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Article

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Design, Synthesis and Biological Evaluation of a Series of Benzo[de][1,7]naphthyridin-7(8H)-ones Bearing a Functionalized Longer Chain Appendage as Novel PARP1 Inhibitors

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[†]*CAS Key Laboratory of Receptor Research, and Synthetic Organic & Medicinal Chemistry Laboratory,* [‡]*State Key Laboratory of Drug Research, and* [ζ]*CAS Key Laboratory of Receptor Research, and Drug Discovery and Design Center, Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences, Shanghai, China 201203.* [§]*These three authors contributed equally to this work.*

[*]To whom correspondence should be addressed. For Ao Zhang, aozhang@mail.shcnc.ac.cn; Tel: 86-21-50806035; Fax: 86-21-50806035; for Ze-Hong Miao, zhmiao@mail.shcnc.ac.cn. Tel.: 86-21-50806820; Fax, 86-21-50806820; For Yechun Xu, ycxu@mail.shcnc.ac. cn; Tel. 86-21 50801267.

[a]Abbreviations: ADP, adenosine 5'-diphosphate; PARP, poly(ADP-ribose) polymerases; NAD+, nicotinamide adenine dinucleotide; PAR, poly(ADP)ribose; BRCA1, breast cancer gene 1; DNA, deoxyribonucleic acid; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, 1-hydroxy-1H-benzotriazole; NOE, Nuclear Overhauser effect; HBTU, 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; PK, pharmacokinetics, Boc, tert-butyloxycarbonyl; DIPEA, diethylpropylamine; DMF, dimethylformamide; TFA, trifluoroacetic acid; DAST, diethylaminosulfur trifluoride; DAPI, 4',6-diamidino-2-phenylindole; MW, microwave; FITC, fluorescein isothiocyanate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DMEM, Dulbecco's Modified Eagle's Medium; SRB, sulforhodamine B; FBS, fetal bovine serum; OPD, o-phenylenediamine.

Abstract.

A series of benzo[de][1,7]naphthyridin-7(8H)-ones possessing a functionalized long-chain appendage have been designed and evaluated as novel PARP1 inhibitors. The initial effort led to the first-generation PARP1 inhibitor **26** bearing a terminal phthalazin-1(2H)-one framework and showing remarkably high PARP1 inhibitory activity (0.31 nM) but only moderate potency in the cell. Further effort generated the second-generation lead **41** showing high potency against both the PARP1 enzyme and *BRCA*-deficient cells, especially for the *BRCA1*-deficient MDA-MB-436 cells (CC_{50} < 0.26 nM). Mechanistic studies revealed that the new PARP1 inhibitors significantly inhibited H_2O_2-triggered PARylation in SKOV3 cells, induced cellular accumulation of DNA double-strand breaks and impaired cell-cycle progression in *BRCA2*-deficient cells. Significant potentiation on the cytotoxicity of temozolomide was also observed. The unique structural character and exceptionally high potency of **41** made it stand out as a promising drug candidate worthy for further evaluation.

INTRODUCTION

Poly(ADP-ribose) polymerases (PARPs)[a] are ADP-ribosyltransferases that cleave nicotinamide adenine dinucleotide (NAD^+) into ADP-ribose and nicotinamide, and then transfer the ADP-ribose units onto a variety of target proteins (such as histones, topoisomerases, DNA polymerases, and DNA ligases) or themselves.[1-3] The PARP family is comprised of 18 members and regulates diverse cellular functions including DNA recombination and repair, cellular proliferation, apoptosis in ischemic condition and necrotic death.[4] Among the PARP superfamily, PARP1 is the most abundant and well-characterized PARP enzyme. It accounts for more than 90% of ADP-ribosylation occurring in cells and is highly evolutionarily conserved in all advanced eukaryotes.[5,6] PARP1 is an enzyme of 113-kDa protein containing three major structural domains, a 42-kDa DNA binding domain at the N-terminal region with two zinc fingers, a 55-kDa catalytic domain at the C-terminal region, and an automodification domain at the central region.[7-9] The DNA binding domain can recognize and locate the damaged DNA single-strand breaks and then bind to them to stimulate polymerization of ADP-ribose leading to the unwinding of DNA from histones and exposing the damaged DNA for repair. The function of the catalytic domain of PARP1 is to utilize NAD^+ as a substrate to construct linear and branched polymers of ADP-ribose onto its targets therefore enabling it to perform a pivotal role in DNA damage repair. Among the PARP family, PARP2 has high structural homology with PARP1 and shares similar functionality. Interestingly, the DNA binding domain of PARP2 is distinct from PARP1 and lacks the central automodification domain. PARP1 and to a lesser extent, PARP2, function as DNA damage sensors by binding with high affinity to the site of single and double strand DNA breaks. PARP1 is over-expressed in a variety of cancers, and its expression has

been associated with overall prognosis in cancers, especially breast and ovarian cancers.[5,8,9]

In view of the key role of PARP1 in maintaining the genomic integrity, in particular the repair of single strand DNA lesions caused by ionizing radiation, chemotherapy, UV light, or products of cellular and oxidative metabolism, PARP1 has emerged as an attractive anticancer drug target anchoring DNA damage.[5,10] The significance of PARP1 in cancer was further strengthened recently by two seminal preclinical studies[11,12] disclosing that cell lines deficient in *BRCA1* and *BRCA2* (two known tumor suppressor genes whose mutations are associated with breast, ovary, prostate, as well as pancreas cancers) were 1000-fold more sensitive to PARP inhibition in comparison to wild-type or heterozygous mutant cells. Such landmark discovery highlighted that PARP1 is the synthetic lethal partner of *BRCA1* and *BRCA2*, and inhibition of PARP1 can induce highly selective killing of *BRCA1/2*-deficient tumor cells. In this regard, many small molecules targeting PARP1 have been developed either as chemosensitizers in combination with ionizing radiation or DNA-damaging chemotherapeutic agents or as stand-alone therapies (synthetic lethal) to kill cancers defective in DNA repair mechanisms (*esp. BRCA1/2*-mutant breast or ovarian tumors).[13-22] Among them, over a dozen are being pursued into different stages of clinical trials, including arylamide **1**[23a,b] (BSI-201, iniparib), azepinoindolone **2**[24] (AG014699, rucaparib), phthalazinone **3**[25] (AZD2281, olaparib) and benzo[d]imidazole-4-carboxamide **4**[26] (ABT-888, veliparib) (Figure 1). All these drugs have shown promising single-agent activity in recurrent epithelial ovarian cancer patients with germline *BRCA1* or *BRCA2* mutations, especially for compound **3** which provided clinical proof-of-concept for the use of PARP1 inhibitors for the treatment of cancers bearing known *BRCA* mutations achieving an

objective response rate of 28-33% and a median duration of response of 28-41 weeks in phase I/II studies.[27-31] However, the enthusiasm of further development of this compound was dampened by the discontinuation announcement made by AstraZeneca[32] very recently after an interim analysis of a Phase II study which indicated that the previously reported progression free survival benefit was not translated into an overall survival benefit, the definitive measure of patient benefit in ovarian cancer. Therefore, although additional efforts on the working mechanism of the inhibitor and the association between PARP-dependent pathways and tumor-specific defects are needed, the final fate of PARP1-targeting strategy has to wait for the clinical outcomes of other PARP1 inhibitors or development of new inhibitors bearing more structural diversity and showing improved efficacy, better safety and pharmacokinetic profiles.[20-22,33,34]

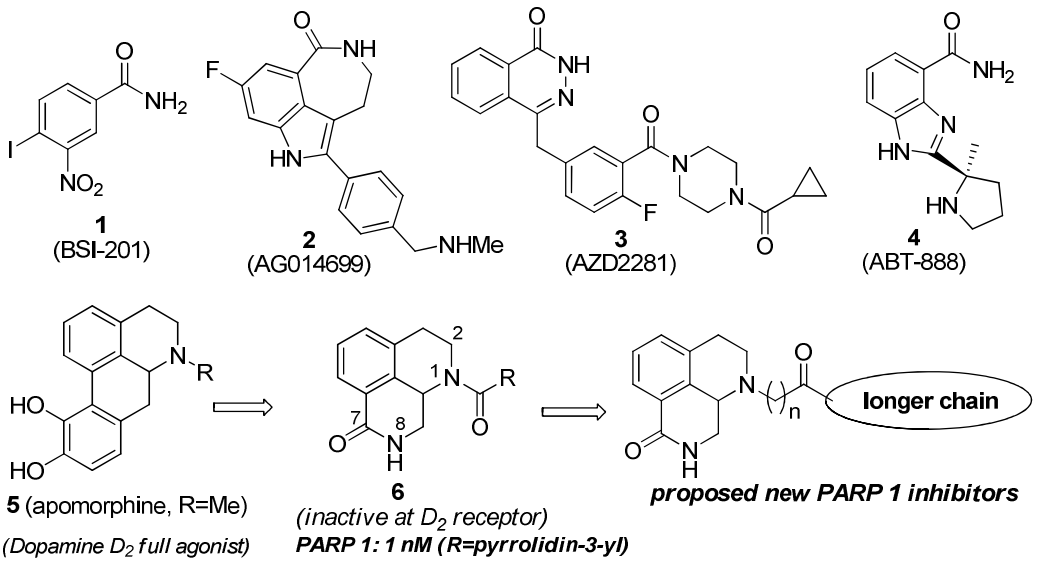


Figure 1. Representative PARP 1 inhibitors in the clinic and our proposed new inhibitors.

Most currently available PARP1 inhibitors were designed to mimic the substrate-protein interactions of NAD^+ with PARP1.[15,18,35,36] 3-Amino/nitro-benzamides (e.g. **1** and **4** in Figure 1) were the earliest PARP1

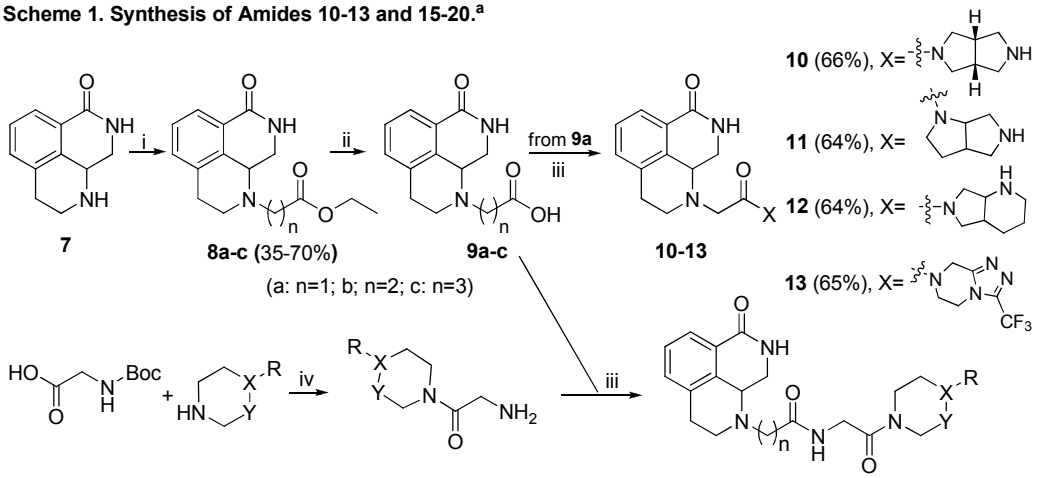
inhibitors structurally analogous to the PARylation by-product, nicotinamide, although these compounds may also hit other enzymes[23b] that bind the nicotinamide derivative NAD^+. The key interactions between these inhibitors and PARP1 include the H-bonding networks formed between the carboxamido component and Ser904 and Gly863 of PARP1, and the π–π stacking between the aryl ring and Tyr907.[15] These compounds generally showed low-to-moderate activity toward PARP1 and non-optimal PK properties. The rest majority of PARP1 inhibitors are bicyclic or tricyclic artworks where the key carboxamido moiety is buried into a ring system to form a lactam. Meanwhile, an auxiliary appendage with a shorter (e.g. **2**) or longer (e.g. **3**) linking chain is generally attached to the polycyclic core as a solvent accessory region.[15,35,36]

Our group[37-39] has long been working on the SAR of aporphine analogues by bioisosteric replacement of the catecholic component of antiparkinsonian drug apomorphine (**5**) to generate new dopamine D_2 receptor agonists with elevated metabolic stability. We recently found that replacement of the catechol function in **5** with a lactam led to benzo[de][1,7]naphthyridin-7(8H)-ones **6** that were completely inactive at any of dopamine receptors. Interestingly, Torrisi and co-authors[40] recently reported that compound **6** with an *N*-acyl group (e.g. R=pyrrolidin-3-yl) displayed one-digit nanomolar PARP1 enzymatic potency but only moderate or no inhibition to the PARylation in whole cells. This result promoted us to switch our focus on the development of novel PARP1 inhibitors bearing the benzo[de][1,7]naphthyridin-7(8H)-one scaffold with a long-chain appendage as that in clinical compound **3**. SAR optimization campaign on the nature and length of the side-chain appendage was expected to achieve novel compounds with high potency both on the PARP1 enzyme and in the *BRCA1* or *BRCA2* mutant cells.

CHEMICAL SYNTHESIS

As outlined in Scheme 1, 1,2,3,8,9,9a-hexahydro-7H-benzo[de]-1,7-naphthyridin -7-one (**7**), prepared[41] from commercially available 1,2,3,4-tetrahydroisoquinoline by following a literature procedure, was used as the key intermediate. Alkylation of **7** with appropriate ethyl bromoalkylates provided corresponding esters **8a-c** in 35-70% yields. Hydrolysis of esters **8a-c** yielded corresponding acids **9a-c**. Compounds **10-13** bearing a bicyclic amine functionality were prepared by treating the key intermediate **9a** with appropriate cyclic amine substrates in the presence of EDCI and HOBt in 65% yields.[42] Likewise, condensation of acids **9a-c** with aminoacetamides **14**, which in turn were prepared by reaction of *N*-Boc-glycine with corresponding amines, yielded compounds **15-20** possessing a long-chain appendage in 65-85% yields.

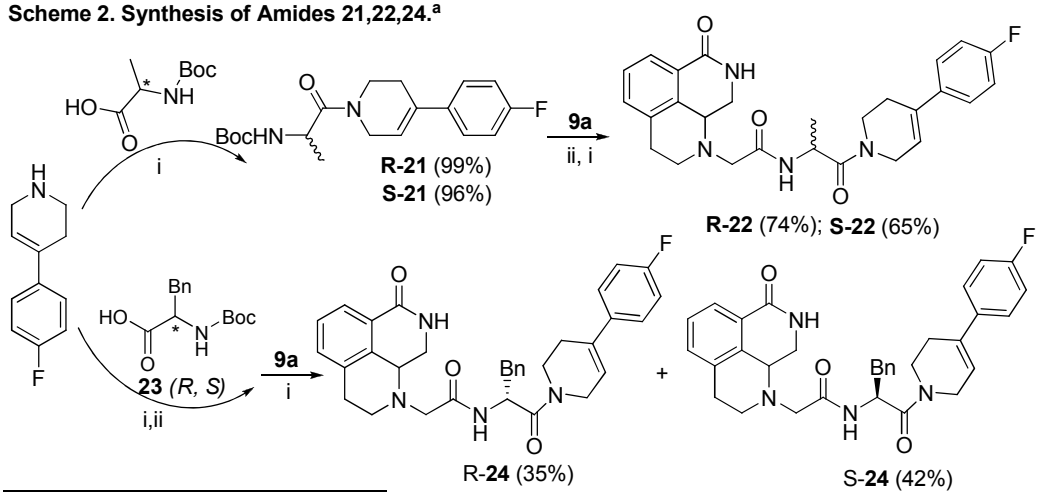
Scheme 1. Synthesis of Amides 10-13 and 15-20.[a]



[a]Reagents and Conditions: i) $Br(CH_2)_nCOOEt$, K_2CO_3, NaI, MeCN, 60 °C, 8h to 24h; ii) LiOH, THF:H_2O = 3:1, 60 °C, 10h; iii) EDCI, HOBt, Et_3N, DMF, rt, 12h; iv) EDCI, HOBt, Et_3N, DMF, rt, 12h, then TFA, CH_2Cl_2, rt, 12h.

Meanwhile, condensation of 4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine with *N*-Boc-D-alanine and *N*-Boc-L-alanine under the same condition provided *R*-**21** and *S*-**21**, subsequent deprotection and re-condensation led to *R*-**22** and *S*-**22** in 74% and 65% overall yield, respectively. Similarly, condensation of acid **9a** with *N*-Boc-D- and L-phenylalanine (*R*-**23**, *S*-**23**) followed by deprotection yielded *R*-**24** and *S*-**24** in 35% and 42% yield, respectively (Scheme 2).

