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Discovery of quinazoline-2,4(1H,3H)-dione derivatives as novel PARP-1/2 inhibitions: design, synthesis and their antitumor activity

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Abstract

Novel quinazoline-2,4(1*H*,3*H*)-dione derivatives bearing 3-amino pyrrolidine moiety were designed and synthesized as PARP-1/2 inhibitors. Structure-activity relationships were conducted and led to a number of potent PARP-1/2 inhibitors with moderate selectivity toward PARP-1 over PARP-2. These compounds had IC_{50} values against PARP-1 at 10^{-9} M level and against PARP-2 at 10^{-8} M level. Among all the synthesized compounds, compounds **10** and **11** displayed strong cytotoxicities either used as a single agent or in combination with temozolomide (TMZ) in MX-1 cells (**10**, IC_{50} < 3.12 μM, PF_{50} >10; **11**, IC_{50} = 3.02 μM, PF_{50} ≈ 10). In vivo tumor growth inhibition was investigated using compound **11** in combination with TMZ, and it was demonstrated that compound **11** could strongly potentiate the cytotoxicity of TMZ in MX-1 xenograft tumor model. The co-crystal structure of compound **11** complexed with PARP-1 was achieved and demonstrated a unique binding mode.

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

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Electronic Supplementary Information (ESI) available: [Synthesis of intermediates, the spectra of ^{1}H-NMR and ^{13}C-NMR of target compounds and the data collection of co-crystal structure were available]. See DOI: 10.1039/x0xx00000x

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1. Introduction

Genomic instability is a hallmark of cancer，which is largely driven by abnormalities in the robust DNA repair mechanisms of human cells[1]. Therefore, targeting DNA damage repair pathway has become an appealing therapeutic strategy in cancer therapy. Poly(ADP-ribose) polymerase-1/2 (PARP-1/2) played a key role in DNA breaks repair and have been extensively studied as targets for cancer treatments in preclinical and clinical models[2-4]. Both of them catalyzed the cleavage of nicotinamide adenine dinucleotides (NAD^+) into nicotinamide and ADP-ribose units, which were transferred to proteins participating in DNA damage repair processes including histone and PARP-1/2 and formed ADP-ribose polymers (PAR) [5,6]. While both PARP-1 and PARP-2 were involved in the DNA breaks repair, their specific roles in the repair pathway have not been well understood [7-9]. PARP-1 and PARP-2 were the closest homologs in this 17-member family and they possessed 69% similarity in the catalytic domain, therefore, most of initially known PARP-1 inhibitors such as AZD-2281, ABT-888, AG014699, MK-4827 and BMN673 also bound PARP-2 with comparable binding affinity [10-16].

It has been demonstrated that PARP-1/2 inhibitors were useful for the treatment of cancers either as a single agent or in the combination therapy. PARP-1/2 inhibitors could potentiate the cytotoxicity of chemotherapeutics such as temozolomide (TMZ) and cisplatin by blocking the repair of DNA breaks resulted from DNA alkylating agents. Specifically, cancer patients with BRCA1/2 mutations benefited from PARP-1/2 inhibitors treatment even more greatly since PARP-1/2 were synthetic lethal with BRCA1/2, which are involved in DNA double strand breaks repair through homologous recombination pathway [17-20].

Since nicotinamide and 3-aminobenzamide were identified as PARP-1 inhibitors in 1980's, tremendous efforts have been devoted and many structurally diverse PARP-1/2 inhibitors were discovered [21-24]. Presently, a number of inhibitors have been advanced into clinical trials [25-28]. Among them, Olaparib (AZD-2281), Rucaparib (AG014699) and Niraparib (MK4827) have been approved by FDA for the treatment of ovarian cancer in patients with or without BRCA mutations during the period of 2014-2017. It has been demonstrated that targeting on PARP-1/2 would be a viable way in developing cancer drugs.

In our efforts to discover novel PARP-1/2 inhibitors, a series of quinazoline-2,4(1*H*,3*H*)-dione derivatives was designed based on the structure-activity relationships (SARs) of known PARP inhibitors and their binding features in the catalytic domain of PARP-1. The quinazoline-2,4(1*H*,3*H*)-dione was chosen as a key structural fragment since it has been identified as a suitable subunit to occupy the nicotinamide-ribose binding site (NI site) in the catalytic domain and formed characteristic hydrogen bonds with residues Ser904 and Gly863; the substituted benzyl group not only could generate π-π stacking interaction with Tyr907, but also served as a linker to direct 1-substituted 3-amino pyrrolidine motifs into the adenine-ribose binding site (AD site)[29, 30], which is rather large and usually used to search for a wide variety of novel inhibitors with the improved potency and pharmacokinetic properties. In this work, we explored the SARs of this series of quinazoline-2,4(1*H*,3*H*)-diones by variation of the substituents on the nitrogen of 3-amino pyrrolidine moiety. Herein, the enzymatic activities against PARP-1/2 and the chemical synthesis of these new quinazoline-2,4(1*H*,3*H*)-dione derivatives were described. The in vitro and in vivo anti-tumor activities of selected compounds were also evaluated. The co-crystal structure of compound **11** with PARP-1 was presented as well to elicit the action mode of the designed molecules.

2. Results and discussion

2.1 Chemical synthesis

The quinazoline-2,4(1*H*,3*H*)-dione derivatives were prepared according to the synthetic route as outlined in **Scheme 1** and their chemical structures were presented in **Tables 1-2**. Upon treatment of quinazoline-2,4(1*H*,3*H*)-dione with methyl 5-(bromomethyl)-2-fluorobenzoate or methyl 5-(bromomethyl)-2-chlorobenzoate and HMDS, the substituted benzyl groups were selectively introduced onto the *N*-1 position of quinazoline-2,4(1*H*,3*H*)-dione, giving rise to compounds **1** and **2** in 42% and 83% yields, respectively. In the presence of lithium hydroxide, compounds **1** and **2** were hydrolyzed into the corresponding carboxylic acids **3** and **4** in 92% and 62% yields, respectively, which were the key intermediates for the preparation of various designed target molecules. The target molecules **5-15** were prepared by direct coupling of compounds **3** and **4** with 1-substituted pyrrolidin-3-amines in moderate to good yields, while target compounds **18-26** were synthesized in two steps starting from compounds **14** and **15** including removal of Boc group

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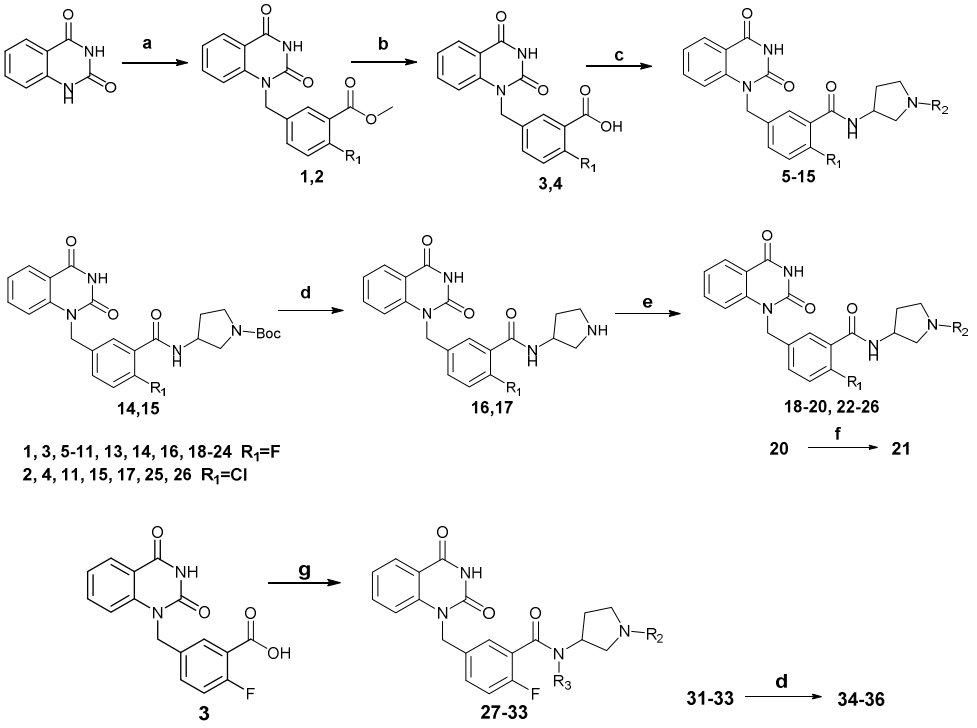
and acylation or alkylation of pyrrolidine ring. The amide nitrogen substituted derivatives **27-33** were prepared by reaction of compound **3** with *N*,1-disubstituted pyrrolidine-3-amines in 26-63% yield. The Boc group was removed from compounds **31-33** giving rise to compounds **34-36** in the presence of TFA.



Scheme 1. Reagents and conditions: a) HMDS, conc. H_2SO_4, toluene, reflux; substituted bromomethylbenzene, 130 °C; MeOH, dioxane, 70 °C; b) LiOH, THF, MeOH, H_2O, r.t.; c) *N*-substitued 3-amino pyrrolidine, EDC, HOBt, Et_3N, DMF, r.t. or HBTU, HOBt, DIEA, DMF, r.t. or HATU, HOBt, DIEA, DMF, r.t.; d) TFA, DCM, rt; e) Acyl chlorides, DCM, Et_3N, or aldehydes or ketones, DIEA, THF, then $NaBH(OAc)_3$; (f) 2N HCl, acetone, r.t.; g) *N*,1-disubstitued pyrrolidin-3-amine, EDC, HOBt, Et_3N, DMF, r.t.

2.2 Enzymatic activity against PARP-1 and PARP-2

The inhibitory activities against PARP-1 and PARP-2 of all target compounds (**5-36**) were evaluated. AZD2281 was used as the reference molecule. The corresponding results were expressed as IC_{50} values and presented in **Tables 1-2**.

