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Development of substituted 6-[4-fluoro-3-(piperazin-1-ylcarbonyl)benzyl]-4,5-dimethylpyridazin-3(2*H*)-ones as potent poly(ADP–ribose) polymerase-1 (PARP-1) inhibitors active in BRCA deficient cells

Federica Ferrigno *, Danila Branca, Olaf Kinzel, Samuele Lillini, Laura Llauger Bufi, Edith Monteagudo, Ester Muraglia, Michael Rowley, Carsten Schultz-Fademrecht, Carlo Toniatti, Caterina Torrisi, Philip Jones

IRBM, Merck Research Laboratories Rome, Via Pontina km 30,600, Pomezia 00040, Rome, Italy

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SAR exploration
Pharmacokinetic properties

ABSTRACT

We describe an extensive SAR study in the 6-[4-fluoro-3-(substituted)benzyl]-4,5-dimethylpyridazin-3(2*H*)-one series which led to the identification of potent PARP-1 inhibitors, capable of inhibiting the proliferation of BRCA-1 deficient cancer cells in the low nanomolar range, and displaying >100-fold selectivity over the BRCA wild type counterparts. The series of compounds was devoid of hERG channel activity, and CYP inhibition and induction liabilities. Several analogs were stable in rat and human liver microsomes and displayed moderate rat clearance, with urinary excretion of parent as the major route of elimination.

Poly(ADP–ribose) polymerase (PARP)-1 is the main isoform of the PARP family, which include some 18 proteins.[1] PARP catalyses the polymerization of NAD^+ into long poly(ADP–ribose) chains. PARP-1 is one of the most abundant nuclear enzymes and has a well established role in DNA repair processes.[2] PARP inhibitors have been used in cancer therapy as both chemo- and radio-sensitizers,[3] and more recently, have been employed alone in treatment of tumors displaying BRCA mutations.[4]

Several PARP inhibitors are currently on-going clinical trials including: AZD-2281,[5] ABT-888,[6] AG-014699,[7] and MK-4827,[8] as well as the undisclosed BS-201 and INO-1001.

We have recently described the identification of a novel series of pyrrolo[1,2-*a*]pyrazin-1(2*H*)-one PARP inhibitors characterized by **A1** and **A2** (Fig. 1).[9]

Both compounds were potent PARP-1 inhibitors (IC_{50} <2.5 nM) with good antiproliferative activity against BRCA-1 deficient HeLa cells (CC_{50} = 48 and 53 nM, respectively) and displaying >100-fold selective cytotoxicity over BRCA wild type cells.

Despite these encouraging characteristics this series suffered from some off-target activities and poor rat pharmacokinetics. In particular, weak binding to the hERG channel, and micromolar CYP inhibition were observed. Furthermore, **A2** also caused modest CYP induction at 10 μM. The pharmacokinetic properties were also suboptimal with moderate–high intrinsic clearance in rat liver microsomes and in rats clearance in excess of hepatic blood flow was measured. With the aim to solve these issues while maintaining PARP inhibition activity, we extensively worked to modify the heterocyclic scaffold, hypothesizing that the electron rich pyrrole motif could be the cause of metabolic instability. We also made an extensive SAR study on the right end side part of our scaffold supposing that the lipophilic substituents caused the unwanted off-target activities.

In this Letter we describe the identification and optimization of a new series of dimethylpyridazin-3(2*H*)-one PARP inhibitors **B** leading to very potent analogs with clean off-target profiles and improved pharmacokinetic properties both in vitro and in vivo in rats.

We prepared two common precursors which allowed us to introduce diversity at the late stage of the synthesis: 5-[(4,5-dimethyl-6-oxo-1,6-dihydropyridazin-3-yl)methyl]-2-fluorobenzoic acid (**5**), and 6-[4-fluoro-3-(piperazin-1-ylcarbonyl)benzyl]-4,5-dimethylpyridazin-3(2*H*)-one (**6**) (Scheme 1).

In brief, the 3,6-dichloro-4,5-dimethylpyridazine (**1**) was condensed with 5-(cyanomethyl)-2-fluorobenzonitrile (**3**) to afford

* Corresponding author.
E-mail address: federica_ferrigno@libero.it (F. Ferrigno).

