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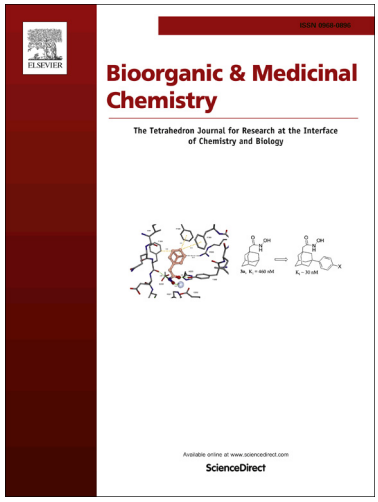

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Discovery of 1-substituted benzyl-quinazoline-2,4(1*H*,3*H*)-dione derivatives as novel poly(ADP-ribose)polymerase-1 inhibitors

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Abstract

Poly(ADP-ribose) polymerase-1 (PARP-1) has emerged as a promising anticancer drug target due to its key role in the DNA repair process. In this work, a novel series of 1-benzyl-quinazoline-2,4(1*H*,3*H*)-dione derivatives were designed and synthesized as human PARP-1 inhibitors, structure-activity relationships were conducted and led to a number of potent PARP-1 inhibitors having IC_{50} values of single or double digit nanomolar level. Compound **7j** was a potent PARP-1 and PARP-2 inhibitor and it could selectively kill the breast cancer cells MX-1 and MDA-MB-468 with mutated BRCA1/2 and PTEN respectively, in comparison with homologous recombination proficient cell types such as breast cancer cells MDA-MB-231. In addition, compound **7j** displayed the strongest potentiation effect on temozolomide in MX-1 cells (PF_{50} = 3.77) in this series of PARP-1 inhibitors.

Key words: PARP-1 inhibitor; PARP-2 inhibitor; quinazoline-2,4(1*H*,3*H*)-dione; antitumor activity.

1. Introduction

Poly (ADP-ribose) polymerase-1 (PARP-1) belongs to the PARP nuclear enzyme superfamily containing 17 members.[1-3] It has three domains including an N-terminal DNA-binding domain with three zinc fingers, a central auto-modification domain and a C-terminal catalytic domain.[4, 5] When the damaged DNA was recognized by PARP-1 through its DNA-binding domain, the enzymatic activity of PARP-1 was rapidly increased to about 500-fold. The activated PARP-1 can

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bind to nicotinamide adenine dinucleotides (NAD^+) through its catalytic binding site, and cleave the NAD^+ to generate nicotinamide and ADP-ribose units, the latter is successively transferred onto the nuclear DNA-binding proteins and PARP-1 itself to form poly(ADP-ribose) (PAR).[6, 7] The poly(ADP-ribosyl)ation of various proteins is believed to be an essential post-translational modification in modulating a plethora of cellular functions, including DNA repair.[3, 8]

It has been demonstrated that PARP-1 plays a pivotal role in the repair of single strand breaks of DNA by the base excision repair (BER) pathway.[9-11] Therefore, the inhibition of PARP-1 activity can augment the cytotoxic effects of the DNA damaging agents (e.g. temozolomide, cyclophosphamide and camptothecin) which cause lesions normally repaired by BER pathway.[12-16] Moreover, given the key role of PARP-1 in DNA repair, PARP-1 inhibitors can induce synthetic lethality in cells defective in homologous recombination (HR) repair, such as tumor cells with mutations in BRCA1 and BRCA2.[17-19] Thus PARP-1 inhibitors are expected to kill BRCA defective tumor cells effectively and selectively. Taken together, these promising results have further sparked tremendous research works to exploit PARP-1 inhibition as a therapeutic approach in cancer treatment.[20, 21]

Nicotinamide and 3-aminobenzamide were identified as PARP-1 inhibitors more than 30 years ago,[22] and since then many structural diversified PARP-1 inhibitors have been investigated.[23-26] The devoted efforts have resulted in a number of drug candidates undergoing clinical trials including AZD-2281, ABT-888, AG014699, MK-4827 and BMN673 (Figure 1).[27-31] These candidates were used either as a single agent or in combination with chemotherapy for the treatment of a wide variety of cancers including breast, ovarian, pancreatic and melanoma tumors with or without BRCA1/2 deficiency. Recently, it has been reported that AZD-2281 was most likely to be beneficial to the patients with platinum-sensitive recurrent serous ovarian cancer with BRCA1/2 mutations.[32] Nonetheless, so far there are no PARP-1 inhibitors as anticancer drugs that have reached the market. It is still a great challenge to develop PARP-1 inhibitors with desired pharmacodynamic and pharmacokinetic properties as novel therapeutic agents in cancer treatment.[33]

The NAD^+ binding site of PARP-1 contains two characteristic binding regions, namely, a nicotinamide-ribose binding site (NI site) and an adenine-ribose binding site (AD site).[34] All of the known PARP-1 inhibitors interacted with the NI site through three key hydrogen bonds formed between the lactam or the carboxamide group of the small molecules and residues Ser904 and

Gly863.[35, 36] In addition, a π-π stacking interaction between the aromatic scaffold of PARP-1 inhibitors and Tyr907 of the NI site was generally observed as another key feature. Compared with the relatively narrow NI site, the AD site of PARP-1 is rather large and may be used to search for a wide variety of novel inhibitors with the improved potency and pharmacokinetic properties. In fact, some of the reported PARP-1 inhibitors such as AZD-2281 and PJ-34 took advantage of this AD site to further improve their binding affinities.[37] In this work, a series of quinazoline-2,4(1*H*,3*H*)-dione derivatives (**7a**-**7s**, **8a**-**8t**) were designed based on the structure–activity relationships (SAR) of the known PARP inhibitors and their binding features in the PARP active site.[26] 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 NI site;[34] a 3-amino and 3-amino-4-fluoro benzyl groups were used as a linker to direct the amino acid motifs into the AD site. We investigated the SARs of this series of quinazoline-2,4(1*H*,3*H*)-dione derivatives by tentatively utilizing various amino acid building blocks to explore the AD site. Herein, the chemical synthesis of these new quinazoline-2,4(1*H*,3*H*)-dione derivatives is described in details. The PARP-1inhibitory activities of these compounds are presented along with their SAR analysis. Some of the potent PARP-1 inhibitors were further evaluated in terms of their PARP-2 inhibitory activities, growth inhibition and potentiation effect on temozolomide (TMZ) in cancer cells.

2. Chemistry

The quinazoline-2,4(1*H*,3*H*)-dione derivatives (**8a**–**8t**) were prepared according to the synthetic route as outlined in **Scheme 1**. The substituted 2-aminobenzoic acids were condensed with urea in a solvent-free condition providing the corresponding quinazoline-2,4(1*H*,3*H*)-dione scaffolds (**2a**-**2e**).[38] In the presence of hexamethyldisilazane (HMDS) and concentric sulfuric acid, compounds **2a-2e** in toluene were first transformed into silylated compounds **3a-3e,** which were then reacted with 3-nitro benzyl bromide or 4-fluoro-3-nitro benzyl bromide to generate the N-1 substituted intermediates **4a-4f**. Upon treatment of the reaction with methanol, compounds **5a-5f** were obtained in 25-77% yields.[39] Compounds **6a-6f**, which were obtained by reducing compounds **5a-5f**, were converted into compounds **7a-7s** and **8t** by coupling with a range of N-Boc protected amino acids in the presence of HBTU/HOBt/DIEA, HATU/HOBt/TEA or EDCI/HOBt/TEA in moderate to good yields. The deprotection of Boc group in compounds **7a-7s** using TFA offered the target compounds **8a-8s** in good yields.

3. Results and discussion

3.1 Enzymatic activity against PARP-1

The inhibitory activities against PARP-1 of all target compounds (**8a-8t**) and their Boc protected derivatives (**7a-7s**) were evaluated. Several potent PARP-1 inhibitors were chosen to further test their inhibition effect on PARP-2. The clinical drug candidates ABT888 and AZD2281 were used as reference molecules. The corresponding results were expressed as IC_{50} values and presented in **Tables 1–4**.

Initially, a number of α-amino acids were introduced onto 3-position of the phenyl ring of the benzyl group by coupling with amines **6a** and **6b**. As shown in **Table 1**, the target compounds (**8a-8g** and **8t**) exhibited greatly varied inhibitory activities against PARP-1 with IC_{50} values ranging from 48.2 nM to 5.29 μM. Compound **8a** containing a glycine moiety showed moderate inhibitory activity against PARP-1 with an IC_{50} value of 171 nM. Mono- or dimethylation on the glycine nitrogen atom (compounds **8b** and **8t**) led to a decrease in activity, and *N*,*N*-dimethyl compound **8t** showed a much weaker inhibition, which was about 25 times less potent than compound **8a**. It was indicated that a tertiary amine was not favorable presumably due to the steric hinderance caused by dimethyl group. Compound **8c** (IC_{50}: 5.29 μM) was the least active of all the target compounds, which indicated that bulky groups such as an isopropyl group substituted at C-2 position of the glycine moiety could be detrimental to the enzymatic potency. When a proline fragment was introduced, the (*R*)- enantiomer (**8d**) displayed comparable activity to compound **8a**, while the *S*-enantiomer (**8f**) was 16 times less potent than **8a** and 5 times less potent than the (*R*)-enantiomer. It was demonstrated that the (*R*)-enantiomer was preferred to the binding interactions. Noticeably, the placement of a fluoro atom at para-position of the benzyl linker resulted in a significant improvement in potency, as compounds **8e** and **8g** had >10 times stronger inhibition than the corresponding des-fluoro derivatives **8d** and **8f**, respectively. Compound **8e** was the most potent inhibitor in this series and displayed inhibitory activity at double digit nanomolar level. This result was consistent with the structure-activity relationships of the known PARP-1 inhibitors.[40] As for the Boc-protected derivatives **7a-7g**, they all displayed dramatic decreases in potency in comparison with their counterparts **8a-8g**. It was indicated that the large bulky group on the nitrogen atom was not beneficial to the binding affinity, which was consistent with the result demonstrated by compound **8t**.

