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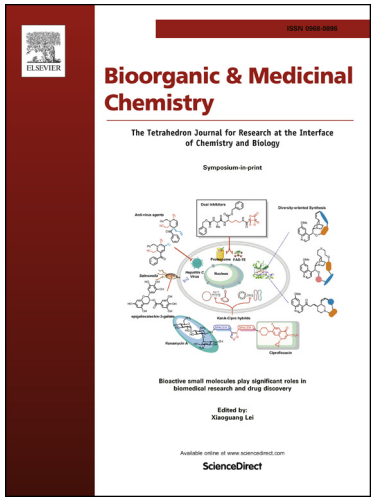

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Discovery of novel quinazoline-2,4(1*H*,3*H*)-dione derivatives as potent PARP-2 selective inhibitors

Hailong Zhao[a,#], Ming Ji[b,#], Guonan Cui[a], Jie Zhou[a], Fangfang Lai[b], Xiaoguang Chen[b,*], Bailing Xu[a,*]

[a]*Beijing Key Laboratory of Active Substance Discovery and Druggability Evaluation, Institute of Materia Medica, Chinese Academy of Medical Sciences &Peking Union Medical College, Beijing, 100050, China*

[b]*State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences &Peking Union Medical College, Beijing, 100050, China*

Abstract: The PARP-2 selective inhibitor is important for clarifying specific roles of PARP-2 in the pathophysiological process and developing desired drugs with reduced off-target side effects. In this work, a series of novel quinazoline-2,4(1*H*,3*H*)-dione derivatives was designed and synthesized to explore isoform selective PARP inhibitors. As a result, compound **11a** (PARP-1 IC_{50} = 467 nM, PARP-2 IC_{50} = 11.5 nM, selectivity PARP-1/PARP-2 = 40.6) was disclosed as the most selective PARP-2 inhibitor with high potency to date. The binding features of compound **11a** within PARP-1 and PARP-2 were investigated respectively to provide useful insights for the further construction of new isoform selective inhibitors of PARP-1 and PARP-2 by using CDOCKER program.

Keywords: Quinazoline-2,4(1*H*,3*H*)-dione; PARP-2 selective inhibitor; Anti-tumor agents.

1. Introduction

ADP-ribosyltransferases (ARTs) represent a large family of enzymes containing 17 members at least, and they can modify their target proteins by ADP-ribosylating. These ARTs bind to nicotinamide adenine dinucleotide (NAD^+) in their catalytic domain, cleave the NAD^+ and transfer ADP-ribose moiety onto specific amino acid residues of acceptor proteins[1-3]. Six members (ARTs 1-6) of this family can function as poly(ADP-ribose)polymerases[2-4] to transfer multiple ADP-ribose moieties consecutively and form the linear or branched ADP-ribose polymers on the target proteins. PARP-1 was first identified in 1963 and its role in the DNA repair pathway was well explored[4, 5] Presently, as PARP-1 inhibitors, Olaparib (AZD-2281), Rucaparib (AG014699) and Niraparib (MK4827) have been approved by FDA for the

[#]The first two authors contributed equally.
[*]Corresponding author. B. Xu, xubl@imm.ac.cn; X. Chen, chxg@imm.ac.cn

treatment of ovarian cancer in patients with or without BRCA mutations[6-8]. In the late 1990's[9, 10] , PARP-2 was confirmed as the second member of this family. In fact, the detection of residual DNA-dependent PARP activity in *PARP-1*$^{-/-}$ deficient mouse fibroblasts led to the discovery of PARP-2. PARP-1 and PARP-2 were the closest homologs and they possessed 69% similarity in the catalytic domain[10]. Therefore, many initially recognized PARP-1 inhibitors such as AZD-2281, ABT-888, AG014699, MK-4827 and BMN673 also showed comparable PARP-2 inhibitory activities, since these inhibitors were designed to compete with NAD^+ in the catalytic domain [6-8, 11-14].

While both PARP-1 and PARP-2 were involved in the DNA breaks repair [1, 15-17], their specific roles in the repair pathway have not been well understood. The delayed and persistent accumulation at UV laser-induced damaged cells[15] demonstrated that PARP-2 participated in later steps of the DNA repair process. Moreover, while PARP-1 can bind with both single-strand breaks and double-strand breaks[18], PARP-2 can bind with single-strand breaks with greater specificity. Meanwhile, the biochemical evidence proved that PARP-2 took part in many other physiological processes, such as spermatogenesis, adipogenesis, and T-cell development[15, 19-21]. Depletion of PARP-2 in mice led to chronic anemia which was not detected in mice lacking PARP-1[22]. Consequently, it was speculated that PARP-1 selective inhibitors might work as anti-cancer drugs, bearing less off-target side effects. Interestingly, PARP-2 has been proved very recently to possess pleiotropic influences on the hallmarks of cancer, such as genomic instability, dysregulated cellular metabolism, angiogenesis, inflammation, tumor invasion and immune evasion[23]. So, the selective inhibition of PARP-2 might cause a multipronged attack on tumorigenesis. Obviously, the development of PARP-1 selective inhibitors and PARP-2 selective inhibitors are highly desired to clarify the biological functions of individual PARPs and to achieve the improved therapeutic agents compared with the known non-selective inhibitors.

By now, several PARP-1 selective inhibitors have been reported[24-27]. In contrast, only one inhibitor (compound **I**) was described as the most selective PARP-2 inhibitor with a 9.3-fold selectivity and an IC_{50} value of 1.5 μM against PARP-2[28].

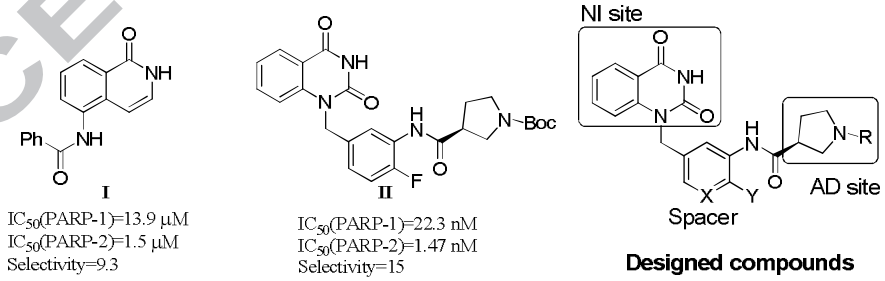
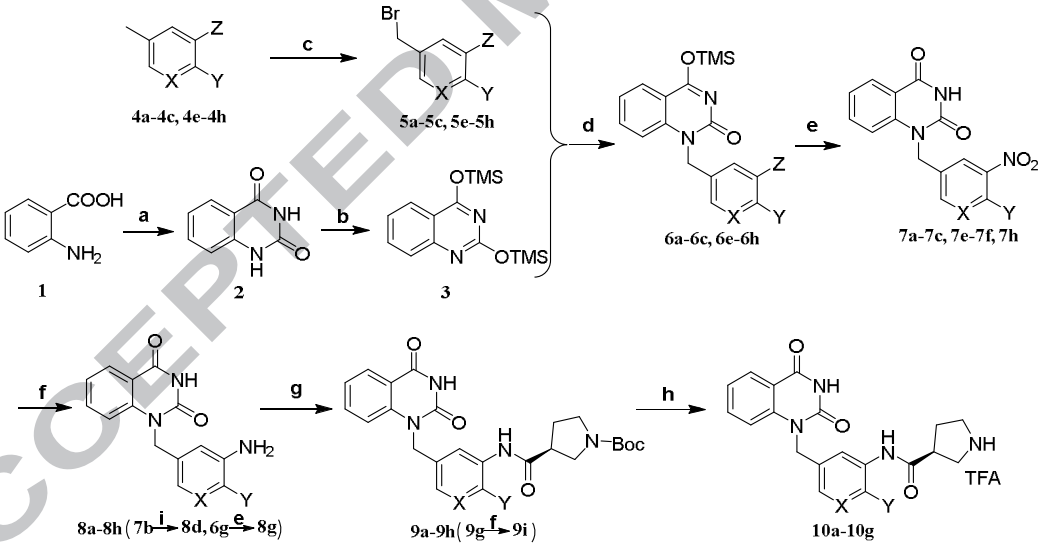


Figure 1. The chemical structures of known PARP-2 selective inhibitors (**I**, **II**), and the general structure of designed compounds