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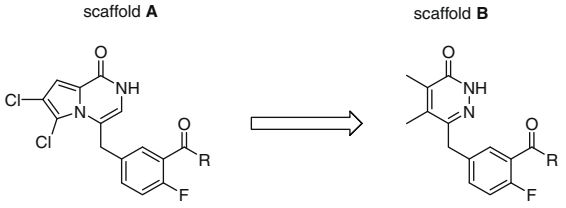
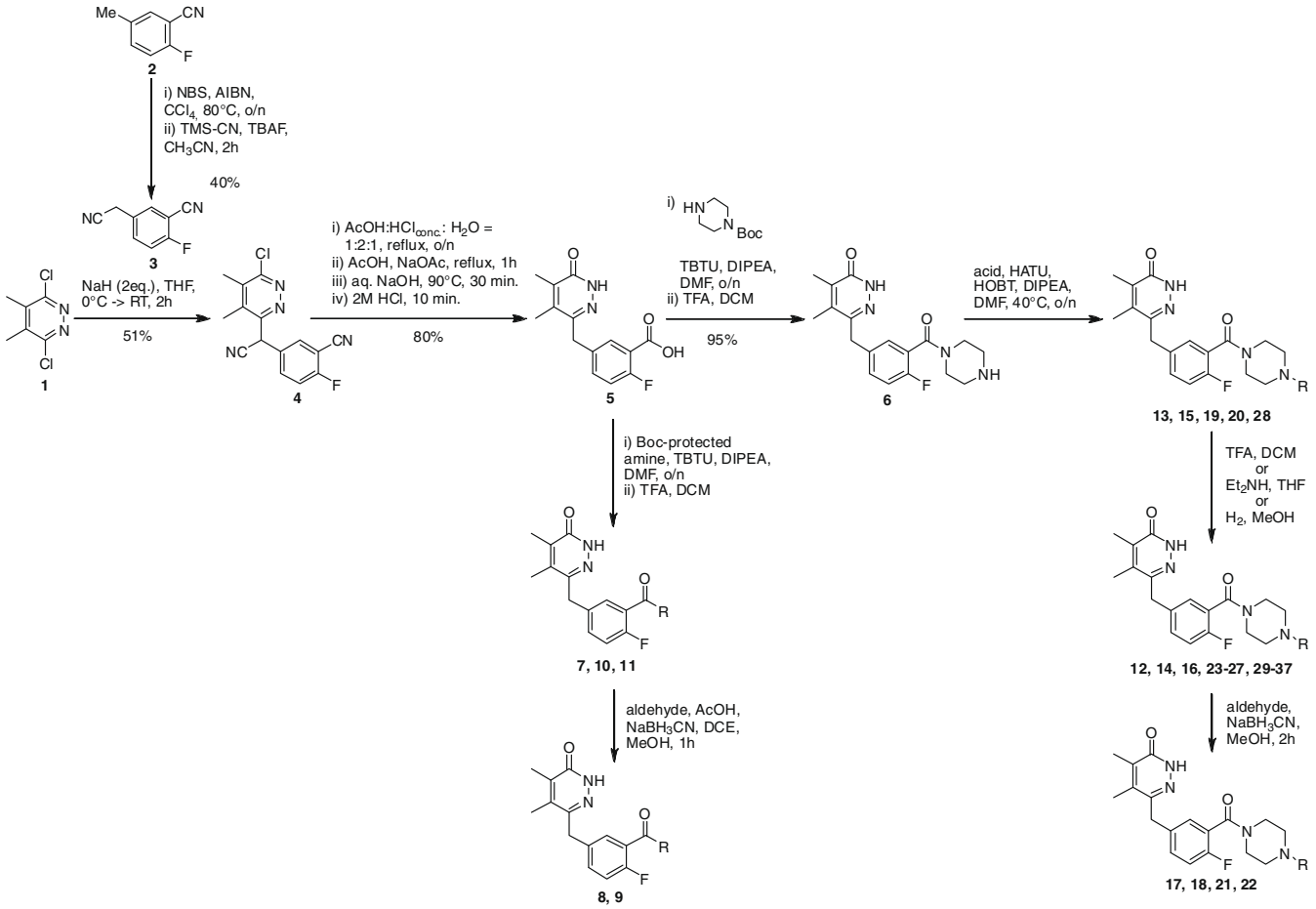


