics were removed by filtration, and the filtrate was concentrated under reduced pressure. The crude residue was dissolved in toluene (50 mL) and heated at reflux for 4 h and then concentrated under reduced pressure and purified by column chromatography on silica eluting with 20% EtOAc/petroleum ether to yield the desired compound (1.15 g, 95%). 1H NMR (300 MHz, DMSO-d6, 300 K) δ 8.91 (1H, d, J = 5.5 Hz), 8.48 (1H, d, J = 5.5 Hz), 8.36 (1H, t, J = 5.5 Hz), 8.16 (1H, t, J = 5.5 Hz), 6.71 (2H, s), 4.48 (2H, q, J = 7.2 Hz), 2.52 (6H, s), 2.29 (3H, s), 1.39 (3H, q, J = 7.2 Hz).

Benzyl 7-Carbamoyl-2-phenylpyrazolo[1,5-a]pyridine-3-carboxylate (25). 1-Amino-(2-ethoxycarbonyl)pyridinium 2,4,6-trimethylbenzenesulfonate (24) (1.15 g, 3.1 mmol), benzyl 3-phenylpropiolate (0.74 g, 3.1 mmol), and K2CO3 (434 mg, 3.1 mmol) in THF (30 mL) were stirred at room temperature for 18 h. The inorganics were removed by filtration, and the filtrate was concentrated under reduced pressure. The crude residue was dissolved in toluene (50 mL) and heated at reflux for 4 h and then concentrated under reduced pressure and purified by column chromatography on silica eluting with 20% EtOAc/petroleum ether to yield the desired compound (1.15 g, 95%). 1H NMR (300 MHz, DMSO-d6, 300 K) δ 8.91 (1H, d, J = 5.5 Hz), 8.48 (1H, d, J = 5.5 Hz), 8.36 (1H, t, J = 5.5 Hz), 8.16 (1H, t, J = 5.5 Hz), 6.71 (2H, s), 4.48 (2H, q, J = 7.2 Hz), 2.52 (6H, s), 2.29 (3H, s), 1.39 (3H, q, J = 7.2 Hz).
pressure gave methyl 2-phenyl[1,2,4]triazolo[1,5-α]pyrimidine-5-carboxylic acid (29) as a brown oil which was used in the next step without further purification. 1H NMR (400 MHz, DMSO-d6, 298 K), δ 9.9 (1H, d, J = 6.4 Hz), 8.25 (1H, d, J = 7.2 Hz), 9.25 (2H, dd, J = 8.0 and 2.0 Hz), 7.94–7.89 (2H, m), 7.31 (2H, d, J = 5.6 Hz), 3.97 (3H, s). MS (ES) C14H12N5O2 requires 253, found 254 (M + H)+.

2-Phenyl[1,2,4]triazolo[1,5-α]pyrimidine-5-carboxamide (30). Methyl 2-phenyl[1,2,4]triazolo[1,5-α]pyrimidine-5-carboxylate (29) (1 mmol) was converted according to method C to give 2-phenyl[1,2,4]triazolo[1,5-α]pyrimidine-5-carboxamide (30) (63 mg, 26% over two steps) as a white solid. 1H NMR (400 MHz, DMSO-d6, 300 K) δ 9.20 (1H, d, J = 6.0 Hz), 8.96 (1H, br s), 8.33–8.28 (3H, m), 8.21 (1H, br s), 7.60–7.55 (3H, m), 7.38 (1H, t, J = 7.2 Hz). HRMS (ESI) m/z calculated for C15H13N4O2 239.0927, found 239.0926. MS (ESI) C15H13N4O2 requires 328, found 239 (M + H)+.