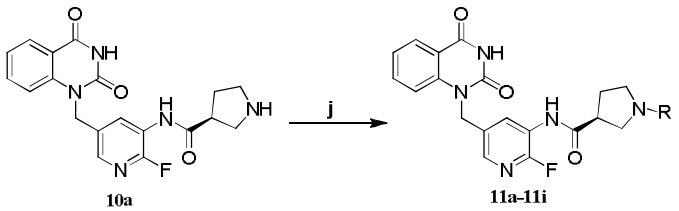
In our previous work, we found that a quinazoline-2,4(1*H*,3*H*)-dione derivative (compound **II**, Figure 1)[29] was about 15-fold more potent against PARP-2 with the IC_{50} value at nanomolar level. The predicated binding mode showed that the

quinazoline-2,4(1*H*,3*H*)-dione scaffold occupied the nicotinamide-ribose binding site (NI site) and the *N*-Boc-pyrrolidin-3-yl fragment extended into the adenine-ribose binding site (AD site)[29]. The benzyl group served as a spacer to direct those fragments into the NI site and AD site, and interacted with the key amino acids in PARP-1, such as Tyr889, Tyr896 and Gly894 and PARP-2, such as Tyr455, Tyr462 and Gly460. By comparison with the highly conserved NI site, the AD site in PARP-1 and PARP-2 differs from each other to some extent. For example, the AD site in PARP-1 consists of Glu763, Asp766 and Leu769 amino acid residues, whereas the corresponding amino acid residues in PARP-2 were Gln332, Glu335 and Gly338. The differentiation of amino acid residues in PARP-1 and PARP-2 could be useful for the design of isoform selective inhibitors[30, 31]. Based on the above structural features of PARP-1 and PARP-2, we envisioned that modifications on the spacer and the *N*-Boc-pyrrolidin-3-yl subunit of compound **II** could tune their interactions with the key amino acids in the spacer site and AD site, and therefore might create novel isoform selective inhibitors. Herein, we present the synthesis of the designed compounds and their enzymatic inhibitory activities against PARP-1 and PARP-2. The structure-activity relationships were investigated preliminarily and led to the discovery of a highly potent and selective PARP-2 inhibitor (Compound **11a**).

2. Chemistry



4a-6a X=N, Y=F, Z=NO_2, **7a-10a** X=N, Y=F;
4b-6b X=N, Y=Cl, Z=NO_2, **7b-10b** X=N, Y=Cl;
4c-6c X=N, Y=Br, Z=NO_2, **7c-10c** X=N, Y=Br;
8d-10d X=N, Y=H;
4e-6e X=C, Y=Cl, Z=NO_2, **7e-10e** X=C, Y=Cl;
4f-6f X=C, Y=Br, Z=NO_2, **7f-10f** X=C, Y=Br;
4g-5g X=C, Y=NO_2, Z=$NBoc_2$, **6g** X=C, Y=NO_2, Z=NH_2, **8g-10g** X=C, Y=NO_2;
4h-5h X=C, Y=OAc, Z=NO_2, **6h** X=C, Y=OH, Z=NO_2, **7h-10h** X=C, Y=OH;
9i X=C, Y=NH_2



Scheme 1 Reagents and conditions: (a) urea, 160 °C, 76.1%; (b) HMDS, conc. H_2SO_4, toluene, reflux; (c) NBS, AIBN, CCl_4, 28.0%-41.5%; (d) substituted bromomethylbenzene, 130 °C; (e) MeOH, dioxane, 70 °C, 27.6%-97.0%; (f) Raney-Ni, H_2, THF, MeOH, 53.6%-93.0%; (g) PPh_3, hexachloroacetone, THF, 22.4%-70.0%; (h)TFA, DCM, 71.4%-88.2%; (i) 10% Pd-C, H_2, THF, MeOH, 62.0%; (j) $NaBH_3CN$, NaOAc, DCM, MeOH or Pd_2dba_3, Xantphos, Cs_2CO_3, Tol, 5.6%-62.2%.

The synthesis of quinazoline-2,4(1*H*,3*H*)-dione derivatives (**9a-9i**, **10a-10g** and**11a-11i**) was accomplished according to the synthetic route as outlined in **Scheme 1**. The condensation of 2-aminobenzoic acid with urea delivered compound **2**[32] under a neat reaction condition. Upon treatment with hexamethyldisilazane (HMDS) and concentric sulfuric acid in toluene, compound **2** was converted into the silylated compound **3;** then, compound **3** reacted with compounds **5a-5c** and **5e-5f**, which were prepared by treating **4a-4c** and **4e-4f** with *N*-bromobutanimide and azodiisobutyronitrile in CCl_4, to generate the *N*-1 substituted intermediates **6a-6c** and **6e-6f**. Removal of the silyl group in **6a-6c** and **6e-6f** in MeOH gave rise to compounds **7a-7c**, **7e-7f** and **7h** in 27%-97% yields. The catalytic hydrogenation of compounds **7a-7c**, **7e-7f** and **7h** provided the corresponding amines **8a-8c**, **8e-8f** and **8h** in 53%-93% yields. In addition, compound **8d** was prepared from **7b** and compound **8g** was prepared from **6g.** In the presence of PPh_3 and hexachloroacetone, the coupling reaction between **8a-8h** with *N*-Boc-β-proline yielded compounds **9a-9h** in a yield of 22%-70%. Under the reaction conditions of Raney-Ni and H_2 in THF, compound **9g** was transformed into compound **9i** in 53% chemical yield. The deprotection of Boc group of compounds **9a-9g** with TFA produced the target compounds **10a**-**10g** in good yields. The alkylation reaction of **10a** afforded **11a-11i** in the reasonable yields.

3. Biological results and discussion

The inhibitory activities of all target compounds (**9a-9i, 10a-10g** and **11a-11i**) were evaluated against PARP-1 and PARP-2. The clinical drug AZD2281 was selected as a reference compound. The corresponding results were expressed as IC_{50} values and presented in **Table 1** and **Table 2**.

Initially, modifications on the benzyl spacer were carried out by changing atom X and substituent Y in order to investigate the impact of these variations on the inhibition and selectivity toward PARP-1 and PARP-2. As shown in **Table 1**, the variation of Y substituent on the pyridine spacer led to the formation of compounds

9a-9d. Compounds **9a** and **9b** with a fluorine or a chlorine atom on the pyridine ring displayed a stronger inhibition toward PARP-2 over PARP-1 (**9a,** PARP-1 IC_{50} = 39.8 nM, PARP-2 IC_{50} = 8.6 nM, selectivity PARP-1/PARP-2 = 4.6; **9b,** PARP-1 IC_{50} = 232 nM, PARP-2 IC_{50} = 12.2 nM, selectivity PARP-1/PARP-2 = 19.0), showing a 4.6-fold and 19-fold selectivity, respectively. In terms of potency and selectivity, these two compounds are comparable with compound **II**. By comparison, using a hydrogen atom or a bromine atom as the Y substituent (compounds **9c** and **9d**) led to a drastic decrease in potency against both PARP-1 and PARP-2.

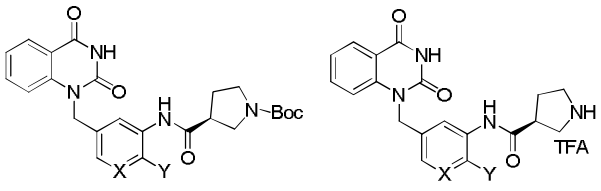
The use of benzyl group as a spacer along with the further variation of Y group yielded compounds **9e-9i**. Compound **9e** with a chlorine atom exhibited the similar inhibitory activities toward PARP-1 and PARP-2 with IC_{50} values in the single digit nanomolar level. The introduction of a bromine atom as the Y group (compound **9f**) lowered the potency toward both PARP-1 and PARP-2, and favored the binding toward PARP-2 with a 5.1-fold selectivity. The incorporation of other substituents (compounds **9g-9i**), such as the nitro, amine and hydroxyl groups into the benzyl ring resulted in the loss of potency.

Taken together, the phenyl group or pyridine ring could be taken as the spacer, which was supposed to interact with the tyrosine residues around it via π-π stacking interactions. The incorporation of a fluorine or a chlorine atom into the spacer could generate highly potent PARP-2 inhibitors (compounds **9a, 9b, 9e** and compound **II**) with IC_{50} values at the nanomolar level. In contrast, regarding the inhibition toward PARP-1, only the fluorine substituted compound **9a** and compound **II** had the comparable potency with IC_{50} at the double digit nanomolar level. The installation of many other substituents was not tolerated on the Y position.