R		
Compds	**A1**	**A2**
PARP-1 IC_{50} (nM)	2.5	2.0
BRCA1- CC_{50} (nM)	48	53
BRCAwt CC_{50} (nM)	>5000	>5000
Rat liver microsomes Cl_{int} (μl/min/mg)	65	122
Rat Clearance (mL/min/kg)	130	>200
hERG IC_{50} (nM)	12000	11000
CYP inhibition (%inh. at 10μM)	2D6: 35	3A4: 25
CYP induction (at 10μM)	-	14% rifampicin response

Figure 1. Profile of pyrrolo[1,2-*a*]pyrazin-1(2*H*)-one PARP inhibitors **A1** and **A2** and the modification to novel series of dimethylpyridazin-3(*2H*)-ones **B**.

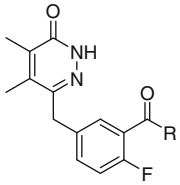


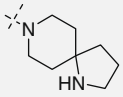
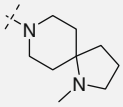
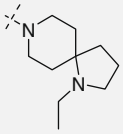
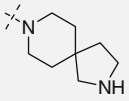
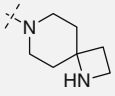
Scheme 1. Synthesis of compounds **1**–**37**.

intermediate **4** using 2 equiv of NaH.[10] Compound **3** was prepared from 2-fluoro-5-methylbenzonitrile (**1**) by radical mono-bromination followed by displacement with TMS-CN in the presence of TBAF. The chloro-dimethylpyridazine **4** was then submitted to full hydrolysis and decarboxylation by a three step sequence which consist of hydrolysis and decarboxylation of the cyano group in the benzylic position, followed by formation of the pyridazinone core, and hydrolysis of the fluorobenzonitrile to give the desired carboxylic acid scaffold **5**.[11] This intermediate was used to obtain the compounds listed in Table 1 using a standard coupling procedure followed by Boc-deprotection and reductive amination. Alternatively, the carboxylic acid intermediate **5** was coupled with *tert*-butyl piperazine-1-carboxylate to install the piperazine moiety and obtain, after Boc-removal, the intermediate **6**. This piperazine was employed for the synthesis of the compounds described in Tables 2 and 3 by coupling reactions with the appropriate carboxylic acids, followed by removal of the N-protecting group and reductive amination to add the alkyl fragments. Compound **22** was obtained from the Fmoc protected amino acid, as the cyclobutyl fragment was discovered to be unstable under acidic conditions required for *N*-Boc cleavage. The coupling reaction of **6** with the sterically hindered carboxylic acids proved to be slow, and was optimized using a combination of HATU and HOBT with gentle heating for extended reaction time.[12]

The replacement of the pyrrolo[1,2-*a*]pyrazin-1(*2H*)-one scaffold **A** by the dimethylpyridazin-3(*2H*)-one **B** was beneficial and we prepared the analog of **A1** (**7**) in the new series (Table 1). The *spiro*-derivative **7** showed comparable activity to **A1** both in the PARP-1 enzyme and in inhibiting the proliferation of BRCA-1 deficient cells (IC_{50} = 2.6 nM, CC_{50} = 49 nM vs IC_{50} = 2.5 nM, CC_{50} = 48 nM). Similarly **7** maintained the excellent selectivity seen with **A1**, displaying

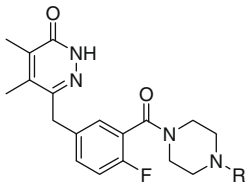
Table 1
Exploration of the *spiro*-bicycle motif in the amide portion of **5**