Based on the preliminary SAR results from *α*-amino acids as depicted above, we then turned our attention to the cyclic *β*-amino acids such as *β*-proline or piperidine-3-carboxylic acid as the key pharmacophoric groups (**Table 2**). These *β*-amino acids were coupled with compounds **6a** and

6b to furnish the target compounds **8h-8m**. Surprisingly, compounds **8h-8k** containing either (*R*)-isomer or (*S*)-isomer of *β*-proline, all were highly active against PARP-1 with IC_{50} values of double digit nanomolar. The fluoro group substituted compounds (**8i** and **8k**) displayed similar activities to the unsubstituted derivatives (**8h** and **8j**) as well. In contrast, when piperidine-3-carboxylic amide was installed at 3-position of benzyl linker, compound **8m** with fluoro atom was >10 times more potent than the unsubstituted derivative **8l**. Of note, all the Boc-protected derivatives (**7h**-**7m**) also exhibited potent inhibitory activities , with an exception of compound **7l**. It was suggested that the nitrogen atom on pyrrolidine or piperidine ring in this series was allowed to modify, and therefore it provided a chance for further developing more potent and druggable candidates. Taken together, these results demonstrated that *β*-proline and piperidine-3-carboxylic amide moieties were more favorable than *α*-proline subunit for PARP-1 inhibitory activity.

The cyclic γ-amino acid, as a key structural fragment, was also primarily explored (**Table 3**). The piperidine-4-carboxylic acid was incorporated through amide bond at 3-position of benzyl group. The introduction of a fluoro atom at para position of the benzyl group greatly increased the inhibitory activity (compound **8n** *vs* **8o**) and as a result compound **8o** had an IC_{50} value of 23.1 nM. The substitution with a fluoro atom on the quinazoline-2,4(1*H*,3*H*)-dione scaffold was also investigated. The 5-, 6- and 8-fluoro substituted analogs (**8p**, **8q** and **8s**) displayed comparable activities to the unsubstituted compound **8o**, and 7-fluoro analog (**8r**) showed about 10 times reduction in potency in comparison with compound **8o**. The corresponding Boc protected derivatives (**7n**-**7s**) were tested as well. In general, the inhibitory potency was decreased to some extent. Surprisingly, compound **7p** showed a 3-fold enhancement in potency (compound **7p** *vs* **8p**), and had an IC_{50} value of 9.51 nM, which was the most potent PARP-1 inhibitor of all the tested compounds.

3.2 Enzymatic activity against PARP-2

Among the numerous members of the PARP superfamily, in addition to PARP-1, PARP-2 is another enzyme that has been characterized to be involved in DNA repair.[41] Due to the high homology of the catalytic domain between PARP-1 and PARP-2, PARP-1 inhibitors usually bind to PARP-2 as well.[42] In fact, many of the reported PARP-1 inhibitors displayed comparable inhibitory activities against PARP-2. In this work, some potent PARP-1 inhibitors were selected to test their inhibition effects on PARP-2 (**Table 4**). As expected, most of them had similar inhibitory activities (less than 10-fold difference) against both PARP-1 and PARP-2. However, compound **7k**

showed its strong preference for binding to PARP-2. It presented the most potent inhibition effect on PARP-2 (IC_{50} = 1.47 nM) and had high selectivity over PARP-1 (IC_{50}: PARP1/PARP2 = 15/1). Further molecular modeling study on compound **7k** may help to develop more selective PARP-2 inhibitors, which had been reported in a very few literatures.[34, 43]

3.3 Cellular Potency

The cellular potency of 18 compounds, which acted as potent PARP-1 inhibitors with IC_{50} values of 10-100 nM, were evaluated for their cytotoxicities as single agents in MX-1 breast cancer cells with mutated BRCA1 and BRCA2. Among them, 10 compounds had cytotoxicities with IC_{50} values lower than 50 μM, which were more potent than ABT-888 (**Table 5**). Others were inactive (IC_{50}, >100 μM) as single agents. The potentiation effects on the DNA damaging agent temozolomide (TMZ) of 9 compounds were evaluated at 5 μM concentration using MX-1 cell lines. Compounds **7j** and **8e** could strongly potentiate TMZ cytotoxicities in comparison with other tested compounds, although their potentiation effects were weaker than that of ABT-888 at this concentration.

It has been reported that tumor cells with defective homologous recombination (HR) repair are highly sensitive to PARP-1 inhibitors. In addition to the well known tumor-suppressor genes BRCA1 and BRCA2, other genes such as PTEN and PIK3CA, which were involved in DNA damage repair, displayed synthetic lethality with PARP-1 as well.[44-46] Accordingly, we further evaluated the cytotoxicities of compound **7j** using a number of cancer cell lines with or without deficient and mutated genes in DNA HR repair. As summarized in **Table 6**, compound **7j** was highly active in MX-1, MDA-MB-468, MDA-MB-453 cancer cells with mutated genes of BRCA1/2, PTEN and PIK3CA, respectively, whereas it was less cytotoxic in BRCA2 deficient pancreatic cancer cell line (Capan-1). In contrast, it was much less cytotoxic in MDA-MB-231 breast cancer cells without deficiency in HR repair, and this result is consistent with that of the known PARP-1 inhibitors. In addition, compound **7j** was more cytotoxic than reference molecule AZD-2281 in all tested cell lines. Surprisingly, compound **7j** showed high growth inhibition in ovarian cancer cells A2780, although this cell line did not carry the mutant gene of HR. Presumably, the off-target effect might exist, and that led to the A2780 cancer cells to be sensitive to compound **7j**.

3.4 Prediction of the binding mode

To gain insights into the binding mode of the designed compounds, compound **7j** which was highly potent in enzymatic activity as well as in cell assay, was docked into the co-crystal

structure of BMN673 in PARP-1 (PDB code, 4PJT). As we expected, the quinazoline-2,4(1*H*,3*H*)-dione scaffold bound to the NI site and the *N*-Boc-pyrrolidin-3-yl fragment extended into the AD site (Figure 2A). As shown in Figure 2B, three characteristic hydrogen bonds were formed between the lactam group in compound **7j** and Gly863 as well as Ser904. In addition, another hydrogen bond was also formed between amide nitrogen on the phenyl ring and Gly894, which was not observed in the known PARP-1 inhibitors. Two key π-π stacking interactions were built up between the quinazolinone and 2-fluorophenyl motif in compound **7j** and the amino acid residues Tyr907 and Tyr896, respectively. These π-π stacking interactions were believed to make significant contributions to the binding affinity as they were frequently exploited by the known PARP-1 inhibitors.[27-31] Furthermore, the carbonyl group in Boc motif was surrounded by the positive charged side chain of Arg878, which could contribute the binding affinity through the charge-dipole interaction. It was noted that the Boc group on the pyrrolidine ring was situated in a spacious subpocket and extended into a solvent exposed surface. Therefore, modifications on the nitrogen of the pyrrolidine ring would offer more chances to further improve the binding affinity and drug-like properties of this series of PARP-1 inhibitors.

4. Conclusion

In summary, a novel series of quinazoline-2,4(1*H*,3*H*)-dione derivatives were designed and synthesized by utilizing an array of amino acid building blocks as key pharmacophoric groups. The SAR studies demonstrated that the β-proline and piperidine-4-carboxylic acid would be the favorable structural fragments in this series of PARP-1 inhibitors because most of compounds with these two subunits demonstrated inhibitory activities with IC_{50} values of single or double digit nanomolar. Compound **7j** showed high inhibitory activity in both enzymatic and cellular level, which could serve as a new structure template for further optimization to explore novel potent PARP-1 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 compounds 8a–8t

5.2.1 Synthesis of quinazoline-2,4(1*H*,3*H*)-diones (2a-2e)

5.2.1.1 Quinazoline-2,4(1H,3H)-dione (**2a**) The mixture of 2-amino-benzoic acid (5 g, 36.46 mmol) and urea (50 g, 83.25 mmol) was stirred at 150℃ for 7 h. The reaction mixture was cooled to 100℃ and then water (50 mL) was added to quench the reaction. The crude product was obtained by filtration, then dissolved in NaOH aq. (6 M, 500 mL). The pH was adjusted to 3 and a precipitate was formed. After filtration and dried under vacuum condition, compound **2a** was obtained as white solid (4.5 g, 76.1%); m.p. >250℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.26 (s, 1H), 11.12 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 7.6 Hz, 1H), 7.14-7.19 (m, 2H); HR-MS (ESI): *m/z*, calcd. for $C_8H_7N_2O_2$ $[M+H]^+$ 163.0502, Found: 163.0500.

5.2.1.2 5-Fluoroquinazoline-2,4(1H,3H)-dione (**2b**) Following the preparation protocol of Section 5.2.1.1, starting from 2-amino-6-fluorobenzoic acid (2 g, 12.9 mmol), the title compound **2b** was obtained as white solid (738 mg, 31.8%); m.p.>250℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.27 (s, 1H), 11.25 (s, 1H), 7.60 (dt, J_1 = 8.4 Hz, J_2 = 5.7 Hz, 1H), 6.88-6.99 (m, 2H); HR-MS (ESI): *m/z*, calcd. for $C_8H_6N_2O_2F$ $[M+H]^+$ 181.0408, Found: 181.0404.

5.2.1.3 6-Fluoroquinazoline-2,4(1*H*,3*H*)-dione (**2c**) Following the preparation protocol of Section 5.2.1.1, starting from 2-amino-5-fluorobenzoic acid (2 g, 12.9 mmol), the title compound **2c** was obtained as white solid (1.76 g, 75.8%); m.p.235-237℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.18 (brs, 2H), 7.47-7.58 (m, 2H), 7.18 (dd, J_1 = 8.7 Hz, J_2 = 4.2 Hz, 1H); HR-MS (ESI): *m/z*, calcd. for $C_8H_6N_2O_2F$ $[M+H]^+$ 181.0408, Found: 181.0404.

5.2.1.4 7-Fluoroquinazoline-2,4(1*H*,3*H*)-dione (**2d**) Following the preparation protocol of Section 5.2.1.1, starting from 2-amino-4-fluorobenzoic acid (1.5 g, 9.67 mmol), the title compound **2d** was obtained as yellow solid (1.13 g, 61.9%); m.p.139-141℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.35 (s, 1H), 11.26 (s, 1H), 7.95 (dd, J_1 = 8.7 Hz, J_2 = 6.6 Hz, 1H), 7.00-7.06 (m, 1H), 6.88-6.92 (m, 1H); HR-MS (ESI): *m/z*, calcd. for $C_8H_6N_2O_2F$ $[M+H]^+$ 181.0408, Found: 181.0404.

5.2.1.5 8-Fluoroquinazoline-2,4(1*H*,3*H*)-dione (**2e**) Following the preparation protocol of Section 5.2.1.1, starting from 2-amino-3-fluorobenzoic acid (500 mg, 3.22 mmol), the title compound **2e** was obtained as yellow solid (350 mg, 60.3%); m.p.241-243℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ

(ppm): 11.43 (s, 1H), 11.28 (s, 1H), 7.72 (d, J = 8.1 Hz, 1H), 7.56 (ddd, J_1 = 10.8 Hz, J_2 = 8.4 Hz , J_3 = 1.2 Hz , 1H), 7.16 (td, J_1 = 8.1 Hz, J_2 = 4.8 Hz, 1H) ; HR-MS (ESI): *m/z*, calcd. for $C_8H_6N_2O_2F$ $[M+H]^+$ 181.0408, Found: 181.0405.