Initially, we incorporated a number of alky groups on the *N*-1 position of pyrrolidine ring to explore the structure-activity relationships. Compounds **5-11** bearing ethyl, propyl, butyl, *iso*-butyl, *iso*-pentyl, pentan-3-yl and cyclopropyl methyl group strongly inhibited PARP-1 enzymatic activity with IC_{50} values ranging from 3.8 nM to 15.1 nM, which were comparable with that of non-substituted compound **16** (IC_{50} = 5.9 nM). Noticeably, compounds **6-11** showed preferable binding to PARP-1 than PARP-2, giving a selectivity of 5-16 folds, while compounds **5** and **16** displayed little preference. These data suggested that bulky alky groups were tolerated on the *N*-1 position of pyrrolidine ring and even more favorable for PARP-1 binding in comparison with PARP-2 binding. In contrast, the placement of cycloalkyl groups such as difluorocyclohexyl and cyclohexanone on the *N*-1 position of pyrrolidine ring led to pronounced reduction in inhibitory activity toward both PARP-1 and PARP-2 (compounds **13** and **19-21**). Presumably, the increased rigidity and the shape of these cyclo fragments are not appropriate for binding in AD pocket of PARP-1/2. While compounds **22-24** with benzyl and substituted benzyl groups on the *N*-1 position exhibited significant inhibition effects on PARP-1 and favorable binding for PARP-1 than PARP-2. Together with alky substituted compounds, these compounds further demonstrated that a large hydrophobic binding pocket was existed in the AD site and a variety of moieties could be introduced to improve the potency and physicalchemical properties.

Substitution on the nitrogen with Boc and cyclopropanecarbonyl fragments resulted in compounds **14** and **18**, which possessed comparable activity against both PARP-1 and PARP-2 with IC_{50} values at double digit nanomolar level. Compared with compound **11**, compound **18** with an acyl group showed over 2-fold reduction in potency toward PARP-1, suggesting that alkylation on the nitrogen was a little more beneficial to the PARP-1 binding affinity than acylation. In addition, we replaced the fluoro group on the benzyl linker with a chloro atom to give rise to compounds **12**, **15**,

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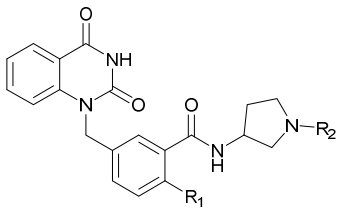
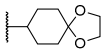
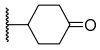
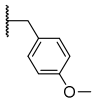
17, **25** and **26**. In comparison with the corresponding fluoro substituted counterparts, these chloro substituted compounds exhibited markedly decreased potency toward PARP-1, while this decrease was not so pronounced toward PARP-2. Therefore, grafting a fluoro atom on the benzyl linker is essential in terms of PARP-1 binding as demonstrated in the literatures [11, 29]. These results also indicated that the binding features of the benzyl linker were somewhat different in the binding site of PARP-1 and PARP-2. And this distinct feature might be used to design iso-form selective inhibitors.

Table 1

The chemical structures and inhibitory activities against PARP-1 and PARP-2 of compounds **5-26**[a]



Compd.	R_1	R_2	PARP-1 IC_{50}(nM)[b]± SD[c]	PARP-2 IC_{50}(nM)[b]± SD[c]	Compd.	R_1	R_2	PARP-1 IC_{50}(nM)[b]± SD[c]	PARP-2 IC_{50}(nM)[b]± SD[c]
5	F		15.1±1.8	15.5±0.9	**16**	F	H	5.9±0.5	15.2±1.2
6	F		6.9±0.8	40.1±3.6	**17**	Cl	H	>100	39.0±2.1
7	F		5.8±0.9	>100	**18**	F		31.5±4.5	40.1±3.1
8	F		3.8±0.6	17.2±4.1	**19**	F		21.8±1.6	90.1±11.2
9	F		5.7±0.8	>100	**20**	F		51.0±11.1	46.4±5.1
10	F		14.2±1.6	>100	**21**	F		>100	43.0±9.9
11	F		13.3±1.4	67.8±10.4	**22**	F		16.0±1.8	28.5±2.1
12	Cl		38.1±3.2	>100	**23**	F		4.7±0.6	28.0±0..6
13	F		>100	45.8±5.7	**24**	F		7.1±1.0	17.1±3.2
14	F		18.7±3.1	35.0±8.1	**25**	Cl		92.5±9.8	>100
15	Cl		>100	>100	**26**	Cl		98.2±11.2	65.2±10.3

[a] The ability of compounds to inhibit PARP-1 enzyme activity were tested using ELISA method and AZD2281 was used as a positive control. The measured IC_{50} against PARP-1 was 2.09 nM, the measured IC_{50} against PARP-2 was 2.26 nM .

[b] Concentrations for 50% inhibition in PARP-1 enzyme assay (IC_{50}).

[c] Standard Deviation

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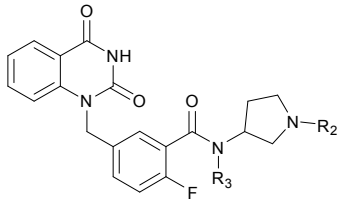
The effects of substitution on the amide nitrogen with R_3 group were also investigated primarily (**Table 2**). Generally, grafting a methyl or an ethyl group onto the nitrogen was tolerated and produced comparable activity with the non-substituted counterparts, while placement of a propyl group greatly deteriorated activity toward PARP-1 and PARP-2 with IC_{50} values of >100 nM, as exemplified by compounds **30**, **33** and **36**, regardless of the substituents on the nitrogen of pyrrolindine ring. It is evidently that bulky alkyl groups were not tolerated to this amide position.

Table 2

The chemical structures and inhibitory activities against PARP-1 and PARP-2 of compounds **27-36**[a]



Compd.	R_2	R_3	PARP-1 IC_{50}(nM)[b] ±SD[c]	PARP-2 IC_{50}(nM)[b] ±SD[c]	Compd.	R_2	R_3	PARP-1 IC_{50}(nM)[b] ±SD[c]	PARP-2 IC_{50}(nM)[b] ±SD[c]
27		CH_3	6.4±0.1	87.5±8.8	**32**			38.8±3.3	30.2±1.3
28		CH_3	32.6±5.6	14.5±2.2	**33**			91.5±14.3	>100
29			67.2±9.3	28.1±2.3	**34**	H	CH_3	5.2±0.8	53.6±4.0
30			>100	>100	**35**	H		4.6±0.6	63.0±6.7
31		CH_3	8.8±3.0	43.8±6.3	**36**	H		>100	>100

[a] The ability of compounds to inhibit PARP-1 enzyme activity were tested using ELISA method and AZD2281 was used as a positive control. The measured IC_{50} against PARP-1 was 2.09 nM, the measured IC_{50} against PARP-2 was 2.26 nM .

[b] Concentrations for 50% inhibition in PARP-1 enzyme assay (IC_{50}).

[c] Standard Deviation

2.3 Cellular potency

The cellular potency of 12 compounds, which acted as highly potent PARP-1 inhibitors with IC_{50} values of 3.8-15.1 nM, was evaluated for their cytotoxicities as single agents and their potentiation effects on TMZ-induced cytotoxicity in MX-1 breast cancer cells with BRCA1 deficiency [15]. As presented in **Table 3**, among the tested derivatives, compounds **10** and **11** demonstrated strong cytotoxities with IC_{50} values of < 3.12 μM and 3.02 μM, respectively, and other compounds had relatively weak cytotoxicities with IC_{50} values >10 μM. The potentiation effects on the DNA damaging agent temozolomide (TMZ) were evaluated at 10 μM concentration using MX-1 cell lines. Compounds **9-11** could strongly potentiate TMZ cytotoxicities in comparison with other tested compounds, the cytotoxicity of TMZ was increased to more than 10 fold. Of note, although all of the tested compounds possessed strong enzymatic inhibition, most of them did not show potent cellular activity. In particular, compound 16 without substituents on both nitrogens showed poor cytotoxicity. Therefore, these data demonstrated that variation of fragments on the nitrogen of pyrrolidine ring would be

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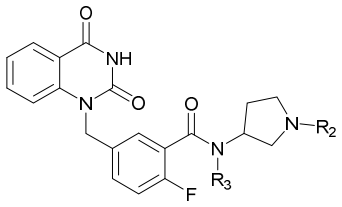
facilitated to improve the cellular activity, presumably, due to the improvement of physicochemical properties of this series of inhibitors.

Table 3

Cytotoxicities and potentiation effects (PF_{50}) of selected compounds on MX-1 cells



Compd.	R_2	R_3	IC_{50} (μM)[a]	PF_{50}[b]	Compd.	R_2	R_3	IC_{50} (μM)[a]	PF_{50}
5		H	91.7	1.3	**11**		H	3.02	~10
6		H	42.2	1.1	**16**	H	H	>100	ND[c]
7		H	52.6	1.3	**27**		CH_3	58.8	2.5
8		H	61.3	1.5	**31**		CH_3	27.6	5.3
9		H	13.1	>10	**34**	H	CH_3	41.5	ND
10		H	<3.12	>10	**35**	H		92.3	ND

[a] Cytotoxicity (IC_{50}): the concentration required to reduce cell proliferation and growth by 50% in single-agent cytotoxicity assay. AZD2281 was used as a positive control and its IC_{50} value was 8.26 μM.

[b] Potentiation factor (PF_{50}): The fold of potentiation was calculated as the ratio of the IC_{50} for TMZ divided by the IC_{50} of TMZ + PARP-1 inhibitor, the test compounds were used at a fixed concentration of 10 μM.

[c] ND: Not Determined.

2.4 In vivo antitumor activity of compound 11

As compound **11** showed pronounced potentiation effect on TMZ-induced cytotoxicity and had potent cytotoxicity (IC_{50} = 3.02 μM) as a single agent in MX-1 cells, we further evaluated its anti-cancer activity as a sensitizer of TMZ using MX-1 xenograft tumor model. The results were shown in **Table 4**. When TMZ (50 mg/kg) was administered orally to BalB/c nude mice bearing MX-1 xenograft tumors, 62% tumor growth inhibition was observed. In contrast, only 13% inhibition was produced when compound **11** was administered at the same dose. Remarkably, when compound **11** was used with TMZ in combination, both dosing at 50 mg/kg, the tumors weight decreased markedly and 91% tumor growth inhibition was achieved. When TMZ treated group was used as the control, the combination dosing still produced 76% inhibition effect. It was demonstrated that compound **11** was an effective chemo-sensitizing agent.