As shown in **Table 1**, the removal of Boc group of compounds **9a-9g** furnishing compounds **10a-10g** generally reduced the inhibitory activity against PARP-2 markedly and had little impact on the PARP-1 inhibition. These results suggested that the incorporation of a variety of substituents on the nitrogen could be beneficial for PARP-2 potency and consequently might produce PARP-2 selective inhibitors. Surprisingly, the removal of Boc group on compound **9d** increased the inhibition toward PARP-1 and gave rise to a PARP-1 selective inhibitor (compound **10d**).

Table 1

The chemical structures and inhibitory activities against PARP-1 and PARP-2 of compounds **9a**–**9i** and **10a**-**10g.**[a,b,c]



9 10

Cpd.	X	Y	PARP-1 IC_{50}/nM ±SD	PARP-2 IC_{50}/nM ±SD	Selectivity PARP-1/2	Cpd.	X	Y	PARP-1 IC_{50}/nM ±SD	PARP-2 IC_{50}/nM ±SD	Selectivity PARP-1/2
9a	N	F	39.8±11.6	8.6±1.7	4.6	**10a**	N	F	13.7±11.3	80±20.4	0.18
9b	N	Cl	232±80.4	12.2±1.8	19.0	**10b**	N	Cl	174±13.4	40±9.4	4.4
9c	N	Br	>100	>100	--	**10c**	N	Br	>100	>100	--
9d	N	H	>100	>100	--	**10d**	N	H	9.9±0.9	220±85	0.04
9e	C	Cl	6.2±0.1	9.1±2.1	0.7	**10e**	C	Cl	>100	>100	--
9f	C	Br	346±45	68±21.5	5.1	**10f**	C	Br	>100	>100	--
9g	C	NO_2	>100	>100	--	**10g**	C	NO_2	>100	>100	--
9h	C	OH	>100	>100	--						
9i	C	NH_2	>100	>100	--						

[a] Concentration for 50% inhibition in PARP-1 enzyme assay (IC_{50}); IC_{50} for AZD-2281 was 8.1 nM.

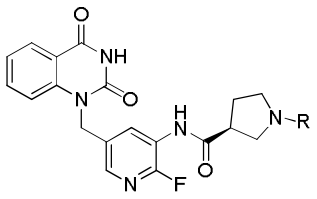
[b] Concentration for 50% inhibition in PARP-2 enzyme assay (IC_{50}); IC_{50} for AZD-2281 was 1.7 nM.

[c]The IC_{50} value was the average of two or three independent experiments; SD, standard deviation.

Based on the results mentioned above, we chose compound **9a** as a template to probe the SAR of the *N*-substituents and to search for the isoform selective inhibitors by changing the Boc group with other various subunits. As shown in **Table 2**, substitution of Boc group with 2,2,2-trifluoroethyl moiety led to a significant drop in potency toward PARP-1 and a little variation in potency toward PARP-2, thus resulting in a highly selective PARP-2 inhibitor (compound **11a**) with about 40-fold selectivity. To the best of our knowledge, compound **11a** showed the highest selectivity toward PARP-2 over PARP-1 with high potency as compared with the known PARP-2 selective inhibitors. Although grafting other alkyl hydrophobic groups such as a trifluoropropyl, cyclopropylmethyl or cyclopropylethyl moiety onto the nitrogen of the pyrrolidine ring could not prodcuce the selective inhibition between PARP-1 and PARP-2 at all, these compounds (**11b-11d**) served as highly potent inhibitors of PARP-1 and PARP-2 with IC_{50} values at low nanomolar level. The placement of a 4,4-difluorocyclohexyl (**11e**) or cyclopropanecarbonyl substituent (**11f**) on the nitrogen atom produced a moderate selectivity toward PARP-2 over PARP-1. Also, we attempted the installation of aromatic groups on the nitrogen atom, and found that compounds (**11g-11i**) showed remarkable inhibition toward PARP-2 with IC_{50} values of 4.1 nM-5.8 nM and less potency against PARP-1 (IC_{50}, 13.3 nM-92.6 nM). Among these compounds examined, compound **11i** exhibited a strong potency and favorable selectivity (PARP-1/PARP-2 = 22.8) toward PARP-2. The chemical modifications on the benzene ring of compound **11i** may further improve the isoform selectivity.

Table 2

The chemical structures and inhibitory activities against PARP-1 and PARP-2 of compounds **11a**-**11i**.[a,b,c]



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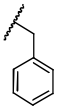
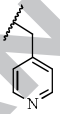
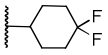
Cpd.	R	PARP-1 IC_{50}/nM ±SD	PARP-2 IC_{50}/nM ±SD	Selectivity PARP-2/1	Cpd.	R	PARP-1 IC_{50}/nM ±SD	PARP-2 IC_{50}/nM ±SD	Selectivity PARP-2/1
11a		467±33.4	11.5±0.9	40.6	**11f**		54±1.3	4.5±0.9	12.0
11b		3.1±0.1	2.9±0.2	1.0	**11g**		13.3±2.5	5.4±0.8	2.5
11c		5.7±0.7	3.6±1.0	1.6	**11h**		62±10.0	5.8±1.3	10.7
11d		1.4±0.5	1.0±0.2	1.8	**11i**		92.6±12.0	4.1±0.5	22.8
11e		81±14.6	11.9±2.3	6.8					

[a] Concentration for 50% inhibition in PARP-1 enzyme assay (IC_{50}); IC_{50} for AZD-2281 was 8.1 nM.

[b] Concentration for 50% inhibition in PARP-2 enzyme assay (IC_{50}); IC_{50} for AZD-2281 was 1.7 nM.

[c]The IC_{50} value was the average of two or three independent experiments; SD, standard deviation.

With an aim to probe the binding features of the most selective PARP-2 inhibitor (**11a**) in the binding site of PARP-1 and PARP-2, we performed the molecular docking by using CDOCER protocol integrated in Accelrys Discovery Studio 2.5[33]. As shown in **Figure 2(A-C)**, the quinazolinedione and the pyridine fragments bound to the NI site of PARP-1 and PARP-2 in a very similar orientation. As anticipated, the quinazolinedione scaffold formed the crucial interactions with PARP-1 through the conserved amino acids Gly863, Ser904 and Tyr907 and with PARP-2 through Gly429, Ser470 and Tyr473. The pyridine spacer resided at the subpocket lined with Tyr889 (Tyr445) and Tyr896 (Tyr462). Although the trifluoroethyl substituted pyrrolidine ring extended into AD site, its orientation in PARP-1 and PARP-2 was noticeably different (**Figure 2, C-E**). Due to the restriction of the amino acid residue Glu763 in PARP-1 through the hydrogen bonds with neighboring residues, the α5 helix was not easily shifted away by the induced fit effect of the trifluoroethyl group[25]. As a

consequence, the trifluoroethyl group stretched into a water-exposed surface and no hydrogen bonding interactions with Arg878 was observed in PARP-1. However, as far as PARP-2 is concerned, the Glu763 residue in PARP-1 was replaced with Gln332. The side chain of Gln332 is more flexible and the α5 helix can be shifted away to make a favorable accommodation for the trifluoroethyl group. Importantly, the trifluoroethyl fragment in this binding orientation could form H-bonding interactions with Arg444 in PARP-2 (**Figure 2, F**). We speculated that this type of distinct binding feature of **11a** within PARP-2 made significant contributions to its inhibition toward PARP-2, which consequently resulted in a high selectivity toward PARP-2 over PARP-1.