5.2.2 Synthesis of 1-benzyl-quinazoline-2,4(1*H*,3*H*)-diones (5a-5f)

5.2.2.1 1-(3-Nitrobenzyl)quinazoline-2,4(1*H*,3*H*)-dione (**5a**) To a suspension of **2a** (140 mg, 0.86 mmol) in toluene (4 mL) and hexamethyldisilazane (HMDS; 347 mg, 2.15 mmol), three drops of sulfuric acid were added with caution. The mixture was heated to reflux and stirred under refluxing for 8 h untill clear solution was obtained. After the removal of toluene and excess HMDS under vacuum distillation, 1-(bromomethyl)-3-nitrobenzene (744 mg, 3.44 mmol) was added to the residue. The reaction mixture was heated to 130°C and was stirred at this temperature for 3 h, the reaction mixture was diluted with 1,4-dioxane (2 mL) at 100°C, and then methanol (3 mL) was added at 70°C for 30 min. The suspension was cooled below 5°C and precipitates were collected by filtriation. After washing with methanol (5 mL) and water (5 mL), the crude product was dried under vacuum condition to afford **5a** as white solid (200 mg, 77.3%); m.p. > 250 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.79 (s, 1H), 8.25 (s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 7.60-7.68 (m, 2H), 7.31 (d, J = 8.8 Hz, 1H), 7.26 (t, J = 7.6 Hz, 1H), 5.46 (s, 2H); HR-MS (ESI): *m/z*, calcd. for $C_{15}H_{12}N_3O_4$ $[M+H]^+$ 298.0822, Found: 298.0816.

5.2.2.2 1-(4-Fluoro-3-nitrobenzyl)quinazoline-2,4(1*H*,3*H*)-dione (**5b**) Following the preparation protocol of Section 5.2.2.1, starting from quinazoline-2,4(1*H*,3*H*)-dione (500 mg, 3.08 mmol), the title compound **5b** was obtained as yellow solid (644 mg, 66.2%); m.p. > 250 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 8.18 (d, J = 6.4 Hz, 1H), 8.03 (d, J = 7.6 Hz, 1H), 7.75 (m, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.51-7.57 (m, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.26 (t, J = 7.6 Hz, 1H), 5.38 (s, 2H); HR-MS (ESI): *m/z*, calcd. for $C_{15}H_{11}N_3O_4F$ $[M+H]^+$ 316.0728, Found: 316.0722.

5.2.2.3 5-Fluoro-1-(4-fluoro-3-nitrobenzyl)quinazoline-2,4(1*H*,3*H*)-dione (**5c**) Following the preparation protocol of Section 5.2.2.1, starting from 5-fluoroquinazoline-2,4(1*H*,3*H*)-dione (320 mg, 1.78 mmol), the title compound **5c** was obtained as gray solid (350 mg, 59.2%); m.p. > 250 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.71 (s, 1H), 8.18 (d, J = 5.4 Hz, 1H), 7.73-7.77 (m, 1H), 7.62 (dt, J_1 = 8.4 Hz, J_2 = 5.7 Hz, 1H), 7.54 (dd, J_1 = 11.1 Hz, J_2 = 8.4 Hz, 1H), 7.00-7.10 (m, 2H), 5.37 (s, 2H); HR-MS (ESI): *m/z*, calcd. for $C_{15}H_{10}N_3O_4F_2$ $[M+H]^+$ 334.0634,

Found: 334.0633.

5.2.2.4 6-Fluoro-1-(4-fluoro-3-nitrobenzyl)quinazoline-2,4(1*H*,3*H*)-dione (**5d**) Following the preparation protocol of Section 5.2.2.1, starting from 6-fluoroquinazoline-2,4(1*H*,3*H*)-dione (500 mg, 2.78 mmol), the title compound **5d** was obtained as white solid (450 mg, 48.6%); m.p. > 250 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.88 (s, 1H), 8.18 (dd, J_1 = 7.2 Hz, J_2 = 1.8 Hz, 1H), 7.70-7.76 (m, 2H), 7.50-7.59 (m, 2H), 7.34 (dd, J_1 = 9.0 Hz, J_2 = 3.9 Hz, 1H), 5.38 (s, 2H); HR-MS (ESI): *m*/*z*, calcd. for $C_{15}H_{10}N_3O_4F_2$ $[M+H]^+$ 334.0634, Found: 334.0632.

5.2.2.5 7-Fluoro-1-(4-fluoro-3-nitrobenzyl)quinazoline-2,4(1*H*,3*H*)-dione (**5e**) Following the preparation protocol of Section 5.2.2.1, starting from 7-fluoroquinazoline-2,4(1*H*,3*H*)-dione (150 mg, 0.84 mmol), the title compound **5e** was obtained as white solid (153 mg, 55.1%); m.p. > 250 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.81 (s, 1H), 8.18 (dd, J_1 = 6.9 Hz, J_2 = 1.8 Hz, 1H), 8.07 (dd, J_1 = 8.4 Hz, J_2 = 6.3 Hz, 1H), 7.75-7.78 (m, 1H), 7.54 (dd, J_1 = 11.1 Hz, J_2 = 8.7 Hz, 1H), 7.24 (dd, J_1 = 11.4 Hz, J_2 = 2.1 Hz, 1H), 7.11 (dt, J_1 = 8.4 Hz, J_2 = 2.1 Hz, 1H), 5.36 (s, 2H); HR-MS (ESI): *m*/*z*, calcd. for $C_{15}H_{10}N_3O_4F_2$ $[M+H]^+$ 334.0634, Found: 334.0634.

5.2.2.6 8-Fluoro-1-(4-fluoro-3-nitrobenzyl)quinazoline-2,4(1*H*,3*H*)-dione (**5f**) Following the preparation protocol of Section 5.2.2.1, starting from 8-fluoroquinazoline-2,4(1*H*,3*H*)-dione (700 mg, 3.89 mmol), the title compound **5f** was obtained as yellow solid (317 mg, 24.6%); m.p. > 250 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.92 (s, 1H), 8.10 (dd, J_1 = 7.2 Hz, J_2 = 1.8 Hz, 1H), 7.90 (dd, J_1 = 7.8 Hz, J_2 = 0.9 Hz , 1H), 7.73-7.76 (m, 1H), 7.50-7.61 (m, 2H), 7.27 (td, J_1 = 8.1 Hz, J_2 = 4.2 Hz, 1H), 5.38 (s, 2H); HR-MS (ESI): *m*/*z*, calcd. for $C_{15}H_{10}N_3O_4F_2$ $[M+H]^+$ 334.0634, Found: 334.0631.

5.2.3 Synthesis of 1-benzyl-quinazoline-2,4(1*H*,3*H*)-diones (6a-6f)

5.2.3.1 1-(3-Aminobenzyl)quinazoline-2,4(1*H*,3*H*)-dione (**6a**) The mixture of compound **5a** (120 mg, 0.40 mmol) and 10% Pd-C (60 mg) in methanol (25 mL) and ethanol (15 mL) was hydrogenated at room temperature and 1 atm for 30 min. Pd-C was filtered, The crude product obtained after concentration was purified with column chromatography to afford compound **6a** as white solid (106 mg, 98.2%); m.p. > 250 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.70 (s, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 7.6 Hz, 1H), 7.18-7.25 (m, 2H), 6.95 (t, J = 7.6 Hz, 1H), 6.39-6.44 (m, 2H), 6.36 (s, 1H), 5.15 (s, 2H), 5.05 (s, 2H); HR-MS (ESI): *m*/*z*, calcd. for $C_{15}H_{14}N_3O_2$ $[M+H]^+$ 268.1080, Found: 268.1076.

5.2.3.2 1-(3-Amino-4-fluorobenzyl)quinazoline-2,4(1*H*,3*H*)-dione (**6b**) Following the preparation protocol of Section 5.2.3.1, starting from **5b** (454 mg, 1.44 mmol), the title compound **6b** was obtained as white solid (290 mg, 70.6%); m.p. > 250 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.76 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 7.6 Hz, 1H), 6.88-7.33 (m, 7H), 5.23 (s, 2H); HR-MS (ESI): *m*/*z*, calcd. for $C_{15}H_{13}N_3O_2F$ $[M+H]^+$ 286.0986, Found: 286.0980.

5.2.3.3 1-(3-Amino-4-fluorobenzyl)-5-fluoroquinazoline-2,4(1*H*,3*H*)-dione (**6c**) Following the preparation protocol of Section 5.2.3.1, starting from **5c** (270 mg, 0.81 mmol), the title compound **6c** was obtained as white solid (220 mg, 89.6%); m.p. > 250 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.74 (s, 1H), 8.10 (dd, J_1 = 14.0 Hz, J_2 = 8.0 Hz, 1H), 7.12 (t, J = 10.0 Hz, 1H), 7.00-7.05 (m, 2H), 6.86-6.93 (m, 2H), 6.43 (brs, 2H), 5.21 (s, 2H); HR-MS (ESI): *m*/*z*, calcd. for $C_{15}H_{12}N_3O_2F_2$ $[M+H]^+$ 304.0892, Found: 304.0886.

5.2.3.4 1-(3-Amino-4-fluorobenzyl)-6-fluoroquinazoline-2,4(1*H*,3*H*)-dione (**6d**) Following the preparation protocol of Section 5.2.3.1, starting from **5d** (300 mg, 0.90 mmol), the title compound **6d** was obtained as white solid (230 mg, 84.3%); m.p. > 250 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.87 (s, 1H), 7.72 (dd, J_1 = 8.4 Hz, J_2 = 3.0 Hz, 1H), 7.56 (dt, J_1 = 8.7 Hz, J_2 = 3.0 Hz, 1H), 7.25 (dd, J_1 = 9.0 Hz, J_2 = 3.9 Hz, 1H), 6.92 (dd, J_1 = 11.4 Hz, J_2 = 8.4 Hz, 1H), 6.58 (d, J = 9.0 Hz, 1H), 6.46-6.48 (m, 1H), 5.15 (s, 2H), 5.12 (s, 2H); HR-MS (ESI): *m*/*z*, calcd. for $C_{15}H_{12}N_3O_2F_2$ $[M+H]^+$ 304.0892, Found: 304.0886.