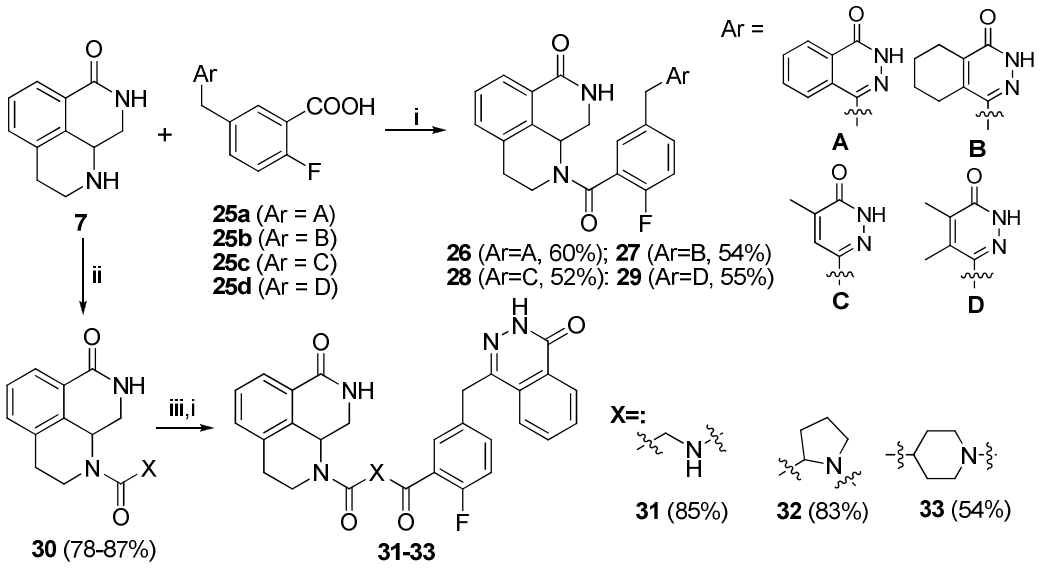
Scheme 2. Synthesis of Amides 21,22,24.[a]



[a]Reagents and Conditions: i) EDCI, HOBt, Et_3N, DMF, rt, 12h; ii) TFA, CH_2Cl_2, rt, 12h.

Amides **26-29** were prepared by condensation of amine **7** with acids **25a – 25d**[43] as a new series of benzo[de][1,7]naphthyridin-7(8H)-ones bearing a bulky chain in 52-60% yields. Inserting varies amino acid moieties (racemic proline was used for preparation of compound **32**) to the connecting linkage yielded compounds **31-33** in 45-70% overall yields (Scheme 3).

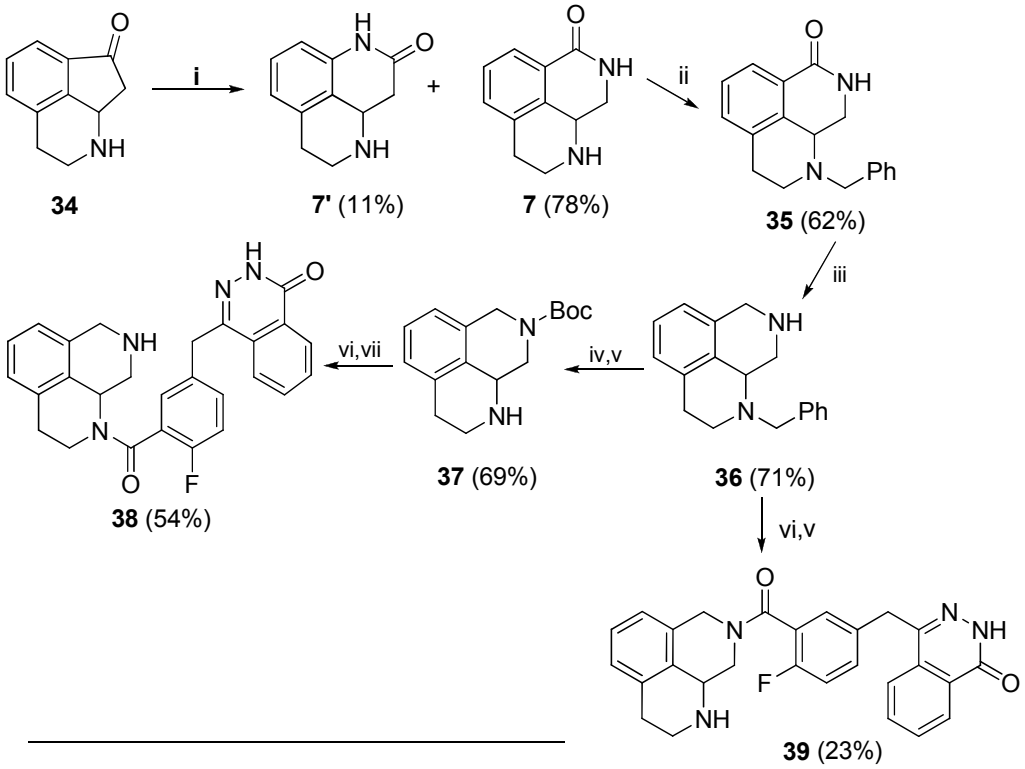
Scheme 3. Synthesis of Amides 26-34 Bearing a Bulky Chain.[a]



[a]Reagents and Conditions: i) **25a**, HBTU, DIPEA, DMF, rt, 12h; ii) N-Boc-analine, N-Boc-proline, or N-Boc-piperidine-4-carboxylic acid, EDCI, HOBt, Et_3N, DMF, rt, 12h; iii) TFA, CH_2Cl_2, rt, 12h.

During the Schmidt rearrangement[44] of cyclopenta[ij]isoquinolin-7(1H)-one **34** under catalytic amount of *conc.* H_2SO_4, the key intermediate **7** was obtained as a minor product in 18% yield, and the regiomer **7'** was produced as a dominant product in 61% yield (Scheme 4). Optimization of the rearrangement conditions with various acid catalysts (*conc.* HCl, CF_3COOH or $MeSO_3H$) established that by using a mixture of solvent system ($MeSO_3H$:CH_2Cl_2 = 1:1), compound **7** was obtained as the major product in 78% yield. *N*-Benzylation of **7** followed by $LiAlH_4$ reduction led to key intermediate **36** in 42% overall yield. *N*-Boc protection of **36** followed by de-benzylation provided **37** in 69% yield. Condensation[45] of acid **25a** with **37** under HBTU/DIPEA/DMF followed by removal of *N*-Boc group yielded amide **38** in 54% overall yield. Similarly, condensation of acid **25a** with amine **36** followed by hydrogenation provided amide **39** in 23% overall yield.

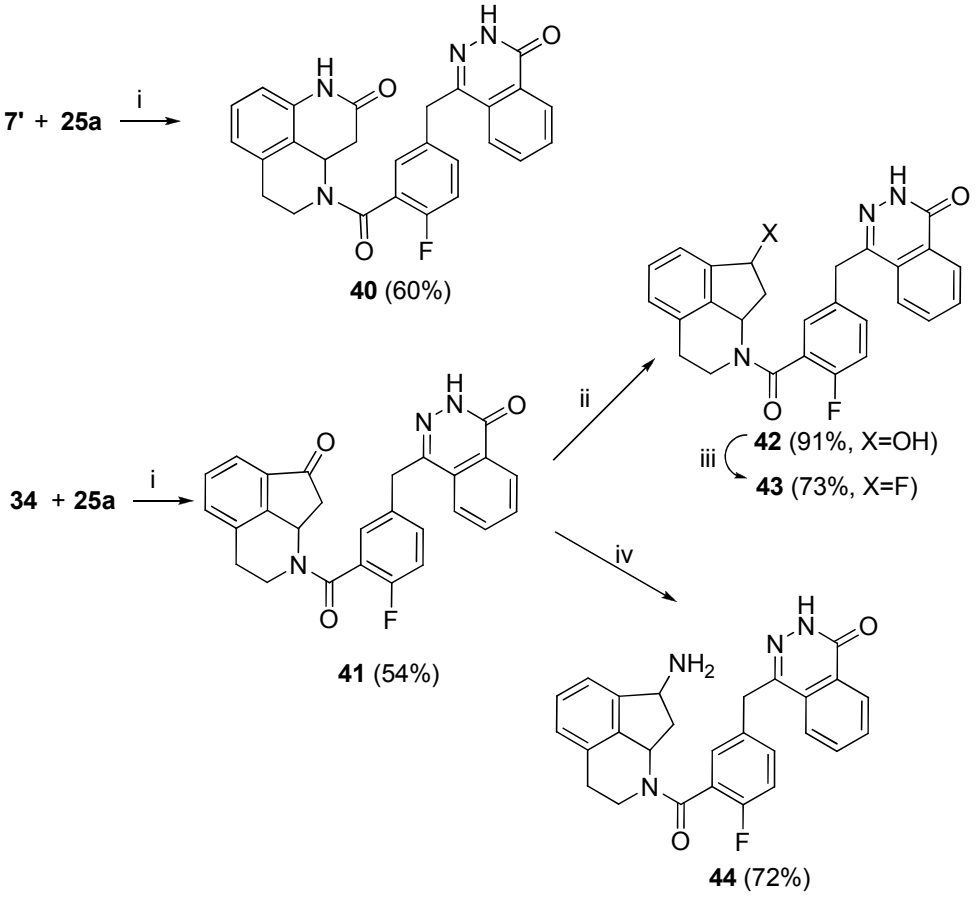
Scheme 4. Synthesis of Compounds 35-39.[a]



[a]Reagents and Conditions. i) NaN_3, $MeSO_3H$:CH_2Cl_2=1:1, 0 °C to rt, 24h; ii) BnBr, K_2CO_3, MeCN, 60 °C, 2h; iii) $LiAlH_4$, THF, reflux, 12h; iv) $(Boc)_2O$, Et_3N, CH_2Cl_2, rt, 1h; v) 10% Pd/C, H_2, AcOH, EtOH, rt, 8h; vi) **25a**, HBTU, DIPEA, DMF, rt, 12h; vii) TFA, CH_2Cl_2, rt, 12h.

To explore the impact of regiochemistry, compound **40** derived from regiomer **7'** was also prepared in a similar manner in 60% yield (Scheme 5). Meanwhile, the precursor **34** used for Schmidt rearrangement was also treated with acid **25a** led to amide **41** lacking the lactam function in 54% yield. Further, reduction of **41** with $NaBH_4$ provided alcohol **42** in 91% yield, which was then treated with DAST at 0 °C afforded fluoride **43** in 73% yield (Scheme 5).[46] Reductive amination of **41** with NH_4OAc and $NaBH_3CN$ under microwave irradiation (5 min, 130 °C)[47] yielded amine **44** in 72% yield.

Scheme 5. Synthesis of Compounds 40-44.[a]

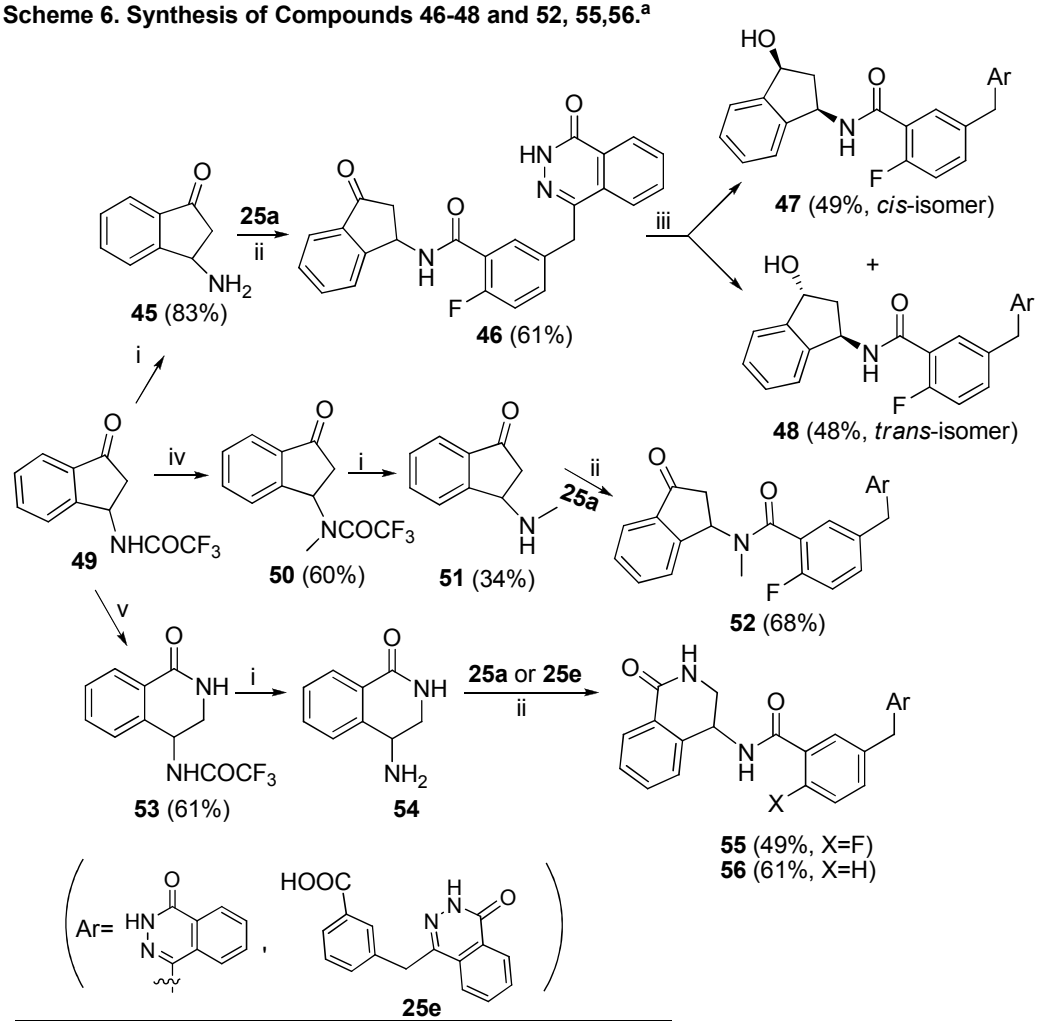


[a]Reagents and Conditions.i) HBTU, DIPEA, DMF, rt, 12h; ii) $NaBH_4$, MeOH, rt, 12h; iii) DAST, CH_2Cl_2, 0 ^oC, 5 min; iv) NH_4OAc, $NaCNBH_3$, EtOH, MW, 130 ^oC, 5 min.

2,2,2-Trifluoro-*N*-(3-oxo-2,3-dihydro-1H-inden-1-yl)acetamide (**49**), devoid of the tetrahydroisoquinoline skeleton was prepared from commercially available D/L-*β*-phenylalanine in three steps following a literature procedure[48] (Scheme 6). Amide **46** was obtained in 51% overall yield by removing the trifluoroacetyl group of **49** followed by condensation with acid **25a**. Reduction of ketone **46** with $NaBH_4$ at rt afforded *cis*-isomer **47** and *trans*-isomer **48** in nearly identical yield (49%). The *cis*- and *trans*-configurations were deduced by comparison of the NMR data with similar compounds,[48] and by NOE NMR experiments (see supporting information).

Methylation of **49** with MeI, K_2CO_3 and Bu_4NBr, followed by removal of trifluoroacetyl group yielded *N*-methylamine **51** in 20% overall yield for two steps. Subsequent condensation of **51** with acid **25a** under HBTU/DIPEA/DMF afforded amide **52** in 68% yields. Schmidt rearrangement of ketone **49** with CF_3COOH as the acid catalyst led to lactam **53**[49] as the major product in 61% yield. After deprotection[50] of **53** with 0.2 N $Ba(OH)_2$ solution followed by standard condensation with acid **25a** or **25e**, corresponding amides **55** and **56** were obtained in 49% and 61% yield, respectively.

Scheme 6. Synthesis of Compounds 46-48 and 52, 55,56.[a]

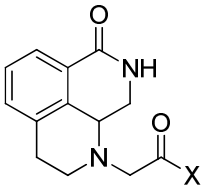
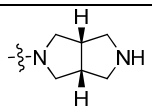
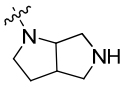
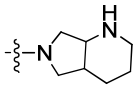
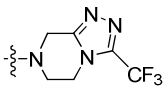
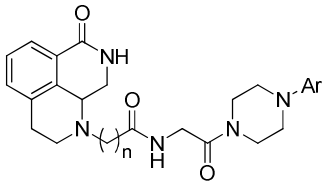
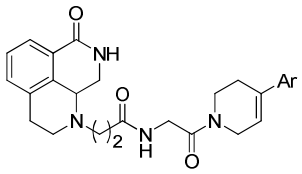
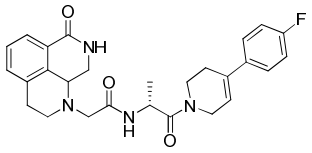
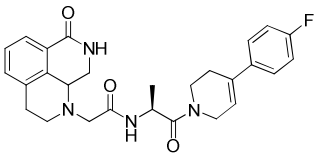


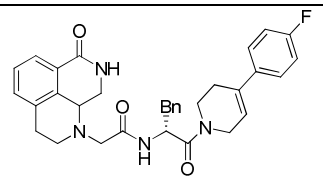
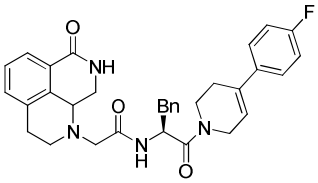
[a]Reagents and Conditions. i) 0.2 N $Ba(OH)_2$, MeOH, rt, 12h; ii) HBTU, DIPEA, DMF, rt, 12h; iii) $NaBH_4$, MeOH, rt, 12h; iv) MeI, K_2CO_3, Bu_4NBr, MeCN, rt, 24h; v) NaN_3, CF_3COOH, reflux, 3d.

RESULTS AND DISCUSSION

PARP1 Inhibition and Lead Generation. All the newly synthesized benzo[de][1,7]naphthyridin-7(8H)-ones were evaluated for their ability to inhibit PARP1 enzymatic activity.[25,40] The clinical compound **3** was used as the reference compound and our data were identical to that reported[25]. First, compounds **10-13** with a bicyclic amino group attached to the N1 of benzo[de][1,7]naphthyridin -7(8H)-one core through a 2-oxo-ethyl (acetamide) linker were prepared to increase molecular flexibility. As shown in Table 1, all the new compounds displayed negligible activity against PARP1 with IC_{50} values of approximately 10 μM. Interestingly, switch of the bicyclic amino appendage to arylpiperazinyl and inserting a second acetamide moiety to the linker afforded compound **15**, showing significant improvement in PARP1 activity with an IC_{50} value of 300 nM. To evaluate the impact of the length of the linker on the PARP1 inhibition, compounds **16** and **17** were designed containing a longer linker. However, both compounds lost PARP1 inhibitory activity. Manipulation of the aryl portion in the arylpiperazinyl fragment with other heterocycles or with various substitutions (e.g. compounds **18** and **19**) led to no improvement at all. It is of note that compound **20** with a 4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine moiety connecting to the benzo[de][1,7]naphthyridin-7(8H)-one core through a bis-acetamido linker showed modest PARP1 activity with an IC_{50} value of 1.1 μM. These results indicated that a lipophilic terminal group (e.g. 4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine) connected to the core template through a bis-acetamido linker was favorable for PARP1 interaction.

