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Identification and SAR of novel pyrrolo[1,2-*a*]pyrazin-1(2*H*)-one derivatives as inhibitors of poly(ADP-ribose) polymerase-1 (PARP-1)

Giovanna Pescatore *, Danila Branca, Fabrizio Fiore, Olaf Kinzel, Laura Llauger Bufi, Ester Muraglia, Federica Orvieto, Michael Rowley, Carlo Toniatti, Caterina Torrisi, Philip Jones

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

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ABSTRACT

Herein we describe the discovery of a novel series of pyrrolo[1,2-*a*]pyrazin-1(2*H*)-one PARP inhibitors. Optimization led to compounds that display excellent PARP-1 enzyme potency and inhibit the proliferation of BRCA deficient cells in the low double-digit nanomolar range showing excellent selectivity over BRCA proficient cancer cells.

Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme involved in the detection and repair of DNA damage. It utilizes NAD^+ to synthesize poly(ADP-ribose) chains either on itself or on a variety of nuclear target proteins: histones, topoisomerases, DNA polymerases, and DNA ligases, in a process called poly(ADP)ribosylation. The addition of these poly(ADP-ribose) units results in a highly negatively charged chromatin, thereby opening up the chromatin and facilitating recruitment of the base excision repair machinery to the site of the DNA damage, resulting in repair.[1] PARP-1 is the principal member of the PARP enzyme family consisting of some 17 proteins,[2] consequently, inhibition of PARP-1 may retard intracellular DNA repair. PARP inhibitors (PARPi) have been demonstrated to function as chemosensitizers, and to enhance and prolong the antitumor effects of certain anticancer therapies.[3] Excessive DNA damage results in PARP-1 overactivation, consumption of the NAD^+ pool that ultimately leads to excessive ATP depletion and necrotic cell death. As a result PARPi have also been demonstrated to work in preclinical models of reperfusion injury (stroke, myocardial infarction), but also in arthritis and other forms of inflammation as well as diabetes.[4] Thus PARP-1 inhibitors may prove useful for the treatment of these diseases. Moreover, recent studies have shown that cells deficient in BRCA1 and BRCA2, proteins involved in DNA double strand break repair by homologous recombination (HR) repair, are highly sensitive to PARP-1 inhibitors.[5] These results indicate that PARPi could have clinical benefits in cancer patients as monotherapy in individuals with cancers bearing specific defects in HR repair, in addition to chemosensitizers in combination with DNA damaging agents as well as in several other therapeutic areas.

The past few decades have witnessed much research in the field of PARPi and most of the compounds in development mimic the nicotinamide moiety of NAD^+. To date several compounds have entered clinical trials, including: ABT-888,[6] AZD-2281,[7] AG014699,[8] and MK-4827[9] (Fig. 1), in addition to several other derivatives whose structures have not been revealed.

A research program was started in our company with the aim of developing PARPi suitable for clinical development in BRCA mutant cancer patients. Although extensive data exists on the design of potent PARP enzyme inhibitors, there is minimal SAR published describing the optimization of cell based activity for the inhibition of BRCA deficient cells, whilst maintaining good selectivity over BRCA wild type cells. This manuscript describes the optimization of a novel series of PARP inhibitors, focusing on the optimization of their cellular activity and selectivity.

On the basis of the proposed binding of NAD^+ to PARP-1, together with X-ray crystal structures of PARP-1 with bound inhibitors,[10] we designed a novel pyrrolo[1,2-*a*]pyrazin-1(2*H*)-one scaffold. We envisaged that this heterocycle could bind in the nicotinamide pocket, the five-membered ring sandwiched between Tyr-896 and Tyr-907 amino acids and the lactam of the pyrazinone making three H-bonds to the enzyme. The pendant fluorobenzyl

* Corresponding author. Fax: +39 06 91093654.
E-mail address: giovanna.pescatore@gmail.com (G. Pescatore).