Methyl 2-(4-formylphenyl)-2H-indazole-7-carboxylate (32). 32 was prepared according to general method D using methyl 2H-indazole-7-carboxylate (31) (0.3 g, 1.7 mmol) and 4-fluorobenzaldehyde (235 μL, 2.2 mmol) to give, after purification by flash column chromatography on silica using a gradient of 30–40% EtOAc/petroleum ether, methyl 2-(4-formylphenyl)-2H-indazole-7-carboxylate (32) (185 mg, 39%). 1H NMR (400 MHz, CDC13, 300 K) δ 10.08 (1H, s), 8.64 (1H, s), 8.20 (2H, d, J = 8.6 Hz), 8.16 (1H, d, J = 7.2 Hz), 8.05 (2H, d, J = 8.6 Hz), 7.96 (1H, d, J = 7.2 Hz), 7.21 (1H, t, J = 7.2 Hz), 7.40 (3H, s). MS (ESI) C21H17N3O2 requires 280, found 281 (M + H)+.

2-(4-Formylphenyl)-2H-indazole-7-carboxamide (35). 35 was prepared according to general method D using 2H-indazole-7-carboxamide (34) (0.8 g, 5.0 mmol) and 4-fluorobenzaldehyde (685 μL, 6.4 mmol) to yield, after purification by flash column chromatography on silica with 10% MeOH/DCM, 2-(4-formylphenyl)-2H-indazole-7-carboxamide (35) (0.59 g, 45%). 1H NMR (400 MHz, DMSO-d6, 300 K) δ 10.10 (1H, s), 9.50 (1H, s), 8.52 (1H, br s), 8.45 (2H, d, J = 8.6 Hz), 8.15 (2H, d, J = 8.6 Hz), 8.08 (1H, d, J = 7.2 Hz), 8.04 (1H, d, J = 7.2 Hz), 7.92 (1H, br s), 7.30 (1H, t, J = 7.2 Hz). MS (ESI) C16H17N4O2 requires 265, found 266 (M + H)+.

2-(4-Acetylphenyl)-2H-indazole-7-carboxamide (36). 36 was prepared following general method D using 2H-indazole-7-carboxamide (34) (322 mg, 2 mmol) and 4-acetylbenzaldehyde (332 mg, 2 mmol) to afford 2-(4-acetylphenyl)-2H-indazole-7-carboxamide (36) (0.50 mg, 0.18 mmol) and 2.0 M NH4OH in MeOH (0.71 mL, 1.4 mmol) to give methyl 2-[4-(dimethylamino)ethyl]phenyl]-2H-indazole-7-carboxylate (33a) which was used in the next step without further purification. MS (ESI) C17H17N4O requires 309, found 310 (M + H)+. Methyl 2-[4-(dimethylamino)ethyl]phenyl]-2H-indazole-7-carboxamide (33a) (55 mg, 0.18 mmol) was treated according to general method C to give, after purification by flash column chromatography on silica with 10% MeOH/DCM, 2-[4-(dimethylamino)ethyl]phenyl]-2H-indazole-7-carboxylate (37) (40 mg, 76%). 1H NMR (300 MHz, CD3CN, 300 K) δ 8.85 (1H, s), 8.78 (1H, br s), 8.17 (1H, d, J = 8.0 Hz), 8.05–8.00 (3H, m), 7.56 (2H, d, J = 8.4 Hz), 7.28 (1H, d, J = 8.0 Hz), 7.26 (1H, br s), 3.56 (2H, t), 2.28 (6H, s). HRMS (ESI) m/z calculated for C18H19N4O requires 315, found 315 (M + H)+.

2-[4-(4-Amino-2-phenyl-1H-indazol-2-yl)phenyl]-N-methylthienamidinium Chloride (43). Methyl 2-[4-(4-phenylamino)phenyl]-
To a solution of 2-[4-[1-(formylamino)-1-methylethyl]-phenyl]-2H-indazole-7-carboxamide (60 mg, 0.19 mmol) in THF (6 mL) was added BH$_3$-THF (1 M in THF, 0.28 mL, 0.28 mmol), and the mixture was stirred for 24 h at room temperature. Then saturated aqueous NaHCO$_3$ solution was added and the mixture was extracted with EtOAc. The combined organic phase was dried over MgSO$_4$. Evaporation of the solvent under reduced pressure gave a residue that was purified by RP-HPLC (column, C$_{18}$, using H$_2$O (0.1% TFA) and MeCN (+0.1% TFA) as eluents, to afforded 2-[4-([aminocarbonyl]-2H-indazol-2-yl)-phenyl]-N-methylpropan-2-amin trifluoroacetate (50) (8 mg, 10%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$, 300 K) $\delta$ 9.38 (s, 1H), 9.14 (1H, br s), 8.53 (1H, br s), 8.27 (2H, d, $J$ = 8.7 Hz), 8.06-8.01 (2H, m), 7.88 (1H, br s), 7.79 (2H, d, $J$ = 8.7 Hz), 7.28 (1H, t, $J$ = 8.2 Hz), 2.36 (3H, s), 1.73 (6H, s). HRMS (ESI) m/z calcd for C$_{19}$H$_{20}$N$_4$O requires 308, found 309 (M + H)$^+$. 