Table 1. PARP1 enzymatic assays of compounds 10-13 and 15-20.[a]

Compound	X	IC_{50} (μM)
	 **10**	8.80
	 **11**	9.38
	 **12**	9.35
	 **13**	>10
	**15** (n=1, Ar=4-MeO-Ph)	0.30
	16 (n=2, Ar=4-MeO-Ph)	8.72
	17 (n=3, Ar=4-MeO-Ph)	8.89
	18 (n=2, Ar=2-F-Ph)	8.96
	19 (n=2, Ar=pyrimidin-2-yl)	8.52
	**20** (Ar=4-F-Ph)	1.10
 ***R*-22**	-	0.21
 ***S*-22**	-	0.019

 ***R*-24**	-	>10
 ***S*-24**	-	0.49
3 (AZD2281)	-	0.004 (0.005[b])

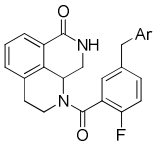
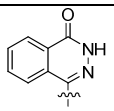
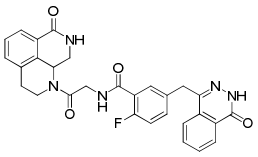
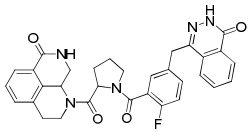
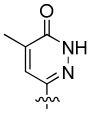
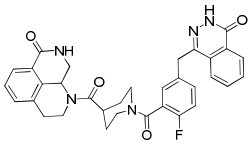
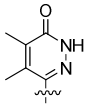
[a] IC_{50}s were calculated by Logit method from the results of at least three independent tests with six concentrations each (standard deviations were within 25% of the mean values); [b]Data from ref 25.

To further explore the impact of the linker bearing a stereogenic center on the PARP1 activity, the second acetamido moiety in the linker of compound **20** was replaced with *R*- and *S*-alanine moieties to afford compounds *R*-**22** and *S*-**22** (Table 1). Both compounds displayed significantly enhanced PARP1 activity, especially *S*-**22** showing an IC_{50} value of 19 nM and being 10-fold more potent than *R*-**22.** However, replacing the second acetamido moiety in the linker of compound **20** with *R*- and *S*-phenylalanine moieties led to compounds *R*-**24** and *S*-**24**, possessing significantly different PARP1 inhibitory activity. Again, *S*-isomer (*S*-**24**) showed higher potency with an IC_{50} value of 490 nM, which was 20-fold more potent than that of *R*-**24** (>10 μM). These results indicated that both the steric effect and stereochemistry of the linker played roles in the ligand-PARP1 interactions. Relatively less bulky linker with *S*-configuration was favored.

Our second series of PARP1 inhibitors were designed by borrowing the core

structure (phthalazin-1(2H)-one) from clinical compound **3** or its bioisosteres as the terminal appendage to mimic the aryl-tetrahydropyridine moiety in *S*-**22**. Meanwhile, a bulky linker - methylbenzoyl was employed for readily synthetic manipulation. As shown in Table 2, compounds **26-29** bearing various terminal functional groups with the same linker displayed high PARP1 potency with IC_{50} values ranging between 0.31—210 nM. Compound **26** bearing the core structure - phthalazin-1(2H)-one of clinical compound **3** displayed remarkably high PARP1 inhibitory activity. It has an IC_{50} value of 0.31 nM and is 13-fold more potent than compound **3** (4.0 nM). Saturation of the aryl moiety in the phthalazin-1(2H)-one framework yielded compound **27** retaining high affinity of 1.8 nM. It is 6-fold less potent than compound **26**, but still 2-fold more potent than compound **3**. Replacing the aryl in the phthalazin-1(2H)-one component with one or two methyl groups led to compounds **28** and **29** showing much reduced enzymatic activity. To increase the flexibility of the linker in compound **26**, a few aminoacyl moieties were inserted to the linkage leading to compounds **31-33**. All the three compounds retained high PARP1 inhibitory activity with IC_{50} values between 1.9—45 nM. The steric effect in the linker seems beneficial to the enzymatic activity. Compound **33** with the most bulky linker showed the highest PARP1 activity among the three compounds with an IC_{50} value of 1.9 nM, compatible to that of **27** and 2-fold more potent than that of **3**.

Table 2. PARP1 enzymatic assays of compounds 26-29 and 31-33.[a]

Compound	Ar	IC_{50} (μM)	Compound	IC_{50} (μM)
	 **26**	0.00031	 **31**	0.033
	 **27**	0.0018	 **32**	0.045
	 **28**	0.21	 **33**	0.0019
	 **29**	0.036		

[a] IC_{50}s were calculated by Logit method from the results of at least three independent tests with six concentrations each (standard deviations were within 25% of the mean values).

Cell Proliferation Inhibition of Potent Compounds and Identification of First-generation Lead Compound 26. From the results above, compounds **26, 27, 29** and **31-33** were identified as potent PARP1 inhibitors with IC_{50} values lower than 50 nM. Therefore, they were further evaluated for their inhibition on the cell proliferation. As shown in Table 3, the selected compounds together with reference compound **3** were tested in a matched pair of previously well-characterized Chinese hamster lung fibroblast V79 (wild type) and V-C8 (*BRCA2* deficient) cells.[51] Compared to its high enzymatic potency, compound **26** showed much less cellular

activity. It has a CC_{50} value of 96.0 nM against the *BRCA2*-deficient V-C8 cells which was 2-fold more potent than that of reference **3**. Similar cellular potency was observed for compound **27** (CC_{50}, 114 nM). Notably, the rest compounds (**29**, **31-33**) did not show appreciable inhibitory activity in the same assay in spite of their potent enzymatic activity. As expected, all the new synthetic compounds together with **3** showed no proliferation inhibition to the wild-type V79 cells confirming that the tested PARP1 inhibitors can selectively kill *BRCA*-deficient cells.[11,12]

Since our compound **26** displayed much higher potency in the PARP1 enzyme but only moderate cytotoxicity in the cell (300-fold discrepancy), a non-optimal profile similar to that of **3**, therefore, a follow-up focused SAR on lead **26** was conducted with the aim to enhance the cellular activity.

Table 3. Enzyme and cellular assays of potent compounds.[a]

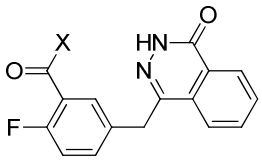
Compound	IC_{50} (enzyme, nM)	CC_{50} (wild-type, V79)	CC_{50} (*BRCA2*,V-C8)
26	0.31	>10 μM	96.0 nM
27	1.80	>10μM	114 nM
29	36.0	>10 μM	>1000 nM
31	32.7	>10 μM	>1000 nM
32	44.7	>10 μM	>1000 nM
33	1.92	>10 μM	>1000 nM
3	4.10	>10 μM	201 nM

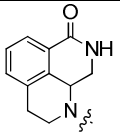
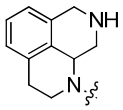
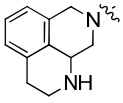
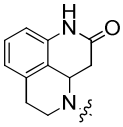
[a] Cytotoxic effect (CC_{50}) means the concentration required to reduce cell proliferation and growth by 50%. Values were calculated by Logit method from the results of at least three independent tests with six concentrations each (standard deviations were within 25% of the mean values).

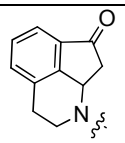
Further SAR on Lead 26 and Discovery of Second-generation PARP1 Inhibitors.

Although we proposed that the benzo[de][1,7]naphthyridin-7(8H)-one network in our lead **26** should interact with the catalytic domain of PARP1 enzyme, it was not clear whether the terminal group phthalazin-1(2H)-one competitively entered the same domain as that[15,25] of compound **3**. To validate the benzo[de][1,7]naphthyridin-7(8H)-one core as an effective scaffold of PARP1 inhibitors, a focused library was designed based on structural modification on the benzo[de][1,7]naphthyridin-7(8H)-one component of **26**, which may be also linked to the non-optimal cellular activity.

Table 4. Structural Modification on Lead 26.[a]



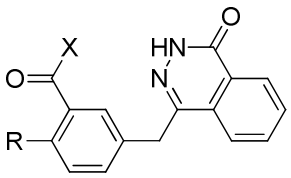
Compd	X	IC_{50} (nM) (enzyme)	CC_{50} (μM) (wild-type, V79)	CC_{50} (nM) (*BRCA2*,V-C8)
26		0.31	>10	96.0
38		156	>10	>10,000
39		1.39	>10	630
40		387	>10	>10,000

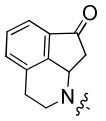
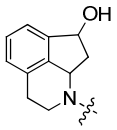
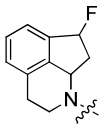
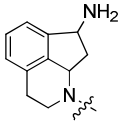
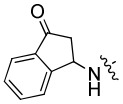
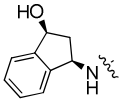
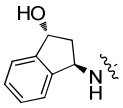
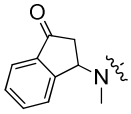
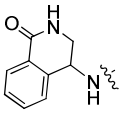
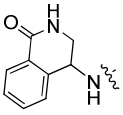
41		3.46	>10	4.53

[a]Values were calculated by Logit method from the results of at least three independent tests with six concentrations each (standard deviations were within 25% of the mean values).

As shown in Table 4, isomerization of the lactam moiety in **26**, the proposed key pharmacophoric group interacting with the PARP1 catalytic domain, led to compound **40** showing over 1000-fold reduction in the PARP1 enzymatic activity. Reduction of the lactam fragment to amine **38** also resulted in 500-fold loss of activity. Moreover, both compounds **40** and **38** had no appreciable proliferation inhibition against *BRCA2*-deficient V-C8 cells, indicating that the lactam component in lead **26** is indeed essential for both enzymatic and cellular activity. Interestingly, shifting the appendage linker in compound **38** from C-ring *N*-atom to B-ring *N*-atom led to compound **39** showing an IC_{50} value of 1.39 nM on the PARP1 enzyme and a CC_{50} value of 630 nM against the *BRCA2*-deficient cells. This compound was only 4- to 6-fold less potent than lead **26** both in the PARP1 enzyme and the V-C8 cells. More surprisingly, compound **41** bearing a cyclopenta[ij]isoquinolin-7(1H)-one skeleton, the ketone precursor of lactam moiety of **26**, showed equally high potency for both the PARP1 enzyme and the *BRCA2*-deficient cells, with IC_{50} and CC_{50} values of 3.46 and 4.53 nM, respectively. Although slightly less potent on the PARP1 enzyme, compound **41** was over 20- and 40-fold more potent against the *BRCA2*-deficient V-C8 cells than our first-generation lead **26** and reference compound **3**, respectively.

Table 5. Structural Modification on Lead 41.[a]



Compd	X	R	IC_{50} (nM) (enzyme)	CC_{50} (μM) (wild-type, V79)	CC_{50} (nM) (*BRCA2*,V-C8)
41		F	3.46	>10	4.53
42		F	86.2	>10	>10,000
43		F	0.56	>10	>10,000
44		F	76.6	>10	>10,000
46		F	83.9	>10	>10,000
47		F	4.81	1.49	418
48		F	0.57	4.26	2269
52		F	11.23	>10	4680
55		F	86.34	>10	>10,000
56		H	145.75	>10	>10,000

[a]Values were calculated by Logit method from the results of at least three independent tests with six concentrations each (standard deviations were within 25% of the mean values).

In view of the readily functional transformation from the ketone moiety in compound **41**, a series of analogues derived from **41** were further designed. As shown in Table 5, reduction of ketone **41** to alcohol **42** yielded 25-fold loss of PARP1 enzymatic activity (86 nM) and complete loss of cellular potency. Conversion of alcohol **42** to fluoride **43** led to a 6-fold enhancement on the enzyme but the cellular potency was abolished. Further conversion of alcohol **42** to amine **44** retained reasonable enzymatic activity (76.6 nM) but again lost cellular activity. Opening the *N*-containing ring of the cyclopenta[ij]isoquinolin-7(1H)-one network in compound **41** led to structurally simplified analog **46** which retained moderate enzymatic activity (83.9 nM). Further reduction of the ketone function in **46** produced *cis*-isomer **47** and *trans*-isomer **48** showing significantly different activity against PARP1 enzyme and the *BRCA2*-deficient V-C8 cells as well. The *cis*-isomer **47** showed high PARP1 enzymatic activity with an IC_{50} value of 4.81 nM, and moderate activity (418 nM) in the cells. Higher enzymatic potency was observed for the *trans*-isomer **48** with an IC_{50} value of 0.57 nM; however, it was much less potent in the cells. In addition, both compounds showed somewhat cytotoxicity against wild-type V79 cells (1.5 *vs* 4.2 μM), indicating their potential non-selective off-target toxicity. *N*-Methylation of compound **46** resulted in analog **52** showing a 7-fold improvement in the enzymatic activity (11.2 nM), and modest cellular activity. Similarly, moderate enzymatic activity was observed on the Schmidt rearranged products **55** and **56** having IC_{50} values of 86 and 146 nM, respectively, but they were inactive against the V-C8 cells.

On the basis of the results above, compound **41** emerged as our second-generation lead compound with high activity both for PARP1 enzyme and the

BRCA2-deficient V-C8 cells.

Inhibition of Cell Proliferation of Potent Compounds on *BRCA1* Deficient Cells. In addition to *BRCA2*-deficient V-C8 cells, both lead compounds **26** and **41** were further evaluated in human breast cancer MDA-MB-436 cells carrying natural *BRCA1* mutations.[25] Meanwhile, compounds **33**, **39**, **43**, **47** and **48** were also assayed for comparison. As summarized in Table 6, the *BRCA1*-deficient MDA-MB-436 cells were hypersensitive to the PARP inhibition by most of the tested compounds, therefore, higher potencies were generally observed in this cell line in comparison with those in *BRCA2*-deficient V-C8 cells. Our first-generation lead **26** had a CC_{50} value of 23.2 nM against MDA-MB-436 cells, 2-fold more potent than that of **3**. Extremely high potency was observed for the second-generation lead **41** which showed a CC_{50} value lower than 0.26 nM, and was respectively 100- and 160-fold more potent than compounds **26** and **3**. This compound may be the most potent PARP1 inhibitors reported in the literature so far against the MDA-MB-436 cell line. It showed much greater potency than any other of the reported clinical candidates in this cell line. Interestingly, compound **33** was inactive in the *BRCA2*-deficient V-C8 cells, but showed moderate cytotoxicity (344 nM) against *BRCA1*-deficient MDA-MB-436 cells. In comparison to their high enzymatic potency, amine **40** only displayed moderate activity and fluoride **43** was inactive in both cell lines. Again, some effects of the stereochemistry were observed on the *cis*- and *trans*-isomers **47** and **48**. The former compound showed moderate potency against the *BRCA2*-deficient V-C8 cells, whereas the latter showed moderate potency against the *BRCA1*-deficient MDA-MB-436 cells.

Table 6. Cell Proliferation Inhibition of Compounds on *BRCA1* and *BRCA 2* Deficient Cells.[a]

Compd	IC_{50} (nM) (PARP1)	CC_{50} (BRCA 2, nM) [a] (V-C8)	CC_{50} (BRCA 1, nM) [a] (MDA-MB-436)
3 (AZD2281)	4.1	201	43.2
26	0.31	96.0	23.3
33	1.92	>10,000	344
39	1.39	630	556
41	3.46	4.53	<0.26
43	0.56	>10,000	4,300
47	4.81	418	4,520
48	0.57	2,270	775

[a] Values were calculated by Logit method from the results of at least three independent tests with six concentrations each (standard deviations were within 25% of the mean values).