Table 4

In vivo tumor growth inhibition of compound **11**

Group	Dose (mg/kg×times)	Number (Start/End)	Weight Change (g)	Tumor Weight (g±SE)	% T/C_{veh} (%TGI)	%T/C_{TMZ} (%TGI)
Vehicle		6/6	5.32	4.09±0.64	NA	NA
TMZ	50**×5**	6/6	0.02	1.57±0.64	38 (62)	NA
Compd. 11 (50)	50**×5**	6/6	4.38	3.57±0.90	87 (13)	NA

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Compd. 11 (25)/TMZ	25×**5**/50×**5**	6/6	-0.78	0.50±0.14	12 (88)	32 (68)
Compd. 11 (50)/TMZ	50×**5**/50×**5**	6/6	0.10	0.38±0.13	9 (91)	24 (76)

NA: not applicable.

2.5. The crystal structure of compound 11 complexed with PARP-1

In order to explore the binding feature of the designed quinazoline-2,4(1*H*,3*H*)-dione derivatives, we obtained and analyzed the co-crystal structure of compound **11** within the catalytic site of PARP-1. As shown in **Figure 1**, as we expected, the quinazolinone scaffold occupied the NI-site and interacted with Ser904, Gly863 and Tyr907 through characteristic hydrogen bonds and π-π stacking interaction. Interestingly, the 2-fluorobenzamide linker formed a π-π interaction with Tyr896 and a hydrogen bond with the backbone of Tyr896. These binding interactions presumably made positive contributions greatly to the binding affinity. The nitrogen on the pyrrolidine ring formed an H-bond with the carbonyl group of Arg878, suggesting that the basic nitrogen was beneficial to the binding interaction. The cyclopropylmethyl group on the pyrrolidine ring was nicely inserted into a deep hydrophobic pocket lined with the side chain of Asp766, Leu769, Asp770 and Arg878 within AD site. This suggested that variation of the size and shape of substituents on the pyrrolidine ring could further increase the binding affinity of this series of inhibitors with PARP-1, and therefore leading to potent PARP-1 selective inhibitors which are pursued by many research groups [31,32].

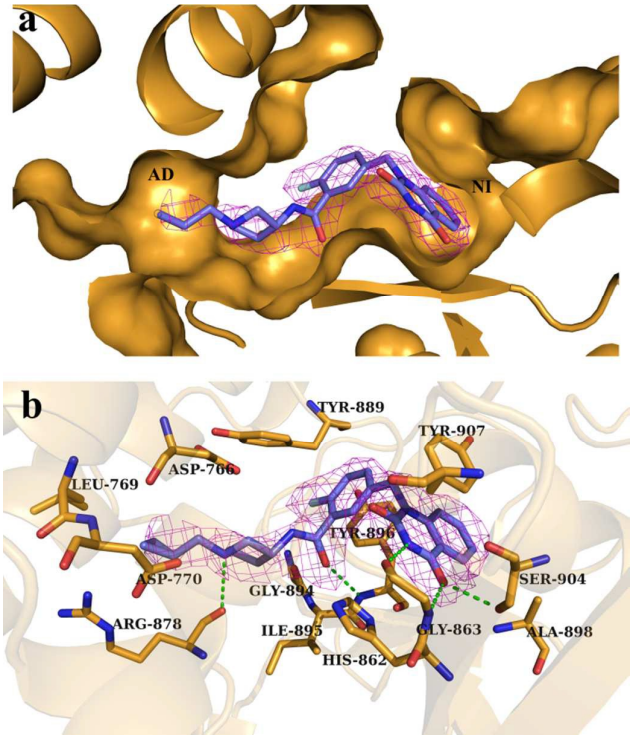

Figure 1. The co-crystal structure of PARP-1 in complex with 11. Protein and ligand are colored in tan and sky blue, respectively. Hydrogen bonds are represented with green lines. The $2F_o–F_c$ electron density map (contoured at 1σ) around the inhibitor is shown as pink mesh (PDB: 5WRY). The image was generated with Pymol [33].

3. Conclusions

In summary, a series of novel quinazoline-2,4(1*H*,3*H*)-dione derivatives were designed and synthesized as PARP-1/2 inhibitors based on the characteristics of the catalytic domain in PARP-1. The SARs were conducted by varying the substituents on the nitrogen of pyrrolidine ring and on the amide nitrogen. The enzymatic inhibition results demonstrated that various alkyl and aromatic hydrophobic groups were well tolerated on the ring nitrogen and bulky alkyl substituents on the amide nitrogen were not tolerated at all. The bulky hydrophobic side chain on the ring nitrogen improved the physicochemical properties and resulted in potent compounds **9-11** in cytotoxicity assay. Furthermore, compound **11** demonstrated high efficacy as a sensitizer of TMZ in MX-1 xenograft tumor model, suggesting that these quinazoline-2,4(1*H*,3*H*)-dione based PARP-1/2 inhibitors which contained 1-substituted 3-amino pyrrolidine moiety could be developed into useful anti-tumor agents. The co-crystal structure of compound **11** located in the catalytic domain of PARP-1 was solved. It has been shown that quinazoline-2,4(1*H*,3*H*)-dione scaffold was situated in the NI site and the benzyl spacer greatly contributed to the binding affinity by forming H-bond and π-π interaction. More importantly, the hydrophobic side chain on the pyrrolidine ring could extend into a deep hydrophobic pocket formed by Asp766, Leu769 and Arg878 and contributed to the binding affinity by Van der Waals interaction. And this distinct binding feature could be used to develop PARP-1 selective inhibitors.

4. Experimental Section

4.1 Chemical Synthesis General

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All reactions involving air- or moisture-sensitive reagents were performed under an argon atmosphere. Melting points were measured on a Yanaco micro melting point apparatus and are uncorrected. 1H NMR (300 MHz and 400 MHz) on a Varian Mercury spectrometer was recorded in DMSO-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

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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).

4.2 Synthesis of compounds

Methyl 5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorobenzoate (1)

To a suspension of quinazoline-2,4(1*H*,3*H*)-dione (411 mg, 2.54 mmol) in toluene (4 mL) and hexamethyldisilazane (HMDS; 819 mg, 5.07 mmol), four drops of sulfuric acid were added with caution. The mixture was heated to reflux and stirred under refluxing for 8 h until clear solution was obtained. After the removal of toluene and excess HMDS under vacuum distillation, methyl 5-(bromomethyl)-2-fluorobenzoate (938 mg, 3.80 mmol) was added to the residue. The reaction mixture was heated to 140 °C and was stirred at this temperature for 3 h, the reaction mixture was diluted with 1,4-dioxane (3 mL) at 100 °C, and then methanol (2 mL) was added at 70 °C for 30 min. The suspension was cooled below 5 °C and precipitates were collected by filtration. After washing with methanol (5 mL) and water (5 mL) the crude product was dried under vacuum condition to afford compound **1** as a white solid (413 mg, 41.6%); m.p. 212-214°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm) : 11.47 (brs, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.86 (d, *J* = 6.0 Hz, 1H), 7.66 (t, *J* = 7.6 Hz, 1H), 7.58-7.61 (m, 1H), 7.23-7.34 (m, 3H), 5.34 (s, 2H), 3.83 (s, 3H); HR-MS (ESI): m/z, calcd. for $C_{17}H_{14}N_2O_4F$ $[M+H]^+$ 329.0932, Found: 329.0929.

Methyl 2-chloro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)benzoate (2)

Following the preparation protocol of compound **1**, starting from methyl 5-(bromomethyl)-2-chlorobenzoate, the title compound **2** was obtained as a white solid (2.3 g, 83.3%); m.p.238-240 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 8.03 (dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz, 1H), 7.78 (d, *J* = 2.0 Hz, 1H), 7.66 (td, J_1 = 9.0 Hz, J_2 = 1.5 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 1H), 7.48 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H), 7.26 (t, *J* = 7.0 Hz, 2H), 5.35 (s, 2H), 3.84 (s, 3H); HR-MS (ESI): m/z, calcd. for $C_{17}H_{14}N_2O_4Cl$ $[M+H]^+$ 345.0637, Found: 345.0633.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) -2-fluorobenzoic acid (3)

To a solution of methyl 2-chloro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)benzoate (200 mg, 0.61 mmol) in H_2O (2 mL), THF (2 mL) and MeOH (4 mL), LiOH (82 mg, 1.94 mmol) was added, and the mixture was stirred at 55°C for 55 min. After the removal of solvent under vacuum distillation, H_2O (10 mL) was added to the residue. The resulting mixture was washed with ethyl acetate (5 mL), and the pH of aqueous layer was adjusted to 2 with aq. HCl (2N). After filtration, the title compound **3** was obtained as a white solid (176 mg, 91.9%); m.p.>250℃; ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm) : 13.31 (s, 1H), 11.76 (s, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.82 (d, *J* = 5.6 Hz, 1H), 7.66 (t, *J* = 7.6 Hz, 1H), 7.55-7.57 (m, 1H), 7.23-7.31 (m, 3H), 5.33 (s, 2H); HR-MS (ESI): m/z, calcd. for $C_{16}H_{12}N_2O_4F$ $[M+H]^+$ 315.0776, Found: 315.0768.