5.2.3.5 1-(3-Amino-4-fluorobenzyl)-7-fluoroquinazoline-2,4(1*H*,3*H*)-dione (**6e**) Following the preparation protocol of Section 5.2.3.1, starting from **5e** (150 mg, 0.45 mmol), the title compound **6e** was obtained as yellow solid (120 mg, 87.9%); m.p. > 250 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.82 (s, 1H), 8.07 (dd, J_1 = 8.7 Hz, J_2 = 6.6 Hz, 1H), 7.04-7.14 (m, 3H), 6.77-6.85 (m, 5H), 5.19 (s, 2H); HR-MS (ESI): *m*/*z*, calcd. for $C_{15}H_{12}N_3O_2F_2$ $[M+H]^+$ 304.0892, Found: 304.0885.

5.2.3.6 1-(3-Amino-4-fluorobenzyl)-8-fluoroquinazoline-2,4(1*H*,3*H*)-dione (**6f**) Following the preparation protocol of Section 5.2.3.1, starting from **5f** (30 mg, 0.09 mmol), the title compound **6f** was obtained as yellow solid (20 mg, 73.3%); m.p. > 250 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.92 (s, 1H), 7.90 (d, J = 7.2 Hz, 1H), 7.56 (dd, J_1 = 14.0 Hz, J_2 = 8.0 Hz, 1H), 7.26 (m, 1H), 6.89 (t, J = 9.6 Hz, 1H), 6.56 (d, J = 8.4 Hz, 1H), 6.35 (m, 1H), 5.24 (s, 2H); HR-MS (ESI):

m/z, calcd. for $C_{15}H_{12}N_3O_2F_2$ $[M+H]^+$ 304.0892, Found: 304.0886.

5.2.4 Synthesis of compounds 7a-7s and 8t

5.2.4.1 *tert*-Butyl (2-((3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)amino)-2-oxoethyl)carbamate (**7a**) To a stired solution of (*tert*-butoxycarbonyl)glycine (162 mg, 0.92 mmol) in DMF was added EDC (176 mg, 0.92 mmol)、HOBt (125 mg, 0.92 mmol) and DMAP (10 mg, 0.077 mmol), then TEA (156 mg, 1.54 mmol) was added dropwise. The mixture was stired at room temperature for 30 min, then **6a** (205 mg, 0.77 mmol) was added, and the reaction mixture was continuously stired at room temperature overnight. The solution was evaporated to dryness and DCM (2 mL) was added to the residue, a crude product was obtained after filtration, and recrystallized with DCM to afford **7a** as white solid (200 mg, 61.2%); m.p. 209-211℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 9.87 (s, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.63 (t, J = 7.6 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.18-7.34 (m, 4H), 6.96-7.02 (m, 2H), 5.28 (s, 2H), 3.65 (d, J = 6.0 Hz, 2H), 1.36 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.22, 161.81, 155.86, 150.59, 140.82, 139.38, 136.97, 135.23, 129.14, 127.57, 122.70, 121.27, 117.82, 116.30, 115.82, 115.11, 77.80, 45.09, 43.71, 28.17; HR-MS (ESI): *m/z*, calcd. For $C_{22}H_{24}N_4NaO_5$ 447.1639 $[M+Na]^+$, Found: 447.1652.

5.2.4.2 *tert*-Butyl (2-((3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)amino)-2-oxoethyl)(methyl)carbamate (**7b**) To a stirred solution of *N*-(*tert*-butoxycarbonyl)-*N*-methylglycine (188 mg, 0.99 mmol) in DMF was added HBTU (372 mg, 0.98 mmol)、HOBt (133 mg, 0.98 mmol) and DMAP (6 mg, 0.049 mmol), then DIEA (127 mg, 0.98 mmol) was added dropwise. The mixture was stirred at room temperature for 5 min, then **6a** (130 mg, 0.49 mmol) was added, and the reaction mixture was continuously stirred at room temperature overnight. The solution was evaporated to dryness and DCM (2 mL) was added to the residue. After filtration a crude product was obtained, and recrystallized with DCM to afford **7b** as white solid (115 mg, 53.6%); m.p. 226-227 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.77 (s, 1H), 9.93 (s, 1H × 0.45), 9.87 (s, 1H × 0.55), 8.03 (d, J = 7.6 Hz, 1H), 7.64 (t, J = 7.6 Hz, 1H), 7.57 (t, J = 7.6 Hz, 1H), 7.19-7.35 (m, 4H), 7.00-7.05 (m, 1H), 5.29 (s, 2H), 3.92 (s, 2H × 0.45), 3.86 (s, 2H × 0.55), 2.85 (s, 3H × 0.45), 2.82 (s, 3H × 0.55), 1.40 (s, 9H × 0.45), 1.26 (s, 9H × 0.55); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): (167.71, 167.53), 161.82, (155.38, 155.08), 150.60, 140.82, 139.32, 137.02, 135.20, 129.17, 127.58, 122.72, (121.44, 121.26), (118.00, 117.76), (116.41, 116.26), 115.83, 115.12, (78.83, 78.61), (52.20, 51.36), 45.09, (35.70, 35.63), (28.02, 27.85); HR-MS (ESI): *m/z*, calcd. For $C_{23}H_{26}N_4NaO_5$ 461.1795 $[M+Na]^+$, Found: 461.1798.

5.2.4.3 *tert*-Butyl (*S*)-(1-((3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (**7c**) To a stirred solution of (*tert*-butoxycarbonyl)-(*S*)-valine (242 mg, 1.13 mmol) in DMF was added HATU (571 mg, 1.50 mmol)、HOBt (203 mg, 1.50 mmol) and DMAP (10 mg, 0.075 mmol) , then TEA (152 mg, 1.50 mmol) was added dropwise. The mixture was stirred at room temperature for 5 min, then **6a** (200 mg, 0.75 mmol) was added, and the reaction mixture was continuously stirred at room temperature overnight. The solution was evaporated to dryness and DCM (20 mL) was added to the residue. The mixture was then washed with $NaHCO_3$ (25 mL×2) and H_2O (25 mL). The organic layer was dried over anhydrous Na_2SO_4. After filtration and concentration, the crude product was obtained and purified with column chromatography on silica gel to afford compound **7c** as white solid (310 mg, 88.7%); m.p.178-180 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.76 (s, 1H), 9.94 (s, 1H), 8.03 (d, J = 7.6 Hz, 1H), 7.59-7.66 (m, 2H), 7.38 (s, 1H), 7.20-7.29 (m, 3H), 7.01 (d, J = 6.8 Hz, 1H), 6.83 (d, J = 8.0 Hz, 1H), 5.29 (s, 2H), 3.85-3.88 (m, 1H), 1.92-1.95 (m, 1H), 1.21-1.36 (m, 9H), 0.84-0.86 (d, J = 5.6 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 170.73, 161.80, 155.52, 150.58, 140.83, 139.30, 136.97, 135.22, 129.10, 127.57, 122.70, 121.34, 117.95, 116.48, 115.80, 115.10, 77.98, 60.53, 45.10, 30.26, 28.15, 19.18, 18.39; HR-MS (ESI): *m/z*, calcd. For $C_{25}H_{31}O_5N_4$ 467.2289 $[M+H]^+$, Found: 467.2287.

5.2.4.4 *tert*-Butyl (*R*)-2-((3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)carbamoyl) pyrrolidine-1-carboxylate (**7d**) Following the preparation protocol of Section 5.2.4.3, starting from **6a** (150 mg, 0.56 mmol) and (*tert*-butoxycarbonyl)-(*R*)-proline (181 mg, 0.84 mmol), the title compound **7d** was obtained as white solid (40 mg, 15.3%); m.p. 128-130 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):9.56 (brs, 1H), 8.75 (brs, 1H), 8.20 (d, J = 7.6 Hz, 1H), 7.54-7.56 (m, 2H), 7.39 (m, 1H), 7.20-7.27 (m, 2H), 7.12 (d, J = 7.6 Hz, 1H), 6.94 (m, 1H), 5.32 (s, 2H), 4.44 (m, 1H), 3.45-3.51 (m, 2H), 2.51 (m, 1H), 1.93 (m, 3H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.14, 161.78, 156.55, 150.68, 140.99, 139.08, 136.33, 135.55, 129.62, 128.66, 123.27, 121.54, 118.95, 117.63, 116.05, 114.98, 80.97, 60.54, 47.21, 46.51, 28.32, 27.10, 24.62; HR-MS (ESI): *m/z*, calcd. For $C_{25}H_{28}N_4O_5Na$ 487.1952 $[M+Na]^+$, Found: 487.1945.

5.2.4.5 *tert*-Butyl (*R*)-2-((5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl) carbamoyl)pyrrolidine-1-carboxylate **(7e)** Following the preparation protocol of Section 5.2.4.2, starting from **6b** (200 mg, 0.70 mmol) and (*tert*-butoxycarbonyl)-(*R*)-proline (227 mg, 1.05 mmol), the title compound **7e** was obtained as yelow solid (75 mg, 22.2%); m.p.215-216℃; ^{1}H NMR (300

MHz, $CDCl_3$) δ (ppm): 9.55 (brs, 1H), 8.71 (s, 1H), 8.45 (d, J = 6.0 Hz, 1H), 8.21 (d, J = 7.5 Hz, 1H), 7.58 (t, J = 7.5 Hz, 1H), 7.21-7.27 (m, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.01 (t, J = 9.3 Hz, 1H), 6.87 (m, 1H), 5.30 (s, 2H), 4.50 (m, 1H), 3.45 (m, 2H), 2.53 (m, 1H), 1.95 (m, 3H), 1.49 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.59, 161.75, 156.05, 151.71 (d, J_{CF} = 243.4 Hz), 150.64, 140.91, 135.59, 131.77, 128.76, 127.27, 123.33, 121.61, 119.87, 116.08, 115.40 (d, J_{CF} = 19.80 Hz), 114.88, 81.18, 60.61, 47.17, 46.19, 28.28, 27.21, 24.56; HR-MS (ESI): *m/z*, calcd. For $C_{25}H_{27}FN_4NaO_5$ 505.1858 $[M+Na]^+$, Found: 505.1860.

5.2.4.6 *tert*-Butyl (*S*)-2-((3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)carbamoyl) pyrrolidine-1-carboxylate (**7f**) Following the preparation protocol of Section 5.2.4.3, starting from **6a** (200 mg, 0.75 mmol) and (*tert*-butoxycarbonyl)-(*S*)-proline (242 mg, 1.13 mmol), the title compound **7f** was obtained as white solid (110 mg, 31.7%); m.p. 124-126 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.76 (s, 1H), 9.94 (s, 1H), 8.02 (s, 1H), 7.52-7.63 (m, 2H), 7.20-7.37 (m, 4H), 7.01-7.06 (m, 1H), 5.31 (s, 2H), 4.10-4.20 (m, 1H), 3.37 (m, 2H), 2.14 (m, 1H), 1.84 (m, 3H). 1.14-1.38 (m, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): (171.43, 171.10); 161.82, 153.07, 150.58, 140.82, 139.44, 136.95, (135.27, 135.16), 129.08, 127.57, 122.70, (121.52, 121.18), (118.18, 117.84), (116.56, 116.30), 115.83, 115.12, 78.35, 60.26, 51.97, 46.52, 45.13, 30.90, 27.80; HR-MS (ESI): *m/z*, calcd. For $C_{25}H_{28}N_4O_5Na$ 487.1952 $[M+Na]^+$, Found: 487.1944.