Pharmacokinetic (PK) Profiles of Leads 26 and 41. Compounds **26** and **41** have molecular weight of less than 500 (468Da and 453Da, respectively), and good lipophilicity (CLogP: 2.06 and 2.67 respectively). As summarized in Table 7, both compounds have similar volume of plasma distribution in rats (Vss =4.3 and 4.6 L/kg, respectively) and similar half-life. Compound **26** has lower plasma clearance (2.28 L/h/kg), good plasma exposure ($AUC_{0-\infty}$, 1818 ng.h/mL) and moderate bioavailability (21%). While compound **41** showed higher plasma clearance (7.67 L/h/kg), moderate lower plasma exposure ($AUC_{0-\infty}$, 985 ng.h/mL) and lower bioavailability (~10%).[52]

Table 7. *In vitro* pharmacokinetic profile of lead compounds in rats

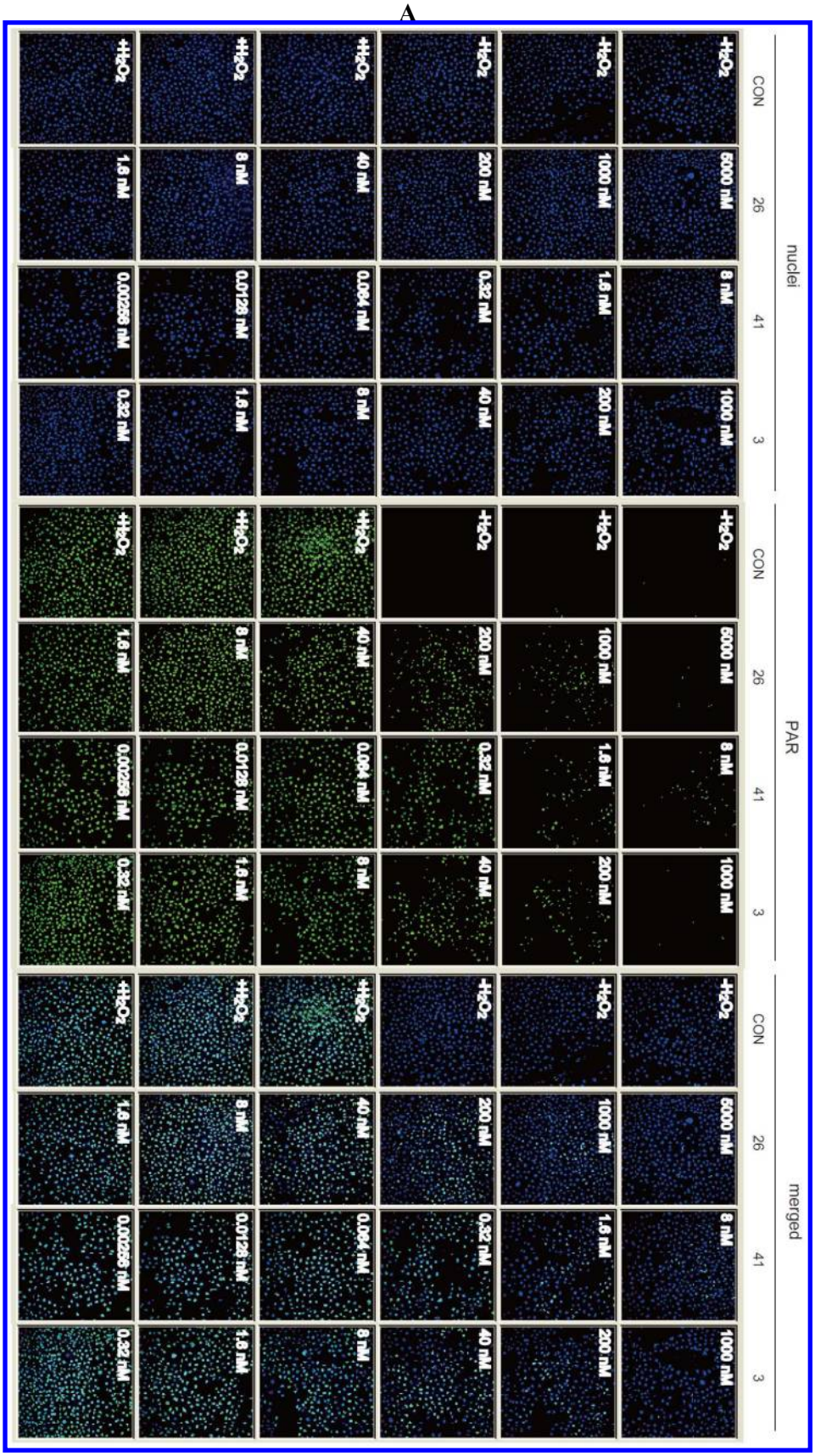
Compd	*iv* (10 mg/kg)[a]			*po* (20 mg/kg)[a]			
	CL ((L/h/kg)	Vss (L/kg)	$T_{1/2}$ (h)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0-\infty}$ (ng·h/mL)	F (%)
26	2.28 ± 0.28	4.27 ± 0.66	1.73 ± 0.12	722 ± 203	0.42±0.14	1818±431	21.0
41	7.67 ± 2.7	4.56 ± 1.86	1.11 ± 0.28	116 ± 10.0	0.44±0.13	980±182	10.0

[a]Values are the average of three runs. Vehicle: DMSO, Tween 80, normal saline. CL, clearance; Vss, volume of distribution; $T_{1/2}$, half-life; Cmax, maximum concentration; T_{max}, time of maximum concentration; $AUC_{0-\infty}$, area under the plasma concentration time curve; F, oral bioavailability.

Meanwhile, compounds **26** and **41** were further evaluated[53] for the inhibitory liability on hERG K+ channels, a major safety concern during the development of therapeutic drugs. Both compounds did not show significant inhibition at the concentration of 5.0 μM (17% and 34%, respectively).

Inhibition of Leads 26 and 41 on the H_2O_2-Triggered PARylation. Since both leads **26** and **41** showed high potency for the PARP1 enzyme and for the *BRCA1*- and *2*-deficient cells, their intrinsic activity was further evaluated. We first compared their ability to inhibit cellular PARP1/2 by detecting the synthesis of poly(ADP-ribose) (PAR) triggered by the DNA damaging agent H_2O_2 in human ovarian cancer BRCA-proficient SKOV3 cells.[15,25] H_2O_2 induces DNA single-strand breaks that causes a series of subsequent responses including activation of PARP, which may lead to PARylation of various proteins, *i.e.*, the synthesis of PAR.[54-56] As shown in Figure 2, H_2O_2 alone resulted in prominent PAR formation in the SKOV3 cells, and the PARylation could be reduced by pretreatment with compound **3**, **26** or **41**. Compound **41** was found significantly more potent to suppress the H_2O_2-driven PAR formation than compound **3** or **26**, with IC_{50} values of 0.49 nM (for **41**) versus 20.9 nM (for **3**) and 66.1 nM (for **26**), respectively.

A


nuclei
PAR
merged
CON
26
41
3
+H2O2
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200 nM
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8 nM
1.6 nM
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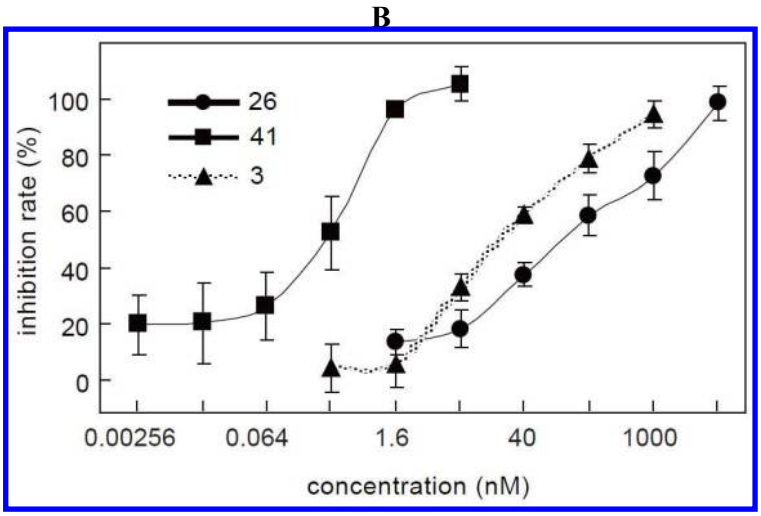

Figure 2. Leads 26 and 41 reduced the PARylation triggered by the DNA-damaging agent H_2O_2. SKOV3 cells were treated and assayed as described in the Methods section. **A**. Representative images of PAR formation inhibition. Nuclei stained with DAPI indicated the cell density; the FITC images revealed the levels of cellular PAR. **B**. The concentration-response curves quantitatively showed the inhibition of **3**, **26** and **41** on the formation of PAR. The data were expressed as mean ± SD, obtained from the images in **A** and from three independent experiments.

Compounds 26 and 41 Caused Cellular Accumulation of DNA Double-strand Breaks and Impaired Cell-cycle Progression in *BRCA2*-deficient Cells. The production and repair of DNA single-strand breaks are common phenomenon in the daily life, and the double-strand breaks would occur and accumulate in the cell if the repair by PARP1/2 is not timely or the repair pathways are deficient, especially *BRCA*-deficient.[56] Since γ-H2AX has been regarded as a molecular marker for DNA double-strand breaks,[57] we therefore examined whether compounds **26** and **41** increased the levels of cellular γ-H2AX. As shown in Figure 3, treatments with compound **3**, **26** and **41** at various concentrations led to significantly enhanced levels of γ-H2AX in *BRCA2*-deficient V-C8 cells, while only slightly altered levels of

γ-H2AX was observed in the *BRCA2*-proficient wild-type V79 cells. Moreover, compound **41** was found more efficacious to enhance the level of γ-H2AX than compound **26** and the reference compound **3**, which was consistent with their capacity of PAR inhibition.

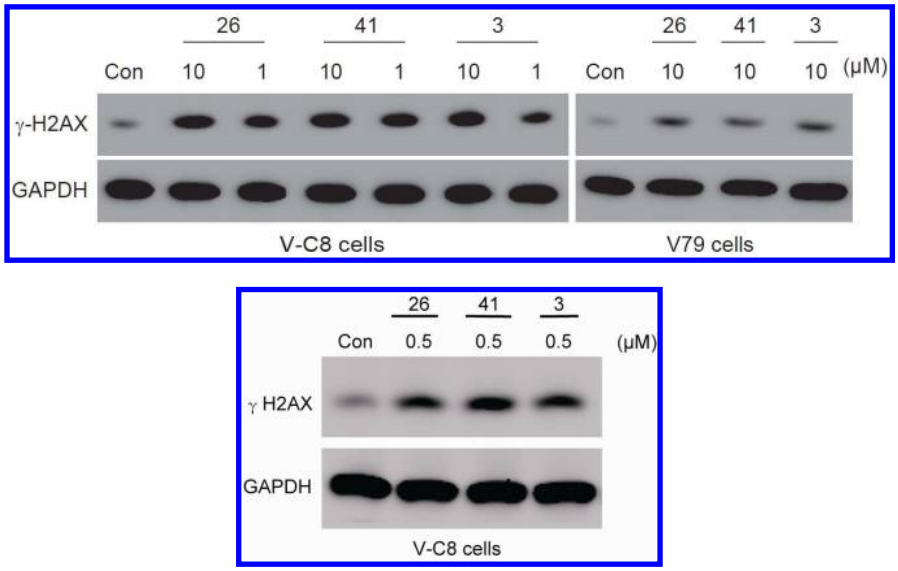

Figure 3. Leads 26 and 41 Increased the Levels of Cellular γ-H2AX. Cells were exposed to the corresponding compound at 37°C for 24 h, and then were subjected to standard Western blotting to detect the levels of cellular γ-H2AX.

Consistently, as shown in Figure 4, exposure of compounds **26** and **41** to the *BRCA2*-deficient V-C8 cells significantly impaired the cell-cycle progression. Moreover, our data also indicated that inhibition of cellular PARP by compounds **26** and **41** likely impaired the capacity of the cell to repair DNA single-strand breaks, thus triggering a series of subsequent biological responses. These results confirmed that our compounds caused DNA double-strand breaks accumulation and this process was induced by PARP1 inhibition.

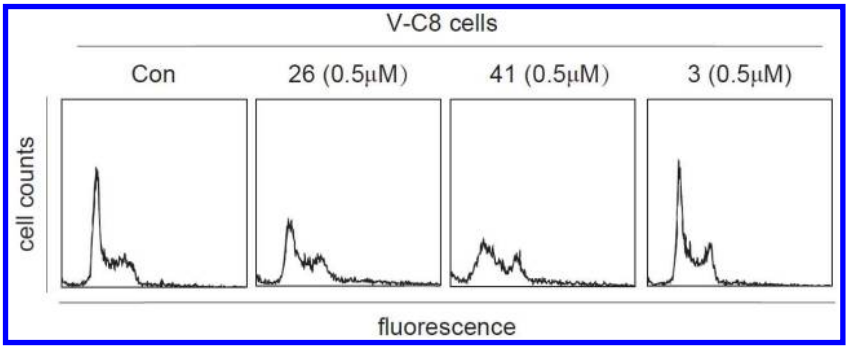

Figure 4. Leads 26 and 41 Changed Cell-cycle Progression. Cells were exposed to the indicated compound at 37°C for 24 h, and then were subjected to routine flow cytometry analyses to detect the cell cycle progression.

Compounds 26 and 41 Potentiated Cytotoxicity of the DNA Damaging Agent Temozolomide. To evaluate the potential application of compounds **26** and **41** as combinations with other antitumor agents, we then explored the impact of these two compounds in parallel with **3** on the cytotoxic efficacy of temozolomide.[25,58-63] As shown in Table 8, at both concentrations of 0.5 and 1.0 μM, all three compounds significantly enhanced the cytotoxicity of temozolomide in SKOV3 cells that have proficient repair for DNA double-strand breaks. In agreement with the previous data, compound **41** displayed higher potentiation on the efficacy of temozolomide than compound **26** and reference compound **3** at the same concentrations.

Table 8. Leads 26 and 41 Potentiated Cytotoxicity of Temozolomide in SKOV3 Cells.

Compound	Temozolomide [IC_{50} (μM) (mean ± SD) (n=3)]		
	alone	**+ 0.5 μM compound**	**+1.0 μM compound**
26	1910 ± 6.45	206 ± 17.2 （9.3-fold*）	180 ± 5.59 （10.6-fold）
41	1910 ± 4.04	98.0 ± 28.6 （19.5-fold）	85.8 ± 18.7 （22.2-fold）

3	1910 ± 6.88	198 ± 36.8 （9.6-fold）	183 ± 55.2 （10.4-fold）

*The fold potentiation was calculation as $IC_{50\ alone}$ / $IC_{50\ plus\ compound}$.

X-Ray Co-Crystal Structures of Compounds *S*-22 and 33 with the PARP1 Catalytic Domain. Since our first-generation lead **26** and second-generation lead **41** were structurally different, they might interact with PARP1 through different modes. Although efforts to obtain co-crystals of the two leads with PARP1 enzyme were unsuccessful, gratifyingly, co-structures of compounds ***S*-22** and **33** with PARP1 catalytic domain were achieved. The complete statistics, as well as the quality of the two solved structures, were shown in Supporting Information (Table S1). In both complex structures, the benzo[de][1,7]naphthyridin-7-one region of the compounds occupied the active site of the catalytic domain while the long chains of the compounds extended from the active site to the other direction until reaching the solvent accessible surface of the protein (Figure S1). Such long binding gorges generated by the two compounds within the catalytic domain of PARP1 were different from the binding modes of most previously reported PARP1 inhibitors but similar to the ones shown in complex structures with pdb codes 1UK0[64] and 1UK1[65]. The detailed interactions between the residues of the catalytic domain and the two compounds were shown in Figure 5. Residues Ser904 and Gly863 were involved into the key H-bonds with the lactam in the benzo[de][1,7]naphthyridin-7-one core while π-stacking interactions were formed between the side-chain of the residue Tyr907 and aromatic rings of the benzo[de][1,7]naphthyridin-7-ones. **S-22** also formed one H-bond with Tyr889, whereas two more H-bonds occurred between the long chain of **33** and residues Ile897 and Ala880. Through a water molecule, S-**22** interacted with residues His862 and Ser864 while **33** only interacted with the residue H862. In

addition, the hydrophobic interactions of residues Tyr889, Tyr896, Ala880, Pro881, Asp766, and Leu769 with S-**22** also contributed to the binding of the compound with PARP1. In the PARP1/**33** complex, hydrophobic interactions mostly were formed between residues Tyr889 and Tyr896 and the benzo[de][1,7]naphthyridin-7-one core. The interactions of *S*-**22** with more amino acid residues than **33** may rationale the 10-fold less potency. Meanwhile, the strong interactions between the active site of the catalytic domain and the benzo[de][1,7]naphthyridin-7-ones of the compounds act as anchors for the binding of these inhibitors into the active site. Meanwhile, either the H-bonds or hydrophobic interactions between residues nearby the protein surface and the long chains of the inhibitors fixed the flexibility of the long chains. It thus makes the long-chain inhibitors S-**22** and **33** perfectly lie down the long binding gorge within the catalytic domain of PARP1.[65]

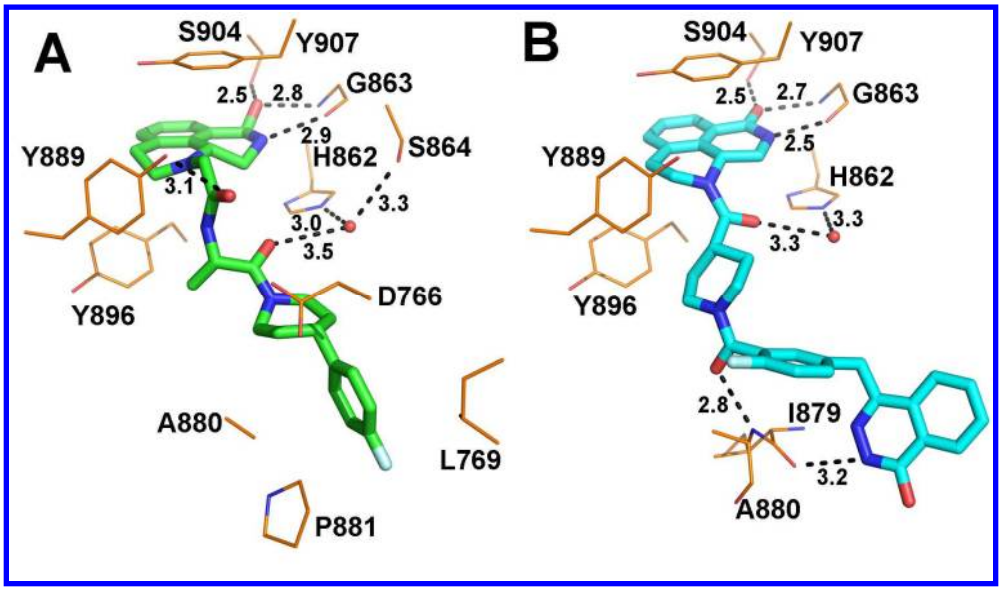

Figure 5. X-ray co-crystal structures of the catalytic domain of PARP1 in complex with compounds S-22 (A) and 33 (B). The deposited codes for the two structures in PDB are 4HHY (PARP1/**33**) and 4HHZ (PARP1/**S-22**).