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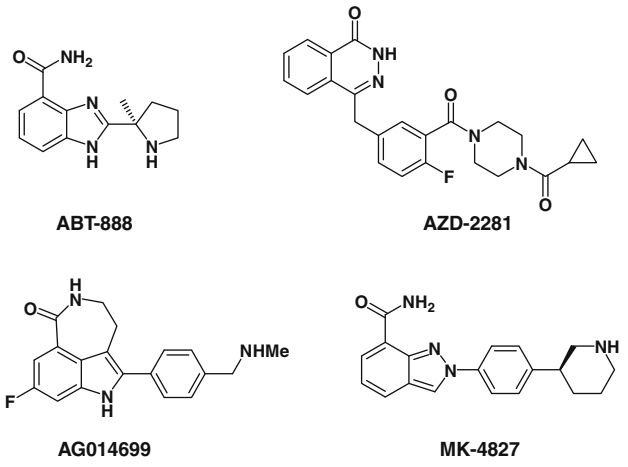


Figure 1. PARP inhibitors currently being evaluated in the clinic.

group could then reach down into the adenine binding pocket allowing any substituent to make further binding interactions there to improve affinity. Gratifyingly as reported in Figure 2, **1** demonstrated that the pyrrolo[1,2-*a*]pyrazin-1(2*H*)-one scaffold is an excellent enzyme inhibitor with IC_{50} = 1.8 nM against PARP-1. Unfortunately this compound failed to demonstrate any anti-proliferative effects in BRCA1 deficient cells at 20 μM.[11]

Knowing that excellent PARP inhibition is required to inhibit proliferation of cells defective in BRCA[9] we sought to improve the activity of this series intrinsically and also to increase the lipophilicity given the hydrophilic nature of **1** (log *D* = −0.89) in the hope to improve cell permeability. We recognized that substitution on the pyrrole ring should be possible, and also allow us to improve the contact surface area with the enzyme. Also given the hydrophobic nature of this part of the enzyme it should be possible to incorporate hydrophobic substituents.

A small improvement in cellular potency was observed when increasing the lipophilic character of the pyrrole moiety by introducing a methyl (**2**) or a chloro group (**3**) in the 7 position (Table 1). The effect due to the chlorine substitution was more evident compared to the methyl, as **3** exhibited improved anti-proliferative effects in BRCA1 deficient cells compared to **2**

Table 1
SAR on the pyrrolo[1,2-*a*]pyrazin-1(2*H*)-one portion

R =

Compd No.	R	PARP1 IC_{50}[a,b] (nM)	BRCA 1- CC_{50}[c,b] (nM)	BRCA wt CC_{50}[d,b] (nM)
1		1.8	>20 μM	>20 μM
2		1.6	3400	>20 μM
3		1.0	2500	>20 μM
4		2.6	170	>20 μM
5		1.6	220	>20 μM
6		4.0	18,000	>20 μM

[a,c–d] The assays were performed as described in Ref. 9.
[b] Values are means of at least four experiments (standard deviations were within 25% of the mean value).