2-Chloro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)benzoic acid (4)

Following the preparation protocol of compound **3**, starting from methyl 2-chloro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)benzoate (1.5 g, 4.35 mmol), the title compound **4** was obtained as a yellow solid (900 mg, 62.5%); m.p.>250 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 13.51 (brs, 1H), 11.76 (s, 1H), 8.04 (d, *J* = 7.5 Hz, 1H), 7.76 (d, *J* = 2.0 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 1H), 7.44 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H), 7.24-7.28 (m, 2H), 5.34 (s, 2H); HR-MS (ESI): m/z, calcd. for $C_{16}H_{12}N_2O_4Cl$ $[M+H]^+$ 331.0480, Found: 331.0476.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) -*N*-(1-ethylpyrrolidin-3-yl)-2-fluorobenzamide (5)

A mixture of 5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorobenzoic acid (120 mg, 0.38 mmol), HBTU (286 mg, 0.76 mmol), HOBt (103 mg, 0.76 mmol), DIEA (0.33 mL, 1.9 mmol) and 1-ethylpyrrolidin-3-amine 2,2,2-trifluoroacetate (260 mg, 0.76 mmol) in DMF (15 mL) was stirred at room temperature for 20 h and then H_2O was added to the mixture. The solution was extracted with the solvent mixture (methylene chloride/methanol = 10:1) (50 mL × 3) and then the organic layer was washed with brine (20 mL). The combined organic layer was dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was obtained and purified with column chromatography (methylene chloride /methanol/Et_3N = 50:1:0.3) to give compound **5** as a white solid (29 mg, 18.7%); m.p. 152-154℃; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.19 (d, *J* = 7.6 Hz, 1H), 7.97 (d, *J* = 6.8 Hz, 1H), 7.70 (brs, 1H), 7.59 (t, *J* = 8.4 Hz, 1H), 7.21-7.30 (m, 2H), 7.10 (d, *J* = 8.8 Hz, 1H), 7.04 (t, *J* = 8.8 Hz, 1H), 5.30 (s, 2H), 4.83 (br s, 1H), 3.37 (brs, 1H), 3.20 (brs, 1H), 2.73-2.85 (m, 3H), 2.60 (brs, 1H), 2.51 (brs, 1H), 2.04 (brs, 1H), 1.28 (t, *J* = 7.2 Hz, 3H); HR-MS (ESI): m/z, calcd. for $C_{22}H_{24}N_4O_3F$ $[M+H]^+$ 411.1827, Found: 411.1832.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) -2-fluoro-*N*-(1-propylpyrrolidin-3-yl)benzamide (6)

Following the preparation protocol of compound **5**, starting from compound 3 and 1-propylpyrrolidin-3-amine 2,2,2-trifluoroacetate, the title compound **6** was obtained as a white solid (97 mg, 60.2%);

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m.p. 187-189 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.19 (d, J = 7.6 Hz, 1H), 7.98 (d, J = 6.8 Hz, 1H), 7.56 (t, J = 8.0 Hz, 1H), 7.43 (t, J = 8.0 Hz, 1H),7.19-7.26 (m, 2H), 7.05 (d, J = 8.4 Hz, 1H), 6.99 (t, J = 9.2 Hz, 1H), 5.27 (s, 2H), 4.68 (s, 1H), 3.05-3.15 (m, 1H), 2.90 (d, J = 10.0 Hz, 1H), 2.55-2.70 (m, 2H), 2.30-2.45 (m, 3H), 1.77 (brs, 1H), 1.53-1.61 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 163.29, 161.81, 158.13 (d, J = 246.5 Hz), 150.73, 140.69, 135.25, 132.62 (d, J = 2.9 Hz), 130.19 (d, J = 8.3 Hz), 128.17 (d, J = 2.9 Hz), 127.68, 124.58 (d, J = 15.3 Hz), 122.81, 116.31 (d, J = 22.6 Hz), 115.99, 115.021, 59.67, 57.28, 52.55, 48.55, 44.35, 30.73, 21.10, 11.84;HR-MS (ESI): m/z, calcd. for $C_{23}H_{26}N_4O_3F$ $[M+H]^+$ 425.1984, Found: 425.1985.

_N_-(1-Butylpyrrolidin-3-yl)-5-((2,4-dioxo-3,4-dihydro quinazolin-1(2_H_)-yl)methyl)-2-fluorobenzamide (7)

Following the preparation protocol of compound **5**, starting from compound **3** and 1-butylpyrrolidin-3-amine 2,2,2-trifluoroacetate , the title compound **7** was obtained as a white solid (160 mg, 83%); m.p. 134-136 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 8.55 (s, 1H), 8.03 (d, J = 7.6 Hz, 1H), 7.66 (t, J = 7.2 Hz, 1H), 7.54-7.56 (m, 1H), 7.43-7.47 (m, 1H), 7.22-7.29 (m, 3H), 5.31 (s, 2H), 4.41 (s, 1H), 3.28 (s, 1H), 2.71-3.07 (m, 5H), 2.20 (brs, 1H), 1.81 (brs, 1H), 1.45-1.55 (m, 2H), 1.26-1.35 (m, 2H), 0.89 (t, J = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 163.39, 161.77, 158.13 (d, J = 246.7 Hz), 150.69, 140.66, 135.23, 132.69 (d, J = 3.1 Hz), 130.45 (d, J = 8.4 Hz), 128.16 (d, J = 2.9 Hz), 127.65, 124.18 (d, J = 15.0 Hz), 122.78, 116.35 (d, J = 22.7 Hz), 115.96, 114.97, 58.69, 54.69, 52.40, 48.24, 44.34, 30.18, 28.74, 19.73, 13.70; HR-MS (ESI): m/z, calcd. for $C_{24}H_{28}N_4O_3F$ $[M+H]^+$ 439.2140, Found: 439.2154.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl) -2-fluoro-_N_-(1-isobutylpyrrolidin-3-yl)benzamide (8)

Following the preparation protocol of compound **5**, starting from compound **3** and 1-isobutylpyrrolidin-3-amine 2,2,2-trifluoroacetate, the title compound **8** was obtained as a white solid (67 mg, 40.3%); m.p. 174-176 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.20 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 6.4 Hz, 1H), 7.58 (t, J = 7.2 Hz, 1H), 7.20-7.35 (m, 2H), 7.10 (d, J = 8.4 Hz, 1H), 7.04 (t, J = 8.8 Hz, 1H), 5.32 (s, 2H), 4.73 (s, 1H), 3.10 (brs, 1H), 2.93 (brs, 1H), 2.68 (brs, 1H), 2.39 (brs, 4H), 1.83 (brs, 2H), 0.96 (d, J = 6.4 Hz, 6H); HR-MS (ESI): m/z, calcd. for $C_{24}H_{28}N_4O_3F$ $[M+H]^+$ 439.2140, Found: 439.2156.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl) -2-fluoro-_N_-(1-isopentylpyrrolidin-3-yl)benzamide (9)

A mixture of 2-fluoro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl)benzoic acid (120 mg, 0.38 mmol), EDC (146 mg, 0.76 mmol), HOBt (103 mg, 0.76 mmol), DIEA (0.33 mL, 1.90 mmol) and 1- _iso_-pentylpyrrolidin-3-amine 2,2,2-trifluoroacetate (189 mg, 0.57 mmol) in DMF (10 mL) was stirred at room temperature for 24 h and then H_2O was added to the mixture. The solution was extracted with the solvent mixture (methylene chloride/methanol = 10:1) (30 mL × 3) and then the organic layer was washed with brine (20 mL × 2). The combined organic layer was dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was obtained and purified with column chromatography (methylene chloride /methanol = 40:1 to 20:1) to give compound **9** as a white solid (90 mg, 52.6%); m.p. 175-177 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.19 (d, J = 7.6 Hz, 1H), 8.00 (d, J = 6.0 Hz, 1H), 7.57 (t, J = 7.2 Hz, 1H), 7.42 (brs, 1H), 7.21-7.27 (m, 2H), 7.06 (d, J = 8.4 Hz, 1H), 7.01 (t, J = 9.2 Hz, 1H), 5.29 (s, 2H), 4.71 (s, 1H), 3.10 (brs, 1H), 2.92 (brs, 1H), 2.65 (brs, 2H), 2.53-2.70 (m, 3H), 1.80 (brs, 1H), 1.52-1.62 (m, 1H), 1.42-1.46 (m, 2H), 0.90 (t, J = 6.4 Hz, 6H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 163.28, 161.81, 158.12 (d, J = 246.5 Hz), 150.73, 140.69, 135.25, 132.62 (d, J = 2.9 Hz), 130.18 (d, J = 8.5 Hz), 128.17 (d, J = 2.8 Hz), 127.67, 124.57 (d, J = 15.5 Hz), 122.80, 116.31 (d, J = 22.9 Hz), 115.98, 115.02, 59.75, 53.57, 52.61, 48.54, 44.35, 36.81, 30.74, 25.76, 22.57; HR-MS (ESI): m/z, calcd. for $C_{25}H_{30}N_4O_3F$ $[M+H]^+$ 453.2296, Found: 453.2294.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl) -2-fluoro-_N_-(1-(pentan-3-yl)pyrrolidin-3-yl)benzamide (10)

Following the preparation protocol of compound **9**, starting from compound **3** and 1-(pentan-3-yl)pyrrolidin-3-amine 2,2,2-trifluoroacetate, the title compound **10** was obtained as a white solid (50 mg, 29.2%); m.p. 143-145 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 12.02 (s, 1H), 8.88 (s, 0.4H), 8.77 (s, 0.6H), 8.18 (d, J = 7.6 Hz, 1H), 7.93 (d, J = 6.0 Hz, 1H), 7.67 (t, J = 7.2 Hz, 1H), 7.33 (brs, 1H), 7.23-7.26 (m, 2H), 7.07 (t, J = 9.6 Hz, 1H), 5.33 (s, 2H), 5.13 (s, 1H), 3.96 (brs, 1H), 3.73 (brs, 1H), 3.24 (brs, 1H), 2.94 (brs, 2H), 2.55 (s, 1H), 2.49 (s, 1H), 1.87 (brs, 4H), 1.09 (d, J = 5.2 Hz, 6H); HR-MS (ESI): m/z, calcd. for $C_{25}H_{30}N_4O_3F$ $[M+H]^+$ 453.2296, Found: 453.2297.

_N_-(1-(Cyclopropylmethyl)pyrrolidin-3-yl)-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl)-2-fluorobenzamide (11)

Following the preparation protocol of compound **9**, starting from compound **3** and 1-(cyclopropylmethyl)pyrrolidin-3-amine 2,2,2-trifluoroacetate, the title compound **11** was obtained as a white solid (40 mg, 24.0%); m.p. 113-115 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.19 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 6.8 Hz, 1H), 7.50-7.57 (m, 2H), 7.23 (t, J = 7.6 Hz, 1H), 7.15-7.17 (m, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.96 (t, J = 8.8 Hz, 1H), 5.26 (s, 2H), 4.71 (brs, 1H), 3.11-3.15 (m, 1H), , 3.01 (d, J = 10.0 Hz, 1H), 2.68-2.77 (m, 2H), 2.54-2.59 (m, 1H), 2.27-2.45 (m, 3H), 1.75-1.78 (m, 1H), 0.90-0.95 (m, 1H), 0.50-0.53 (m, 2H), 0.14-0.15(m, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 163.29, 161.76, 158.11 (d, J = 246.7 Hz), 150.69, 140.66, 135.21,

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132.59 (d, *J* = 3.0 Hz), 130.18 (d, *J* = 8.6 Hz), 128.16 (d, *J* = 2.7 Hz), 127.64, 124.50 (d, *J* = 15.4 Hz), 122.77, 116.28 (d, *J* = 22.6 Hz), 115.96, 114.98, 59.88, 59.54, 52.52, 48.48, 44.33, 30.63, 9.22, 3.65; HR-MS (ESI): m/z, calcd. for $C_{24}H_{26}N_4O_3F$ $[M+H]^+$ 437.1983, Found: 437.1976.