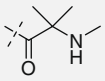
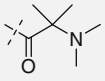
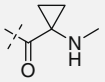
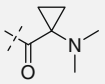
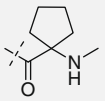
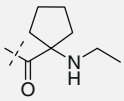
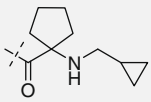
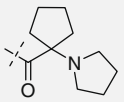
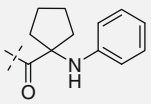
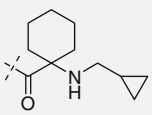
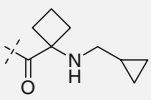


Compds	R	PARP-1 IC_{50}[a,b] (nM)	BRCA1-CC_{50}[c,b] (nM)	BRCAwt CC_{50}[d,b] (nM)
7		2.6	49	>5000
8		4.5	100	>5000
9		9.7	170	>5000
10		4.7	>5000	>5000
11		4.6	>5000	>5000

[a,c,d] The assays were performed as described in Ref. [8].
[b] Values are means of at least two experiments.

Table 2
SAR study in the α,α-disubstituted amino acid series



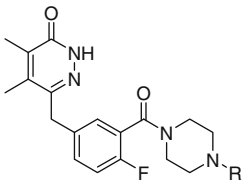
Compds	R	PARP-1 IC_{50}[a,b] (nM)	BRCA1-CC_{50}[c,b] (nM)	BRCAwt CC_{50}[d,b] (nM)
12		3.7	280	>5000
13		1.8	360	> 2500
14		3.6	240	>5000
15		5.7	350	7700
16		2.7	81	>5000
17		3.1	91	4000
18		2.4	71	2100
19		2.7	89	1500
20		3.9	130	870
21		3.5	170	1900
22		3.2	24	450

[a–d] See footnotes of Table 1.

>100-fold selective cytotoxicity for BRCA-1 deficient cells compared to their wild type counterparts (CC_{50} >5 μM). In contrast, unlike **A1** which displayed multiple off-targets activities, **7** was devoid of binding to the hERG channel and CYP inhibition or induction at 10 μM. Furthermore, **7** was found to be extremely stable in rat and human liver microsomes (Cl_{int} <1 μl/min/mg). In spite of this, when profiled in vivo in rats, **7** showed a high plasma clearance (Cl = 57 mL/min/kg) with a $t_{1/2}$ of 3.7 h mainly due to a high volume of distribution (Vd_{ss} = 6.7 L/kg). Routes of elimination studies revealed that excretion of parent to urine was an important route of clearance, with 37% of the dose excreted intact in the first 24 h following iv dosing.

Starting from **7** we tried to modify the *spiro*-bicycle motif with the aim to improve first the cellular activity, and eventually the PK

Table 3
SAR study in the α-substituted proline derivative series



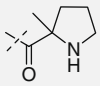
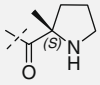
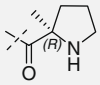
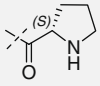
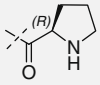
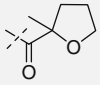
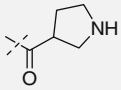
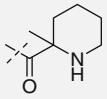
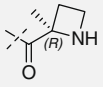
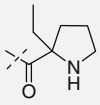
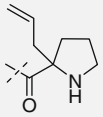
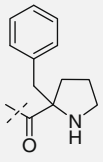
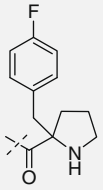
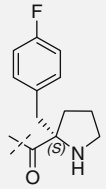
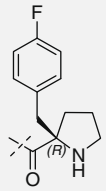
Compds	R	PARP-1 IC_{50}[a,b] (nM)	BRCA1-CC_{50}[c,b] (nM)	BRCAwt CC_{50}[d,b] (nM)
23		2.8	90	>5000
24		3.8	780	>40,000
25		1.3	45	>5000
26		3.1	3000	>10,000
27		2.7	220	>10,000
28		2.8	380	11,000
29		3.9	>5000	>5000
30		12	2000	>20,000
31		3.1	78	>40,000
32		3.8	24	16,000
33		1.8	31	19,000
34		3.3	53	>5000
35		3.6	9.1	2700

Table 3 (*continued*)

Compds	R	PARP-1 IC_{50}[a,b] (nM)	BRCA1-CC_{50}[c,b] (nM)	BRCAwt CC_{50}[d,b] (nM)
36		4.2	290	15,000
37		2.5	15	3300