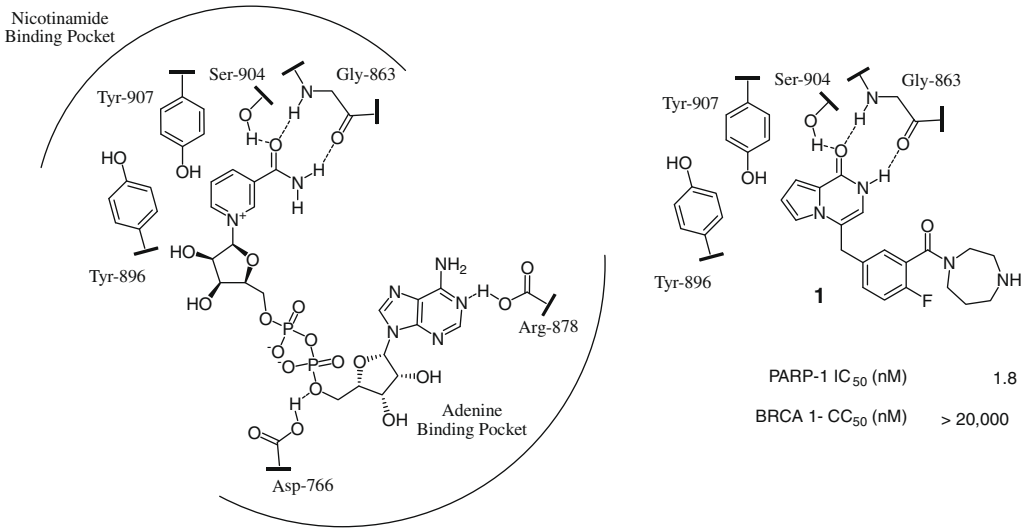


Figure 2. Binding mode of NAD^+ in PARP active site,[10] and proposed binding mode of the pyrrolo[1,2-*a*]pyrazin-1(2*H*)-one scaffold. PARP and BRCA1- assays conducted as described in Refs. 9 and 11.

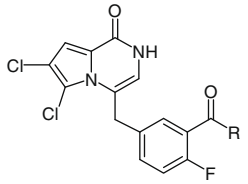
(CC_{50} = 2.5 μM vs 3.4 μM) reflective the larger pi value of the chlorine group (pi = 0.71 vs 0.56). The incorporation of a second chlorine at either the 6 (**4**) or 8 (**5**) position confirmed the observed trend and significantly improved cellular activity with both compounds displaying CC_{50} <250 nM in BRCA1 deficient cells. This finding tracks the lipophilicity as confirmed by increased log *D* values (log *D* = 0.58 and 0.91 for **3** and **4**, respectively). Remarkably, the 6,7-dichloropyrrolo analogue (**4**) was 15-fold more potent than **3** on BRCA1 deficient cells (CC_{50} = 170 nM) and displayed optimal selectivity with no anti-proliferative activity in BRCA wild type HeLa cells at 20 μM. Surprisingly, the 6,8-dichloropyrrolo analogue (**6**) though maintaining PARP-1 inhibitory activity, significantly lost cellular activity (CC_{50} = 18 μM).

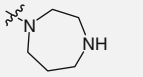
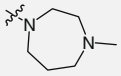
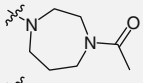
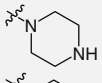
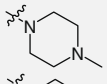
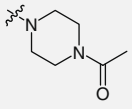
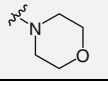
On the basis of the encouraging cellular data for **4**, the 6,7-dichloropyrrolo[1,2-*a*]pyrazin-1(2*H*)-one was selected for further investigation. Optimization focused on the homopiperazine substituent, and in a logical extension substituted homopiperazine and a number of corresponding piperazine analogs were explored (Table 2). The unsubstituted homopiperazine and piperazine (**4** and **9**) showed similar PARP-1 inhibitory activity in the nanomolar range and similar potency in the cell based assays. Alkylation in the homopiperazine series (**7**) was tolerated but a significant loss in cellular activity was observed (CC_{50} = 1.1 μM), while in the piperazine series it resulted in a marked loss of enzymatic activity (**10**: IC_{50} = 41 nM).

Acylation to produce the 4-acetyl-homopiperazine **8** resulted in sevenfold decrease in PARP-1 inhibitory activity compared to the unsubstituted analogue **4** and the acetamide showed only marginal activity in cells, losing 30-fold. Instead the 4-acetyl-piperazine **11** still displayed good PARP-1 inhibitory activity and submicromolar cellular activity (IC_{50} = 6.3 nM, CC_{50} = 700 nM). Removal of the nitrogen with the piperidine **12** and the morpholine **13** was detrimental as both compounds lost more than 20-fold enzymatic activity.