2-Chloro-*N*-(1-(cyclopropylmethyl)pyrrolidin-3-yl)-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) benzamide (12)

Following the preparation protocol of compound **9**, starting from compound **4** and 1-(cyclopropylmethyl)pyrrolidin-3-amine 2,2,2-trifluoroacetate, the title compound **12** was obtained as a light yellow solid (92 mg, 61.3%); m.p. 105-107 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.25 (brs, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 7.50-7.53 (m, 2H), 7.19-7.24 (m, 2H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 9.0 Hz, 1H), 5.19 (d, *J* = 17.0 Hz, 1H), 4.92 (d, *J* = 16.0 Hz, 1H), 4.84 (brs, 1H), 3.38-3.44 (m, 2H), 2.94 (dd, J_1 = 12.5 Hz, J_2 = 6.5 Hz, 1H), 2.68 (t, *J* = 9.0 Hz, 1H), 2.66-2.71 (m, 1H), 2.48-2.55 (m, 1H), 2.36-2.39 (m, 1H), 2.29-2.33 (m, 1H), 1.88-1.90 (m, 1H), 0.97-1.02 (m, 1H), 0.52-0.61 (m, 2H), 0.18-0.23 (m, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 165.78, 161.77, 150.69, 140.66, 137.11, 135.62, 135.22, 129.69, 128.69, 128.54, 127.64, 127.07, 122.78, 115.99, 114.96, 59.87, 59.44, 52.52, 48.38, 44.42, 30.50, 9.22, 3.70, 3.62; HR-MS (ESI): m/z, calcd. for $C_{24}H_{26}N_4O_3Cl$ $[M+H]^+$ 453.1688, Found: 453.1675.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) -2-fluoro-*N*-(1-(oxetan-3-yl)pyrrolidin-3-yl)benzamide (13)

Following the preparation protocol of compound **9**, starting from compound **3** and 1-(oxetan-3-yl)pyrrolidin-3-amine 2,2,2-trifluoroacetate, the title compound **13** was obtained as a light yellow solid (25 mg, 41.6%); m.p. 117-119 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 9.02 (s, 1H), 8.21 (d, *J* = 7.6 Hz, 1H), 8.05 (d, *J* = 7.2 Hz, 1H), 7.57 (t, *J* = 7.2 Hz, 1H), 7.34-7.38 (m, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 7.03-7.10 (m, 3H), 5.33 (s, 2H), 4.61-4.73 (m, 5H), 3.65-3.72 (m, 1H), 2.90-2.98 (m, 1H), 2.63-2.75 (m, 2H), 2.32-2.43 (m, 2H), 1.75-1,80 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 163.36, 161.81, 158.10 (d, *J* = 246.5 Hz), 150.73, 140.69, 135.25, 132.63 (d, *J* = 2.8 Hz), 130.16 (d, *J* = 8.8 Hz), 128.14 (d, *J* = 2.8 Hz), 127.68, 124.67 (d, *J* = 15.6 Hz), 122.81, 116.30 (d, *J* = 22.5 Hz), 115.99, 115.02, 74.64, 57.11, 56.26, 48.99, 48.63, 44.35, 30.81; HR-MS (ESI): m/z, calcd. for $C_{23}H_{24}N_4O_4F$ $[M+H]^+$ 439.1776, Found: 439.1761.

***tert*-Butyl 3-(5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorobenzamido) pyrrolidine -1-carboxylate (14)**

A mixture of 5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorobenzoic acid (200 mg, 0.64 mmol), HATU (487 mg, 1.28 mmol), HOBt (173 mg, 1.28 mmol), DIEA (0.22 mL, 1.28 mmol) and *N*-Boc-3-aminopyrrolidine (178 mg, 0.96 mmol) in DMF (15 mL) was stirred at room temperature for 20 h and then H_2O was added to the mixture. The solution was extracted with the solvent mixture (ethyl acetate/methanol = 10:1) (30 mL × 3) and then the organic layer was washed with brine (20 mL × 2). The combined organic layer was dried over anhydrous $MgSO_4$. After filtration and concentration, the crude product was obtained and purified with column chromatography to give compound **14** as a white solid (189 mg, 61.7%); m.p. 171-173°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.73 (s, 1H), 8.57 (d, *J* = 6.4 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.66 (t, *J* = 7.2 Hz, 1H), 7.54 (d, *J* = 5.6 Hz, 1H), 7.42 (brs, 1H), 7.20-7.29 (m, 3H), 5.31 (s, 2H), 4.34-4.39 (m, 1H), 3.45-3.55 (m, 1H), 3.25-3.39 (m, 2H), 3.12-3.19 (m, 1H), 2.02-2.11 (m, 1H), 1.78-1.87 (m, 1H), 1.39 (s, 9H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 163.66, 161.75, 158.03 (d, *J* = 246.7 Hz), 153.46, 150.69, 140.66, 135.20, 132.63 (d, *J* = 3.1 Hz), 130.18 (d, *J* = 7.9 Hz), 128.14, 127.65, 124.57 (d, *J* = 15.8 Hz), 122.76, 116.28 (d, *J* = 22.5 Hz), 115.97, 114.96, 78.30, 50.82 (50.48), 49.40 (48.64), 44.32, 43.98 (43.72), 30.84 (29.86), 28.15; HR-MS (ESI): m/z, calcd. for $C_{25}H_{27}N_4O_5FNa$ $[M+Na]^+$ 505.1858, Found: 505.1853.

***tert*-Butyl 3-(2-chloro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)benzamido)**

pyrrolidine-1-carboxylate (15)

Following the preparation protocol of compound **9**, starting from compound **4** and *N*-Boc-3-aminopyrrolidine, the title compound **15** was obtained as a white solid(1.06 g, 85.5%); m.p. 197-199°C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 11.74 (s, 1H), 8.71 (brs, 1H), 8.03 (d, *J* = 7.0 Hz, 1H), 7.66 (t, *J* = 7.5 Hz, 1H), 7.42-7.44 (m, 2H), 7.33 (d, *J* = 8.5 Hz, 1H), 7.23-7.28 (m, 2H), 5.31 (s, 2H), 4.32-4.36 (m, 1H), 3.42-3.52 (m, 1H), 3.32-3.40 (m, 1H), 3.28 (brs, 1H), 3.15-3.24 (m, 1H), 2.01-2.11 (m, 1H), 1.84 (brs, 1H), 1.39 (s, 9H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 166.07, 161.82, 153.49, 150.72, 140.67, 137.01, 135.72, 135.28, 129.72, 128.70, 128.63, 127.66, 127.13, 122.83, 116.00, 115.04, 78.28, 50.92 (50.57), 49.39 (48.58), 44.42, 44.02 (43.74), 30.68 (29.68), 28.19; HR-MS (ESI): m/z, calcd. for $C_{25}H_{28}N_4O_5Cl$ $[M+H]^+$ 499.1743, Found: 499.1757.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) -2-fluoro-*N*-(pyrrolidin-3-yl)benzamide (16)

To a solution of compound **14** (500 mg, 1.04 mmol) in DCM (15.0 mL), TFA (2 mL, 31.3 mmol) was added and then the reaction mixture was stirred at room temperature for 2.5 h. After removal of the solvent under vacuum distillation, H_2O (10 mL) was added to the residue. The resulting mixture was washed with ethyl acetate (5 mL), and the pH of aqueous layer was adjusted to 8 with aqueous *ammonia*. The solution was extracted with the solvent mixture (methylene chloride/methanol = 10:1) (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$. After filtration and

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concentration, the title compound **16** was obtained as a white solid (380 mg, 96%); m.p. 170-172 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 8.31 (d, J = 7.2 Hz, 1H), 8.03 (dd, J_1 = 7.6 Hz, J_2 = 2.0 Hz, 1H), 7.63-7.68 (m, 1H), 7.54 (dd, J_1 = 6.8 Hz, J_2 = 2.0 Hz, 1H), 7.38-7.43 (m, 1H), 7.19-7.29 (m, 3H), 5.31 (s, 2H), 4.23-4.28 (m, 1H), 2.92-2.97 (m, 1H), 2.83-2.89 (m, 1H), 2.70-2.76 (m, 1H), 2.59-2.63 (m, 1H), 1.91-2.00 (m, 1H), 1.55-1.63 (m, 1H); HR-MS (ESI): m/z, calcd. for $C_{20}H_{20}N_4O_3F$ $[M+H]^+$ 383.1514, Found: 383.1508.

2-Chloro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-*N*-(pyrrolidin-3-yl)benzamide (17)

Following the preparation protocol of compound **16**, starting from compound **15**, the title compound **17** was obtained as a white solid (620 mg, 86.4%); m.p. 162-164 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 11.79 (brs, 1H), 8.90 (brs, 1H), 8.83 (d, J = 6.5 Hz, 1H), 8.06 (d, J = 7.5 Hz, 1H), 7.69 (t, J = 7.0 Hz, 1H), 7.45-7.52 (m, 2H), 7.41 (dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.27 (d, J = 8.5 Hz, 1H), 5.35 (s, 2H), 4.46-4.51 (m, 1H), 3.51 (dd, J_1 = 12.0 Hz, J_2 = 7.0 Hz, 1H), 3.24-3.36 (m, 3H), 3.12 (dd, J_1 = 12.0 Hz, J_2 = 5.5 Hz, 1H), 2.17-2.25 (m, 1H), 1.92-1.99 (m, 1H); HR-MS (ESI): m/z, calcd. for $C_{20}H_{20}N_4O_3Cl$ $[M+H]^+$ 399.1218, Found: 399.1212.