[a-d] See footnotes of Table 1.

profile. Compared to the lead **7**, the N-methylated derivative **8** was a less potent PARP-1 inhibitor and displayed reduced antiproliferation effect on BRCA-1 deficient cells. The introduction of larger lipophilic substituents, as in **9**, further decreased the activity both in the enzyme and the cell based assays and confirmed the important role of the free *NH* moiety. Shifting the *NH* to the β position of the *spiro*-centre resulted in **10**, which although maintained enzyme activity (IC_{50} = 4.7 nM), was devoid of activity in cells at 5 μM. The same result was obtained by changing the five-membered cycle to a fused azetidine, as in **11**.

Although **7** displayed an encouraging profile, we were unable to further improve the antiproliferation activity of this series, so we decided to turn our attention to the amino acid derivative **A2,** and accordingly analog **12** was prepared (Table 2).

As seen previously with the pair **A1** and **7**, **12** displayed comparable enzyme activity to the pyrrolo[1,2-*a*]pyrazin-1(*2H*)-one **A2** (IC_{50} = 3.7 and 2.0 nM, respectively), although it was fivefold weaker than **A2** in inhibiting the proliferation of BRCA-1 deficient cells (CC_{50} = 280 nM compared to 53 nM for **A2**). More importantly the pyridazinone **12** was also devoid of off-target cardiac ion channel activity, and CYP inhibition or induction at 10 μM. Similarly to **7**, the amino acid derivative **12** displayed good stability in rat and human liver microsomes (Cl_{int} = 7 and 10 μl/min/mg) but high plasma clearance in rats (Cl = 64 mL/min/kg). Equally to **7**, there was an extensive excretion of the parent in urine, 34% of the iv dose excreted as parent in first 24 h.

Driven by the results of **12** an extensive SAR study was undertaken. Unsurprisingly, the cyclopropyl analog **14** was comparable to **12** both in enzyme and cell based assays, but the enlargement to the cyclopentyl **16** resulted in an improvement in activity (IC_{50} = 2.7 nM) and encouragingly **16** displayed double digit antiproliferation activity against BRCA1 deficient cells (CC_{50} = 81 nM) with greater that 60-fold selectivity. The addition of a second methyl on the nitrogen in **13** and **15** was slightly detrimental for the activity, demonstrating once again the importance of a secondary amine moiety in this region of the molecule. The homologation of the methyl group of **16** to an ethyl in **17,** or to a cyclopropyl in **18** caused only slight changes in enzyme potency and cell based activity. Unfortunately the introduction of a larger and more lipophilic substituent in this region of the molecule, as the methylcyclopropyl group, resulted in a loss of selectivity between BRCA-1 deficient and wild type cells, with **18** displaying only 30-fold selectivity compared to the 60-fold selectivity of **16**. This trend was confirmed by the pyrrolidine analog **19**, which showed only 15-fold selectiv-

ity, and the *N*-phenyl derivative **20**, which was the least selective compound of this series, displaying only sixfold selectivity. The replacement of the cyclopentyl fragment with the corresponding cyclohexyl in **21** was detrimental for both enzyme and cellular activity. Reducing the ring size to the corresponding cyclobutyl **22** produced instead a more potent compound. In fact **22** displayed a threefold improvement in the BRCA-1 deficient cells assay compared to **18**, but with only 20-fold selectivity.

The threefold boost in cellular activity seen when we cyclised the *gem*-dimethyl group of **12** to the corresponding cyclopentyl of **16** drove us to explore if a similar result could be obtained by cyclising the *gem*-dimethyl group onto the *N*-methyl amino group, thereby forming a pyrrolidine or related derivative (Table 3).

Indeed, the α-methyl proline derivative **23** was a more potent PARP-1 inhibitor than **12** (IC_{50} = 2.8 and 3.7 nM, respectively), and, similarly to what we previously observed by cyclising to **16**, **12** showed a threefold improvement in cell based activity (CC_{50} = 90 nM for **23** compared to 280 nM for **12**). Moreover, **23** was completely stable in presence of rat and human liver microsomes (Cl_{int} <1 μl/min/mg), and, when dosed in rats, it showed only a moderate plasma clearance (Cl = 38 mL/min/kg). Further studies revealed that the compound was essentially cleared intact with 82% of the iv dose being eliminated in the urine as parent within 72 h, and a further 3% in the bile. Trace amounts of **6** were also detected as a minor elimination route. Given that the clearance value is significantly higher than the renal glomerular filtration rate an active transport mechanism is suspected.