Further elaboration of the piperazine series was then undertaken with a view to pick up additional binding interactions in the adenine pocket and thereby improve activity further (Table 3). Addition of

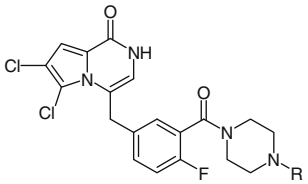
Table 2
First SAR on the pendant anilide

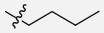
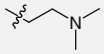
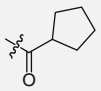
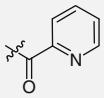
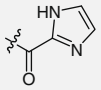
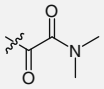
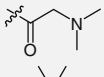
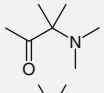
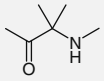


Compd No.	R	PARP1 IC_{50}[a,b] (nM)	BRCA1-CC_{50}[c,b] (nM)	BRCA wt CC_{50}[d,b] (nM)
4		2.6	170	>20 μM
7		5.0	1100	>20 μM
8		19	5700	>20 μM
9		2.3	180	>20 μM
10		41	ND	ND
11		6.3	700	>10 μM
12		200	ND	ND
13		59	ND	ND

[a–d] See footnotes of Table 1. ND = not determined.

Table 3
SAR study on the piperazine portion: alkyl- and acyl-substituted analogues



Compd No.	R	PARP1 IC_{50}[a,b] (nM)	BRCA 1-CC_{50}[c,b] (nM)	BRCA wt CC_{50}[d,b] (nM)
14		14	250	>10,000
15		13	1600	>10,000
16		14	970	>5000
17		18	>10,000	>10,000
18		13	>5000	>10,000
19		3.2	1200	>5000
20		2.1	150	>5000
21		1.0	340	>5000
22		3.7	270	>5000
23		2.4	150	>5000
24		3.1	500	>5000
25		7.2	670	>10,000
26		9.0	3000	>5000
27		2.4	910	>10,000
28		2.0	1800	>10,000
29		2.5	3500	>10,000
30		5.1	200	>5000
31		2.1	53	>5000

[a–d] See footnotes of Table 1.

larger lipophilic chains to the piperazine such as **14–16** resulted in a more than fivefold loss of enzymatic activity and an even larger loss of cellular activity.

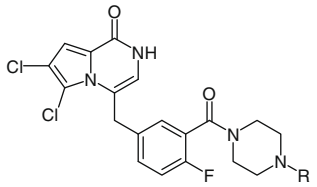
In contrast, amides **19**–**31** all showed single digit nanomolar inhibition of PARP-1. Although aliphatic amides **20**–**22** show anti-proliferative effects in BRCA deficient cells in the 150–350 nM range, no further improvement could be achieved. The isopropyl derivative **20** is the most encouraging with CC_{50} = 150 nM and more than 30-fold selectivity over BRCA wild type cells. The addition of a weakly basic center, either a basic heterocycle, like pyridine **23** and imidazole **24**, as well as aliphatic amine, like **29**–**31,** was promising. In particular the 2-pyridyl derivative (**23**) displayed good anti-proliferative activity in BRCA1 deficient cells with CC_{50} = 150 nM, and showing 30-fold selectivity. The amino acid derivative **31** displayed double-digit nanomolar anti-proliferative against BRCA1 deficient cells (CC_{50} = 53 nM) with more than 100-fold selectivity over BRCA proficient (CC_{50} >5 μM) and this particular analogue was one of the most interesting derivatives.