***N*-(1-(Cyclopropanecarbonyl)pyrrolidin-3-yl)-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorobenzamide (18)**

Following the preparation protocol of compound **14**, starting from compound **16** and cyclopropanecarboxylic acid, the title compound **18** was obtained as a light yellow solid (55 mg, 73.0%); m.p. 136-138 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 8.66 (d, J = 6.8 Hz, 0.5H), 8.60 (d, J = 6.8 Hz, 0.5H), 8.03 (d, J = 8.0 Hz, 1H), 7.66 (t, J = 8.0 Hz, 1H), 7.53-7.56 (m, 1H), 7.39-7.45 (m, 1H), 7.21-7.29 (m, 3H), 5.32 (s, 2H), 4.46-4.52 (m, 0.5H), 4.36-4.43 (m, 0.5H), 3.88-3.93 (m, 0.5H), 3.65-3.74 (m, 1H), 3.24-3.58 (m, 2.5H), 2.16-2.23 (m, 0.5H), 2.04-2.12 (m, 0.5H), 1.93-1.99 (m, 0.5H), 1.83-1.88 (m, 0.5H), 1.69-1.77 (m, 1H), 0.69-0.73 (m, 4H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 170.95 (170.87), 163.73 (163.71), 161.81, 158.05 (d, J = 246.6 Hz), 150.73, 140.69, 135.25, 132.69 (d, J = 3.9 Hz) (132.67 (d, J = 3.1 Hz)), 130.29 (d, J = 9.1 Hz) (130.20 (d, J = 9.4 Hz)), 128.21 (d, J = 2.3 Hz) (128.12 (d, J = 2.8 Hz)), 127.68, 124.63 (d, J = 16.4 Hz) (124.58 (d, J = 15.8 Hz)), 122.81, 116.34 (d, J = 22.3 Hz) (116.32 (d, J = 21.9 Hz)), 116.00, 115.01, 51.15 (50.59), 49.69 (48.19), 44.34 (44.32), 43.78, 31.16 (29.43), 12.03 (11.72), 7.10 (7.00), 6.98; HR-MS (ESI): m/z, calcd. for $C_{24}H_{24}N_4O_4F$ $[M+H]^+$ 451.1776, Found: 451.1796.

***N*-(1-(4,4-Difluorocyclohexyl)pyrrolidin-3-yl)-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorobenzamide (19)**

To a solution of **16** (100 mg, 0.40 mmol) in dichloromethane (4.0 mL) and methanol (1 mL), 4,4-difluorocyclohexan-1-one (110 mg, 0.78 mmol) and sodium acetate (87 mg, 1.04 mmol) were added. The resulting solution was stirred at 37 °C for 2.5 h. Then sodium cyanoborohydride (52 mg, 0.78 mmol) was added and the mixture was stirred for 2 d. The dichloromethane (40 mL) and methanol (4 mL) were added and washed with saturated aqueous sodium bicarbonate (10 mL×2) and brine (10 mL×2), dried over anhydrous magnesium sulfate. After filtration and concentration, the crude product was obtained and purified with column chromatography (methylene chloride /methanol = 50:1 to 30:1) to give compound **19** as a white solid (95 mg, 72.5%); m.p. 211-213°C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 11.74 (s, 1H), 8.42 (d, J = 8.0 Hz, 1H), 8.03 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H), 7.66 (td, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H), 7.52 (dd, J_1 = 6.5 Hz, J_2 = 2.5 Hz, 1H), 7.40-7.43 (m, 1H), 7.20-7.29 (m, 3H), 5.31 (s, 2H), 4.30-4.35 (m, 1H), 2.76-2.81 (m, 1H), 2.65-2.71 (m, 1H), 2.43-2.48 (m, 2H), 1.96-2.24 (m, 4H), 1.55-1.82 (m, 7H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 163.23, 161.80, 158.10 (d, J = 246.3 Hz), 150.72, 140.68, 135.24, 132.63 (d, J = 3.0 Hz), 130.14 (d, J = 7.8 Hz), 128.14 (d, J = 2.8 Hz), 127.67, 124.70 (d, J = 15.1 Hz), 124.09 (t, J = 243.3 Hz), 122.80, 116.30 (d, J = 22.6 Hz), 115.98, 115.01, 58.47, 57.53, 49.98, 48.58, 44.34, 30.78, 30.41 (t, J = 23.6 Hz) (30.35 (t, J = 23.9 Hz)), 26.95; HR-MS (ESI): m/z, calcd. for $C_{26}H_{28}N_4O_3F_3$ $[M+H]^+$ 501.2108, Found: 501.2096.

***N*-(1-(1,4-Dioxaspiro[4.5]decan-8-yl)pyrrolidin-3-yl)-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)methyl)-2-fluorobenzamide (20)**

Following the preparation protocol of compound **19**, starting from compound **16** and 1,4-dioxaspiro[4.5]decan-8-one, the title compound **20** was obtained as a white solid (240 mg, 58.7%); m.p. 149-151 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 11.73 (s, 1H), 8.40 (d, J = 5.0 Hz, 1H), 8.03 (d, J = 7.5 Hz, 1H), 7.66 (t, J = 8.0 Hz, 1H), 7.53 (d, J = 5.5 Hz, 1H), 7.41 (brs, 1H), 7.18-7.33 (m, 3H), 5.31 (s, 2H), 4.31 (s, 1H), 3.83 (s, 4H), 2.80 (brs, 1H), 2.65 (brs, 1H), 2.46 (brs, 2H), 2.10 (brs, 2H), 1.64-1.79 (m, 5H), 1.42-1.49 (m, 4H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 163.19, 161.76, 158.10 (d, J = 246.3 Hz), 150.69, 140.66, 135.21, 132.57 (d, J = 3.2 Hz), 130.08 (d, J = 8.3 Hz), 128.17 (d, J = 2.9 Hz), 127.64, 124.64 (d, J = 15.7 Hz), 122.76, 116.26 (d, J = 22.6 Hz), 115.96, 114.98, 107.79, 63.60, 63.56, 60.13, 57.50, 49.95, 48.55, 44.33, 31.91, 30.72, 28.04; HR-MS (ESI): m/z, calcd. for $C_{28}H_{32}N_4O_5F$ $[M+H]^+$ 523.2351, Found: 523.2335.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) -2-fluoro-*N*-(1-(4-oxocyclohexyl)pyrrolidin-3-yl)benzamide (21)

To a solution of compound **20** (100 mg, 0.19 mmol) in acetone (10.0 mL), 2N HCl (15 mL) was added and then the reaction mixture was stirred at room temperature for 24 h. After removal of the solvent under vacuum distillation, the solvent mixture (methylene chloride/methanol = 10:1) (30 mL) was added to the residue. Then

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the pH of mixture was adjusted to alkaline with saturated aqueous sodium bicarbonate. The solution was extracted with the solvent mixture (methylene chloride/methanol = 10:1) (20 mL × 3) and then the organic layer was washed with brine (20 mL). The combined organic layer was dried over anhydrous $MgSO_4$. After filtration and concentration, the title compound **21** was obtained as a light yellow solid (52 mg, 57.1%); m.p. 201-203 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 11.79 (s, 1H), 8.49 (d, J = 7.0 Hz, 1H), 8.05 (d, J = 7.5 Hz, 1H), 7.68 (t, J = 7.5 Hz, 1H), 7.55 (d, J = 5.0 Hz, 1H), 7.44 (brs, 1H), 7.21-7.33 (m, 3H), 5.34 (s, 2H), 4.39 (brs, 1H), 2.87 (brs, 1H), 2.73-2.83 (m, 1H), 2.51-2.57 (m, 2H), 2.37-2.49 (m, 3H), 2.13-2.28 (m, 3H), 1.94 (br, 2H), 1.79-1.89 (m, 2H), 1.69-1.78 (m, 1H); HR-MS (ESI): m/z, calcd. for $C_{26}H_{28}N_4O_4F$ $[M+H]^+$ 479.2089, Found: 479.2088.

_N_-(1-Benzylpyrrolidin-3-yl)-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl)-2-fluorobenzamide (22)

Following the preparation protocol of compound **19**, starting from compound **16** and benzaldehyde, the title compound **22** was obtained as a white solid (65 mg, 52.8%); m.p. 175-177 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ (ppm): 11.74 (s, 1H), 8.44 (d, J = 7.0 Hz, 1H), 8.02-8.04 (m, 1H), 7.64-7.68 (m, 1H), 7.51-7.53 (m, 1H), 7.40-7.42 (m, 1H), 7.19-7.31 (m, 8H), 5.30 (s, 2H), 4.32 (brs, 1H), 3.54-3.61 (m, 2H), 2.76 (brs, 1H), 2.60 (brs, 1H), 2.45 (brs, 1H), 2.39 (brs, 1H), 2.12-2.16 (m, 1H), 1.70 (brs, 1H); HR-MS (ESI): m/z, calcd. for $C_{27}H_{26}N_4O_3F$ $[M+H]^+$ 473.1984, Found: 473.1973.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl) -2-fluoro-_N_-(1-(4-methoxybenzyl) pyrrolidin-3-yl)benzamide (23)

Following the preparation protocol of compound **19**, starting from compound **16** and 4-methoxybenzaldehyde, the title compound **23** was obtained as a white solid (82 mg, 62.1%); m.p. 171-173 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm):11.75 (s, 1H), 8.43 (d, J = 6.8 Hz, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.65 (t, J = 8.0 Hz, 1H), 7.50-7.54 (m, 1H), 7.37-7.43 (m, 1H), 7.18-7.30 (m, 5H), 6.87 (d, J = 8.4 Hz, 2H), 5.30 (s, 2H), 4.25-4.35 (m, 1H), 3.73 (s, 3H), 3.51 (brs, 2H), 2.75 (brs, 1H), 2.56 (brs, 1H), 2.45 (brs, 1H), 2.36 (brs, 1H), 2.08-2.18 (m, 1H), 1.67-1.71 (m, 1H); HR-MS (ESI): m/z, calcd. for $C_{28}H_{28}N_4O_4F$ $[M+H]^+$ 503.2089, Found: 503.2080.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl) -2-fluoro-_N_-(1-(3-methylbenzyl)pyrrolidin-3-yl)benzamide (24)

Following the preparation protocol of compound **19**, starting from compound **16** and 3-methylbenzaldehyde, the title compound **24** was obtained as a white solid (72 mg, 48%); m.p. 185-187 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.74 (s, 1H), 8.44 (d, J = 6.8 Hz, 1H), 8.02 (dd, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H), 7.61-7.70 (m, 1H), 7.52 (dd, J_1 = 6.8 Hz, J_2 = 2.4 Hz, 1H), 7.38-7.43 (m, 1H), 7.17-7.30 (m, 4H), 7.03-7.14 (m, 3H), 5.30 (s, 2H), 4.31 (brs, 1H), 3.54 (brs, 2H), 2.76 (brs, 1H), 2.58 (brs, 1H), 2.47 (brs, 1H), 2.38 (brs, 1H), 2.29 (s, 3H), 2.09-2.19 (m, 1H), 1.64-1.74 (m, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 163.26, 161.75, 158.09 (d, J = 246.4 Hz), 150.68, 140.66, 138.77, 137.17, 135.20, 132.58 (d, J = 3.2 Hz), 130.10 (d, J = 8.3 Hz), 129.16, 128.15 (d, J = 3.0 Hz), 128.03, 127.64, 127.51, 125.62, 124.59 (d, J = 15.6 Hz), 122.76, 116.26 (d, J = 22.6 Hz), 115.96, 114.97, 59.64, 59.19, 52.37, 48.66, 44.33, 30.70, 20.98; HR-MS (ESI): m/z, calcd. for $C_{28}H_{28}N_4O_3F$ $[M+H]^+$ 487.2140, Found: 487.2141.