Given the encouraging profile of **23**, we first elucidated which features of the proline residue were essential for the activity. Preparation of the enantiomers of **23** revealed that the (*R*)-**25** was more active than the enantiomer **24** both in PARP-1 enzyme (IC_{50} = 1.3 vs 3.8 nM) and antiproliferation (CC_{50} = 45 and 780 nM, respectively) assays. The (*R*)-enantiomer **25** was one of the most interesting compounds and displayed more than 100-fold selectivity between BRCA-1 deficient and wild type cells. The same difference was observed for other pairs of enantiomers with the (*R*)-enantiomer displaying at least 10-fold improved activity in the BRCA-1 deficient cells assay (**26** and **27**; **36** and **37**). Removal of the α-methyl group produced in **27** a fivefold drop in antiproliferation activity compared to **25**, demonstrating the importance of the steric crowding in the α-position of the carbonyl. Other important features in this series for both the enzyme and the cell based assays activity included the presence of the free *NH*, as shown by the replacement of the *NH* with an oxygen in tetrahydrofuran **28**, which was fourfold less potent than **23** in cells and only displayed 30-fold selectivity between BRCA deficient and proficient cells. Similarly, the position of the nitrogen in the cycle was critical for cell based activity, as pyrrolidin-3-yl derivative **29** was devoid of antiproliferation activity in BRCA-1 deficient cells at 5 μM. As in the previous series the size of the ring was important for activity, where the six-membered ring analog **30** was fourfold less potent in the PARP-1 assay compared to pyrrolidine **23**. Interestingly, the azetidine analog **31** retained most of the activity of pyrrolidine derivative **25**, being twofold less active in cells with CC_{50} = 78 nM, but displaying excellent selectivity. Moreover **31** was stable when incubated with rat and liver microsomes (Cl_{int} <1 and 3 μl/min/mg, respectively) and it displayed moderate plasma clearance in rats (Cl = 23 mL/min/kg), with 11% of the iv dose excreted intact in the urine as parent in the first 24 h.

Given the excellent cell based activity and selectivity seen with the α-methyl proline derivative **25**, a further exploration was conducted for the replacement of the α-methyl substituent. Here we obtained compounds, such as the ethyl derivative **32** and the allyl analog **33**, which improved cellular activity compared to the racemic compound **23**, with both **32** and **33** displaying low nanomolar antiproliferation activity in BRCA-1 deficient cells (CC_{50} = 24 and 31 nM, respectively). The ethyl analog **32** displayed good stability in presence of rat and human liver microsomes (Cl_{int} = 6 and 13 μl/min/mg, respectively), but unfortunately it showed high plasma clearance in rats (Cl = 63 mL/min/kg). The introduction of the simple benzyl substituent in **34** caused a modest improvement in cellular activity compared to the racemic α-methyl derivative **23**, with **34** displaying CC_{50} = 53 nM and more than 100-fold selectivity. Further exploration of **34** demonstrated that when we installed the *para*-fluoro benzyl group in **35** we obtained a further improvement in cellular activity with antiproliferation activity in the BRCA-1 deficient cells in the low nanomolar range (CC_{50} = 9.1 nM). Again the majority of the activity resided in one enantiomer and, when the corresponding enantiomers **36** and **37** were prepared, the (*R*)-enantiomer displayed superior cellular activity compared to the (*S*)-enantiomer (CC_{50} = 10 and 290 nM, respectively). Unfortunately this class of compounds, bearing an α-benzyl proline residue, was metabolically unstable, displaying high intrinsic clearance when compounds were incubated with rat and human liver microsomes (**34**: Cl_{int} = 101 and 50 μl/min/mg, respectively). Probably the additional lipophilicity was also the source of some CYP3A4 inhibition (**35**: 73% inh. at 10 μM), although **35** remained devoid of hERG activity and CYP induction liabilities.