5.2.4.7 *tert*-Butyl (*S*)-2-((5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl) carbamoyl)pyrrolidine-1-carboxylate **(7g)** Following the preparation protocol of Section 5.2.4.2, starting from **6b** (200 mg, 0.70 mmol) and (*tert*-butoxycarbonyl)-(*S*)-proline (227 mg, 1.05 mmol), the title compound **7g** was obtained as yellow solid (35 mg, 10.4%); m.p. 211-212℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.56 (brs, 1H), 8.64 (s, 1H), 8.44 (m, 1H), 8.21 (d, J = 7.2 Hz, 1H), 7.56-7.59 (m, 1H), 7.23-7.27 (m, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.01 (m, 1H), 6.87 (m, 1H), 5.30 (s, 2H), 4.50 (m, 1H), 3.46 (m, 2H), 2.53 (m, 1H), 1.95 (m, 3H), 1.49 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 170.44, 161.43, 151.71 (d, J_{CF} = 243.5 Hz), 150.39, 140.92, 135.65, 131.60, 128.78, 127.22, 123.38, 121.50, 119.82, 116.06, 115.42 (d, J_{CF} = 21.1 Hz), 114.90, 81.20, 60.66, 47.19, 46.26, 28.30, 27.15, 24.59; HR-MS (ESI): *m/z*, calcd. For $C_{25}H_{27}FN_4NaO_5$ 505.1858 $[M+Na]^+$, Found: 505.1849.

5.2.4.8 *tert*-Butyl (*R*)-3-((3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)carbamoyl) pyrrolidine-1-carboxylate (**7h**) Following the preparation protocol of Section 5.2.4.2, starting from **6a** (186 mg, 0.70 mmol) and (*R*)-1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid (227 mg,

1.05 mmol), the title compound **7h** was obtained as white solid (125 mg, 65.2%); m.p. 244-245 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.66 (brs, 1H), 8.07-8.13 (m, 2H), 7.74 (m, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.41 (s, 1H), 7.22-7.32 (m, 3H), 7.04 (d, J = 7.8 Hz, 1H), 5.30 (s, 2H), 3.55-3.67 (m, 3H), 3.31-3.35 (m, 1H), 3.04-3.08 (m, 1H), 2.04-2.15 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 170.99, 161.80, 153.33, 150.58, 140.82, 139.51, 136.97, 135.22, 129.09, 127.57, 122.70, 121.40, 117.97, 116.43, 115.82, 115.11, 78.27, 48.27, 45.37, 45.11, 44.27, 43.37, 28.13; HR-MS (ESI): *m/z*, calcd. For $C_{25}H_{28}N_4O_5Na$ 487.1952 $[M+Na]^+$, Found: 487.1946.

5.2.4.9 *tert*-Butyl (*R*)-3-((5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl) carbamoyl)pyrrolidine-1-carboxylate (**7i**) Following the preparation protocol of Section 5.2.4.3, starting from **6b** (170 mg, 0.53 mmol) and (*R*)-1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid (173 mg, 0.84 mmol), the title compound **7i** was obtained as white solid (153 mg, 68.0%); m.p. 188-190℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.74 (s, 1H), 8.44 (d, J = 6.8 Hz, 1H), 8.21 (d, J = 8.0 Hz, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.52 (s, 1H), 7.23 (d, J = 7.2 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H), 7.00-7.06 (m, 1H), 6.91 (m, 1H), 5.30 (s, 2H), 3.69-3.75 (m, 1H), 3.58-3.64 (m, 2H), 3.37-3.44 (m, 1H), 3.03-3.11 (m, 1H), 2.17-2.30 (m, 2H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): (171.52, 171.36), 161.77, 153.34, 152.90 (d, J_{CF} = 243.8 Hz), 150.64, 140.73, 135.22, 132.52 (d, J_{CF} = 3.1 Hz), 127.62, 126.16 (d, J_{CF} = 12.1 Hz), 123.49, 122.75, 122.39, 115.90, 115.67 (d, J_{CF} = 19.9 Hz), 115.04, 78.31, (48.34, 48.25), (45.38, 45.20), 44.60, (43.70, 42.80), (29.21, 28.38), 28.15; HR-MS (ESI): *m/z*, calcd. For $C_{25}H_{27}FN_4NaO_5$ 505.1858 $[M+Na]^+$, Found: 505.1847.

5.2.4.10 *tert*-Butyl (*S*)-3-((3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)-carbamoyl)pyrrolidine-1-carboxylate (**7j**) Following the preparation protocol of Section 5.2.4.2, starting from **6a** (186 mg, 0.70 mmol) and (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid (227 mg, 1.05 mmol), the title compound **7j** was obtained as white solid (124 mg, 65.2%); m.p. 227-228 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.74 (s, 1H), 8.16 (s, 1H), 8.10 (d, J = 7.2 Hz, 1H), 7.74 (m, 1H), 7.59 (m, 1H), 7.40 (s, 1H), 7.20-7.26 (m, 3H), 7.03 (m, 1H), 5.28 (s, 2H), 3.05-3.65 (m, 5H), 2.02-2.14 (m, 2H), 1.37-1.43 (m, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 170.82, 161.89, 154.37, 151.27, 140.91, 138.88, 136.20, 135.77, 129.68, 128.58, 123.55, 122.52, 119.43, 118.02, 116.08, 114.94, 79.53, 48.69, 46.71, 46.49, 28.48; HR-MS (ESI): *m/z*, calcd. For $C_{25}H_{28}N_4O_5Na$ 487.1952 $[M+Na]^+$, Found: 487.1944.

5.2.4.11 *tert*-Butyl (*S*)-3-((5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl) carbamoyl)pyrrolidine-1-carboxylate (**7k**) Following the preparation protocol of Section 5.2.4.3, starting from **6b** (170 mg, 0.53 mmol) and (*S*)-1-(tert-butoxycarbonyl)pyrrolidine-3-carboxylic acid (173 mg, 0.84 mmol), the title compound **7k** was obtained as yellow solid (145 mg, 64.4%); m.p. 209-211℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.76 (s, 1H), 8.43 (d, J = 6.8 Hz, 1H), 8.21 (d, J = 7.6 Hz, 1H), 7.60 (t, J = 7.6 Hz, 1H), 7.53 (s, 1H), 7.22-7.27 (m, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.04 (t, J = 9.2 Hz, 1H), 6.91 (m, 1H), 5.30 (s, 2H), 3.59-3.72 (m, 3H), 3.37-3.45 (m, 1H), 3.05-3.10 (m, 1H), 2.21-2.25 (m, 2H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): (171.52, 171.36), 161.77, 153.34, 152.93 (d, J_{CF} = 243.8 Hz), 150.64, 140.73, 135.22, 132.52 (d, J_{CF} = 3.0 Hz), 127.62, 126.16 (d, J_{CF} = 12.1 Hz), 123.48, 122.75, 122.40, 115.90, 115.68 (d, J_{CF} = 19.9 Hz), 115.04, 78.31, (48.34, 48.25), (45.20, 44.95), 43.70, (42.79, 42.41), (29.21, 28.38), 28.15; HR-MS (ESI): *m/z*, calcd. For $C_{25}H_{27}FN_4NaO_5$ 505.1858 $[M+Na]^+$, Found: 505.1850.

5.2.4.12 *tert*-Butyl 3-((3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl) carbamoyl) piperidine-1-carboxylate (**7l**) Following the preparation protocol of Section 5.2.4.3, starting from **6a** (200 mg, 0.75 mmol) and 1-(*tert*-butoxycarbonyl)piperidine-3-carboxylic acid (260 mg, 1.13 mmol), the title compound **7l** was obtained as white solid (255 mg, 71.0%); m.p. 136-138 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.77 (s, 1H), 9.95 (s, 1H), 8.03 (d, J = 7.5 Hz, 1H), 7.56-7.67 (m, 2H), 7.38 (s, 1H), 7.19-7.27 (m, 3H), 7.01 (d, J = 6.9 Hz, 1H), 5.28 (s, 2H), 3.81-3.95 (m, 2H), 2.69-2.77 (m, 2H), 2.38 (m, 1H), 1.85-1.90 (m, 1H), 1.50-1.65 (m, 2H), 1.37 (m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 172.55, 162.51, 154.48, 151.29, 141.53, 140.33, 137.62, 135.92, 129.74, 128.27, 123.40, 121.96, 118.58, 117.07, 116.52, 115.81, 79.37, 46.00, 45.83, 43.61, 28.71, 28.18; HR-MS (ESI): *m/z*, calcd. For $C_{26}H_{30}N_4O_5Na$ 501.2108 $[M+Na]^+$, Found: 501.2101.

5.2.4.13 *tert*-Butyl 3-((5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl) carbamoyl)piperidine-1-carboxylate (**7m**) Following the preparation protocol of Section 5.2.4.3, starting from **6b** (180 mg, 0.56 mmol) and 1-(*tert*-butoxycarbonyl)piperidine-3-carboxylic acid (193 mg, 0.84 mmol), the title compound **7m** was obtained as white solid (190 mg, 60.6%); m.p. 188-190℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.85 (s, 1H), 8.41 (d, J = 5.7 Hz, 1H), 8.21 (d, J = 7.2 Hz, 1H), 7.56-7.62 (m, 1H), 7.22-7.27 (m, 1H), 7.13 (d, J = 8.4 Hz, 1H), 6.98-7.05 (m, 1H), 6.88 (m, 1H), 5.29 (s, 2H), 4.12-4.18 (m, 1H), 3.88-3.93 (m, 1H), 3.14-3.22 (m, 1H), 2.95 (m, 1H), 2.52 (m, 1H), 1.67-2.00 (m, 3H), 1.46-1.55 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 171.87, 161.77, 154.89, 151.79 (d, J_{CF} = 242.8 Hz), 150.69, 140.87, 135.62, 131.84 (d, J_{CF} = 2.6

Hz), 128.76, 126.59 (d, J_{CF} = 10.8 Hz), 123.37, 122.07 (d, J_{CF} = 7.8 Hz), 120.40, 116.10, 115.43 (d, J_{CF} = 19.8 Hz), 114.81, 80.21, 77.20, 46.14, 46.02, 44.06, 28.37, 27.70, 24.05; HR-MS (ESI): *m/z*, calcd. For $C_{26}H_{29}FN_4NaO_5$ 519.2014 $[M+Na]^+$, Found: 519.2007.