Conclusion

In summary, extensive SAR studies were conducted to develop novel PARP1 inhibitors possessing the benzo[de][1,7]naphthyridin-7(8H)-one network as the core scaffold and a functionalized long-chain as the appendage. Our initial effort led to the first-generation PARP1 inhibitor **26** bearing a terminal phthalazin-1(2H)-one framework. This compound displayed remarkably high PARP1 affinity with an IC_{50} value of 0.31 nM, but only a moderate CC_{50} value of 96.0 nM against the *BRCA2*-deficient V-C8 cells. Further efforts generated our second-generation lead compound **41** showing equally high potency both for the PARP1 enzyme and for the *BRCA2*-deficient cells with IC_{50} and CC_{50} values of 3.46 and 4.53 nM, respectively. This compound was respectively over 20- and 40-fold more potent in the cells than our first-generation lead **26** and reference compound **3**. It was further found that both compounds **26** and **41** were hypersensitive against the *BRCA1*-deficient MDA-MB-436 cells, with compound **41** showing extremely high potency of less than 0.26 nM. Mechanistic studies revealed that our PARP1 inhibitors **26** and **41** significantly inhibited H_2O_2-triggered PARylation, induced cellular accumulation of DNA double-strand breaks, and impaired cell-cycle progression in *BRCA2*-deficient cells. Meanwhile, significant potentiation on the cytotoxicity of temozolomide by these compounds was also observed in SKOV3 cells. All these data confirmed that compounds **26** and **41** were potent PARP1 inhibitors possessing high potency both for the PARP1 enzyme and for the *BRCA1*/*2*-deficient cells. Compound **41** lacking the usual lactam pharmacophoric function as in the known PARP1 inhibitors and showing exceptionally high potency has been selected as a drug candidate for further *in vivo* studies.

Experimental Sections

Chemistry. ^{1}H NMR spectral data were recorded in $CDCl_3$ or $CDCl_3$ + CD_3OD or D_6-DMSO on Varian Mercury 300 or 400 NMR spectrometer and ^{13}C NMR was recorded in $CDCl_3$ on Varian Mercury 400 or 500 NMR spectrometer. Low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded at an ionizing voltage of 70 eV on a Finnigan/MAT95 spectrometer. Optical rotations were determined with a digital polarimeter and were the average of three measurements. The thermometers were used without additional correction. Column chromatography was carried out on silica gel (200–300 mesh). All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. Compounds **7**, **14**, **25a-e**, **34** and **49** were prepared according to corresponding literature procedures. HPLC analysis was conducted for all bioassayed compounds on an Agilent Technologies 1260 series LC system (Agilent ChemStation Rev.A.10.02; Eclipse XDB-CN, 4.6 mm x 250 mm, 5 μM, i-PrOH/n-Hex, rt) with two ultraviolet wavelengths (uv 254 and 214 nM). All the assayed compounds displayed a chemical purity of 95%-99% in both wavelengths.

General procedure for the synthesis of esters 8a-c

2,3,9,9a-Tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-one **7** (600mg, 3.19 mmol), an appropriate ethyl 2-bromoalkanoate (4.78 mmol), K_2CO_3 (6.38 mmol) and sodium iodide (*cat.*) were mixed in acetonitrile (20 mL). The resulting mixture was stirred at rt for 8h (for **8a**) or refluxed for 24h (for **8b, c**). After removal of the solvent, the crude material was dissolved in CH_2Cl_2 (30 mL) and water (20 mL). After filtration, the solvent was evaporated and the residue was subjected to chromatography to give corresponding esters **8a-c**.

Ethyl 2-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl) acetate (8a). Bright yellow oil (613 mg, 70.1%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (m, 2H), 7.30 – 7.22 (m, 2H), 4.18 (q, J = 7.1 Hz, 2H), 4.08 (dd, J = 12.5, 4.8 Hz, 1H), 3.69 (dt, J = 11.2, 5.4 Hz, 1H), 3.50 (q, J = 16.9 Hz, 2H), 3.32 (t, J = 12.0 Hz, 1H), 3.21 – 2.93 (m, 3H), 2.78 (dd, J = 14.3, 8.1 Hz, 1H), 1.27 (t, J = 7.1 Hz, 3H).

Ethyl 3-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)-

propanoate (8b). Yellow oil (414 mg, 45.0%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (m, 2H), 7.36 – 7.16 (m, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.86 – 3.62 (m, 2H), 3.31 – 2.90 (m, 4H), 2.83 – 2.62 (m, 2H), 2.61 – 2.38 (m, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).

Ethyl 4-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)-butanoate (8c). Brown oil (339 mg, 35.1%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, *J* = 5.9 Hz, 1H), 7.30 (m, 3H), 4.10 (q, *J* = 7.2 Hz, 2H), 3.73 (dd, *J* = 16.1, 7.3 Hz, 2H), 3.33 – 2.95 (m, 3H), 2.90 – 2.73 (m, 2H), 2.58 – 2.29 (m, 4H), 1.95 – 1.80 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 3H).

General procedure for the synthesis of 2-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo -[de][1,7]naphthyridin-1-yl)acetamides 10-13.

A solution of lithium hydroxide (2.0 mmol) in water (1 mL) was added to the solution of ester **8a-c** (1.0 mmol) in tetrahydrofuran (3 mL). The mixture was stirred overnight. After removal of the solvent, the residue was diluted with water (2 mL), acidified with 5N HCl to pH 6-7, and then extracted with $CHCl_3$ and MeOH (20:1, 10 mL × 3). The combined organic layer was washed with brine (10 mL), dried, filtrated, and then evaporated. The corresponding crude products **9a-c** was yielded as solid.

An appropriate bicycle-amine (0.13 mmol)**,** EDCI (49 mg, 0.25 mmol), HOBt (17 mg, 0.12 mol), Et_3N (60 uL, 0.44 mmol) were added successively to a solution of crude **9a** (31 mg, 0.13 mmol) in DMF (4 mL). The mixture was stirred overnight at rt, diluted with water (8 mL), and then extracted with CH_2Cl_2 (15 mL × 2). The combined organic phase was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH:30/1/0.2) to give corresponding acetamides **10-13.**

1-(*cis*-2-Hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)-2-oxoethyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-one (10). White foam (28 mg, 66 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.35-7.17 (m, 2H), 7.01 (s, 1H), 4.05-3.43 (m, 8H), 3.40-2.61 (m, 11H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.5, 168.4, 166.0, 135.7, 135.6, 133.2, 132.5, 127.1, 127.0, 125.7, 58.4, 58.2, 57.9, 57.8, 52.7, 52.6, 52.4,

52.3, 51.0, 50.9, 50.8, 50.7, 50.5, 43.9, 43.8, 41.5, 41.4, 28.5. MS (*EI*, M^+) *m/z* 340; HR-MS (*EI*) calcd for $C_{19}H_{24}N_4O_2$: 340.1899, found: 340.1898.

1-(2-(Hexahydropyrrolo[3,4-b]pyrrol-1(2H)-yl)-2-oxoethyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-one (11). White foam (27 mg, 64 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 6.2, 2.4 Hz, 1H), 7.34 – 7.22 (m, 2H), 6.90 (s, 1H), 4.44 (s, 1H), 4.01 – 3.87 (m, 1H), 3.79 – 3.54 (m, 3H), 3.42 – 3.03 (m, 6H), 1.93-2.61 (m, 4H), 2.34 – 1.98 (m, 4H), 1.82 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.4, 168.2, 166.1, 166.0, 135.7, 135.6, 133.2, 133.2, 132.5, 132.4, 127.1, 125.7, 63.3, 63.2, 58.6, 58.4, 58.2, 58.0, 57.8, 54.2, 54.0, 52.6, 50.9, 50.5, 47.1, 46.9, 43.9, 41.7, 41.6, 30.7, 30.5, 29.7, 28.6, 28.5, 28.3. MS (*EI*, M^+) *m/z* 340; HR-MS (*EI*) calcd for $C_{19}H_{24}N_4O_2$: 340.1899, found: 340.1892.

1-(2-Oxo-2-(tetrahydro-1H-pyrrolo[3,4-b]pyridin-6(2H,7H,7aH)-yl)ethyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-one (12). White foam (29 mg, 64 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.28 (d, *J* = 13.4 Hz, 2H), 7.08 (s, 1H), 4.12 – 3.88 (m, 1H), 3.79 – 3.44 (m, 5H), 3.42 – 2.70 (m, 8H), 2.62 (t, *J* = 10.7 Hz, 1H), 2.37-2.08 (m, 1H), 1.91 (s, 2H), 1.69 (m, 3H), 1.47 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 168.9, 168.8, 166.2, 166.1, 135.8, 135.7, 133.3, 133.2, 132.4, 127.1, 127.0, 126.9, 126.8, 125.5, 58.4, 58.2, 58.0, 57.9, 57.8, 57.7, 57.4, 57.3, 56.8, 56.6, 54.8, 53.4, 53.3, 53.2, 50.6, 50.5, 50.3, 47.3, 47.2, 47.1, 46.9, 45.0, 44.9, 44.6, 43.9, 43.8, 37.6, 35.1, 28.4, 23.1, 22.9, 21.4, 21.3, 21.2. MS (*EI*, M^+) *m/z* 354; HR-MS (*EI*) calcd for $C_{20}H_{26}N_4O_2$: 354.2056, found: 354.2048.

1-(2-Oxo-2-(3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl)ethyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-one (13). White foam (35 mg, 65 %). ^{1}H NMR (300 MHz, DMSO) δ 8.12 (d, *J* = 5.2 Hz, 1H), 7.72 – 7.53 (m, 1H), 7.30 (d, *J* = 5.1 Hz, 2H), 5.15 – 4.74 (m, 2H), 4.42 – 3.55 (m, 7H), 3.43 (s, 2H), 3.03 (dd, *J* = 38.0, 25.8 Hz, 2H), 2.75 (dd, *J* = 32.4, 17.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 169.8, 164.6, 151.4, 136.3, 133.6, 132.3, 128.0, 127.2, 125.2, 120.0, 117.8, 58.1, 58.0, 57.3, 57.1, 50.0, 44.2, 43.5, 43.3, 43.2, 42.2, 41.6,

39.0, 38.0, 28.5. MS (*EI*, M^+) *m/z* 420; HR-MS (*EI*) calcd for $C_{19}H_{19}N_6O_2F_3$: 420.1522, found: 420.1514.

General procedure for the synthesis of *N*-(2-(alkylamino)-2-oxoethyl)-3-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)alkylamides 15-20.

An appropriate amine **14** (0.13 mmol)**,** EDCI (49 mg, 0.25 mmol), HOBt (17 mg, 0.12 mol), Et_3N (60 uL, 0.44 mmol) were added successively to a solution of corresponding crude **9a-c** (0.13 mmol) in DMF (4 mL). The reaction was stirred overnight at rt and then diluted with water (8 mL). The mixture was extracted with CH_2Cl_2 (15 mL × 2), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH:30/1/0.2) to give the expected amides **15-20.**

***N*-(2-(4-(4-Methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-2-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)acetamide (15)**. White foam (49 mg, 80 %).^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.85 (d, *J* = 6.5 Hz, 1H), 7.31 (m, 2H), 6.92 – 6.74 (m, 5H), 4.24 – 4.06 (m, 2H), 3.88 – 3.71 (m, 6H), 3.60 – 3.34 (m, 5H), 3.26 – 2.99 (m, 7H), 2.89 – 2.75 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6, 166.3, 154.5, 144.9, 135.0, 132.8, 132.4, 127.3, 127.2, 125.9, 119.0, 114.5, 58.9, 58.8, 55.5, 52.1, 51.0, 50.7, 44.6, 44.2, 42.1, 40.7, 28.8. MS (*EI*, [M^+]) *m/z* 477; HR-MS (*EI*) calcd for $C_{26}H_{31}N_5O_4$: 477.2376, found: 477.2370.

***N*-(2-(4-(4-Methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-3-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)propanamide (16)**. White foam (54 mg, 85 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 6.7, 2.1 Hz, 1H), 7.67 (t, *J* = 4.0 Hz, 1H), 7.35 – 7.29 (m, 2H), 6.94 – 6.78 (m, 5H), 4.14 (d, *J* = 4.2 Hz, 2H), 3.85 – 3.72 (m, 7H), 3.61 – 3.55 (m, 2H), 3.37 (t, *J* = 11.8 Hz, 1H), 3.33 – 3.22 (m, 2H), 3.17 – 2.99 (m, 5H), 2.82 (d, *J* = 16.4 Hz, 1H), 2.73 – 2.46 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.8, 166.4, 166.0, 154.5, 145.0, 135.6, 133.1, 132.3, 127.3, 127.0, 125.8, 119.0, 114.5, 58.3, 55.5, 51.0, 50.7, 49.9, 48.8, 44.6, 44.0, 42.1, 41.2, 33.0,

28.4. MS (*EI*, [M^+]) *m/z* 491; HR-MS (*EI*) calcd for $C_{27}H_{33}N_5O_4$: 491.2533, found: 491.2523.

***N*-(2-(4-(4-Methoxyphenyl)piperazin-1-yl)-2-oxoethyl)-4-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)butanamide (17)**. White foam (53 mg, 82 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (dd, *J* = 5.9, 2.9 Hz, 1H), 7.26 (dd, *J* = 3.2, 2.8 Hz, 2H), 6.99 (d, *J* = 4.5 Hz, 1H), 6.94 – 6.76 (m, 5H), 4.10 (d, *J* = 4.1 Hz, 2H), 3.83 – 3.60 (m, 7H), 3.59 – 3.47 (m, 2H), 3.30 – 3.11 (m, 2H), 3.09 – 2.94 (m, 5H), 2.90 – 2.72 (m, 2H), 2.57 – 2.39 (m, 2H), 2.33 (m, 2H), 1.98 – 1.84 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.7, 166.6, 166.2, 154.5, 144.9, 136.0, 133.3, 132.3, 127.2, 126.9, 125.6, 119.0, 114.5, 58.0, 55.5, 53.2, 51.0, 50.7, 49.1, 44.5, 44.1, 42.1, 41.1, 33.6, 28.5, 21.9. MS (*EI*, [M^+]) *m/z* 505; HR-MS (*EI*) calcd for $C_{28}H_{35}N_5O_4$: 505.2689, found: 505.2676.

***N*-(2-(4-(2-Fluorophenyl)piperazin-1-yl)-2-oxoethyl)-3-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)propanamide (18)**. Bright yellow foam (40 mg, 65 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, *J* = 6.1 Hz, 1H), 7.69 (s, 1H), 7.27 (d, *J* = 6.3 Hz, 2H), 7.13 – 6.84 (m, 5H), 4.12 (d, *J* = 3.8 Hz, 2H), 3.87 – 3.66 (m, 4H), 3.57 (s, 2H), 3.38 – 3.18 (m, 3H), 3.17 – 2.94 (m, 5H), 2.78 (m, 1H), 2.71 – 2.44 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 168.0, 167.6, 157.1 (d, *J* = 244.6 Hz), 140.7 (d, *J* = 8.6 Hz), 137.1, 134.6, 133.7, 128.7, 128.4, 127.1, 126.0 (d, *J* = 3.4 Hz), 124.8 (d, *J* = 7.9 Hz), 120.7, 117.7 (d, *J* = 20.4 Hz), 59.6, 52.0, 51.6, 51.4, 50.2, 46.0, 45.4, 43.5, 42.6, 34.3, 29.8. MS (*EI*, [M^+]) *m/z* 479; HR-MS (*EI*) calcd for $C_{26}H_{30}N_5O_3F$: 479.2333, found: 479.2340.

3-(7-Oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)-N-(2-oxo-2-(4-(pyrimidin-2-yl)piperazin-1-yl)ethyl)propanamide (19). White foam (41 mg, 68 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (d, *J* = 4.6 Hz, 2H), 7.82 (d, *J* = 5.7 Hz, 1H), 7.70 (s, 1H), 7.25 (s, 2H), 7.07 (d, *J* = 4.7 Hz, 1H), 6.52 (t, *J* = 4.6 Hz, 1H), 4.12 (d, *J* = 3.5 Hz, 2H), 3.92 – 3.58 (m, 8H), 3.47 (s, 2H), 3.41 – 3.16 (m, 3H), 3.06 (d, *J* = 11.2 Hz, 1H), 2.78 (d, *J* = 16.0 Hz, 1H), 2.71 – 2.41 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.9, 166.8, 166.1, 161.3, 157.7, 135.6, 133.1, 132.2, 127.3, 127.0,

125.7, 110.6, 58.2, 49.9, 48.8, 44.2, 44.0, 43.4, 43.2, 41.8, 41.2, 32.9, 28.4. MS (*EI*, $[M^+]$) *m/z* 463; HR-MS (*EI*) calcd for $C_{24}H_{29}N_7O_3$: 463.2332, found: 463.2327.

***N*-(2-(4-(4-Fluorophenyl)-5,6-dihydropyridin-1(2H)-yl)-2-oxoethyl)-3-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)propanamide (20)**. White foam (50 mg, 82 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 – 7.79 (m, 1H), 7.65 (d, *J* = 3.8 Hz, 1H), 7.35 – 7.21 (m, 4H), 7.00 (td, *J* = 8.6, 1.6 Hz, 2H), 6.78 (s, 1H), 5.95 (d, *J* = 18.6 Hz, 1H), 4.27 – 3.99 (m, 4H), 3.88 – 3.66 (m, 3H), 3.61 (t, *J* = 5.7 Hz, 1H), 3.42 – 3.19 (m, 3H), 3.17 – 3.02 (m, 1H), 2.79 (d, *J* = 16.7 Hz, 1H), 2.74 – 2.37 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 166.9, 166.6, 166.0, 136.1, 136.0, 135.8, 135.6, 134.1, 133.2, 132.3, 127.3, 127.2, 127.0, 126.9, 126.8, 126.7, 126.6, 126.5, 125.8, 125.7, 120.1, 118.3, 115.4, 115.2, 58.2, 49.9, 48.8, 44.0, 43.6, 42.3, 41.6, 41.5, 41.3, 38.7, 33.0, 28.4, 27.6, 26.9. MS (*EI*, M^+) *m/z* 476; HR-MS (*EI*) calcd for $C_{27}H_{29}N_4O_3F$: 476.2224, found: 476.2217.