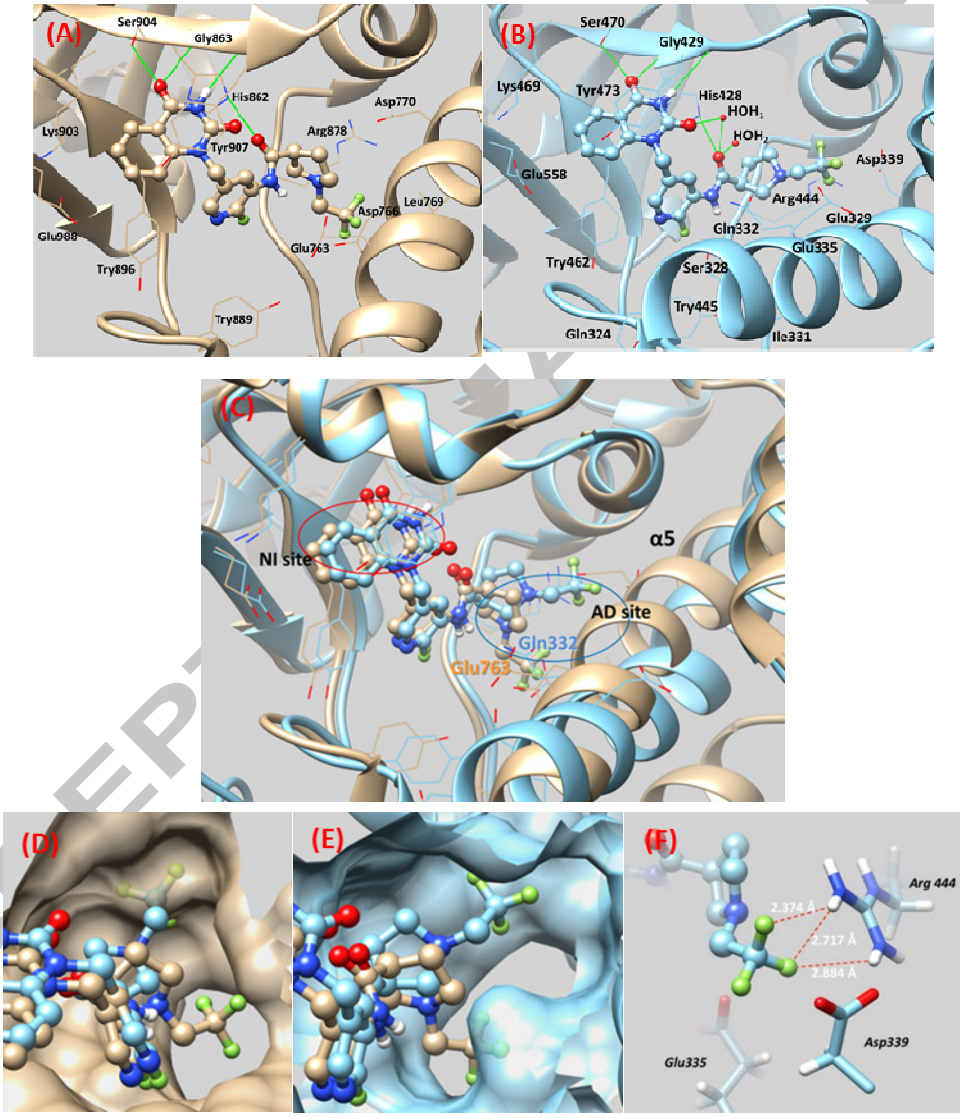


Figure 2.CDOCKER-modeled binding mode of compound **11a** within PARP-1(carbon atoms colored gold) and PARP-2 (carbon atoms colored blue). (A) The binding interactions of compound **11a** within the binding site of PARP-1; (B) The binding interactions of compound **11a** within the binding site of PARP-2; (C) Comparison of the binding poses of **11a** in PARP-1 and PARP-2, the α5 helix shifted away in PARP-2 in comparison with PARP-1; (D) Close-up view of the binding

orientation of the trifluoroethyl group in PARP-1; (E) Close-up view of the binding orientation of the trifluoroethyl group in PARP-2; (E) The hydrogen bonding network was observed between the trifluoroethyl moiety and Arg444 in PARP-2. Molecular image was generated with UCSF Chimera[34].

4. Conclusion

In summary, a series of structurally novel quinazoline-2,4(1*H*,3*H*)-dione derivatives were designed and synthesized by varying the spacer and the substituents on the pyrrolidine ring of our lead compound, which showed selectivity toward PARP-2 over PARP-1 to some degree. Introducing the pyridine ring into the spacer improved the structural novelty and conferred this series of inhibitors with distinct physicochemical properties. Among all the target molecules, three compounds (**11b-11d**) strongly inhibited PARP-1 and PARP-2 with IC_{50} in the single digit nanomolar level, although no selectivity was observed. Compound **11i** possessed the very potent activity as well as a moderate PARP-2 selectivity. Remarkably, compound **11a** was discovered as the most selective PARP-2 inhibitor with high potency for the present. The molecular docking of **11a** with PARP-1 and PARP-2 offered an insight into its preference for PARP-2 binding. These results will deepen our understanding on the distinct features of the AD site within PARP-1 and PARP-2, and facilitate to develop more isoform selective PARP inhibitors.

5. Experimental section

5.1. General

Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. ^{1}H NMR (300 MHz or 400 MHz) on a Varian Mercury 300 or 400 spectrometer was recorded in DMSO-d_6, acetone-d_6 or $CDCl_3$. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). High resolution mass spectra (HRMS) were obtained on an Agilent Technologies LC/MSD TOF spectrometer. All chemicals and solvents used were of reagent grade without purified or dried before use. All the reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel G plates at 254 nm under a UV lamp. Column chromatography separations were performed with silica gel (200–300 mesh).

5.2. Synthesis of target compounds 9a-9i, 10a-10g, 11a-11i

5.2.1. (*S*)-*Tert*-butyl 3-((5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoropyridin-3-yl)carbamoyl)pyrrolidine-1-carboxylate (9a)

A mixture of (3*S*)-1-(*tert*-butoxycarbonyl)-3-pyrrolidinecarboxylic acid (70 mg, 0.32 mmol) and triphenylphosphine (170 mg, 0.63 mmol) in dried tetrahydrofuran (4.0 mL) was stirred under argon and cooled to 0 ℃. Hexachloroacetone (85 mg, 0.32 mmol) in dried tetrahydrofuran (2.0 mL) was added dropwise and the mixture was stirred for 1h.The acyl chloride solution was then treated with a solution of **8a** (60 mg, 0.21 mmol) in dried tetrahydrofuran (4.0 mL)dropwise followed by triethylamine (32 mg, 0.32 mmol) in dried tetrahydrofuran (1.0 mL). The reaction mixture was then allowed to reach room temperature and stirred for 5 h and then the mixture was evaporated. The crude product was obtained and purified with column chromatography (petroleum ether/methylene chloride/ethyl acetate = 1:1:1 to methylene chloride/methanol/tetrahydrofuran = 40:1:1) to give the corresponding product **9a** as a light yellow solid (50 mg, 49.5%); mp 212-214 ℃; $[\alpha]_D^{25}$+22.00 (*c*=0.43, $CHCl_3$:MeOH=10:1); ^{1}HNMR (400 MHz, $CDCl_3$)δ (ppm): 8.83 (d, *J* = 9.2 Hz, 1H), 8.63 (s, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 7.82 (brs, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.52 (brs, 1H), 7.29 (d, *J* = 7.6 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 5.33 (s, 2H), 3.55-3.76 (m, 3H), 3.35-3.46 (m, 1H), 3.02-3.12 (m, 1H), 2.16-2.26 (m, 2H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 161.72,153.45 (d, *J* = 234.6 Hz), 153.31, 150.65, 140.53, 135.28, 131.53, 130.79 (d, *J* = 4.2 Hz),127.70, 122.83, 121.46 (d, *J* = 27.5 Hz), 119.44, 115.99, 114.79, 78.31, 48.24 (48.14), 45.32 (45.15), 43.71 (42.84), 42.33, 29.12 (28.29), 28.13; HR-MS (ESI): *m*/*z*, calcd. for $C_{24}H_{26}N_5O_5FNa[M+Na]^+$506.1810, Found: 506.1791.

5.2.2. (*S*)-*Tert*-butyl 3-((2-chloro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)pyridin-3-yl)carbamoyl)pyrrolidine-1-carboxylate (9b)

Following the preparation protocol of compound **9a**, starting from **8b** (87 mg, 0.29 mmol), the title compound **9b** was obtained as a white solid (80 mg, 58.0%); mp 234-236 ℃;$[\alpha]_D^{25}$+16.11 (*c*=0.42, $CHCl_3$:MeOH=10:1);^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 9.79 (s, 1H), 8.27 (s, 1H), 8.09 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.67 (t, *J* = 8.0 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.27 (t, *J* = 7.6 Hz, 1H),5.35 (s, 2H), 3.46-3.54 (m, 1H), 3.32-3.41 (m, 2H), 3.22-3.29 (m, 2H), 1.96-2.14 (m, 2H), 1.40 (s, 9H); HR-MS (ESI): *m*/*z*, calcd. for $C_{24}H_{26}N_5O_5ClNa[M+Na]^+$522.1515, Found: 522.1504.