Methyl 2-[4-[1-(tert-Butyloxycarbonyl)piperidin-3-ylphenyl]-2H-indazole-7-carboxylate (7i). 7i was prepared following the general methods A and B using methyl 3-formyl-2-nitrobenzoate (5) (1.0 g, 4.8 mmol) and N-Boc-3-(4-aminophenyl)piperidine (1.45 g, 5.3 mmol). The crude residue was purified by flash column chromatography on silica using a gradient of 20–40% EtOAc/petroleum ether to yield the desired 7i (0.95 g, 46%) as a yellow solid. $^1$H NMR (400 MHz, CDCl$_3$, 300 K) $\delta$ 8.51 (1H, d, $J$ = 6.8 Hz), 7.91 (1H, d, $J$ = 6.8 Hz), 7.70 (2H, m), $J$ = 8.2 Hz), 7.31 (2H, m), 7.00 (1H, br s), 4.40-4.05 (2H, m), 2.90-2.70 (3H, m), 2.15-2.00 (1H, m), 1.85-1.75 (1H, m), 1.67-1.55 (9H, s). MS (ESI) C$_{27}$H$_{29}$N$_4$O$_3$ requires 435, found 436 (M + H)$^+$. 

tert-Butyl 3-[4-[7-(Aminocarbonyl)-2H-indazol-2-yl]phenyl]piperidine-1-carboxylate (55). The title compound was prepared according to general method C from 7i (0.95 g, 2.18 mmol), and the crude product was purified by crystallization with EtOAc to give the desired product (55) (0.75 g, 82%) as a yellow solid. $^1$H NMR (400 MHz, CDCl$_3$, 300 K) $\delta$ 0.004, MeOH). HRMS (ESI) m/z calcd for C$_{19}$H$_{21}$N$_4$O requires 320, found 321 (M + H)$^+$. 

Alkaloid preparation of (3R)-3-[4-[7-(Aminocarbonyl)-2H-indazol-2-yl]phenyl]piperidininium Chloride (57) and (3S)-3-[4-[7-(Aminocarbonyl)-2H-indazol-2-yl]phenyl]piperidininium Chloride (56). The racemate 16 was separated by chiral SFC purification using CO$_2$ as supercritical eluent: column, Chiralpak AS-H, 1 mm x 25 mm.; flow = 10 mL/min; $T_{	ext{chiral}}$ = 35 °C; $P_{\text{chiral}}$ = 100 bar; modifier, 55% MeOH containing 4% Et$_3$NH. Retention time of the first eluting enantiomer was 48.80 min. Evaporation of the solvent followed by lyophilization gave 2-[(3R)-piperidin-3-ylphenyl]-2H-indazole-7-carboxamide as a white powder (99.0% ee). Retention time of the second eluting enantiomer was 65.11 min. Evaporation of the solvent followed by lyophilization gave 2-[(3S)-piperidin-3-ylphenyl]-2H-indazole-7-carboxamide as a white powder (99.25% ee). $^1$H NMR (400 MHz, DMSO-$d_6$, 300 K) $\delta$ 9.28 (1H, s), 8.57 (1H, br s), 8.06 (2H, d, $J$ = 7.2 Hz), 8.04 (2H, d, $J$ = 8.4 Hz), 7.78 (1H, br s), 7.49 (2H, d, $J$ = 8.4 Hz), 7.27 (1H, dd, $J$ = 8.4, 7.2 Hz), 3.08-2.94 (2H, m), 2.77-2.67 (1H, m), 2.64-2.52 (1H, m), 1.98-1.90 (1H, m), 1.75-1.47 (4H, m). MS (ESI) C$_{19}$H$_{20}$N$_4$O requires 320, found 321 (M + H)$^+$. The chlorohydrate salts of both enantiomers were prepared by stirring a solution of the free base in 1 N HCl for 15 min and lyophilization of the resulting solution, affording the corresponding hydrochloride salts as a pale-yellow solids.