***N*-(1-(4-(4-Fluorophenyl)-5,6-dihydropyridin-1(2H)-yl)-1-oxopropan-2-yl)-2-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)acetamides *R*-22 and *S*-22**.

N-Boc-Ala (72mg, 0.38 mmol)**,** EDCI (148 mg, 0.76 mmol), HOBt (52 mg, 0.36 mol), Et_3N (160 uL, 1.16 mmol) were added successively to a solution of 4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine (67 mg, 0.38 mmol) in DMF (4 mL). The reaction was stirred overnight at rt and diluted with water (8 mL). The mixture was extracted with CH_2Cl_2 (15 mL × 2), dried over Na_2SO_4, and concentrated under reduced pressure to give corresponding crude amides ***R*-21** and ***S*-21**.

(*R*)-*tert*-Butyl 1-(4-(4-fluorophenyl)-5,6-dihydropyridin-1(2H)-yl)-1-oxoprop-an-2-ylcarbamate (*R*-21). White foam (130 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.26 – 7.17 (m, 2H), 6.95 – 6.86 (m, 2H), 5.89 (d, *J* = 14.1 Hz, 1H), 5.60 (d, *J* = 7.4 Hz, 1H), 4.60 (d, *J* = 7.0 Hz, 1H), 4.27 – 3.95 (m, 2H), 3.78 – 3.51 (m, 2H), 2.47 (s, 1H), 1.34 (d, *J* = 1.4 Hz, 9H), 1.26 – 1.18 (m, 3H).

(*S*)-*tert*-Butyl 1-(4-(4-fluorophenyl)-5,6-dihydropyridin-1(2H)-yl)-1-oxoprop-

an-2-ylcarbamate (*S*-21). White foam (127 mg, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.28 – 7.15 (m, 2H), 6.98 – 6.83 (m, 2H), 5.89 (d, *J* = 12.8 Hz, 1H), 5.58 (s, 1H), 4.58 (dd, *J* = 12.4, 6.9 Hz, 1H), 4.10 (t, *J* = 15.1 Hz, 2H), 3.82 – 3.48 (m, 2H), 2.44 (d, *J* = 17.9 Hz, 2H), 1.40 – 1.28 (m, 9H), 1.24 (m, 3H).

To the solution of crude amide ***R*-21** or ***S*-21** (69 mg, 0.20 mmol) in CH_2Cl_2 (10 mL) was added TFA (1.5 mL). The mixture was stirred at rt for 24h. The solvent was removed, and the residue was taken-up in water (2 mL), alkalified with *conc.* NH_4OH to pH > 7. The mixture was extracted with $CHCl_3$ and MeOH (20:1, 10 mL × 3). The combined organic layer was washed with brine (10 mL), dried, and then evaporated to yield the amine intermediate as brown oil.

To a solution of the amine intermediate (62 mg, 0.17 mmol) in DMF (4 mL) was added **9a** (43 mg, 0.17 mmol)**,** EDCI (68 mg, 0.35 mmol), HOBt (24 mg, 0.17 mol), and Et_3N (125 uL, 0.88 mmol) successively. The mixture was stirred overnight at rt, diluted with water (8 mL), and extracted with CH_2Cl_2 (15 mL × 2). The combined organic phase was dried over Na_2SO_4, and concentrated. The residue was subjected to column chromatography on silica gel (30:1:0.2 CH_2Cl_2/MeOH/NH_4OH) to give corresponding acetamides ***R*-22** and ***S*-22**.

***N*-((*R*)-1-(4-(4-Fluorophenyl)-5,6-dihydropyridin-1(2H)-yl)-1-oxopropan-2-yl)-2-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)acetamide (*R*-22)**. White foam (60 mg, 74 %). $[\alpha]^{23}_D$ -10.8°(*c* 0.86, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (m, 1H), 7.83 (t, *J* = 6.0 Hz, 1H), 7.27 (d, *J* = 8.1 Hz, 5H), 7.00 (t, *J* = 7.5 Hz, 2H), 5.97 (d, *J* = 9.2 Hz, 1H), 5.07 – 4.87 (m, 1H), 4.31 – 4.07 (m, 2H), 3.90 – 3.68 (m, 3H), 3.61 – 3.04 (m, 6H), 2.90 – 2.68 (m, 2H), 2.55 (d, *J* = 25.4 Hz, 2H), 1.35 (dd, *J* = 17.7, 10.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.9, 170.8, 170.7, 170.6, 169.7, 169.6, 166.2, 163.3, 163.2, 161.3, 161.2, 136.0, 135.8, 135.1, 134.0, 133.9, 133.0, 132.8, 132.5, 132.3, 127.4, 127.3, 127.2, 126.6, 126.5, 126.4, 125.9, 125.8, 120.1, 118.9, 118.7, 115.4, 115.2, 59.2, 59.1, 58.8, 58.7, 52.2, 52.0, 45.2, 45.1, 44.9, 44.8, 44.2, 44.1, 42.6, 42.5, 42.3, 39.4, 39.2, 28.8, 27.9, 27.1, 27.0, 19.1,

18.9. MS (*EI*, M^+) *m/z* 476; HR-MS (*EI*) calcd for $C_{27}H_{29}N_4O_3F$: 476.2223, found: 476.2209.

***N*-((*S*)-1-(4-(4-Fluorophenyl)-5,6-dihydropyridin-1(2H)-yl)-1-oxopropan-2-yl)-2-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)acetamide (*S*-22)**. White foam (53 mg, 65 %). $[\alpha]^{23}_D$ +18.8°(*c* 1.05, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, *J* = 8.3 Hz, 1H), 7.86 (s, 1H), 7.31 (s, 3H), 7.02 (t, *J* = 8.6 Hz, 2H), 6.49 (s, 1H), 5.99 (d, *J* = 11.8 Hz, 1H), 5.01 (s, 1H), 4.20 (d, *J* = 17.3 Hz, 2H), 3.79 (dd, *J* = 31.2, 6.6 Hz, 3H), 3.61 – 3.32 (m, 3H), 3.17 (dd, *J* = 37.2, 16.8 Hz, 3H), 2.89 – 2.76 (m, 2H), 2.56 (d, *J* = 20.0 Hz, 2H), 1.37 (dd, *J* = 17.5, 10.7 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8, 170.6, 169.7, 165.9, 136.0, 135.1, 134.1, 134.0, 133.0, 132.7, 132.6, 132.5, 127.4, 127.2, 127.1, 126.7, 126.6, 126.5, 126.1, 126.0, 120.2, 118.8, 118.7, 115.5, 115.3, 59.3, 59.2, 58.9, 58.8, 52.3, 52.1, 45.2, 45.0, 44.9, 44.4, 44.3, 42.6, 42.4, 39.3, 28.8, 28.0, 27.1, 19.2, 19.0. MS (*EI*, M^+) *m/z* 476; HR-MS (*EI*) calcd for $C_{27}H_{29}N_4O_3F$: 476.2223, found: 476.2214.

***N*-(1-(4-(4-Fluorophenyl)-5,6-dihydropyridin-1(2H)-yl)-1-oxo-3-phenylpropan-2-yl)-2-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)acetamide (*R*-24)**. This compound was prepared as white foam in 35% yield by following a procedure similar to that of preparation of ***R*-22**. $[\alpha]^{25}_D$ -30.4° (*c* 1.10, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.4 Hz, 1H), 7.86 (d, *J* = 6.4 Hz, 1H), 7.36 – 7.11 (m, 10H), 6.99 (td, *J* = 8.7, 3.0 Hz, 2H), 5.94 (s, 0.5H), 5.73 (s, 0.5H), 5.24 (dd, *J* = 25.8, 7.6 Hz, 1H), 4.17 (m, 2H), 3.81 – 3.67 (m, 2H), 3.66 – 3.53 (m, 1H), 3.51 – 3.27 (m, 3H), 3.24 – 2.94 (m, 6H), 2.75 (m, 2H), 2.45 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.8, 169.7, 166.0, 163.3, 163.2, 160.9, 160.8, 135.8, 135.7, 135.1, 134.9, 134.0, 132.7, 132.3, 129.3, 129.2, 128.4, 128.3, 127.2, 127.1, 127.0, 126.9, 126.4, 126.3, 126.2, 125.7, 119.6, 118.7, 115.2, 115.0, 58.6, 51.9, 49.7, 49.2, 44.7, 43.8, 42.4, 39.3, 39.2, 38.9, 28.6, 27.6, 26.8. MS (*EI*, M^+) *m/z* 550; HR-MS (*EI*) calcd for $C_{33}H_{33}N_4O_3F$: 552.2537, found: 552.2532.

***N*-(1-(4-(4-Fluorophenyl)-5,6-dihydropyridin-1(2H)-yl)-1-oxo-3-phenylpropan-2-yl)-2-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)acetamide (*S*-24)**. This compound was prepared as white foam in 42% yield by following a procedure similar to that of preparation of ***R*-22**. $[\alpha]^{25}_{D}$ +61.7° (*c* 0.83, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.3 Hz, 1H), 7.81 (d, *J* = 6.0 Hz, 1H), 7.33 – 7.15 (m, 9H), 6.99 (td, *J* = 8.8, 3.3 Hz, 3H), 5.92 (s, 0.5H), 5.73 (s, 0.5H), 5.25 (dd, *J* = 26.9, 7.3 Hz, 1H), 4.14 (s, 2H), 3.81-3.65 (m, 2H), 3.64-3.53 (m, 1H), 3.53-3.20 (m, 4H), 3.18-2.94 (m, 4H), 2.88 (d, *J* = 4.3 Hz, 1H), 2.81 – 2.67 (m, 2H), 2.44 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.6, 169.5, 169.4, 165.8, 163.0, 161.1, 161.0, 135.9, 135.8, 135.7, 135.1, 134.9, 133.9, 132.4, 132.2, 129.3, 129.2, 128.4, 128.3, 127.2, 127.1, 127.0, 126.9, 126.4, 126.3, 126.2, 125.8, 119.6, 118.7, 115.3, 115.2, 115.1, 115.0, 58.6, 58.5, 58.4, 51.8, 49.6, 49.1, 44.7, 43.9, 42.4, 42.3, 39.3, 39.2, 38.9, 28.5, 27.5, 26.8. MS (*EI*, M^+) *m/z* 550; HR-MS (*EI*) calcd for $C_{33}H_{33}N_4O_3F$: 552.2537, found: 552.2501.

General procedure for the synthesis of 1-(5-substitute-2-fluorobenzoyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-ones 26-29.

To a solution of **7** (69 mg, 0.36 mmol) in DMF (4 mL), an appropriate acid **25a – 25d** (0.33 mol), HBTU (161 mg, 0.42 mmol), and DIPEA (130 uL, 0.72 mmol) were added. The mixture was stirred for 12h, diluted with water (8 mL), and then extracted with $CHCl_3$ (15 mL × 2). The combined organic phase was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH:25/1/0.1) to give corresponding products **26-29**.

1-(2-Fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-one (26). White solid (92 mg, 60%). Mp: 265-267 °C. ^{1}H NMR (300 MHz, DMSO) δ 12.59 (s, 1H), 10.36 (s, 1H), 8.30 – 8.09 (m, 2H), 7.95 (d, *J* = 7.7 Hz, 1H), 7.85 (m, 2H), 7.72 (s, 1H), 7.39 (m, 3H), 7.24 (t, *J* = 9.2 Hz, 1H), 5.27 (d, *J* = 7.3 Hz, 1H), 4.33 (s, 2H), 3.77 – 3.47 (m, 2H), 3.36 – 3.07 (m, 2H), 2.75 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 165.8, 164.0, 159.9,

157.0 (d, *J* = 245.3 Hz), 145.3, 135.4, 134.9, 134.4, 133.9, 132.0, 132.0, 129.5, 129.0, 128.3, 127.4, 126.5, 126.1, 125.9, 124.5, 124.4, 116.5, 116.3, 49.7, 43.7, 41.4, 36.9, 29.7. MS (*EI*, [M^+]) *m/z* 468; HR-MS (*EI*) calcd for $C_{27}H_{21}N_4O_3F$: 468.1598, found: 468.1589.

1-(2-Fluoro-5-((4-oxo-3,4,5,6,7,8-hexahydrophthalazin-1-yl)methyl)benzoyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-one (27). White solid (84 mg, 54%). Mp: 238-239 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.84 (s, 1H), 7.95 (d, *J* = 6.1 Hz, 1H), 7.39 (s, 1H), 7.33 – 7.21 (m, 4H), 7.04 (t, *J* = 8.7 Hz, 1H), 5.53 (d, *J* = 7.3 Hz, 1H), 4.07 (s, 1H), 3.92 (s, 2H), 3.81 (d, *J* = 12.1 Hz, 1H), 3.39 (t, *J* = 11.7 Hz, 1H), 3.24 (s, 1H), 2.89 (s, 1H), 2.71 (d, *J* = 14.7 Hz, 1H), 2.56 (s, 2H), 2.38 (s, 2H), 1.69 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.3, 165.4, 162.5, 157.1 (d, *J* = 246.8 Hz), 146.6, 140.5, 138.1, 134.2, 133.8, 131.9, 129.0, 128.3, 127.2, 126.8, 124.2, 124.1, 116.1, 115.9, 49.5, 44.0, 41.8, 37.5, 30.2, 25.5, 22.9, 21.2, 20.6. MS (*EI*, [M^+]) *m/z* 472; HR-MS (*EI*) calcd for $C_{27}H_{25}N_4O_3F$: 472.1911, found: 472.1913.

1-(2-Fluoro-5-((5-methyl-6-oxo-1,6-dihydropyridazin-3-yl)methyl)benzoyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-one (28). White solid (74 mg, 52%). Mp: 208-209 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.96 (s, 1H), 7.94 (d, *J* = 6.2 Hz, 1H), 7.57 (s, 1H), 7.28 (m, 4H), 7.04 (t, *J* = 8.7 Hz, 1H), 6.98 (s, 1H), 5.59 – 5.46 (m, 1H), 4.08 (m, 1H), 3.88 (s, 2H), 3.81 (d, *J* = 12.6 Hz, 1H), 3.35 (m, 2H), 2.76 (m, 2H), 2.14 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 165.6, 163.1, 157.2 (d, *J* = 246.0 Hz), 147.1, 140.9, 134.6, 133.8, 131.9, 131.1, 129.3, 128.3, 127.3, 126.8, 124.4, 124.2, 116.3, 116.1, 49.5, 43.8, 41.8, 39.8, 30.2, 16.2. MS (*EI*, [M^+]) *m/z* 432; HR-MS (*EI*) calcd for $C_{24}H_{21}N_4O_3F$: 432.1598, found: 432.1601.

1-(5-((4,5-Dimethyl-6-oxo-1,6-dihydropyridazin-3-yl)methyl)-2-fluorobenzoyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-one (29). White solid (81 mg, 55%). Mp: 230-232 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.96 (s, 1H), 7.95 (d, *J* = 6.5 Hz, 1H), 7.39 – 7.21 (m, 5H), 7.04 (t, *J* = 8.8 Hz, 1H), 5.52 (dd, *J* = 12.0, 4.9 Hz, 1H), 4.17 – 4.02 (m, 1H), 3.98 (s, 2H), 3.80 (d, *J* = 11.0 Hz, 1H), 3.35 (m, 2H), 2.77 (m, 2H), 2.14 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$+ CD_3OD) δ

166.6, 165.5, 162.3, 157.1 (d, J = 247.0 Hz), 147.1, 139.7, 137.2, 134.3, 133.6, 132.1, 128.8, 128.0, 127.4, 126.7, 124.0, 123.8, 116.2, 116.0, 49.1, 43.8, 41.5, 38.5, 30.0, 15.4, 11.8. MS (*EI*, [M^+]) *m/z* 446; HR-MS (*EI*) calcd for $C_{25}H_{23}N_4O_3F$: 446.1754, found: 446.1755.

General procedure for the synthesis of 1-(1-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)substitute-2-carbonyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-ones 31-33.

To a solution of intermediate **30** (69 mg, 0.20 mmol, prepared by following a procedure similar to that of compounds (**26-29**) in CH_2Cl_2 (10 mL), was added TFA (1.5 mL). The mixture was stirred at rt for 24h and then evaporated. The residue was taken-up in water (2 mL), alkalified with *conc.* NH_4OH to pH > 7, and extracted with the mixture of $CHCl_3$ and MeOH (20:1, 10 mL × 3). The combined organic layer was washed with brine (10 mL), dried, filtrated, and then evaporated to yield the amine intermediate as white solid.

To a solution of the white solid prepared above (0.17 mmol) in DMF (4 mL), DIPEA (130 uL, 0.72 mmol), acid **25a** (55 mg, 0.18 mol), and HBTU (161 mg, 0.42 mmol) were added, and the reaction was stirred for 12h. The mixture was diluted with water (8 mL), extracted with $CHCl_3$ (15 mL×2), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH:25/1/0.2) to give corresponding products **31-33**.