5.2.3. (*S*)-*Tert*-butyl 3-((2-bromo-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)pyridin-3-yl)carbamoyl)pyrrolidine-1-carboxylate (9c)

Following the preparation protocol of compound **9a**, starting from **8c** (90 mg, 0.26 mmol), the title compound **9c** was obtained as a white solid (70 mg, 70.0%); mp 239-241 °C;$[\alpha]_D^{25}$+15.13 (*c*=0.26, $CHCl_3$:MeOH=10:1); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 9.76 (s, 1H), 8.27 (s, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.92 (s, 1H), 7.67 (t, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 8.8Hz, 1H), 7.26 (t, *J* = 7.6 Hz, 1H), 5.33 (s, 2H), 3.45-3.54 (m, 1H), 3.31-3.42 (m, 2H), 3.24 (brs, 2H), 1.95-2.15 (m, 2H), 1.39 (s, 9H); HR-MS (ESI): *m*/*z*, calcd. for $C_{24}H_{26}N_5O_5BrNa[M+Na]^+$566.1010, Found: 566.0991.

5.2.4. (*S*)-*Tert*-butyl 3-((5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)pyridin-3-yl)carbamoyl)pyrrolidine-1-carboxylate (9d)

Following the preparation protocol of compound **9a**, starting from **8d** (77 mg, 0.29 mmol), the title compound **9d** was obtained as a white solid (30 mg, 22.4%); mp 163-165 °C; $[\alpha]_D^{25}$ +10.94 (*c*=0.79, $CHCl_3$:MeOH=10:1); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.80 (s, 1H), 10.20 (s, 1H), 8.76 (s, 1H), 8.34 (s, 1H), 8.04 (d, *J* = 7.6 Hz, 1H), 7.81 (s, 1H), 7.68 (t, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.27 (t, *J* = 7.6 Hz, 1H), 5.34 (s, 2H), 3.45-3.52 (m, 1H), 3.33-3.40 (m, 2H), 3.18-3.28 (m, 1H), 3.09 (brs, 1H), 1.91-2.13 (m, 2H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm):161.74, 153.31, 150.62, 142.91, 140.59, 139.72, 135.76, 135.34, 132.18, 127.69, 123.71, 122.85, 119.44, 115.89, 114.88, 78.31, 48.15 (48.06), 45.33 (45.15), 44.18 (43.30), 42.81, 29.12 (28.28), 28.13; HR-MS (ESI): *m*/*z*, calcd. for $C_{24}H_{28}N_5O_5[M+H]^+$466.2085, Found: 466.2080.

5.2.5. (*S*)-*Tert*-butyl 3-((2-chloro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (9e)

Following the preparation protocol of compound **9a**, starting from **8e** (100 mg, 0.33 mmol), the title compound **9e** was obtained as a white solid (135 mg, 52.0%); mp 158-160 °C;$[\alpha]_D^{25}$ +7.12 (*c*=0.39, $CHCl_3$:MeOH=10:1); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 9.64 (s, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.63-7.67 (m, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.23-7.27 (m, 2H), 7.13 (d, *J* = 8.0 Hz, 1H),5.29 (s, 2H), 3.47-3.54 (m, 1H), 3.33-3.43 (m, 2H), 3.19-3.29 (m, 2H), 1.95-2.15 (m, 2H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 171.34, 161.74, 153.33, 150.62,

140.68, 136.04, 135.21, 134.92, 129.68, 127.62, 126.15, 124.69, 122.76, 115.90, 114.97, 78.29, 48.23 (48.18), 45.33 (45.16), 44.54, 43.61(42.72), 29.11 (28.33), 28.14; HR-MS (ESI): *m/z*, calcd. for $C_{25}H_{27}N_4O_5ClNa[M+Na]^+$521.1562, Found: 521.1536.

5.2.6. (*S*)-*Tert*-butyl 3-((2-bromo-5-((2,4-dioxo-3,4-dihydroquinazolin-1(*2H*)-yl)methyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (9f)

Following the preparation protocol of compound **9a**, starting from **8f** (100 mg, 0.29 mmol), the title compound **9f** was obtained as a white solid (90 mg, 57.0%); mp 163-165 °C;$[\alpha]_D^{25}$+7.84 (*c*=0.1, $CHCl_3$:MeOH=10:1); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 9.61 (s, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.51 (brs, 1H), 7.23-7.27 (m, 2H), 7.08 (d, *J* = 8.0 Hz, 1H), 5.28 (s, 2H), 3.46-3.55 (m, 1H), 3.34-3.43 (m, 2H), 3.14-3.29 (m, 2H), 1.96-2.16 (m, 2H), 1.40 (s, 9H); HR-MS (ESI): *m/z*, calcd. for $C_{25}H_{27}N_4O_5BrNa[M+Na]^+$565.1057, Found: 565.1030.

5.2.7. (*S*)-*Tert*-butyl 3-((5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-nitrophenyl)carbamoyl)pyrrolidine-1-carboxylate (9g)

Following the preparation protocol of compound **9a**, starting from **8g** (200 mg, 0.64 mmol), the title compound **9g** was obtained as a gray solid (90 mg, 27.6%); mp 142-144 °C;$[\alpha]_D^{25}$+5.48 (*c*=0.15, $CHCl_3$:MeOH=10:1); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 10.62 (s, 1H), 8.92 (s, 1H), 8.69 (s, 1H),8.24 (d, *J* = 8.0 Hz, 1H), 8.19 (d, *J* = 8.8 Hz, 1H), 7.60 (t, *J* = 8.0 Hz, 1H), 7.26-7.31 (m, 1H), 7.03 (d, *J* = 8.4 Hz, 2H), 5.40 (s, 2H), 3.54-3.83 (m, 3H), 3.45 (brs, 1H), 3.16 (brs, 1H), 2.21-2.30 (m, 2H), 1.48 (s, 9H); HR-MS (ESI): *m/z*, calcd. for $C_{25}H_{27}N_5O_7Na[M+Na]^+$532.1803, Found: 532.1797.

5.2.8. (*S*)-*Tert*-butyl3-((5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-hydroxyphenyl)carbamoyl)pyrrolidine-1-carboxylate (9h)

Following the preparation protocol of compound **9a**, starting from **8h** (80 mg, 0.28 mmol), the title compound **9h** was obtained as a gray solid (80 mg, 59.0%); mp 208-210 °C;$[\alpha]_D^{25}$+6.35 (*c*=0.15, $CHCl_3$:MeOH=10:1); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.71 (s, 1H), 9.76 (s, 1H), 9.32 (s, 1H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.74 (s, 1H), 7.64 (t, *J* = 8.0 Hz, 1H), 7.20-7.30 (m, 2H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H),5.18 (s, 2H), 3.49 (t, *J* = 8.4 Hz, 1H), 3.16-3.41 (m, 4H), 1.90-2.12 (m, 2H),1.40 (s, 9H); HR-MS (ESI): *m/z*, calcd. for $C_{25}H_{28}N_4O_6Na[M+Na]^+$503.1901,

Found: 503.1882.

5.2.9. (*S*)-*Tert*-butyl 3-((2-amino-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (9i)

The mixture of compound **9g** (60 mg, 0.12 mmol) and Raney nickel (40 mg) in methanol (8.0 mL) and tetrahydrofuran (8.0 mL) was hydrogenated at room temperature and 1 atm for 18 h. Tetrahydrofuran (30.0 mL) was added and the Raney nickel was filtered over a pad of Celiteand, the solution was concentrated to afford the crude compound. The crude product was purified with column chromatography (DCM/MeOH = 50:1-30:1) to afford compound **9i** as a white solid (30mg, 53.6%); mp 149-151 °C;$[\alpha]_D^{25}$+8.40 (*c*=0.13, $CHCl_3$:MeOH=10:1); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm):11.68 (s, 1H), 9.26 (s, 1H), 8.00 (d, *J* = 7.6 Hz, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.23 (t, *J* = 7.2 Hz, 1H), 7.15 (s, 1H), 6.89 (d, *J* = 7.6 Hz, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 5.14 (brs, 2H), 4.82 (brs, 2H), 3.51 (t, *J* = 9.2 Hz, 1H),3.34-3.41 (m, 2H), 3.07-3.28 (m, 2H), 1.93-2.14 (m, 2H), 1.40 (s, 9H); HR-MS (ESI): *m*/*z*, calcd. for $C_{25}H_{30}N_5O_5[M+H]^+$480.2242, Found: 480.2245.