Nevertheless the issue of interesting analogs, as **23**, **31** and **32**, was the lack of oral bioavailability (*F* <3%). This was probably due to the lack of absorption, as confirmed for compound **23** that remained undetected after sampling of the hepatic portal vein of rats dosed orally.

In summary, we have developed a new class of PARP-1 inhibitors with good enzyme activity and demonstrating low nanomolar antiproliferative activity in BRCA-1 deficient cells. Our compounds showed excellent selectivity between BRCA1 deficient and wild type cells, displaying no off-targets activities on cardiac ion channels and no CYP liabilities. Several compounds showed good stability in liver microsome incubations and acceptable clearance in rats, being eliminated unchanged in the urine and bile.

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10. *5-[(6-Chloro-4,5-dimethylpyridazin-3-yl)(cyano)methyl]-2-fluorobenzonitrile* (**4**): To a ice-cold solution of **1** (13.3 g, 75 mmol) and **3** (10 g, 62 mmol) in dry THF (200 mL), was added portion wise NaH (5.0 g, 125 mmol). The reaction was stirred at 0 °C for 15 min and at rt for 2 h, then quenched with a satd aq $NaHCO_3$ solution. Aqueous work up, followed by crystallization from EtOAc afforded **4** (9.5 g, 51%) as white solid. MS (ES) $C_{15}H_{10}ClFN_4$ requires 300, 302, found: 301, 303 $(M+H)^+$.
11. *5-[(4,5-Dimethyl-6-oxo-1,6-dihydropyridazin-3-yl)methyl]-2-fluorobenzoic acid* (**5**): **4** (9.5 g, 32 mmol) was dissolved in AcOH, concd HCl, H_2O (1:2:1, 180 mL) and the mixture was stirred at 95 °C for 12 h. Volatiles were removed under reduced pressure and the crude product was dissolved in AcOH (180 mL). NaOAc (3.7 g, 63 mmol) was added and the mixture was stirred at 100 °C for 1 h. The reaction mixture was cooled to rt and extracted with EtOAc. The crude product was suspended in 4 M aq NaOH solution (50 mL), and stirred at 90 °C for 30 min. After cooling to rt, 2 M HCl solution was added until pH 4, and filtration gave **5** (7.0 g, 80%) as yellow powder. ^1H NMR (300 MHz, DMSO-d_6) δ: 13.23 (br s, 1H), 12.66 (br s, 1H), 7.71–7.63 (m, 1H), 7.47–7.39 (m, 1H), 7.29–7.19 (m, 1H), 3.98 (s, 2H), 2.01 (s, 3H), 1.99 (s, 3H). MS (ES) $C_{14}H_{13}FN_2O_3$ required: 276 found: 277 $(M+H)^+$.
12. *6-(4-Fluoro-3-{[4-(2-methylprolyl)piperazin-1-yl]carbonyl}benzyl)-4,5-dimethylpyridazin-3(2H)-one* (**23**): a solution of Boc-2-methyl-D,L-proline (50 mg, 0.22 mmol), HOBT (33 mg, 0.22 mmol) and HATU (83 mg, 0.22 mmol) in DMF (1.4 mL) was stirred at rt for 30 min. then **6** (50 mg, 0.15 mmol) and DIPEA (38 μL, 0.22 mmol) were added. The mixture was stirred at 40 °C for 12 h, then diluted with EtOAc. Aqueous work up, followed by preparative RP-HPLC purification (column: Waters X-Terra C_{18}) afforded **23** (38 mg, 51%) as white powder. ^1H NMR (400 MHz, DMSO-d_6) δ: 12.67 (br s, 1H), 9.19 (br s, 1H), 7.36–7.17 (m, 3H), 3.96 (s, 2H), 3.75–3.42 (m, 6H), 3.33–3.03 (m, 4H), 2.28–2.09 (m, 2H), 2.06–1.82 (m, 8H), 1.63-1.46 (m, 3H). MS (ES) $C_{24}H_{30}FN_5O_3$ requires 455, found: 456 $(M+H)^+$.