5.2.4.14 *tert*-Butyl 4-((3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)carbamoyl) piperidine-1-carboxylate (**7n**) Following the preparation protocol of Section 5.2.4.2, starting from **6a** (200 mg, 0.75 mmol) and 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (260 mg, 1.13 mmol), the title compound **7n** was obtained as white solid (215 mg, 66.6%); m.p. 224-226 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.77 (s, 1H), 9.87 (s, 1H), 8.03 (d, J = 7.2 Hz, 1H), 7.58-7.65 (m, 2H), 7.37 (s, 1H), 7.19-7.25 (m, 3H), 7.00 (d, J = 6.8 Hz, 1H), 5.27 (s, 2H), 3.95-3.98 (m, 2H), 2.72 (m, 2H), 2.44-2.50 (m, 1H), 1.70-1.74 (m, 2H), 1.40 (m, 11H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.01, 161.81, 153.81, 150.58, 140.82, 139.74, 136.90, 135.21, 129.03, 127.60, 122.69, 121.19, 117.86, 116.31, 115.82, 115.10, 78.60, 45.72, 45.12, 42.60, 28.04; HR-MS (ESI): *m/z*, calcd. For $C_{26}H_{30}N_4O_5Na$ 501.2108 $[M+Na]^+$, Found: 501.2100.

5.2.4.15 *tert*-Butyl 4-((5-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl) carbamoyl)piperidine-1-carboxylate (**7o**) Following the preparation protocol of Section 5.2.4.3, starting from **6b** (170 mg, 0.53 mmol)and 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (214 mg, 0.93 mmol), the title compound **7o** was obtained as white solid (200 mg, 65.0%); m.p. 189-191℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 9.68 (s, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.82 (d, J = 6.8 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.16-7.27 (m, 3H), 7.07 (m, 1H), 5.26 (s, 2H), 3.95-3.98 (m, 2H), 2.60-2.74 (m, 3H), 1.73-1.77 (m, 2H), 1.40-1.48 (m, 11H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.37, 161.77, 153.83, 153.23 (d, J_{CF} = 243.6 Hz), 150.63, 140.73, 135.23, 132.44 (d, J_{CF} = 3.0 Hz), 127.61, 126.38 (d, J_{CF} = 12.1 Hz), 123.25 (d, J_{CF} = 7.3 Hz), 122.75, 122.22, 115.88, 115.60 (d, J_{CF} = 20.0 Hz), 115.05, 78.61, 44.61, 40.13, 28.18, 28.06; HR-MS (ESI): *m/z*, calcd. For $C_{26}H_{29}FN_4NaO_5$ 519.2014 $[M+Na]^+$, Found: 519.2007.

5.2.4.16 *tert*-Butyl 4-((2-fluoro-5-((5-fluoro-2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) phenyl)carbamoyl)piperidine-1-carboxylate (**7p**) Following the preparation protocol of Section 5.2.4.3, starting from **6c** (220 mg, 0.66 mmol) and 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (227 mg, 0.99 mmol), the title compound **7p** was obtained as white solid (90 mg, 24.1%); m.p. 233-235 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.43-8.46 (m, 2H), 7.51 (dt, J_1 = 8.1 Hz, J_2 = 6.0 Hz, 1H), 7.46 (m, 1H), 7.04 (dd, J_1 = 10.5 Hz, J_2 = 8.4 Hz, 1H), 6.87-6.95 (m, 3H), 5.27 (s, 2H), 4.18 (m, 2H), 2.76-2.85 (m, 2H), 2.41-2.49 (m, 1H), 1.90-1.94 (m, 2H), 1.67-1.81 (m, 2H),

1.47 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.38, 161.72 (d, J_{CF} = 260.1 Hz), 158.97 (d, J_{CF} = 3.1 Hz), 153.84, 152.89 (d, J_{CF} = 243.6 Hz), 150.36, 142.52 (d, J_{CF} = 2.5 Hz), 135.92 (d, J_{CF} = 11.2 Hz), 132.14 (d, J_{CF} = 3.2 Hz), 126.38 (d, J_{CF} = 12.1 Hz), 123.17 (d, J_{CF} = 7.6 Hz), 122.16, 115.61 (d, J_{CF} = 19.9 Hz), 111.17, 110.12 (d, J_{CF} = 20.6 Hz), 105.50 (d, J_{CF} = 8.7 Hz), 78.62, 45.25, 41.87, 28.18, 28.06; HR-MS (ESI): *m/z*, calcd. For $C_{26}H_{28}F_2N_4NaO_5$ 537.1920 $[M+Na]^+$, Found: 537.1914.

5.2.4.17 *tert*-Butyl 4-((2-fluoro-5-((6-fluoro-2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) phenyl)carbamoyl)piperidine-1-carboxylate (**7q**) Following the preparation protocol of Section 5.2.4.3, starting from **6d** (400 mg, 1.32 mmol) and 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (454 mg, 1.98 mmol), the title compound **7q** was obtained as white solid (210 mg, 30.9%); m.p. 182-184 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.89 (s, 1H), 8.44 (d, J = 6.6 Hz, 1H), 7.87 (dd, J_1 = 7.8 Hz, J_2 = 2.7 Hz, 1H), 7.26-7.34 (m, 1H), 7.12 (dd, J_1 = 9.0 Hz, J_2 = 3.6 Hz, 1H), 7.00-7.08 (m, 1H), 6.88 (m, 1H), 5.28 (s, 2H), 4.17-4.22 (m, 2H), 2.76-2.86 (m, 2H), 2.42-2.50 (m, 1H), 1.90-1.95 (m, 2H), 1.68-1.81 (m, 2H), 1.47 (m, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.40, 160.98 (d, J_{CF} = 2.5 Hz), 157.51 (d, J_{CF} = 240.1 Hz), 153.85, 152.91 (d, J_{CF} = 243.8 Hz), 150.41, 137.47, 132.23 (d, J_{CF} = 3.0 Hz), 126.41 (d, J_{CF} = 12.0 Hz), 123.23 (d, J_{CF} = 7.4 Hz), 122.73 (d, J_{CF} = 20.0 Hz), 122.22, 117.54 (d, J_{CF} = 7.6 Hz), 117.31 (d, J_{CF} = 7.6 Hz), 115.63 (d, J_{CF} = 19.9 Hz), 112.82 (d, J_{CF} = 23.7 Hz), 78.64, 44.88, 41.88, 28.19, 28.07; HR-MS (ESI): *m/z*, calcd. For $C_{26}H_{28}F_2N_4NaO_5$ 537.1920 $[M+Na]^+$, Found: 537.1913.

5.2.4.18 *tert*-Butyl 4-((2-fluoro-5-((7-fluoro-2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) phenyl)carbamoyl)piperidine-1-carboxylate (**7r**) Following the preparation protocol of Section 5.2.4.3, starting from **6e** (350 mg, 1.16 mmol) and 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (399 mg, 1.74 mmol), the title compound **7r** was obtained as white solid (200 mg, 33.7%); m.p. 115-117 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.82 (s, 1H), 9.69 (s, 1H), 8.04-8.10 (m, 1H), 7.83 (d, J_1 = 7.5 Hz, 1H), 7.07-7.24 (m, 4H), 5.26 (s, 2H), 3.95-3.99 (m, 2H), 2.64-2.74 (m, 3H), 1.73-1.78 (m, 2H), 1.40-1.46 (m, 11H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.38, 166.09 (d, J_{CF} = 249.3 Hz), 160.97, 153.83, 152.93 (d, J_{CF} = 243.8 Hz), 150.67, 142.92 (d, J_{CF} = 12.3 Hz), 132.06 (d, J_{CF} = 3.1 Hz), 130.73 (d, J_{CF} = 11.2 Hz), 126.38 (d, J_{CF} = 12.1 Hz), 123.38 (d, J_{CF} = 7.5 Hz), 122.39, 115.63 (d, J_{CF} = 19.8 Hz), 112.80, 110.48 (d, J_{CF} = 22.8 Hz), 102.26 (d, J_{CF} = 27.7 Hz), 79.09, 44.88, 41.88, 28.18, 28.06; HR-MS (ESI): *m/z*, calcd. For $C_{26}H_{28}F_2N_4NaO_5$ 537.1920 $[M+Na]^+$, Found: 537.1912.

5.2.4.19 *tert*-Butyl 4-((2-fluoro-5-((8-fluoro-2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl) phenyl)carbamoyl)piperidine-1-carboxylate (**7s**) Following the preparation protocol of Section 5.2.4.3, starting from **6f** (300 mg, 0.99 mmol) and 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (340 mg, 1.49 mmol), the title compound **7s** was obtained as white solid (150 mg, 29.5%); m.p. 143-145 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.56 (s, 1H), 8.37 (d, J = 6.9 Hz, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.32-7.41 (m, 2H), 7.20 (td, J_1 = 7.8 Hz, J_2 = 3.6 Hz, 1H), 6.99-7.06 (m, 1H), 6.91 (m, 1H), 5.47 (s, 2H), 4.16-4.21 (m, 2H), 2.75-2.84 (m, 2H), 2.38-2.46 (m, 1H), 1.88-1.93 (m, 2H), 1.67-1.80 (m, 2H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.38, 160.97 (d, J_{CF} = 2.6 Hz), 153.83, 151.43, 150.73, 150.33, 149.10 (d, J_{CF} = 245.4 Hz), 133.81, 129.68 (d, J_{CF} = 6.9 Hz), 126.14 (d, J_{CF} = 12.2 Hz), 123.95 (d, J_{CF} = 2.7 Hz), 123.87 (d, J_{CF} = 8.1 Hz), 122.91 (d, J_{CF} = 23.4 Hz), 122.36 (d, J_{CF} = 7.4 Hz), 121.47, 119.08, 115.37 (d, J_{CF} = 19.8 Hz), 78.61, 47.98, 47.85, 41.88, 28.19, 28.06; HR-MS (ESI): *m/z*, calcd. For $C_{26}H_{28}F_2N_4NaO_5$ 537.1920 $[M+Na]^+$, Found: 537.1912.