2-Chloro-_N_-(1-(4,4-difluorocyclohexyl)pyrrolidin-3-yl)-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl)benzamide (25)

Following the preparation protocol of compound **19**, starting from compound **17** and 4,4-difluorocyclohexan-1-one, the title compound **25** was obtained as a white solid (110 mg, 56.7%); m.p. 164-166 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.74 (s, 1H), 8.61 (d, J = 7.2 Hz, 1H), 8.03 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H), 7.63-7.68 (m, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.40 (d, J = 2.0 Hz, 1H), 7.32 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H),7.24-7.28 (m, 2H), 5.31 (s, 2H), 4.29 (brs, 1H), 2.79 (brs, 1H), 2.68 (brs, 1H), 2.53 (brs, 1H), 2.45 (brs, 1H), 1.97-2.22 (m, 4H), 1.65-1.87 (m, 5H), 1.51-1.64 (m, 2H); HR-MS (ESI): m/z, calcd. for $C_{26}H_{28}N_4O_3ClF_2$ $[M+H]^+$ 517.1812, Found: 517.1803.

_N_-(1-Benzylpyrrolidin-3-yl)-2-chloro-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)methyl)benzamide (26)

Following the preparation protocol of compound **19**, starting from compound **17** and benzaldehyde, the title compound **26** was obtained as a white solid (97 mg, 52.7%); m.p. 188-190 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.74 (s, 1H), 8.62 (d, J = 6.8 Hz, 1H), 8.03 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H), 7.63-7.68 (m, 1H), 7.38-7.43 (m, 2H), 7.30-7.33 (m, 5H), 7.22-7.28 (m, 3H), 5.30 (s, 2H), 4.29 (brs, 1H), 3.62 (d, J = 12.4 Hz, 1H), 3.54 (d, J = 13.2 Hz, 1H), 2.76 (brs, 1H), 2.58 (brs, 1H), 2.40-2.48 (m, 2H), 2.08-2.19 (m, 1H), 1.65-1.76 (m, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 166.78, 161.81, 150.72, 140.69, 139.04, 137.13, 135.64, 135.24, 129.69, 128.70, 128.52, 128.16, 127.67, 127.08, 126.86, 122.81, 116.01, 114.99, 59.60, 59.23, 52.47, 48.60, 44.42, 30.58; HR-MS (ESI): m/z, calcd. for $C_{27}H_{26}N_4O_3Cl$ $[M+H]^+$ 489.1688, Found: 489.1692.

_N_-(1-Butylpyrrolidin-3-yl)-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)methyl)-2-fluoro-_N_-methylbenzamide (27)

Following the preparation protocol of compound **9**, starting from compound **3** and 1-butyl-_N_-methylpyrrolidin-3-amine, the title compound **27** was obtained as a white solid (60 mg, 41.9%); m.p. 104-106 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 9.05 (brs, 1H), 8.23

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(s, 1H), 7.60 (s, 1H), 7.27 (brs, 2H), 7.08 (brs, 2H), 5.32 (s, 2H), 4.18 (s, 1H), 3.08 (s, 2H), 2.92 (s, 1H), 2.30-2.73 (m, 6H), 2.00-2.50 (m, 4H), 1.87 (brs, 1H), 1.61 (brs, 1H), 1.20-1.50 (m, 4H), 0.91 (brs, 3H); HR-MS (ESI): m/z, calcd. for $C_{25}H_{30}N_4O_3F$ $[M+H]^+$ 453.2297, Found: 453.2310.

_N_-(1-(Cyclopropylmethyl)pyrrolidin-3-yl)-5-((2,4-dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl)-2-fluoro-N-methylbenzamide (28)

Following the preparation protocol of compound **9**, starting from compound **3** and 1-(cyclopropylmethyl)-*N*-methylpyrrolidin-3-amine, the title compound **28** was obtained as a white solid (95 mg, 55.5%); m.p. 114-116 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.73 (s, 1H), 8.01-8.03 (m, 1H), 7.62-7.68 (m, 1H), 7.37-7.47 (m, 1H), 7.23-7.30 (m, 4H), 5.31 (s, 2H), 5.07-5.13 (m, 0.4H), 3.96 (brs, 0.6H), 2.93 (s, 3H), 2.60-2.80 (m, 2H), 1.60-2.40 (m, 6H), 0.76-0.89 (m, 1H), 0.42-0.49 (m, 2H), 0.06-0.14 (m, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 165.48 (164.87), 161.82, 158.68 (d, J = 243.0 Hz) (156.55 (d, J = 245.4 Hz)), 150.72, 140.72 (140.67), 135.21 (135.15), 132.20 (d, J = 3.0 Hz) (132.11 (d, J = 2.6 Hz)), 129.40 (d, J = 7.8 Hz), 127.66, 126.82 (d, J = 4.0 Hz), 124.82 (d, J = 18.4 Hz), 122.76, 116.25 (d, J = 21.0 Hz), 116.06 (116.04), 115.01, 59.72, 56.86, 53.16 (52.93), 51.59, 44.41 (44.35), 28.04, 27.67, 9.35, 3.78 (3.73), 3.58 (3.51); HR-MS (ESI): m/z, calcd. for $C_{25}H_{28}N_4O_3F$ $[M+H]^+$ 451.2140, Found: 451.2133.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl) -N-ethyl-_N_-(1-ethylpyrrolidin-3-yl)-2-fluorobenzamide (29)

Following the preparation protocol of compound **9**, starting from compound **3** and *N*,1-diethylpyrrolidin-3-amine, the title compound **30** was obtained as a white solid (45 mg, 26%); m.p. 64-66 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.91 (brs, 1H), 8.23 (d, J = 7.6 Hz, 1H), 7.58 (t, J = 7.2 Hz, 1H), 7.36 (brs, 1H), 7.22-7.30 (m, 2H), 7.02-7.14 (m, 2H), 5.33 (s, 2H), 4.17-4.40 (m, 1H), 3.76 (brs, 1H), 3.40-3.58 (m, 3H), 3.08-3.22 (m, 3H), 2.43-2.76 (m, 3H), 1.64-1.94 (m, 2H), 1.46 (t, J = 7.2 Hz, 2H), 1.33 (t, J = 6.0 Hz, 1H), 1.14 (brs, 1H), 1.06 (t, J = 6.4 Hz, 3H), ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 165.95 (165.05), 161.85, 156.71 (d, J = 242.9 Hz) (156.65 (d, J = 243.0 Hz)), 150.74, 140.69 (140.66), 135.23 (135.16), 133.18 (133.16), 129.58 (d, J = 7.5 Hz) (129.37 (d, J = 7.0 Hz)), 127.66, 126.37 (126.07), 124.89 (d, J = 18.1 Hz), 122.77, 116.29 (d, J = 21.4 Hz), 116.06, 115.09 (114.98), 56.34, 53.81, 52.46 (51.42), 49.16 (48.98), 44.42 (43.15), 36.59, 27.65, 14.80 (14.33), 10.92; HR-MS (ESI): m/z, calcd. for $C_{24}H_{28}N_4O_3F$ $[M+H]^+$ 439.2140, Found: 439.2155.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl) -N-(1-ethylpyrrolidin-3-yl)-2-fluoro-_N_-propylbenzamide (30)

Following the preparation protocol of compound **9**, starting from compound **3** and 1-ethyl-*N*-propylpyrrolidin-3-amine, the title compound **30** was obtained as a light yellow solid (45 mg, 26%); m.p. 105-107 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 8.02 (dd, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 7.61-7.68 (m, 1H), 7.40-7.52 (m, 1H), 7.23-7.34 (m, 4H), 5.33 (s, 2H), 4.51 (brs, 0.5H), 4.06 (brs, 0.5H), 3.20-4.00 (m, 4H), 2.98-3.09 (m, 3H), 2.27-2.34 (m, 0.5H), 2.10-2.18 (m, 0.5H), 1.50-1.90 (m, 2H), 1.25-1.31 (m, 2H), 1.17-1.21 (m, 1.5H), 1.06 (brs, 1.5H), 0.89 (t, J = 7.2 Hz, 1.5H), 0.47 (t, J = 7.2 Hz, 1.5H); HR-MS (ESI): m/z, calcd. for $C_{25}H_{30}N_4O_3F$ $[M+H]^+$ 453.2296, Found: 453.2283.