(3R)-3-[4-[7-(Aminocarbonyl)-2H-indazol-2-yl]phenyl]piperidininium Chloride (57), [c$a_{3}$$_{00}$] +133.3 (+0.004, MeOH). HRMS (ESI) m/z calcd for C$_{19}$H$_{21}$N$_4$O$_3$ 321.1710, found 321.1711.

(3S)-3-[4-[7-(Aminocarbonyl)-2H-indazol-2-yl]phenyl]piperidininium Chloride (56), [c$a_{3}$$_{00}$] −137.9 (-0.004, MeOH). $^1$H NMR (600 MHz, DMSO-$d_6$, 300 K) $\delta$ 9.32 (1H, s), 9.30 (1H, br s), 8.13 (2H, d, $J$ = 8.5 Hz), 8.06 (1H, d, $J$ = 7.0, 0.8 Hz), 8.02 (1H, d, $J$ = 8.3, 0.8 Hz), 7.90 (1H, br s), 7.55 (2H, d, $J$ = 8.5 Hz), 7.27 (1H, d, J = 8.3, 7.0 Hz), 3.35–3.29 (2H, m), 3.16–3.06 (2H, m), 2.92 (1H, m), 1.95–1.74 (4H, m). $^1$C NMR (150 MHz, DMSO-$d_6$, 300 K) $\delta$ 165.5, 146.1, 142.2, 138.2, 129.7, 128.5, 125.3, 123.5, 123.4, 121.9, 121.5, 120.9, 47.5, 42.8, 38.7, 29.4, 22.1. HRMS (ESI) m/z calcd for C$_{19}$H$_{20}$N$_4$O$_3$ 321.1712, found 321.1712. MS (ESI) C$_{19}$H$_{20}$N$_4$O$_3$ requires 320, found 321 (M + H)$^+$. 

Chlorohydrate solution of the free base in 1 N HCl for 15 min and lyophilization of the resulting solution, affording the corresponding hydrochloride salts as a pale-yellow solids.
form. The mixture was heated to reflux and then left to cool to room temperature and allowed to stand for 2 days. The resulting crystals (21.7 g) were filtered off and dried. Three recrystallizations from the EtOAc solution of the original material provided a yield of 38.8% (2.3 g, 517 mg) of (3S)-3-[(1-tert-Butyloxy carbonyl)piperidin-3-yl]phenyl]-2H-indazole-7-carboxylate (7k) (3.4 g, 46% over two steps) as a yellow solid. 1H NMR (400 MHz, CDCl3, 300 K)  9.04 (1H, br s), 8.51 (1H, s), 8.31 (1H, d, J = 6.8 Hz), 7.91 (1H, d, J = 8.3 Hz), 7.84 (2H, d, J = 8.2 Hz), 7.42 (2H, d, J = 8.2 Hz), 7.31—7.22 (1H, m), 5.95 (1H, br s), 4.70–4.05 (2H, m), 2.90–2.70 (3H, m), 2.15–2.00 (1H, m), 1.85–1.75 (1H, m), 1.75–1.50 (2H, m), 1.48 (9H, s). MS (ES) C26H26N2O4 requires 420, found 421 (M + H)+.  

Supporting Information Available: Additional experimental procedures for the biological assays including protocols for the PARP isoforms and proliferation assays in additional cell lines; general experimental details for the chemistry procedures including synthetic procedures for compounds 9–15, 17, 38–42, 44–47, 52, and 54; purity analysis results of the final compounds; and Mosher’s amide analysis of the stereochimistry of 56. This material is available free of charge via the Internet at http://pubs.acs.org.

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Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose)polymerase.


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