5.2.4.20 2-(Dimethylamino)-*N*-(3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl) acetamide (**8t**) Following the preparation protocol of Section 5.2.4.2, starting from **6a** (200 mg, 0.75 mmol) and dimethylglycine (116 mg, 1.12 mmol), the title compound **8t** was obtained as yellow solid (210 mg, 79.6%); m.p. 251-252℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.79 (s, 1H), 10.47 (s, 1H), 9.71 (brs, 1H), 8.04 (d, J = 7.5 Hz, 1H), 7.65 (t, J = 7.8 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.16-7.39 (m, 5H), 5.32 (s, 2H), 4.04 (s, 2H), 2.82 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.13, 161.85, 150.60, 140.79, 138.32, 137.36, 135.30, 129.44, 127.64, 122.81, 122.67, 118.25, 116.50, 115.82, 115.10, 58.03, 44.99, 43.45; HR-MS (ESI): *m/z*, calcd. For $C_{19}H_{21}N_4O_3$ 353.1608 $[M+Na]^+$, Found: 353.1603.

5.2.5 Synthesis of compunds 8a-8s

5.2.5.1 2-Amino-*N*-(3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)acetamide 2,2,2-trifluoroacetate (**8a**) To a stired solution of **7a** (100 mg, 0.24 mmol) in DCM (2 mL) was added TFA (2 mL) dropwise, the reaction mixture was then allowed to stir at room temperature overnight. DCM and excessive TFA was then removed under reduced pressure, ethyl ether (2 mL) was then added to the residue. After filtration and dried under vacuum condition, compound **8a** was obtained as white solid without further purification (83 mg, 80.4%); m.p. 216-218 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.78 (s, 1H), 10.40 (s, 1H), 8.10 (brs, 3H), 8.04 (d, J = 7.6 Hz, 1H), 7.64 (t, J = 7.6 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.39 (s, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.21-7.28 (m, 2H), 7.15 (d, J = 7.6 Hz, 1H), 5.32 (s, 2H), 3.71 (s, 2H); ^{13}C NMR (100 MHz,

DMSO-d_6) δ (ppm): 164.83, 161.86, 150.60, 140.81, 138.73, 137.29, 135.30, 129.41, 127.63, 122.79, 122.23, 117.86, 116.19, 115.82, 115.12, 45.01, 40.96; HR-MS (ESI): *m/z*, calcd. For $C_{17}H_{17}N_4O_3$ 325.1295 $[M+H]^+$, Found: 325.1290.

5.2.5.2 *N*-(3-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)-2-(methylamino)-acetamide 2,2,2-trifluoroacetate (**8b**) Following the preparation protocol of Section 5.2.5.1, starting from **7b** (40 mg, 0.09 mmol), the title compound **8b** was obtained as white solid (33 mg, 83.1%); m.p.> 250 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.78 (s, 1H), 10.46 (s, 1H), 8.82 (brs, 2H), 8.04 (d, *J* = 7.6 Hz, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.33-7.38 (m, 2H), 7.21-7.28 (m, 2H), 7.16 (d, *J* = 7.2 Hz, 1H), 5.32 (s, 2H), 3.86 (s, 2H), 2.59 (s, 3H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.96, 161.85, 150.60, 140.79, 138.52, 137.32, 135.29, 129.42, 127.63, 122.79, 122.41, 117.98, 116.30, 115.81, 115.12, 49.64, 44.97, 32.67; HR-MS (ESI): *m/z*, calcd. For $C_{18}H_{19}N_4O_3$ 339.1452 $[M+H]^+$, Found: 339.1440.

5.2.5.3 (*S*)-2-Amino-*N*-(3-((2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)-3-methyl-butanamide 2,2,2-trifluoroacetate (**8c**) Following the preparation protocol of Section 5.2.5.1, starting from **7c** (160 mg, 0.34 mmol), the title compound **8c** was obtained as white solid (156 mg, 94.7%); m.p. 167-169 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.78 (s, 1H), 10.44 (s, 1H), 8.20 (brs, 3H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.42 (s, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.22-7.28 (m, 2H), 7.15 (d, *J* = 7.6 Hz, 1H), 5.32 (s, 2H), 3.68 (m, 1H), 2.11-2.16 (m, 1H), 0.94 (t, *J* = 7.6 Hz, 6H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.93, 161.85, 150.61, 140.83, 138.48, 137.32, 135.29, 129.37, 127.64, 122.80, 122.47, 118.35, 116.71, 115.82, 115.12, 58.23, 45.05, 29.86, 18.43, 17.60; HR-MS (ESI): *m/z*, calcd. For $C_{20}H_{23}O_3N_4$ 367.1765 $[M+H]^+$, Found: 367.1761.

5.2.5.4 (*R*)-*N*-(3-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**8d**) Following the preparation protocol of Section 5.2.5.1, starting from **7d** (65 mg, 0.14 mmol), the title compound **8d** was obtained as white solid (50 mg, 74.7%); m.p. 145-147 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.77 (brs, 1H), 10.50 (s, 1H), 8.96 (brs, 2H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.64 (t, *J* = 7.6 Hz, 1H), 7.51 (d, *J* = 8.4Hz, 1H), 7.43 (s, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.20-7.27 (m, 2H), 7.17 (d, *J* = 7.6 Hz, 1H), 5.31 (s, 2H), 4.23-4.27 (m, 1H), 3.17-3.29 (m, 2H), 2.29-2.37 (m, 1H), 1.85-1.97 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 167.35, 162.32, 151.07, 141.28, 139.10, 137.83, 135.77, 129.86, 128.10, 123.27, 123.05, 118.68, 116.95, 116.29, 115.58, 60.17, 46.23, 45.53, 30.00, 24.05; HR-MS (ESI):

m/z, calcd. For $C_{20}H_{21}N_4O_3$ 365.1608 $[M+H]^+$, Found: 365.1599.

5.2.5.5 (*R*)-*N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**8e**) Following the preparation protocol of Section 5.2.5.1, starting from **7e** (40 mg, 0.08 mmol), the title compound **8e** was obtained as white solid (30 mg, 92.4%); m.p. 208-210℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.76 (s, 1H), 10.37 (s, 1H), 9.29 (brs, 1H), 8.65 (brs, 1H), 8.03 (d, J = 7.2 Hz, 1H), 7.79 (d, J = 6.3 Hz, 1H), 7.63-7.66 (m, 1H), 7.27-7.32 (m, 4H), 5.30 (s, 2H), 4.40 (m, 1H), 3.23 (m, 2H), 2.35 (m, 1H), 1.90 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 167.56, 161.82, 152.73 (d, J_{CF} = 244.0 Hz), 150.60, 140.71, 135.30, 132.83 (d, J_{CF} = 3.2 Hz), 127.66, 125.37 (d, J_{CF} = 12.0 Hz), 124.59 (d, J_{CF} = 7.6 Hz), 122.83, 121.48, 116.05, 115.85, 115.04, 59.51, 45.82, 44.57, 29.70, 23.56; HR-MS (ESI): *m/z*, calcd. For $C_{20}H_{20}N_4O_3F$ 383.1514 $[M+H]^+$, Found: 383.1507.

5.2.5.6 (*S*)-*N*-(3-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**8f**) Following the preparation protocol of Section 5.2.5.1, starting from **7f** (65 mg, 0.14 mmol), the title compound **8f** was obtained as white solid (50 mg, 74.7%); m.p. 138-140 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.77 (s, 1H), 10.52 (s, 1H), 9.40 (brs, 1H), 8.65 (brs, 1H), 8.04 (d, J = 7.2 Hz, 1H), 7.65 (t, J = 7.2 Hz, 1H), 7.51 (d, J = 7.2 Hz, 1H), 7.43 (s, 1H), 7.36 (t, J = 7.6 Hz, 1H), 7.21-7.28 (m, 2H), 7.17 (d, J = 7.2 Hz, 1H), 5.32 (s, 2H), 4.27 (m, 1H), 3.25 (m, 2H), 2.32-2.34 (m, 1H), 1.91 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.88, 161.85, 150.61, 140.81, 138.64, 137.35, 135.30, 129.38, 127.63, 122.80, 122.56, 118.21, 116.49, 115.82, 115.11, 59.69, 45.75, 45.07, 29.54, 23.58; HR-MS (ESI): *m/z*, calcd. For $C_{20}H_{21}N_4O_3$ 365.1608 $[M+H]^+$, Found: 365.1604.

5.2.5.7 (*S*)-*N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl)pyrrolidine-2-carboxamide 2,2,2-trifluoroacetate (**8g**) Following the preparation protocol of Section 5.2.5.1, starting from **7g** (20 mg, 0.04 mmol), the title compound **8g** was obtained as white solid (18 mg, 87.4%); m.p. 209-211℃; ^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 8.06 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 7.2 Hz, 1H), 7.57 (t, J = 7.6 Hz, 1H), 7.19-7.23 (m, 2H), 7.10-7.15 (m, 2H), 5.30 (s, 2H), 4.37-4.41 (m, 1H), 3.25-3.41 (m, 2H), 2.41-2.49 (m, 1H), 1.98-2.11 (m, 3H); ^{13}C NMR (150 MHz, CD3OD) δ (ppm): 168.36, 163.88, 154.34 (d, J_{CF} = 244.8 Hz), 152.44, 142.23, 136.41, 133.81 (d, J_{CF} = 3.5 Hz), 128.93, 126.68 (d, J_{CF} = 12.0 Hz), 125.44 (d, J_{CF} = 7.8 Hz), 124.12, 122.80, 117.22, 116.76 (d, J_{CF} = 20.1 Hz), 116.12, 61.46, 47.29, 46.18, 30.94, 24.86; HR-MS (ESI): *m/z*, calcd. For $C_{20}H_{20}N_4O_3F$ 383.1514 $[M+H]^+$, Found: 383.1504.

5.2.5.8 (*R*)-*N*-(3-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)pyrrolidine-3-carboxamide 2,2,2-trifluoroacetate (**8h**) Following the preparation protocol of Section 5.2.5.1, starting from **7h** (80 mg, 0.17 mmol), the title compound **8h** was obtained as white solid (72 mg, 87.4%); m.p. 133-135 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.78 (s, 1H), 10.20 (s, 1H), 8.95 (brs, 2H), 8.03 (d, J = 7.5 Hz, 1H), 7.64 (t, J = 7.5 Hz, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.39 (s, 1H), 7.19-7.33 (m, 3H), 7.09 (d, J = 6.9 Hz, 1H), 5.29 (s, 2H), 3.20-3.42 (m, 5H), 2.17-2.24 (m, 1H), 1.98-2.05 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 170.40, 161.86, 150.63, 140.84, 139.37, 137.10, 135.27, 129.19, 127.62, 122.77, 121.83, 118.11, 116.48, 115.85, 115.12, 46.88, 45.15, 44.88, 43.02, 28.89; HR-MS (ESI): *m/z*, calcd. For $C_{20}H_{21}N_4O_3$ 365.1608 $[M+H]^+$, Found: 365.1603.