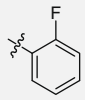
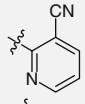
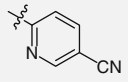
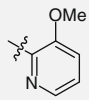
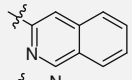
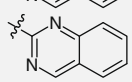
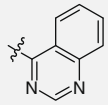
Fujisawa (Astellas) have previously described *N*-aryl piperazines to be fragments that significantly improve the potency of PARPi,[12] therefore an investigation around this fragment was performed (Table 4). Substituted phenyl derivatives exemplified by *ortho*-fluorophenyl **32** showed weaker enzyme potency and lost significant cellular activity. Installation of a polar heterocycle such as 2-pyridyl recovered this activity and **33** displayed high enzymatic inhibitory activity and moderate potency in cells (IC_{50} = 2.8 nM and CC_{50} = 430 nM) while the isomeric 3-pyridyl (**34**) lost fourfold in enzymatic activity and was inactive in cell based assay at 5 μM.

The corresponding 2-pyrimidine **35** showed a further improvement in the anti-proliferative effect on BRCA1 deficient cells; however activity was also observed against the BRCA wild type cell line (CC_{50} = 2800 nM), and **35** displayed only 22-fold selectivity. A fourfold increase in enzymatic activity and sevenfold increase in cellular potency was gained when a CN group was introduced in 3 position of the pyridyl analogue (**36**, IC_{50} = 0.7 nM and CC_{50} = 62 nM), but again this derivative displayed only 25-fold selectivity between BRCA1 deficient and proficient cells.

Substitution in the 5 position of the pyridine was less tolerated and significantly decreased the enzymatic potency (**37** vs **36**, IC_{50} = 14 nM vs 0.7 nM) although some cellular activity was maintained (CC_{50} = 200 nM). Other modifications such as the introduc-

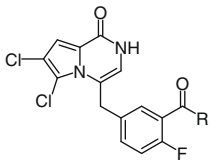
Table 4
SAR study on the piperazine portion: aryl- and heteroaryl-substituted analogues

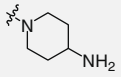
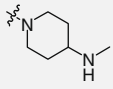
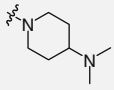
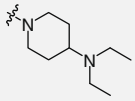
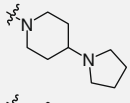
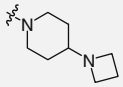
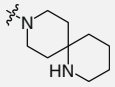
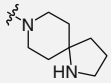
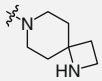
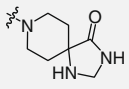


Compd No.	R	PARP1 IC_{50}[a,b] (nM)	BRCA 1- CC_{50}[c,b] (nM)	BRCA wt CC_{50}[d,b] (nM)
32		24	>10,000	>10,000
33		2.8	430	>10,000
34		12	>5000	>5000
35		3.1	130	2800
36		0.7	62	1600
37		14	200	4200
38		15	2700	>5000
39		1.8	320	1200
40		1.0	190	770
41		1.4	60	920

[a–d] See footnotes of Table 1.

Table 5
SAR study on the benzamide portion: piperidinyl-substituted analogues and spiro-substituted analogues



Compd No.	R	PARP1 IC_{50}[a,b] (nM)	BRCA 1- CC_{50}[c,b](nM)	BRCA wt CC_{50}[d,b](nM)
42		7.1	3800	>10,000
43		2.3	400	>5000
44		2.6	1600	>10,000
45		22	1100	>5000
46		5.4	120	>5000
47		1.0	180	>10,000
48		6.2	220	>5000
49		2.5	48	>10,000
50		8.2	2300	>5000
51		9.0	>2500	>5000

[a–d] See footnotes of Table 1.

Table 6
Profile of compounds **31**, **46** and **49**

Compounds	**31**	**46**	**49**
PARP-1 IC_{50} (nM)	2.1	5.4	2.5
BRCA1-CC_{50} (nM)	53	120	48
BRCAwt CC_{50} (nM)	>5000	>5000	>10,000
Rat liver microsomes			
CL_{int} (μl/min/mg)	122	135	65
Rat Clearance (mL/min/kg)	>200	94	130
hERG IC_{50} (nM)	11,000	8000	12,000
CYP inhibition			
(% at 10 μM)	3A4: 25	3A4: 30	2D6: 35