5.2.10. (*S*)-*N*-(5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoropyridin-3-yl)pyrrolidine-3-carboxamide2,2,2-trifluoroacetate(10a)

To a stirred solution of **9a** (45 mg, 0.09 mmol) in DCM (4.0 mL) was added TFA (0.5 mL) dropwise, the reaction mixture was then allowed to stir at room temperature overnight. DCM and excessive TFA were then removed under reduced pressure. The crude product was purified by crystallization from a solvent mixture of methyl alcohol and diethyl ether to afford compound **10a** as a yellow solid (34 mg, 77.3%); mp 205-207 °C; $[\alpha]_D^{25}$+3.11 (*c*=0.26, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.76 (s, 1H), 10.23 (s, 1H), 8.84 (brs, 2H), 8.37 (d, *J* = 9.2 Hz, 1H), 8.09 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.68 (t, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.27 (t, *J* = 7.6 Hz, 1H), 5.34 (s, 2H), 3.34-3.45 (m, 3H), 3.14-3.24 (m, 2H), 2.16-2.28 (m, 1H), 1.97-2.08 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 171.64, 161.77, 158.30 (q, *J* = 31.4 Hz), 153.41 (d, *J* = 234.4 Hz), 150.66, 141.55, 140.54, 140.10 (d, *J* = 14.9 Hz), 135.37, 132.49 (q, *J* = 245.3 Hz), 131.22, 130.87 (d, *J* = 4.1 Hz), 127.73, 122.92, 121.36 (d, *J* = 27.7 Hz), 115.95, 114.82, 46.78, 44.90, 42.50, 42.31, 28.80; HR-MS (ESI): *m*/*z*, calcd. for $C_{19}H_{19}N_5O_3F$ $[M+H]^+$384.1466, Found: 384.1456.

5.2.11.

(*S*)-*N*-(2-Chloro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)pyridin-3-yl)pyrrolidine-3-carboxamide2,2,2-trifluoroacetate(10b)

Following the preparation protocol of compound **10a**, starting from **9b** (40 mg, 0.08 mmol), the title compound **10b** was obtained as a white solid (30 mg, 77.0%); mp 207-209 °C; $[\alpha]_D^{25}$+12.76 (*c*=0.19, MeOH); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.76 (s, 1H), 9.99 (s, 1H), 8.82 (brs, 2H), 8.35 (s, 1H), 8.01-8.06 (m, 2H), 7.67 (t, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 8.8 Hz, 1H), 7.27 (t, *J* = 7.6 Hz, 1H),5.36 (s, 2H), 3.32-3.44 (m, 3H),3.20 (t, *J* = 7.2 Hz, 2H), 2.21-2.29 (m, 1H), 2.01-2.09 (m, 1H); HR-MS (ESI): m/z, calcd. for $C_{19}H_{19}N_5O_3Cl$ $[M+H]^+$400.1171, Found: 400.1169.

5.2.12.

(*S*)-*N*-(2-Bromo-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)pyridin-3-yl)pyrrolidine-3-carboxamide2,2,2-trifluoroacetate(10c)

Following the preparation protocol of compound **10a**, starting from **9c** (40 mg, 0.07 mmol), the title compound **10c** was obtained as a white solid (33 mg, 84.6%); mp 201-202 °C; $[\alpha]_D^{25}$+13.01 (*c*=0.33, MeOH); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.76 (s, 1H), 9.98-10.00 (m, 1H), 8.88 (brs, 2H), 8.35 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.87 (s, 1H), 7.67 (t, *J* = 7.6 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H),7.27 (t, *J* = 7.6 Hz, 1H), 5.34 (s, 2H), 3.37 (brs, 3H), 3.16-3.26 (m, 2H), 2.19-2.30 (m, 1H), 2.01-2.12 (m, 1H); HR-MS (ESI): m/z, calcd. for $C_{19}H_{19}N_5O_3Br$ $[M+H]^+$444.0666, Found: 444.0651.

5.2.13.

(*S*)-*N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)pyridin-3-yl)pyrrolidine-3-carboxamide2,2,2-trifluoroacetate(10d)

Following the preparation protocol of compound **10a**, starting from **9d** (15 mg, 0.03 mmol), the title compound **10d** was obtained as a white solid (10 mg, 71.4%); mp 143-145 °C; $[\alpha]_D^{25}$+5.59 (*c*=0.14, MeOH); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.80 (s, 1H), 10.40 (s, 1H), 8.81 (brs, 2H), 8.74 (s, 1H), 8.42 (s, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.81 (s, 1H), 7.67 (t, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.27 (t, *J* = 7.6 Hz, 1H), 5.36 (s, 2H), 3.32-3.41 (m, 2H), 3.15-3.27 (m, 3H), 2.14-2.26 (m, 1H), 1.98-2.08 (m, 1H); HR-MS (ESI): m/z, calcd. for $C_{19}H_{20}N_5O_3$ $[M+H]^+$366.1561, Found: 366.1553.

5.2.14.

(*S*)-*N*-(2-Chloro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)pyrrolidine-3-carboxamide2,2,2-trifluoroacetate(10e)

Following the preparation protocol of compound **10a**, starting from **9e** (40 mg, 0.08 mmol), the title compound **10e** was obtained as a white solid (32 mg, 82.0%); mp 223-225 °C; $[\alpha]_D^{25}$+2.52 (*c*=0.32, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.76 (s, 1H), 9.86 (s, 1H), 8.93 (s, 2H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.57 (s, 1H), 7.48 (d, *J* = 8.0 Hz, 1H),7.18-7.30 (m, 3H), 5.30 (s, 2H), 3.34-3.43 (m, 3H), 3.15-3.27 (m, 2H), 2.20-2.31 (m, 1H), 1.99-2.11 (m, 1H); HR-MS (ESI): m/z, calcd. for $C_{20}H_{20}N_4O_3Cl[M+H]^+$ 399.1218, Found: 399.1206.

5.2.15.

(*S*)-*N*-(2-Bromo-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)pyrrolidine-3-carboxamide2,2,2-trifluoroacetate(10f)

Following the preparation protocol of compound **10a**, starting from **9f** (35 mg, 0.06 mmol), the title compound **10f** was obtained as a pink solid (30 mg, 88.2%); mp 145-147 °C; $[\alpha]_D^{25}$+3.24 (*c*=0.21, MeOH); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 9.83 (s, 1H), 8.84 (brs, 2H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.65 (t, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 2.0 Hz, 1H), 7.22-7.28 (m, 2H), 7.16 (d, *J* = 8.4 Hz, 1H), 5.28 (s, 2H), 3.33-3.41 (m, 3H), 3.15-3.25 (m, 2H), 2.21-2.31 (m, 1H), 2.02-2.14 (m, 1H); HR-MS (ESI): m/z, calcd. for $C_{20}H_{20}N_4O_3Br$ $[M+H]^+$443.0713, Found: 443.0702.

5.2.16.

(*S*)-*N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-nitrophenyl)pyrrolidine-3-carboxamide2,2,2-trifluoroacetate(10g)

Following the preparation protocol of compound **10a**, starting from **9g** (40 mg, 0.08 mmol), the title compound **10g** was obtained as a yellow solid (32 mg, 82.5%); mp 147-149 °C; $[\alpha]_D^{25}$+21.57 (*c*=0.21, MeOH); ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 11.82 (s, 1H), 10.60 (s, 1H), 8.86 (brs, 2H), 8.05 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 8.8 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.20-7.34 (m, 3H), 5.41 (s, 2H), 3.11-3.41 (m, 5H), 2.16-2.26 (m, 1H), 1.97-2.05 (m, 1H); HR-MS (ESI): m/z, calcd. for $C_{20}H_{20}N_5O_5$ $[M+H]^+$410.1459, Found: 410.1440.

5.2.17.

(*S*)-*N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoropyridin-3-yl)-1-(2,2,2-trifluoroethyl)pyrrolidine-3-carboxamide (11a)

To a stirred solution of **10a** (80 mg, 0.16 mmol) in tetrahydrofuran (6.0 mL) were added Et_3N (98 mg, 1.01 mmol) and 2,2,2-trifluoroethyl trifluoromethanesulfonate (198 mg, 0.81 mmol). The reaction mixture was heated at 36 °C for 12 h and then was evaporated. After concentration, the crude product was obtained and purified with column chromatography (methylene chloride/methanol = 50:1-30:1) to give compound **11a** as a white solid (36 mg, 46.8%); mp 260-262°C; $[\alpha]_D^{25}$+2.27 (*c*=0.41,$CHCl_3$:MeOH=10:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 9.92 (s, 1H), 8.40 (dd, J_1 = 9.6 Hz, J_2 = 2.4 Hz, 1H), 8.03 (dd, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 7.99 (brs, 1H), 7.65-7.71 (m, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.24-7.29 (m, 1H), 5.33 (s, 2H), 3.14-3.31 (m, 3H), 3.01 (t, J = 8.8 Hz, 1H), 2.72-2.84 (m, 2H), 2.64-2.71 (m, 1H), 1.89-2.05 (m, 2H); HR-MS (ESI): m/z, calcd. for $C_{21}H_{20}N_5O_3F_4[M+H]^+$466.1497, Found: 466.1486.