2-Fluoro-*N*-(2-oxo-2-(7-oxo-2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridin-1-yl)ethyl)-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzamide (31). White solid (76 mg, 85%). Mp: 260-262 °C. ^{1}H NMR (300 MHz, DMSO) δ 12.58 (s, 1H), 8.35 (s, 1H), 8.27 – 8.21 (m, 1H), 8.11 (s, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.91 – 7.77 (m, 2H), 7.69 (dd, J = 9.0, 4.2 Hz, 2H), 7.54 – 7.45 (m, 1H), 7.44 – 7.29 (m, 2H), 7.24 (dd, J = 10.8, 8.5 Hz, 1H), 5.11 (d, J = 7.9 Hz, 1H), 4.34 (s, 2H), 4.27 (s, 2H), 4.09 (d, J = 10.6 Hz, 1H), 3.58 – 3.47 (m, 1H), 3.16 (m, 2H), 2.82 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 168.1, 164.0, 163.6, 159.8, 158.7 (d, J = 248.2 Hz), 145.4,

135.2, 135.0, 134.6, 134.0, 133.6, 132.0, 130.9, 129.5, 129.1, 128.3, 127.3, 126.5, 125.9, 116.9, 116.7, 49.8, 42.5, 41.5, 41.2, 36.9, 30.0. MS (*EI*, [M^+]) *m/z* 525; HR-MS (*EI*) calcd for $C_{29}H_{24}N_5O_4F$: 525.1812, found: 525.1807.

1-(1-(2-Fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)pyrrolidine-2-carbonyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-one (32). White foam (80 mg, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.58 (s, 1H), 11.92 (s, 1H), 8.43 (s, 1H), 7.99 – 7.87 (m, 1H), 7.70 (s, 3H), 7.53 – 7.45 (m, 1H), 7.39 – 7.27 (m, 3H), 6.98 (t, *J* = 8.9 Hz, 1H), 5.48 (m, 1H), 5.17 – 5.01 (m, 1H), 4.39 (m, 1H), 4.26 (s, 2H), 3.94 – 3.15 (m, 6H), 2.82 (m, 2H), 2.32 (m, 1H), 2.07 – 1.87 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 171.3, 165.5, 165.0, 161.1, 157.2 (d, *J* = 247.4 Hz), 145.6, 135.0, 134.4, 134.2, 134.1, 133.7, 133.5, 132.4, 131.8, 131.7, 131.4, 129.5, 129.0, 128.4, 128.2, 128.1, 127.4, 127.0, 126.9, 126.5, 125.1, 124.9, 124.7, 116.3, 116.1, 57.4, 56.9, 51.2, 49.8, 49.4, 48.7, 46.9, 43.6, 42.3, 41.8, 38.2, 37.6, 30.3, 29.8, 24.8, 22.7. MS (*EI*, [M^+]) *m/z* 565; HR-MS (*EI*) calcd for $C_{32}H_{28}N_5O_4F$: 565.2126, found: 565.2122.

1-(1-(2-Fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperidine-4-carbonyl)-2,3,9,9a-tetrahydro-1H-benzo[de][1,7]naphthyridin-7(8H)-one (33). White solid (53 mg, 54%). Mp: 144-146 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.88 (s, 1H), 8.40 (d, *J* = 7.6 Hz, 1H), 7.99 – 7.83 (m, 1H), 7.70 (m, 3H), 7.39 – 7.15 (m, 5H), 6.97 (t, *J* = 8.9 Hz, 1H), 5.33 (m, 1H), 4.67 (m, 1H), 4.27 (s, 2H), 4.11 (d, *J* = 12.6 Hz, 1H), 3.85 (m, 1H), 3.60 (m, 1H), 3.05 (m, 7H), 1.77 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 165.4, 164.9, 161.0, 157.0 (d, *J* = 246.0 Hz), 145.6, 134.3 (d, *J* = 7.9 Hz), 133.7, 133.6, 131.8, 131.5, 131.2 (d, *J* = 7.7 Hz), 129.5, 129.0, 128.4, 128.2, 127.2, 127.0, 126.8, 125.0, 124.2 (d, *J* = 18.2 Hz), 116.2, 116.0, 49.4, 46.4, 42.0, 41.9, 41.2, 39.0, 37.6, 30.6, 28.3, 27.8. MS (*EI*, [M^+]) *m/z* 579; HR-MS (*EI*) calcd for $C_{33}H_{30}N_5O_4F$: 579.2282, found: 579.2282.

4-(4-Fluoro-3-(2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridine-1-carbonyl)benzyl)phthalazin-1(2H)-one (38).

To a solution of *N*-benzyl **35** (104 mg, 0.37 mmol) in anhydrous THF (6 mL) at 0°C, was added $LiAlH_4$ (45 mg, 1.12 mmol) by portions. The reaction was stirred at rt for 30 min and then refluxed overnight. The mixture was cooled to 0°C and then added H_2O (1 mL), 10% NaOH (1.5 mL) and H_2O (2 mL) successively. The white solid was removed and the filtrate was dried over Na_2SO_4. After removal of the solvent, crude amine **36** was obtained as brown oil (70 mg, 71%).

To a solution of crude amine **36** (70 mg, 0.27 mmol, prepared above) and triethylamine (74 uL, 0.53 mmol) in dry CH_2Cl_2 (8 mL), was added $(Boc)_2O$ (55 mg, 0.32 mmol). The mixture was stirred at rt for 1h and then washed successively with *aq*. NH_4Cl, *aq* $NaHCO_3$ and brine. After dried over Na_2SO_4, the filtrate was concentrated under reduced pressure to give crude yellow oil. To the crude oil (85 mg, 0.23 mmol) in MeOH (20 mL) and AcOH (1 mL), was added Pd/C (16 mg). The mixture was hydrogenated under a pressure of 1.5 *psi* for 4h. The mixture was filtered through a plug of celite, and the filtrate was concentrated. The residue was basified (pH 9) with ammonia and the liberated base was extracted with CH_2Cl_2 (3 × 10 mL), washed with brine (15 mL), and concentrated to give **37** (50 mg, 69% for two step) as a yellow oil.

To a solution of **37** (50 mg, 0.18 mmol) prepared above in DMF (4 mL), DIPEA (73 uL, 0.41 mmol), acid **25a** (50 mg, 0.17 mmol), and HBTU (91 mg, 0.24 mmol) were added. The mixture was stirred for 12h and then diluted with water (8 mL), extracted with $CHCl_3$ (15 mL × 2), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was then de-Boced with TFA/CH_2Cl_2 by following a general procedure, and purified by silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH:15/1/0.1) to give target **38** as white foam (20 mg, 54%). ^1H NMR (300 MHz, $CDCl_3$) δ 8.50 – 8.40 (m, 1H), 7.75 (m, 3H), 7.36 (m, 2H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.00 (m, 3H), 5.15 (m, 1H), 4.30 (s, 2H), 4.09 (s, 2H), 3.91 (dd, *J* = 11.5, 5.0 Hz, 1H), 3.71 (d, *J* = 9.9 Hz, 1H), 3.14 (s, 1H), 2.83 – 2.54 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 160.8, 157.3 (d, *J* = 245.6 Hz), 145.6, 136.3, 134.8, 134.3, 133.6, 131.5, 129.5, 129.2, 128.3, 127.1, 126.3, 125.9, 125.1, 124.9, 124.1,

116.2, 116.0, 51.1, 46.9, 45.0, 44.3, 37.7, 31.1. MS (*ESI*, $[M+1]^+$) *m/z* 455; HR-MS (*ESI*) calcd for $C_{27}H_{24}N_4O_2F$: 455.1883, found: 455.1851.

4-(4-Fluoro-3-(2,3,7,8,9,9a-hexahydro-1H-benzo[de][1,7]naphthyridine-8-carbonyl)benzyl)phthalazin-1(2H)-one (39). To a solution of **36** (82 mg, 0.31 mmol) prepared above in DMF (5 mL), DIPEA (130 μL, 0.74 mmol), acid **25a** (111 mg, 0.37 mmol), and HBTU (165 mg, 0.43 mmol) were added. The mixture was stirred for 12h, and then diluted with water (8 mL), extracted with $CHCl_3$ (15 mL×2), and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH:60/1/0.1) to give the crude amide as yellow foam (110 mg, 55%).

To the amide (58 mg, 0.11 mmol) prepared above was dissolved in MeOH (15 mL) and AcOH (1 mL), Pd/C (25 mg) was added, and the mixture was hydrogenated under a pressure of 1.5 *psi* for 8h. The mixture was filtered, and the filtrate was concentrated. The residue was basified (pH 9) with ammonia, and the liberated base was extracted with CH_2Cl_2 (3×10 mL), washed with brine (15 mL), and concentrated. Amide **39** was obtained as white foam (20 mg, 23% for two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.72 (s, 1H), 8.51 – 8.42 (m, 1H), 7.87 – 7.69 (m, 3H), 7.43 – 7.27 (m, 2H), 7.18 (d, *J* = 7.5 Hz, 0.7H), 7.13 – 6.99 (m, 3H), 6.72 (d, *J* = 7.1 Hz, 0.3H), 5.16 (d, *J* = 17.9 Hz, 1H), 4.77 (d, *J* = 10.5 Hz, 0.4H), 4.62 (d, *J* = 17.7 Hz, 1H), 4.47 (s, 0.6H), 4.29 (s, 2H), 4.10-3.78 (m, 2H), 3.47 – 3.32 (m, 1H), 3.19 – 2.89 (m, 3H), 2.80 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.2, 165.6, 160.4, 158.5, 158.2, 156.6, 156.2, 145.5, 144.9, 134.3, 134.2, 133.9, 133.7, 133.6, 131.6, 131.5, 130.8, 130.7, 130.0, 129.6, 129.6, 128.3, 128.3, 127.6, 127.3, 127.2, 127.1, 126.8, 125.1, 124.6, 124.4, 124.0, 122.8, 116.4, 116.3, 116.2, 116.1, 53.0, 52.4, 49.7, 48.5, 45.8, 45.4, 43.3, 42.9, 37.7, 37.2, 28.7, 28.4. MS (*ESI*, M^+) *m/z* 454; HR-MS (*EI*) calcd for $C_{27}H_{23}N_4O_2F$: 454.1805, found: 454.1800.

4-(2-Fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)-3a,4,5,6-tetrahydro-1H-benzo[de][1,6]naphthyridin-2(3H)-one (40). This compound was prepared from **7'** and **25a** as white solid in 60% yield by following a procedure similar to that

of preparation of compounds **26-29**. Mp: 202-203 °C. ^{1}H NMR (300 MHz, DMSO) δ 12.59 (s, 1H), 10.33 (s, 1H), 8.24 (d, *J* = 7.59 Hz, 1H), 8.03 – 7.74 (m, 3H), 7.41 (d, *J* = 5.64 Hz, 2H), 7.24 (t, *J* = 8.91 Hz, 1H), 7.12 (t, *J* = 7.51 Hz, 1H), 6.80 (d, *J* = 7.58 Hz, 2H), 5.22 (d, *J* = 10.13 Hz, 1H), 4.33 (s, 2H), 4.09 (m, 1H), 3.15 (d, *J* = 5.28 Hz, 1H), 2.68 (m, 4H). ^{13}C NMR (126 MHz, DMSO) δ 169.6, 165.3, 159.9, 157.0 (d, *J* = 245.7 Hz), 145.4, 137.4, 135.3, 134.0, 132.0, 129.5, 128.2, 127.8, 126.5, 125.9, 124.5, 124.4, 122.5, 120.1, 116.5, 116.3, 113.8, 47.6, 42.8, 36.9, 35.1, 29.5. MS (*EI*, [M^+]) *m/z* 468; HR-MS (*EI*) calcd for $C_{27}H_{21}N_4O_3F$: 468.1598, found: 468.1598.

1-(2-Fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)-2,3,8,8a-tetrahydrocyclopenta[ij]isoquinolin-7(1H)-one (41). This compound was prepared from **34** and **25a** (98 mg, 0.33 mmol) as pale yellow solid (81 mg, 54%) by following a procedure similar to that of preparation of compounds **26-29**. Mp: 228-230 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.63 (s, 1H), 8.45 (d, *J* = 8.1 Hz, 1H), 7.68 (d, *J* = 38.7 Hz, 4H), 7.51 – 7.29 (m, 4H), 7.02 (t, *J* = 8.8 Hz, 1H), 5.21 (m, 1H), 4.31 (s, 2H), 3.90 – 3.57 (m, 2H), 3.24 (m, 1H), 3.05 – 2.93 (m, 1H), 2.87 – 2.56 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.1, 167.3, 160.9, 157.5 (d, *J* = 246.9 Hz), 150.2, 145.6, 136.1, 135.2, 134.3, 133.6, 131.9, 131.5, 129.5, 129.1, 128.2, 127.1, 125.0, 124.6, 124.4, 121.4, 116.3, 116.1, 51.3, 48.4, 45.8, 37.7, 29.3. MS (*EI*, [M^+]) *m/z* 453; HR-MS (*EI*) calcd for $C_{27}H_{20}N_3O_3F$: 453.1489, found: 453.1486.

4-(4-Fluoro-3-(7-hydroxy-1,2,3,7,8,8a-hexahydrocyclopenta[ij]isoquinoline-1-carbonyl)benzyl)phthalazin-1(2H)-one (42). To a solution of **41** (220 mg, 0.49 mmol) in dry MeOH (15 mL) under argon was added $NaBH_4$ (92 mg, 2.43 mmol). The mixture was stirred at rt for 8h, quenched with ice water (5 mL), and then filtered. Evaporation of the solvent provided alcohol **42** as white solid (200 mg, 91%). Mp: 233-235 °C. ^{1}H NMR (300 MHz, DMSO) δ 12.59 (s, 1H), 8.24 (d, *J* = 7.4 Hz, 1H), 8.04 – 7.69 (m, 3H), 7.41 (d, *J* = 5.3 Hz, 2H), 7.29 – 6.96 (m, 4H), 5.53 (s, 1H), 5.11 (m, 1H), 4.80 (m, 2H), 4.32 (s, 2H), 3.55 (m, 1H), 3.00 – 2.62 (m, 3H). ^{13}C NMR (126 MHz, DMSO) δ 166.1, 159.8, 157.0 (d, *J* = 245.9 Hz), 145.4, 145.1, 138.4, 135.2, 134.5, 133.9, 132.0, 129.4, 128.3, 128.0, 126.5, 125.9, 125.4, 125.2, 121.8,

116.4, 116.3, 72.0, 53.5, 47.2, 36.8, 29.0. MS (*ESI*, $[M+Na]^+$) *m/z* 478; HR-MS (*ESI*) calcd for $C_{27}H_{22}N_3O_3FNa$: 478.1543, found: 478.1545.

4-(4-Fluoro-3-(7-fluoro-1,2,3,7,8,8a-hexahydrocyclopenta[ij]isoquinoline-1-carbonyl)benzyl)phthalazin-1(2H)-one (43). DAST (13 μL, 0.10 mmol) was added dropwise to a suspension of **42** (30 mg, 0.07 mmol) in anhydrous CH_2Cl_2 (10 mL) at -10 °C. The reaction mixture was stirred for 10 min at rt, then washed successively with *aq.* NH_4Cl, *aq.* $NaHCO_3$ and brine. After removal of the solvent, the residue was subjected to column chromatography on silica gel (CH_2Cl_2/MeOH:60/1) to give target compound **43** as white foam (22 mg, 73.3%). ^1H NMR (300 MHz, $CDCl_3$) δ 11.68 (s, 1H), 8.46 (d, *J* = 8.1 Hz, 1H), 7.76 (t, *J* = 8.7 Hz, 3H), 7.56-7.22 (m, 4H), 7.15 (dd, *J* = 12.8, 7.3 Hz, 1H), 7.00 (t, *J* = 8.7 Hz, 1H), 6.23 – 5.76 (m, 1H), 5.42 (s, 0.6H), 5.20 – 4.67 (m, 1H), 4.31 (s, 2H), 3.81 – 3.66 (m, 1H), 3.51 (td, *J* = 16.3, 5.6 Hz, 0.6H), 3.11 (s, 1H), 2.86 (m, 1.4H), 2.66 (d, *J* = 15.1 Hz, 1H), 2.27 – 2.14 (m, 0.4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.1, 166.9, 161.0, 157.4 (d, *J* = 248.7 Hz), 145.7, 141.9, 137.7, 137.6, 134.9, 134.4, 134.3, 133.6, 131.5, 129.5, 128.7, 128.5, 128.2, 128.0, 127.5, 127.1, 126.5, 125.1, 125.0, 124.1, 123.9, 122.3, 116.2, 116.0, 95.0, 94.0, 93.7, 92.5, 55.6, 53.4, 45.9, 43.8, 43.6, 37.7, 29.7, 29.3. MS (*ESI*, $[M+Na]^+$) *m/z* 480; HR-MS (*ESI*) calcd for $C_{27}H_{21}N_3O_2F_2Na$: 480.1500, found: 480.1531.

4-(3-(7-Amino-1,2,3,7,8,8a-hexahydrocyclopenta[ij]isoquinoline-1-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one (44). Ammonium acetate (514 mg, 3.33 mmol) and sodium cyanoborohydride (34 mg, 0.53 mmol) were added to a solution of ketone **41** (100 mg, 0.22 mmol) in EtOH (2.4 mL) in a 10 mL microwave vial. The mixture was stirred and heated at 130°C for 5 min in a microwave reactor (CEM Creator). After cooled to rt, the reaction mixture was concentrated. The residue was treated with 2 N NaOH until pH >10, and extracted with EtOAc (2×15 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH:15/1/0.1) to give **44** as white foam (72 mg, 72.0%). ^1H NMR (400 MHz, $CDCl_3$) δ 11.65 (s, 1H), 8.48 (d, *J* = 7.9 Hz, 1H), 7.96-7.65 (m, 3H), 7.47 – 7.18 (m, 4H), 7.15 – 6.98 (m, 2H),

5.07 – 4.80 (m, 1H), 4.65 – 4.43 (m, 1H), 4.32 (s, 2H), 4.11 (m, 1/2H), 3.82 – 3.50 (m, 3/2H), 3.36-2.54 (m, 3H), 2.02 (m, 2H), 1.60 (dd, J = 21.2, 10.2 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 160.8, 160.7, 157.4 (d, J = 246.0 Hz), 145.6, 145.4, 145.3, 144.8, 138.7, 138.3, 135.1, 134.2, 133.9, 133.6, 133.5, 131.5, 131.4, 129.7, 129.5, 128.3, 128.1, 128.0, 127.1, 126.3, 125.3, 125.1, 124.8, 121.2, 120.7, 116.2, 116.0, 54.7, 54.6, 54.4, 48.4, 45.6, 41.9, 37.8, 37.2, 29.3, 28.6. MS (*ESI*, $[M+1]^+$) *m/z* 455; HR-MS (*ESI*) calcd for $C_{27}H_{24}N_4O_2F$: 455.1883, found: 455.1867.