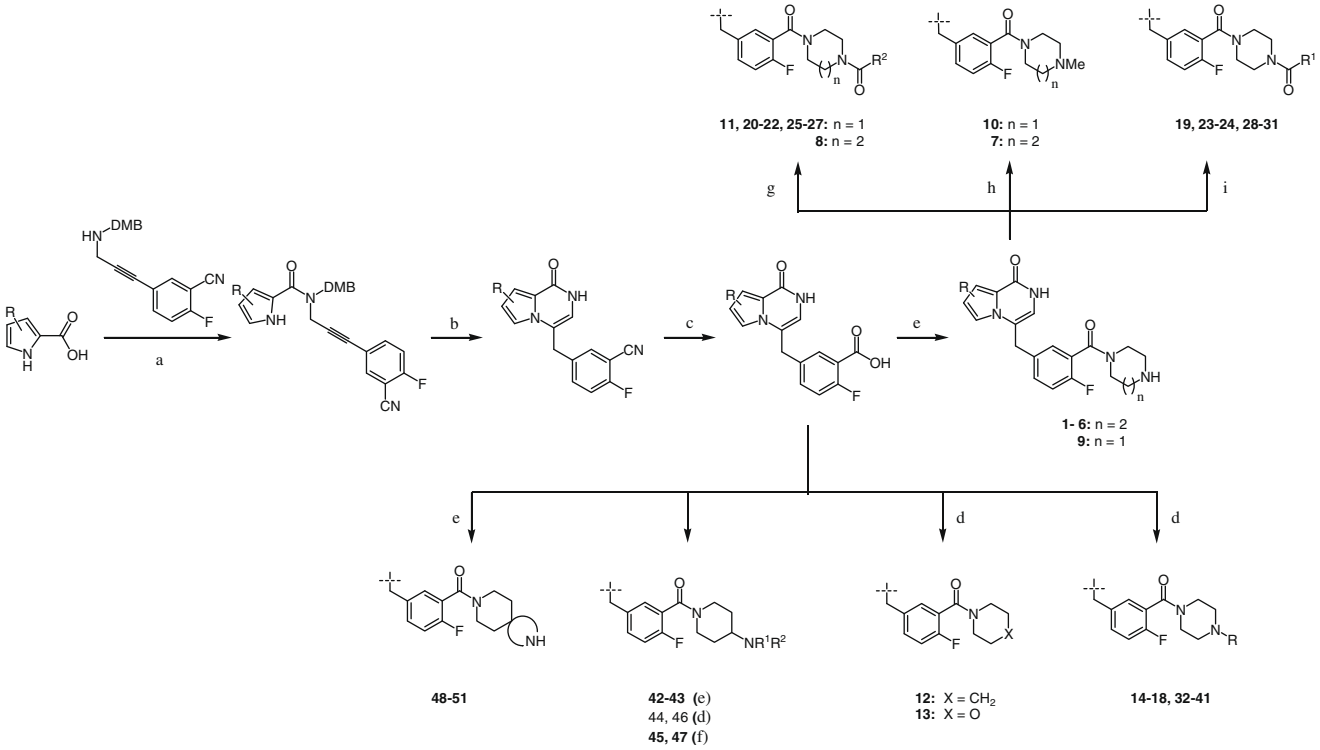
tion of a methoxy group were detrimental, as **39** displayed significantly lower activity both in enzyme and cellular assays (IC_{50} = 15 nM and CC_{50} = 2700 nM). Similarly, benzo fusion of **34** and **35** was also detrimental, and although isoquinoline **39** and isomeric quinazolines **40** and **41** proved to be very potent PARP-1 inhibitors, these compounds showed an undesirable profile in cells with minimal selectivity between BRCA deficient and wild type cells. In contrast to **34** and **35** which displayed 20-fold selectivity the benzofused analogs demonstrated only around fourfold selectivity suggesting underlying off-target activity.