5.2.18.

(*S*)-*N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoropyridin-3-yl)-1-(3,3,3-trifluoropropyl)pyrrolidine-3-carboxamide (11b)

Following the preparation protocol of compound **11a**, starting from **10a** (90mg, 0.18mmol) and 3,3,3-trifluoropropyl trifluoromethanesulfonate (234mg, 0.94mmol), the title compound **11b** was obtained as a white solid (56mg, 62.2%); mp 236-238 °C; $[\alpha]_D^{25}$ +1.78 (*c*=0.41,$CHCl_3$:MeOH=10:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm):11.77 (s, 1H), 9.92 (s, 1H), 8.41 (d, J = 9.2 Hz, 1H), 8.03 (d, J = 7.6 Hz, 1H), 7.99 (s, 1H), 7.65-7.72 (m, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.27 (t, J = 7.2 Hz, 1H), 5.34 (s, 2H), 3.14 (brs, 1H), 2.83 (t, J = 8.0 Hz,1H), 2.55-2.68 (m, 5H), 2.38-2.48 (m, 2H), 1.89-2.04 (m, 2H);^{13}C NMR (150 MHz, DMSO-d_6) δ(ppm): 174.24, 162.21, 153.77 (d, J = 233.85 Hz), 151.12, 141.01, 139.85 (d, J = 14.25 Hz), 135.78, 131.53, 131.26 (d, J = 3.6 Hz), 128.18, 127.50 (q, J = 274.95 Hz), 123.33, 122.20 (d, J = 27.45 Hz), 116.45, 115.28, 57.24, 53.65, 47.99 (d, J = 2.7 Hz), 43.46, 42.82, 32.60 (q, J = 26.4 Hz), 28.24; HR-MS (ESI): m/z, calcd. for $C_{22}H_{22}N_5O_3F_4[M+H]^+$480.1653, Found: 480.1650.

5.2.19.

(*S*)-1-(Cyclopropylmethyl)-*N*-(5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoropyridin-3-yl)pyrrolidine-3-carboxamide (11c)

To a suspension of **10a** (100 mg,0.21 mmol) in dichloromethane (4.0 mL),

cyclopropanecarbaldehyde (49 mg, 0.63 mmol), sodium acetate (84 mg, 1.04 mmol), andmethanol (1.0 mL) were added. The resulting solution was stirred at 37 °C for 10 h. Then sodium cyanoborohydride (42 mg, 0.63 mmol) was addedand the mixture was stirred for 8 h. The dichloromethane (30 mL) and methanol (3 mL) were added and washed with saturated aqueous sodium bicarbonate (10 mL×2)and brine (10 mL×2), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (methylene chloride/methanol = 50:1-30:1) to give the title compound **11c** (45 mg, 45.9%); yellow-green solid; mp 215-217 °C; $[\alpha]_D^{25}$+3.31 (*c*=0.23,$CHCl_3$:MeOH=10:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.76 (brs, 1H), 10.02 (s, 1H), 8.44 (d, J = 7.6 Hz, 1H), 8.03 (dd, J_1= 7.6 Hz, J_2= 1.2 Hz, 1H), 7.97 (s, 1H), 7.65-7.71 (m, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.27 (t, J = 7.6 Hz, 1H), 5.33 (s, 2H), 3.05-3.15 (m, 1H), 2.82 (t, J = 8.4 Hz,1H), 2.53-2.65 (m, 3H), 2.22-2.33 (m, 2H), 1.87-2.04 (m, 2H), 0.79-0.89 (m, 1H), 0.40-0.48 (m, 2H), 0.06-0.12 (m, 2H);^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):174.07, 161.73, 153.15 (d, J = 233.8 Hz), 150.64, 140.53, 139.15 (d, J = 15.2 Hz), 135.30, 130.78 (d, J = 4.2 Hz), 127.70, 122.84, 121.83 (d, J = 27.3 Hz), 115.96, 114.80, 59.68, 56.95, 53.20, 43.09, 42.33, 27.62, 9.71, 3.62, 3.52; HR-MS (ESI): m/z, calcd. for $C_{23}H_{25}N_5O_3F$ $[M+H]^+$438.1936, Found: 438.1919.

5.2.20.

(*S*)-1-(2-Cyclopropylethyl)-*N*-(5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoropyridin-3-yl)pyrrolidine-3-carboxamide (11d)

Following the preparation protocol of compound **11a**, starting from **10a** (96 mg, 0.2 mmol) and 2-cyclopropylethyl trifluoromethanesulfonate (220mg, 1.0mmol), the title compound **11d** was obtained as a white solid (42mg, 46.7%); mp 197-199 °C; $[\alpha]_D^{25}$ +4.73 (*c*=0.34,$CHCl_3$:MeOH=10:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm):11.76 (s, 1H), 10.04 (s, 1H), 8.40 (dd, J_1 = 9.2 Hz, J_2 = 2.0 Hz, 1H), 8.01 (dd, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 7.99 (s, 1H), 7.64-7.70 (m, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.26 (t, J = 7.6 Hz, 1H), 5.33 (s, 2H), 3.28-3.47 (m, 1H), 3.17 (s, 1H), 2.52-2.94 (m, 5H), 1.88-2.11 (m, 2H), 1.32-1.42 (m, 2H), 0.62-0.73 (m, 1H), 0.32-0.42 (m, 2H), 0.04-0.06 (m, 2H); HR-MS (ESI): m/z, calcd. for $C_{24}H_{27}N_5O_3F$ $[M+H]^+$452.2092, Found: 452.2091.

5.2.21.

(*S*)-1-(4,4-Difluorocyclohexyl)-*N*-(5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoropyridin-3-yl)pyrrolidine-3-carboxamide (11e)

Following the preparation protocol of compound **11c**, starting from **10a** (100 mg, 0.21 mmol) and 4,4-difluorocyclohexanone(84 mg, 0.63 mmol), the title compound **11e** was obtained as a white solid (46 mg, 44.2%); mp 189-191 °C; $[\alpha]_D^{25}$+2.20 (*c*=0.42, $CHCl_3$:MeOH=10:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.76 (s, 1H), 9.97 (s,

1H), 8.43 (d, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.98 (s, 1H), 7.68 (t, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.27 (t, *J* = 7.6 Hz, 1H), 5.33 (s, 2H), 3.10 (brs, 1H), 2.81 (brs, 1H), 2.55-2.69 (m, 3H), 2.23 (s, 1H), 1.72-2.09 (m, 8H), 1.57 (brs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm):173.94, 161.73, 150.64, 140.54, 139.14 (d, *J* = 15.0 Hz),135.30, 130.80 (d, *J* = 4.4 Hz), 127.70, 122.85, 121.65 (d, *J* = 27.5 Hz), 119.45, 115.96, 114.82, 58.60, 54.43, 50.73, 43.06, 42.34, 30.56 (t, *J* = 24.2 Hz), 27.68, 27.05; HR-MS (ESI): m/z, calcd. for $C_{25}H_{27}N_5O_3F_3[M+H]^+$502.2060, Found: 502.2085.

5.2.22.

(*S*)-1-(Cyclopropanecarbonyl)-*N*-(5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl) methyl)-2-fluoropyridin-3-yl)pyrrolidine-3-carboxamide (11f)

To a stirred solution of **10a** (90 mg, 0.19 mmol) in methylene chloride (15.0 mL), Et_3N (49 mg, 0.47 mmol) and cyclopropanecarbonyl chloride (23 mg, 0.21 mmol) were added at 0°C. The reaction mixture was then allowed to stir at room temperature overnight. The dichloromethane(30 mL) and methanol(3 mL) were added and washed with water (10 mL×2)and brine (10 mL×2), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (methylene chloride/methanol = 50:1-30:1) to give the title compound **11f** (49 mg, 57.69%); light yellow solid;mp 208-210 °C; $[\alpha]_D^{25}$+49.3 (*c*=0.5,$CHCl_3$:MeOH=10:1);^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.77 (s, 1H), 10.12 (s, 0.5H), 10.09 (s, 0.5H),8.42 (d, *J* = 9.6 Hz, 1H), 8.01 (t, *J* = 7.6 Hz, 2H), 7.64-7.71 (m, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.26 (t, *J* = 7.6 Hz, 1H), 5.33 (s, 2H), 3.84-3.90 (m, 0.5H), 3.67-3.78 (m, 1H), 3.55-3.66 (m, 1H), 3.36-3.48 (m, 1.5H), 3.21-3.30 (m, 1H), 2.05-2.26 (m, 1.5H), 1.91-2.00 (m, 0.5H), 1.69-1.72 (m, 1H), 0.67-0.74 (m, 4H); HR-MS (ESI): m/z, calcd. for $C_{23}H_{23}N_5O_4F$ $[M+H]^+$452.1729, Found: 452.1718.