2-Fluoro-N-(3-oxo-2,3-dihydro-1H-inden-1-yl)-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzamide (46). To the solution of **49** (150 mg, 0.62 mmol) in MeOH (10 mL), a solution of $Ba(OH)_2$ (0.2 N, 5 mL) was added. The mixture was stirred for 6h, and then evaporated. Water (8 mL) was added and the solution was extracted with EtOAc (3×100 mL). The combined organic phase was dried (Na_2SO_4), evaporated to give a crude brown oil **45** (76 mg, 83%). Crude amine **45** was then treated with acid **25a** by following a procedure similar to that of preparation of compounds **26-29** to yield target compound **46** as white solid (61%). Mp: 263-264 °C. ^{1}H NMR (300 MHz, DMSO) δ 12.57 (s, 1H), 8.87 (d, J = 8.7 Hz, 1H), 8.24 (dd, J = 7.7, 1.3 Hz, 1H), 7.95 (d, J = 7.7 Hz, 1H), 7.84 (m, 2H), 7.77 – 7.55 (m, 4H), 7.49 (m, 2H), 7.19 (dd, J = 10.3, 8.6 Hz, 1H), 5.68 (m, 1H), 4.32 (s, 2H), 3.10 (dd, J = 18.0, 8.5 Hz, 1H), 2.59 (dd, J = 18.7, 3.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 203.7, 164.3, 159.8, 158.3 (d, J = 247.6 Hz), 155.4, 145.4, 136.8, 135.7, 134.8, 134.0, 133.2, 132.0, 130.5, 129.5, 129.3, 128.3, 126.4, 125.9, 124.1, 122.9, 116.7, 116.5, 47.8, 43.7, 36.9. MS (*EI*, $[M^+]$) *m/z* 427; HR-MS (*EI*) calcd for $C_{25}H_{18}N_3O_3F$: 427.1332, found: 427.1340.

2-Fluoro-N-(3-hydroxy-2,3-dihydro-1H-inden-1-yl)-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzamides 47 and 48.

To a solution of **46** (110 mg, 0.26 mmol) in dry MeOH (15 mL) under argon was added $NaBH_4$ (49 mg, 1.29 mmol). The mixture was stirred at rt for 8h, quenched with ice water and extracted with $CHCl_3$ (2×30 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and evaporated. The yellow oily residue was

purified by flash column chromatography (CH_2Cl_2/MeOH (40:1) to give *cis*-isomer **47** (54 mg, 49%) and *trans*-isomer **48** (53 mg, 48.2%) as white solid.

2-Fluoro-*N*-(*syn*-3-hydroxy-2,3-dihydro-1H-inden-1-yl)-5-((4-oxo-3,4-dihydr ophthalazin-1-yl)methyl)benzamide (47). Mp: 224-225 °C. ^{1}H NMR (300 MHz, DMSO) δ 12.59 (s, 1H), 8.67 (d, *J* = 8.0 Hz, 1H), 8.25 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.97 (d, *J* = 7.6 Hz, 1H), 7.84 (m, 2H), 7.64 – 7.56 (m, 1H), 7.47 – 7.33 (m, 2H), 7.32 – 7.14 (m, 4H), 5.55 (d, *J* = 5.9 Hz, 1H), 5.30 (dd, *J* = 16.4, 8.3 Hz, 1H), 4.98 (dd, *J* = 13.6, 7.0 Hz, 1H), 4.32 (s, 2H), 2.76 (dt, *J* = 12.2, 7.0 Hz, 1H), 1.78 (dd, *J* = 20.3, 9.2 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 165.2, 160.8, 159.2 (d, *J* = 247.9 Hz), 147.2, 146.3, 143.8, 135.6, 134.9, 133.7 (d, *J* = 8.2 Hz), 132.9, 131.3, 130.4, 129.2, 128.9, 127.4, 126.9, 125.6 (d, *J* = 15.1 Hz), 125.2, 124.7, 117.6, 117.4, 72.4, 51.8, 44.5, 37.8. MS (*ESI*, $[M+Na]^+$) *m/z* 452; HR-MS (*ESI*) calcd for $C_{25}H_{20}N_3O_3FNa$: 452.1386, found: 452.1408.

2-Fluoro-*N*-(*anti*-3-hydroxy-2,3-dihydro-1H-inden-1-yl)-5-((4-oxo-3,4-dihydr ophthalazin-1-yl)methyl)benzamide (48). Mp: 136-138 °C. ^{1}H NMR (300 MHz, DMSO) δ 12.58 (s, 1H), 8.58 (d, *J* = 8.20 Hz, 1H), 8.24 (d, *J* = 7.19 Hz, 1H), 7.96 (d, *J* = 7.91 Hz, 1H), 7.84 (m, 2H), 7.58 – 7.52 (m, 1H), 7.37 (dd, *J* = 4.49, 16.62 Hz, 2H), 7.27 (d, *J* = 3.16 Hz, 2H), 7.22 – 7.12 (m, 1H), 5.63 (d, *J* = 7.60 Hz, 1H), 5.22 (d, *J* = 5.68 Hz, 1H), 5.18 – 5.10 (m, 1H), 4.31 (s, 2H), 2.26 – 2.12 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 169.9, 165.5, 163.9 (d, *J* = 248.2 Hz), 151.8, 151.1, 149.8, 140.3, 139.6, 138.3, 137.7, 135.6 (d, *J* = 11.3 Hz), 135.1, 134.3, 133.9 (d, *J* = 7.6 Hz), 132.2, 131.6, 130.9, 130.4 (d, *J* = 15.4 Hz), 130.2, 122.3, 122.1, 78.3, 58.4, 48.5, 42.5. MS (*ESI*, $[M+1]^+$) *m/z* 430; HR-MS (*ESI*) calcd for $C_{25}H_{20}N_3O_3FNa$: 452.1386, found: 452.1371.

2-Fluoro-*N*-methyl-N-(3-oxo-2,3-dihydro-1H-inden-1-yl)-5-((4-oxo-3,4-dihydrop hthalazin-1-yl)methyl)benzamide (52). K_2CO_3 (680 mg, 4.94 mmol), $Bu_4N^+Br^-$ (*cat.*) and MeI (915 μL, 14.8 mmol) were added to the solution of **49** in MeCN (20 mL). The mixture was stirred at rt for 24h and evaporated. Water (20 mL) was added, and the solution was extracted with CH_2Cl_2 (3×20 mL), dried over Na_2SO_4, and

concentrated. The residue was purified by silica gel chromatography (PE/EtOAc:5/1) to give *N*-methyl amine **50** as yellow oil (380 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 – 7.67 (m, 2H), 7.61 – 7.41 (m, 2H), 6.37 (d, *J* = 5.2 Hz, 1H), 5.76 (s, 1H), 3.12 (dd, *J* = 19.4, 7.8 Hz, 1H), 2.83 – 2.48 (m, 4H). This compound was converted to 3-(methylamino)-2,3-dihydro-1H-inden-1-one (**51**) in 34% by following a procedure similar to that of preparation of compound **45**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (dt, *J* = 14.3, 7.5 Hz, 3H), 7.38 (t, *J* = 7.1 Hz, 1H), 4.36 (dd, *J* = 6.7, 3.0 Hz, 1H), 2.90 (dd, *J* = 18.8, 6.8 Hz, 1H), 2.47 (q, *J* = 3.1 Hz, 4H).

Condensation of amine **51** (81 mg, 0.50 mmol) and acid **25a** (149 mg, 0.50 mmol) was conducted by following a procedure similar to that of preparation of compounds **26-29** and the target compound **52** was obtained as white foam (150 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.77 (s, 1H), 8.44 (dd, *J* = 6.9, 1.3 Hz, 1H), 7.82 – 7.59 (m, 5H), 7.58 – 7.28 (m, 4H), 7.04 (dd, *J* = 18.6, 9.4 Hz, 1H), 6.57 (d, *J* = 5.1 Hz, 1/2H), 5.30 (s, 1/2H), 4.31 (s, 2H), 3.13 (dd, *J* = 19.4, 8.0 Hz, 1H), 2.78 – 2.41 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.9, 167.5, 166.8, 160.9, 156.9 (d, *J* = 245.4 Hz), 151.9, 151.0, 145.5, 145.4, 137.5, 137.1, 135.4, 134.6, 134.3, 133.5, 131.4, 129.5, 129.4, 129.2, 128.9, 128.1, 127.0, 125.9, 124.9, 123.6, 116.2, 116.0, 56.7, 51.5, 40.8, 40.1, 37.6, 30.8, 27.6. MS (*EI*, [M^+]) *m/z* 441; HR-MS (*EI*) calcd for $C_{26}H_{20}N_3O_3F$: 441.1489, found: 441.1496.

2-Fluoro-*N*-(1-oxo-1,2,3,4-tetrahydroisoquinolin-4-yl)-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzamide (55). This compound was prepared as white solid in 49% yield by condensation of amine **54** and acid **25a** following a procedure similar to that of preparation of compounds **26-29**. Mp: 154-156 °C. ^{1}H NMR (300 MHz, DMSO) δ 12.57 (s, 1H), 8.87 (d, *J* = 8.2 Hz, 1H), 8.25 (d, *J* = 7.7 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 2H), 7.92 – 7.76 (m, 3H), 7.56 (t, *J* = 7.3 Hz, 2H), 7.48 – 7.36 (m, 3H), 7.23 – 7.15 (m, 1H), 5.30 (dd, *J* = 13.3, 7.8 Hz, 1H), 4.32 (s, 2H), 3.55 – 3.34 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.1, 168.9, 164.5, 163.0 (d, *J* = 246.7 Hz), 150.0, 144.0, 139.4, 138.7, 137.7 (d, *J* = 8.6 Hz), 137.2, 136.7, 135.1, 134.2, 134.0, 133.0 (d, *J* = 7.7 Hz), 132.4, 131.2, 130.6, 129.1, 128.9, 121.4, 121.1, 51.1, 48.8, 41.6. MS (*EI*,

[M^+]) *m/z* 442; HR-MS (*EI*) calcd for $C_{25}H_{19}N_4O_3F$: 442.1441, found: 442.1443.

***N*-(1-Oxo-1,2,3,4-tetrahydroisoquinolin-4-yl)-3-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzamide (56).** This compound was prepared as white solid in 61% yield by following a procedure similar to that of preparation of compounds **26-29**. Mp: 150-151 °C. ^{1}H NMR (300 MHz, DMSO) δ 12.59 (s, 1H), 8.92 (d, *J* = 8.2 Hz, 1H), 8.24 (d, *J* = 7.5 Hz, 1H), 8.02 – 7.68 (m, 7H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.49 – 7.28 (m, 4H), 5.34 (m, 1H), 4.35 (s, 2H), 3.46 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 166.4, 163.9, 159.4, 145.0, 139.0, 138.3, 134.1, 133.5, 132.0, 131.7, 131.5, 129.1, 129.0, 128.4, 128.0, 127.8, 127.2, 126.2, 126.0, 125.6, 125.5, 45.9, 43.7, 37.4. MS (*EI*, [M^+]) *m/z* 424; HR-MS (*EI*) calcd for $C_{25}H_{20}N_4O_3$: 424.1535, found: 424.1544.

Biology. Temozolomide was purchased from Beijing J&K Chemical (China). Antibodies against γ-H2AX (sc-101696) and PAR [pADPr (10H) (sc-56198)] were from Santa Cruz Biotechnology. The antibody against GAPDH was from Zhejiang Haimeng Pharmaceutical Factory (China). The Alexa Fluor® 488 Antibody (A11029) was from Invitrogen. Histone was from Shanghai Yuanye Biotech Co., Ltd (China). NAD^+ was from Sigma (St. Louis, MO, USA). Activator deoxyoligonucleotide (sequence: CGGAATTCCG) and *o*-phenylenediamine (OPD) was from Shanghai Sangon Biotech Co., Ltd (China). Goat anti-mouse IgG horseradish peroxidase antibody was from Merk/Calbiochem (Darmstadt, Germany).

Enzyme-linked-immunosorbent assays (ELISA) for PARP1 enzymatic activity *in vitro*. Recombinant human PARP1 (1 μg/mL) was expressed and purified in our laboratory. The inhibition of the tested compounds on PARP1 enzymatic activity in a cell-free system was determined by ELISA in 96-well plates. Each well was pre-coated with histone (20 μg/ml) diluted in 100μl PBS buffer (10 mM NaH_2PO_4, 10 mM Na_2HPO_4, 150 mM NaCl, pH 7.4) by incubation at 37°C overnight. NAD^+ (8 μM) and activator deoxyoligonucleotide (100 μg/mL) diluted in 70 μL reaction buffer (50 mM Tris, 2 mM $MgCl_2$, pH 8.0) were added into each well, and then 10 μL compound or solvent control was added at varying concentrations. The reaction was initiated by the addition of 20 μL PARP1 (10 ng/well) at 37 °C for 1 h. The primary

antibody pADPr (10H) (sc-56198) was incubated at 37°C for another 1 h. And then the second antibody Goat anti-mouse IgG horseradish peroxidase was added and incubated at 37°C for additional 30 min. Finally, 100 μL of solution (0.03% H_2O_2, 2 mg/mL OPD in citrate buffer 0.1 M, pH 5.4) was added and incubated at room temperature for 15 min. The reaction was stopped by the addition of 2 M H_2SO_4 (50 μL), and A_{490} was measured using a multiwell spectrophotometer (SPECTRA MAX 190). The inhibition rate of PARP1 enzymatic activity was calculated as ($A_{490\ control}$ -$A_{490\ treated}$/$A_{490\ control}$) x 100%. The concentration required for 50% inhibition of PARP1 enzymatic activity (IC_{50}) was determined with the Logit method.

Cell culture. V-C8 and V79 cells were from Leiden University, Holland. SKOV3 cells were obtained from the American Type Culture Collection. V-C8 cells were cultured in F12 and V79 cells were cultured in RPMI-1640 while SKOV3 cells were cultured in DMEM, supplemented with 10% FBS at 37 ^oC in a humidified atmosphere containing 5% CO_2.

Cytotoxicity Assays. SKOV3 cells were exposed to gradient concentrations of temozolomide with or without compounds **3**, **26** or **41** for 7 days. The cytotoxicity was assessed by sulforhodamine B (SRB) assays.[66] Experiments were repeated three times.

Western blotting analyses. V-C8 cells (4×10^5/mL) and V79 cells (1×10^5/mL) were seeded in a 6-well plate and incubated overnight. The cells were exposed to the indicated compound at 37 °C for 24 h. Then the cells were harvested for standard Western blotting to detect the levels of cellular γ-H2AX.

Poly(ADP-ribose) (PAR) synthesis inhibition analyses. SKOV3 cells (1.8×10^4/mL) were seeded in a 96-well plate and incubated overnight. Following pretreatments with compounds **3**, **26** or **41** at the indicated concentrations for 4 h, the cells were exposed to 10 mM H_2O_2 for 5 min. The cells were fixed with methanol at -20°C for 20 min followed by incubation with the primary antibody against PAR at 4°C for 1 h and then with the second antibody Alexa Fluor® 488 (A11029) at 4°C for additional 20 min. The images were captured under IN Cell Analyzer 2000 (GE Healthcare). The data were analyzed with the IN Cell Analyzer workstation software. The concentration required for 50% PAR synthesis inhibition (IC_{50}) in SKOV-3 cells was determined

from the concentration-response curve. The experiments were repeated three times, and the IC_{50} values were expressed as mean ± SD.

Flow cytometry assays. V-C8 cells were treated with compounds **3**, **26** or **41** (0.5 μM) at 37°C for 24 h, and subsequently harvested, fixed, and stained with 10 μg/mL propidium iodide (PI). All samples were analyzed using a FACSCalibur cytometer (BD Biosciences). At least 10,000 events were counted for each sample.

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Supporting Information Available. Experimental details for the intermediates and final compounds, as well as the copies of their ^{1}H and ^{13}C spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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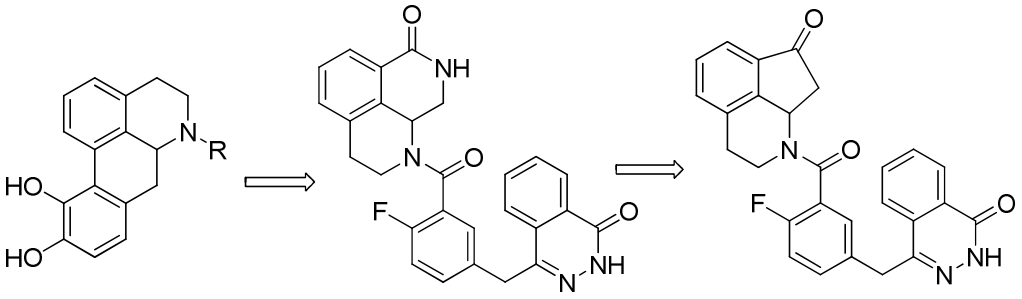
Table of Contents graphic



apomorphine (R=Me)

(Dopamine D_2 full agonist)

1st generation lead

IC_{50} (PARP1): 0.31 nM

CC_{50} (BRCA2): 96.0 nM

CC_{50} (BRCA1): 23.3 nM

2nd generation lead

IC_{50} (PARP1): 3.46 nM

CC_{50} (BRCA2): 4.53 nM

CC_{50} (BRCA1): 0.26 nM