Knowing the amino group on the piperazine was important for activity, exploration was also undertaken in a related series, the 4-amino piperidines, to explore the effect of a nitrogen in an exocyclic position, and a series of analogs were investigated including alkyl substitution on the external nitrogen (**43**–**45**), or incorporation of the nitrogen into a cycle (**46**–**47**) or a spiro-structure (**48**–**51**) (Table 5). Simple 4-amino piperidine (**42)** was tolerated displaying PARP-1 IC_{50} = 7.1 nM and micromolar activity in BRCA deficient cells (CC_{50} = 3.8 μM). Enzymatic activity could be improved threefold by alkylation, as **43** displayed IC_{50} = 2.3 nM and showed around 10-fold improvement in cellular activity (CC_{50} = 400 nM). Dialkylation to give **44** resulted in no improvement in enzymatic activity but was detrimental for cellular activity (CC_{50} = 1.6 μM) and homologation to diethylamino **45** resulted in a 10-fold loss in enzymatic activity (IC_{50} = 28 nM). Noticing this steric constraint the dimethylamino was constrained in an azetidine (**47**) or pyrrolidine (**46**), and encouragingly both derivatives displayed CC_{50} <200 nM in BRCA deficient cells with more than 50-fold selectivity over BRCA wild type cells. The cyclization strategy could also be applied to the 4-position of the piperidine to give spirocycles **48**–**51**. The spiro-pyrrolidine **49** proved to have the best cellular activity with CC_{50} = 48 nM being fivefold more active than piperidine **48** and 40-fold more active than the spiro azetidine **50**. Incorporation of further functionality into the spiro ring as in the case of **51** was not tolerated loosing all cellular activity at 2.5 μM.

The discovery of the 6,7-dichloropyrrolo[1,2-*a*]pyrazin-1(2*H*)-one class of PARPi enabled several sub-classes of compounds to be developed that showed good levels of PARP inhibition, and which are able to inhibit the proliferation of BRCA1-silenced HeLa cells with CC_{50} <200 nM, for example, **31**, **46**, and **49**. These compounds demonstrate excellent selectivity over BRCA proficient cells, typically at least 50-fold. However, the pharmacokinetic properties of these compounds were suboptimal and despite modest stability in rat microsomes, **31**, **46**, and **49** were characterized with high plasma clearance in rats (Table 6). They also demonstrated micromolar binding to the hERG ion channel and weak CYP inhibition.

The general synthesis of the key intermediate dichloropyrrolo[1,2-*a*]pyrazin-1(2*H*)-one acid scaffold is outlined in Scheme 1 and was constructed according to a novel DBU-catalyzed intramolecular hydroamination reaction which was recently developed in our laboratories.[13,14] Further synthetic manipulations then enabled construction of the desired final compounds as illustrated.

In summary we have reported the identification of a series of pyrrolo[1,2-*a*]pyrazin-1(2*H*)-ones PARP inhibitors; the physical



Scheme 1. Reagents and conditions: (a) TBTU, TEA, DMF; (b) (i) 30 mol % DBU, DCM, reflux; (ii) iPr$_3$SiH, TFA, 110 °C; (c) 10% aq NaOH, reflux; (d) amine, TBTU, DIPEA, DMF; (e) (i) Boc-amine, TBTU, DIPEA, DMF; (ii) TFA/DCM; (f) (i) piperidin-4-one, TBTU, DIPEA, DMF; (ii) NHR^{1}R^2, Ti(O-iPr)$_4$ NaBH$_3$CN, DCE, MeOH; (g) R^{2}COCl, TEA, DCM; (h) H_2CO, NaBH$_3$CN, NaOAc, MeOH; (i) R^{1}CO$_2$H, HBTU, DIPEA, DCM.

chemical properties were modified to improve the cellular activity of these compounds resulting in compounds that inhibit the proliferation of BRCA deficient cells in the low double-digit nanomolar range and show excellent selectivity over BRCA proficient cancer cells. The efforts to improve the pharmacokinetics of these compounds will be the subject of a future paper.

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