***tert*-Butyl 3-(5-((2,4-dioxo-3,4-dihydroquinazolin-1(2H)-yl)methyl)-2-fluoro-N-methylbenzamido) pyrrolidine-1-carboxylate (31)**

Following the preparation protocol of compound **9**, starting from compound **3** and *tert*-butyl 3-(methylamino)pyrrolidine-1-carboxylate, the title compound **31** was obtained as a white solid (37 mg, 46.8%); m.p. 107-109 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.73 (s, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.61-7.68 (m, 1H), 7.23-7.45 (m, 5H), 5.25-5.39 (m, 2H), 4.95-5.01 (m, 0.4H), 4.02 (brs, 0.6H) 3.39-3.53(m, 1H), 3.17-3.35 (m, 3H), 2.88 (s, 1.8H), 2.71(s, 1.2H), 1.80-2.10 (m, 2H), 1.38-1.41 (m, 9H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 165.81 (165.30), 161.78, 156.67 (d, J = 242.9 Hz) (156.55 (d, J = 243.3 Hz)), 153.43 (153.23), 150.69, 140.69 (140.64), 135.16 (135.01), 132.18 (d, J = 3.1 Hz), 129.47 (129.39), 127.62, 126.93, 124.87 (d, J = 18.9 Hz) (124.48 (d, J = 18.5 Hz)) , 122.73 (122.66), 116.30 (d, J = 21.5 Hz), 116.06 (115.98), 114.98 (114.95), 78.50, 57.06 (56.29), 52.44 (51.71), 46.71 (46.36), 44.40 (44.34), 44.02 (43.82), 31.38 (29.31), 28.09; HR-MS (ESI): m/z, calcd. for $C_{26}H_{29}N_4O_5FNa$ $[M+Na]^+$ 519.2014, Found: 519.2033.

***tert*-Butyl 3-(5-((2,4-dioxo-3,4-dihydroquinazolin-1(2_H_)-yl)methyl)-_N_-ethyl-2-fluorobenzamido) pyrrolidine-1-carboxylate (32)**

Following the preparation protocol of compound **9**, starting from compound **3** and *tert*-butyl 3-(ethylamino)pyrrolidine-1-carboxylate, the title compound **32** was obtained as a light yellow solid (37 mg, 46.8%); m.p. 107-109 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.73 (s, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.64 (t, J = 7.2 Hz, 1H), 7.22-7.42 (m, 5H), 5.23-5.40 (m, 2H), 4.53-4.62 (m, 0.4H), 3.96 (brs, 0.6H), 3.07-3.59 (m, 6H), 1.68-2.23 (m, 2H), 1.37-1.41 (m, 9H), 1.14 (t, J = 6.8 Hz, 1.2H), 0.83 (t, J = 7.2 Hz, 1.8H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 165.89 (165.11), 161.77, 156.66 (d, J = 242.6 Hz) (156.39 (d, J = 242.8 Hz)), 153.45 (153.25), 150.72, 140.64, 135.07, 133.21 (133.10), 129.34 (129.12), 127.62, 126.21, 125.37 (d, J = 18.9 Hz) (124.75 (d, J = 19.6 Hz)) , 122.71 (122.67), 116.29 (d, J = 21.2 Hz), 116.05, 114.98, 78.56 (78.46), 57.20 (56.42), 54.20 (53.50), 47.17 (46.81), 44.33 (43.97), 41.28, 35.94, 28.13 (28.09), 15.18 (14.33); HR-MS (ESI): m/z, calcd. for $C_{27}H_{31}N_4O_5FNa$ $[M+Na]^+$ 533.2171, Found: 533.2194.

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tert-Butyl 3-(5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoro-*N*-propylbenzamido) pyrrolidine-1-carboxylate (33)

Following the preparation protocol of compound **5**, starting from compound **3** and _tert_-butyl 3-(propylamino)pyrrolidine-1-carboxylate, the title compound **33** was obtained as a white solid (160 mg, 63.7%); m.p. 117-119 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.80 (s, 1H), 8.23 (d, J = 8.0 Hz, 1H), 755-7.62 (m, 1H), 7.25-7.32 (m, 2H), 7.06-7.10 (m, 2H), 5.33 (s, 2H), 4.74-4.78 (m, 0.5H), 4.09 -4.12 (m, 1H), 3.72-3.76 (m,0.5H), 3.04-3.59 (m, 5H), 1.63-2.18 (m, 4H), 1.38-1.46 (m, 9H), 0.96 (t, J = 5.6 Hz, 2H), 0.60 (t, J = 6.4 Hz, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 165.98 (165.40), 161.76, 156.62 (d, J = 248.2 Hz) (156.39 (d, J = 242.7 Hz)), 153.40 (153.20), 150.72, 140.63 (140.58), 135.04, 133.21 (133.10), 129.40 (129.33), 127.62, 126.35, 125.33 (d, J = 18.9 Hz) (125.78 (d, J = 18.8 Hz)) , 122.71 (122.68), 116.31 (d, J = 19.1 Hz), 116.04, 114.99 (114.91), 78.54 (78.50), 57.19 (56.43), 54.56 (53.83), 48.39, 47.08 (46.88), 44.30 (43.96), 43.51 (42.80), 28.12 (28.09), 22.92 (21.86), 11.26 (10.65); HR-MS (ESI): m/z, calcd. for $C_{28}H_{33}N_4O_5F$ $[M+Na]^+$547.2215, Found: 547.2231.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluoro-*N*-methyl-*N*-(pyrrolidin-3-yl)benzamide 2,2,2-trifluoroacetate (34)

To a solution of compound **31** (45 mg, 0.12 mmol) in DCM (2.0 mL), TFA (2 mL) was added and then the reaction mixture was stirred at room temperature for 5 h. Ether (20 mL) was added into the mixture. After filtration, the title compound **34** was afforded as a white solid (40 mg, 86.5%); m.p. 116-118 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.74 (s, 1H), 9.00 (brs, 0.5H), 8.91 (brs, 1H), 8.67 (brs, 0.5H), 8.03 (d, J = 8.0 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.46 (brs, 1H), 7.24-7.38 (m, 4H), 5.32 (s, 2H), 4.89-4.97 (m, 0.5H), 4.26 (brs, 0.5H), 3.59 (brs, 2H), 3.17-3.24 (m, 2H), 2.90 (s, 1.5H), 2.76 (s, 1.5H), 2.00-2.10 (m, 2H); HR-MS (ESI): m/z, calcd. for $C_{21}H_{22}N_4O_3F$ $[M+H]^+$ 397.1671, Found: 397.1699.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) -N-ethyl-2-fluoro-*N*-(pyrrolidin-3-yl)benzamide 2,2,2-trifluoroacetate (35)

Following the preparation protocol of compound **34**, starting from compound **32**, the title compound **35** was obtained as a white solid (40 mg, 86.5%); m.p. 111-112 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.74 (s, 1H), 9.01 (brs, 1H), 8.64 (brs, 1H), 8.04 (s, 1H), 7.64 (s, 1H), 7.25-7.48 (m, 4H), 5.33 (s, 2H), 4.20-4.34 (m, 1H), 2.90-3.54 (m, 6H), 2.32 (s, 1H), 2.16 (s, 1H), 0.88-1.18 (m, 3H); HR-MS (ESI): m/z, calcd. for $C_{22}H_{24}N_4O_3F$ $[M+H]^+$ 411.1827, Found: 411.1838.

5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) -2-fluoro-*N*-propyl-N-(pyrrolidin-3-yl)benzamide 2,2,2-trifluoroacetate (36)

Following the preparation protocol of compound **34**, starting from compound **33**, the title compound **36** was obtained as a white solid (85 mg, 93.4%); m.p. 114-116 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.72 (s, 1H), 8.98 (brs, 1H), 8.60 (brs, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.49 (s, 1H), 7.21-7.41 (m, 4H), 5.31 (s, 2H), 4.02-4.33 (m, 1H), 3.50-3.65 (m, 3H), 2.97-3.25(m, 3H), 2.25-2.35 (m, 1H), 2.10-2.18 (m, 1H), 1.56 (brs, 0.5H), 1.29 (q, J = 7.2 Hz, 1.5H), 0.87 (t, J = 7.2 Hz, 1H), 0.47 (t, J = 7.2 Hz, 2H); HR-MS (ESI): m/z, calcd. for $C_{23}H_{26}N_4O_3F$ $[M+H]^+$ 425.1983, Found: 425.1968.

Note: Except for target compounds, the synthesis and characterization of all other compounds mentioned in the Scheme 1 were described in the supporting information.

4.3 Biological evaluation

4.3.1 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 [34]. The ability of compounds to inhibit PARP1/2 enzyme activity were tested using ELISA method as described [35,36]. IC_{50} values were calculated using GraphPad Prism 5 software.

4.3.2 The cytotoxicity assay

MX-1 were purchased from National Infrastruture of Cell Line Resources, Cells were seeded in a density in 96-well plates (2,000cells/well). Cells were treated in their recommended growth media containing increasing concentrations of PARP inhibitors 24 h later. After 72 h treatment, cell survival was determined by MTT assay. IC_{50} values were calculated using GraphPad Prism 5 software.

4.3.3 The PF_{50} assay in MX-1 cells

In chemosensitization assays, MX-1 cells were seeded in a density (2,000 cells/well) in 96-well plates. Cells were treated with PARP inhibitors at a fixed concentration of 10 μmol/L and temozolomide (TMZ) at different concentration (0-0.5 mmol/L). After 72 h treatment, cell survival was determined by MTT assay. IC_{50} values were calculated using GraphPad Prism5 software. Potentiation factor (PF_{50}) was calculated as the ratio of the IC_{50} for TMZ divided by the IC_{50} of combination (TMZ + PARP inhibitor).

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4.3.4 Xenograft experiment

The anti-cancer activity as a sensitizer of TMZ using MX-1 xenograft tumor model was tested as described in our previous work [35]. TMZ and compound **11** were orally administrated respectively or combined for 5 days. %T/C is calculated as (tumor weight of treatment group/Vehicle or TMZ treatment group) ×100, while %TGI is calculated as (100-%T/C). Statistical analyses were performed by GraphPad Prism5 software and the significance levels were evaluated using one-way ANOVA model.

All animal experiments were approved by the Ethics Committee for Animal Experiments of the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College (No: 00001207) and conducted in accordance with the Guidelines for Animal Experiments of Peking Union Medical College.

4.4 The X-ray crystallographic experiment

The protein expression, purification and the crystallographic experiment were performed as described in our previous work [36].

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Discovery of quinazoline-2,4(1*H*,3*H*)-dione derivatives as novel PARP-1/2 inhibitions: design, synthesis and their antitumor activity

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Novel quinazoline-2,4(1*H*,3*H*)-dione derivatives bearing 3-amino pyrrolidine motif were identified as potent PARP-1/2 inhibitors with distinct binding features.