5.2.23.

(*S*)-1-Benzyl-*N*-(5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoropy ridin-3-yl)pyrrolidine-3-carboxamide (11g)

Following the preparation protocol of compound **11c**, starting from **10a** (100 mg, 0.21 mmol) and benzaldehyde(68 mg, 0.63 mmol), the title compound **11g** was obtained as a white solid (53 mg, 54.1%); mp 218-220 °C; $[\alpha]_D^{25}$+34.65 (*c*=0.18, $CHCl_3$:MeOH=10:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.76 (s, 1H), 9.88 (s, 1H), 8.41 (dd, J_1= 9.6 Hz, J_2= 2.4 Hz, 1H), 8.03 (dd, J_1= 7.6 Hz, J_2= 1.6 Hz, 1H), 7.97 (brs, 1H), 7.66-7.70 (m, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.21-7.34 (m, 6H), 5.33 (s, 2H), 3.50-3.65 (m, 2H), 3.07-3.18 (m, 1H), 2.78 (t, *J* = 8.4 Hz, 1H), 2.52-2.62 (m, 2H), 2.42-2.49 (m, 1H), 1.92-2.02 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):173.86, 161.78, 152.09,150.68, 140.56, 139.31 (d, *J* = 17.1 Hz), 135.34, 131.02,

130.80 (d, *J* = 4.1 Hz),128.56, 128.17, 127.73, 126.88, 122.88, 121.77(d, *J* = 27.3 Hz), 115.99, 114.84, 59.08, 56.88, 53.31, 43.06, 42.35, 27.56; HR-MS (ESI): m/z, calcd. for $C_{26}H_{25}N_5O_3F$ $[M+H]^+$474.1936, Found: 474.1916.

5.2.24.

(*S*)-*N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoropyridin-3-yl)-1-(pyridin-4-ylmethyl)pyrrolidine-3-carboxamide (11h)

Following the preparation protocol of compound **11c**, starting from **10a** (100 mg, 0.21 mmol) and *iso*-nicotinaldehyde (72 mg, 0.63 mmol), the title compound **11h** was obtained as a light yellow solid (52 mg, 52.5%); mp 167-169 °C; $[\alpha]_D^{25}$+36.20 (*c*=0.44,$CHCl_3$:MeOH=10:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.77 (s, 1H), 9.88 (s, 1H), 8.50 (d, *J* = 5.6 Hz, 2H), 8.41 (d, *J* = 8.0 Hz, 1H), 8.03 (d, *J* = 6.8 Hz, 1H), 7.99 (s, 1H), 7.68 (t, *J* = 7.2 Hz, 1H), 7.37 (d, *J* = 8.8 Hz, 1H), 7.33 (d, *J* = 5.6 Hz, 2H), 7.27 (t, *J* = 7.6 Hz, 1H), 5.33 (s, 2H), 3.56-3.70 (m, 2H), 3.18 (p, *J* = 7.6 Hz, 1H), 2.81 (t, *J* = 8.4 Hz, 1H), 2.54-2.63 (m, 3H), 1.94-2.06 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 173.68,168.51, 161.73, 159.75, 150.64, 149.49, 140.53, 139.39 (d, *J* = 14.1 Hz), 135.30, 131.19, 130.79, 127.70, 123.48, 122.85, 121.69 (d, *J* = 27.2 Hz),115.96, 114.80, 57.76, 56.82, 53.39, 43.02, 42.31, 27.67; HR-MS (ESI): m/z, calcd. for $C_{25}H_{24}N_6O_3F$ $[M+H]^+$475.1888, Found: 475.1885.

5.2.25.

(*S*)-*N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoropyridin-3-yl)-1-phenylpyrrolidine-3-carboxamide (11i)

To a stirred solution of **10a** (200 mg, 0.52 mmol) in anhydrous toluene (3.0 mL) were added Pd_2dba_3 (72 mg, 0.08mmol), Xantphos (132 mg, 0.24 mmol), bromobenzene (250 mg, 1.56 mmol) and Cs_2CO_3 (676 mg, 2.08mmol). The reaction mixture was heated by MW (100 W, 300 min, 120 °C, 150 psi) and then was evaporated. After concentration, the crude product was obtained and purified with column chromatography (methylene chloride/methanol/tetrahydrofuran= 50:1:1) to give compound **11i** as a light yellow solid (10 mg, 5.6%); mp 252-254 °C; $[\alpha]_D^{25}$+17.34 (*c*=0.44, $CHCl_3$:MeOH=10:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.76 (s, 1H), 10.10 (s, 1H), 8.44 (dd, J_1= 9.6 Hz, J_2= 2.0 Hz, 2H),7.97-8.04 (m, 2H), 7.64-7.71 (m, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.26 (t, *J* = 7.6 Hz, 1H), 7.12-7.19 (m, 2H), 6.60 (t, *J* = 7.2 Hz, 1H), 6.54 (d, *J* = 8.0 Hz, 2H), 5.33 (s, 2H), 3.21-3.53 (m, 5H), 2.11-2.30 (m, 2H); HR-MS (ESI): m/z, calcd. for $C_{25}H_{23}N_5O_3F$ $[M+H]^+$460.1779, Found: 460.1772.

5.3. The assay for PARP-1 and PARP-2 inhibition

Plasmid pET32a-PARP1 was a gift from Prof. Satoh (Canada). Human recombinant

PARP1/2 were expressed and purified as described[35]. The ability of compounds to inhibit PARP1/2 enzyme activity were tested using ELISA method and the chemical quantitation of NAD^+ method as described[35,36]. IC_{50} values were calculated using GraphPad Prism 5 software.

5.4. Computational studies

All molecular computation studies were performed using CDOCER protocol integrated in Accelrys Discovery Studio Client 2017 (Accelrys Software Inc., San Diego, CA). The co-crystal structures of NMS118-PARP-1 complex (PDB ID: 5A00) and NMS118-PARP-2 complex (PDB ID: 4ZZY) were chosen for molecular modeling. The docking protocol of compound **11a** with PARP-1 was chosen as a representative. Using Prepare Protein tool of DS, the water molecules in protein were removed and the protein were added hydrogens, corrected the incomplete residues and refined with CHARMm force field. To define the binding site more rationally, we superimpose 5WRQ (the co-crystal structure of 7TX-PARP1 complex) onto 5A00 and then choose 7TX as the center to construct the binding site within 13 Å. Compound **11a**was minimized using Prepare Ligands tool of DS and refined with CHARMm force field. Then it was docked into the prepared PARP-1 and PARP-2 protein with CDOCKER using the default parameters, respectively. The 40 final docked conformations were ranked according to their binding free energy. The docking mode was chosen on the basis of binding rationality.

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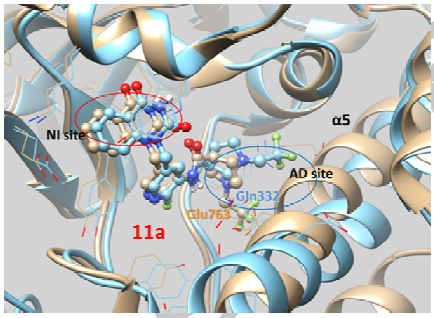
Graphical Abstract:

Discovery of novel quinazoline-2,4(1*H*,3*H*)-dione derivatives as potent PARP-2 selective inhibitors

Hailong Zhao, Ming Ji, Guonan Cui , Jie Zhou, Fangfang Lai, Xiaoguang Chen , Bailing Xu

A series of novel quinazoline-2,4(1*H*,3*H*)-dione derivatives was prepared and their inhibitory activities were evaluated against both PARP-1 and PARP-2. Compound **11a** was identified as the highly potent and selective PARP-2 inhibitor.



PARP-1: IC_{50} = 467 nM
PARP-2: IC_{50} = 11.5 nM
Selectivity: PARP-1/PARP-2 = 40.6