5.2.5.9 (*R*)-*N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl)pyrrolidine-3-carboxamide 2,2,2-trifluoroacetate (**8i**) Following the preparation protocol of Section 5.2.5.1, starting from **7i** (40 mg, 0.08 mmol), the title compound **8i** was obtained as yellow solid (35 mg, 85.0%); m.p. 100-102℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 10.01 (s, 1H), 8.85 (brs, 2H), 8.02 (m, 1H), 7.79 (m, 1H), 7.65 (m, 1H), 7.18-7.25 (m, 4H), 5.28 (s, 2H), 3.19-3.36 (m, 5H), 2.22 (m, 1H), 2.02 (m, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 170.90, 161.78, 152.84 (d, J_{CF} = 244.2 Hz), 150.60, 140.72, 135.24, 132.59, 127.63, 125.96 (d, J_{CF} = 11.9 Hz), 123.97 (d, J_{CF} = 7.7 Hz), 122.79, 121.92, 115.86, 115.75 (d, J_{CF} = 20.0 Hz), 115.02, 46.89, 44.95, 42.39, 41.45, 28.89; HR-MS (ESI): *m/z*, calcd. For $C_{20}H_{20}N_4O_3F$ 383.1514 $[M+H]^+$, Found: 383.1505.

5.2.5.10 (*S*)-*N*-(3-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)pyrrolidine-3-carboxamide 2,2,2-trifluoroacetate (**8j**) Following the preparation protocol of Section 5.2.5.1, starting from **7j** (67 mg, 0.14 mmol), the title compound **8j** was obtained as white solid (65 mg, 94.2%); m.p. 132-134 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.78 (s, 1H), 10.20 (s, 1H), 8.90 (brs, 2H), 8.04 (d, J = 7.2 Hz, 1H), 7.56-7.65 (m, 2H), 7.39 (s, 1H), 7.20-7.31 (m, 3H), 7.10 (m, 1H), 5.30 (s, 2H), 3.20-3.36 (m, 5H), 2.02-2.20 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 170.41, 161.87, 150.63, 140.84, 139.37, 137.11, 135.28, 129.19, 127.62, 122.77, 121.83, 118.10, 116.46, 115.85, 115.13, 46.88, 45.14, 44.88, 43.02, 28.90; HR-MS (ESI): *m/z*, calcd. For $C_{20}H_{21}N_4O_3$ 365.1608 $[M+H]^+$, Found: 365.1597.

5.2.5.11 (*S*)-*N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl)pyrroli-

dine-3-carboxamide 2,2,2-trifluoroacetate (**8k**) Following the preparation protocol of Section 5.2.5.1, starting from **7k** (50 mg, 0.10 mmol), the title compound **8k** was obtained as white solid (48 mg, 93.3%); m.p. 130-132℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 10.02 (s, 1H), 8.92 (brs, 2H), 8.02 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 6.4 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.17-7.27 (m, 4H), 5.28 (s, 2H), 3.37 (m, 3H), 3.20 (m, 2H), 2.22-2.24 (m, 1H), 2.01-2.03 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 170.91, 161.81, 152.88 (d, J_{CF} = 244.0 Hz), 150.63, 140.74, 135.27, 132.61 (d, J_{CF} = 3.1 Hz), 127.64, 126.01 (d, J_{CF} = 12.0 Hz), 123.95 (d, J_{CF} = 7.5 Hz), 122.80, 122.01, 115.88, 115.67, 115.05, 46.85, 44.93, 44.62, 42.44, 28.93; HR-MS (ESI): m/z, calcd. For $C_{20}H_{20}N_4O_3F$ 383.1514 $[M+H]^+$, Found: 383.1508.

5.2.5.12 *N*-(3-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)piperidine-3-carboxamide 2,2,2-trifluoroacetate (**8l**) Following the preparation protocol of Section 5.2.5.1, starting from **7l** (100 mg, 0.21 mmol), the title compound **8l** was obtained as white solid (90 mg, 87.4%); m.p. 135-137 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.77 (s, 1H), 10.14 (s, 1H), 8.69 (brs, 2H), 8.03 (d, J = 7.6 Hz, 1H), 7.64 (t, J = 7.6 Hz, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.38 (s, 1H), 7.19-7.31 (m, 3H), 7.06 (d, J = 7.6 Hz, 1H), 5.28 (s, 2H), 3.26-3.30 (m, 1H), 3.02-3.19 (m, 2H), 2.89 (m, 1H), 2.76 (m, 1H), 1.96-1.99 (m, 1H), 1.79 (m, 1H), 1.58-1.66 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 170.77, 161.84, 150.61, 140.84, 139.31, 137.07, 135.26, 129.16, 127.61, 122.76, 121.73, 118.03, 116.42, 115.83, 115.13, 45.66, 45.11, 44.05, 42.95, 26.14, 21.13; HR-MS (ESI): m/z, calcd. For $C_{21}H_{23}N_4O_3$ 379.1765 $[M+H]^+$, Found: 379.1757.

5.2.5.13 *N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl)piperidine-3-carboxamide 2,2,2-trifluoroacetate (**8m**) Following the preparation protocol of Section 5.2.5.1, starting from **7m** (130 mg, 0.26 mmol), the title compound **8m** was obtained as white solid (125 mg, 93.5%); m.p. 131-133℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 9.97 (s, 1H), 8.62 (brs, 2H), 8.03 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 6.8 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.15-7.27 (m, 4H), 5.28 (s, 2H), 3.27-3.31 (m, 1H), 3.15-3.19 (m, 1H), 2.91-3.05 (m, 3H), 1.99-2.02 (m, 1H), 1.80 (m, 1H), 1.53-1.67 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 171.18, 161.81, 153.04 (d, J_{CF} = 244.1 Hz), 150.65, 140.74, 135.27, 132.57 (d, J_{CF} = 3.0 Hz), 127.65, 125.92 (d, J_{CF} = 12.1 Hz), 123.92 (d, J_{CF} = 7.4 Hz), 122.80, 122.34, 115.90, 115.67, 115.05, 44.61, 44.01, 42.93, 26.31, 21.17; HR-MS (ESI): m/z, calcd. For $C_{21}H_{22}N_4O_3F$ 397.1670 $[M+H]^+$, Found: 397.1664.

5.2.5.14 *N*-(3-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl)piperidine-4-

carboxamide 2,2,2-trifluoroacetate (**8n**) Following the preparation protocol of Section 5.2.5.1, starting from **7n** (110 mg, 0.23 mmol), the title compound **8n** was obtained as white solid (77 mg, 68.0%); m.p. 134-136 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.78 (s, 1H), 11.01 (s, 1H), 8.68 (brs, 1H), 8.39 (brs, 1H), 8.04 (d, J = 6.9 Hz, 1H), 7.58-7.65 (m, 2H), 7.37 (s, 1H), 7.20-7.28 (m, 3H), 7.04 (d, J = 6.6 Hz, 1H), 5.29 (s, 2H), 3.30-3.40 (m, 2H), 2.88-2.92 (m, 2H), 2.50-2.58 (m, 1H), 1.74-1.94 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 172.10, 161.85, 150.63, 140.85, 139.56, 137.00, 135.27, 129.12, 127.61, 122.76, 121.44, 118.02, 116.47, 115.85, 115.14, 45.64, 45.13, 42.45, 25.08; HR-MS (ESI): *m/z*, calcd. For $C_{21}H_{23}N_4O_3$ 379.1765 $[M+H]^+$, Found: 379.1758.

5.2.5.15 *N*-(5-((2,4-Dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)-2-fluorophenyl)piperidine-4-carboxamide 2,2,2-trifluoroacetate (**8o**) Following the preparation protocol of Section 5.2.5.1, starting from 7o (50 mg, 0.10 mmol), the title compound **8o** was obtained as white solid (59 mg, 114.8%); m.p. 151-153℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H), 9.78 (s, 1H), 8.58 (brs, 2H), 8.32 (brs, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.78 (d, J = 6.4 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.18-7.27 (m, 3H), 7.12 (m, 1H), 5.27 (s, 2H), 3.30-3.36 (m, 2H), 2.88-2.91 (m, 2H), 2.74 (m, 1H), 1.91-1.96 (m, 2H), 1.71-1.80 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 172.43, 161.78, 153.02 (d, J_{CF} = 243.9 Hz), 150.65, 140.74, 135.25, 132.52, 127.63, 126.15 (d, J_{CF} = 12.1 Hz), 123.62 (d, J_{CF} = 6.2 Hz), 122.78, 122.38, 115.89, 115.69 (d, J_{CF} = 19.9 Hz), 115.05, 44.59, 42.47, 25.12; HR-MS (ESI): *m/z*, calcd. For $C_{21}H_{22}N_4O_3F$ 397.1670 $[M+H]^+$, Found: 397.1664.

5.2.5.16 *N*-(2-Fluoro-5-((5-fluoro-2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl) piperidine-4-carboxamide 2,2,2-trifluoroacetate (**8p**) Following the preparation protocol of Section 5.2.5.1, starting from **7p** (20 mg, 0.04 mmol), the title compound **8p** was obtained as brown solid (14 mg, 68.1%); m.p. 181.5-183.5 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.72 (s, 1H), 9.80 (s, 1H), 8.62 (brs, 1H), 8.34 (brs, 1H), 7.77 (d, J = 6.3 Hz, 1H), 7.61-7.64 (m, 1H), 6.99-7.24 (m, 4H), 5.25 (s, 2H), 3.30-3.35 (m, 2H), 2.74-3.02 (m, 3H), 1.91-2.04 (m, 2H), 1.70-1.78 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 172.40, 161.71 (d, J_{CF} = 260.1 Hz), 158.95, 153.04 (d, J_{CF} = 243.8 Hz), 150.35, 142.50, 135.92 (d, J_{CF} = 11.2 Hz), 132.20, 126.12 (d, J_{CF} = 12.2 Hz), 123.54 (d, J_{CF} = 7.5 Hz), 122.31, 115.68 (d, J_{CF} = 19.8 Hz), 111.13, 110.13 (d, J_{CF} = 20.4 Hz), 105.50 (d, J_{CF} = 8.6 Hz), 45.20, 42.44, 25.08; HR-MS (ESI): *m/z*, calcd. For $C_{21}H_{21}N_4O_3F_2$ 415.1576 $[M+H]^+$, Found: 415.1568.

5.2.5.17 *N*-(2-Fluoro-5-((6-fluoro-2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl) piperidine-4-carboxamide 2,2,2-trifluoroacetate (**8q**) Following the preparation protocol of Section 5.2.5.1, starting from 7q (45 mg, 0.09 mmol), the title compound **8q** was obtained as white solid (35 mg, 75.7%); m.p. 62-64 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.90 (s, 1H), 9.82 (s, 1H), 8.65 (brs, 1H), 8.38 (brs, 1H), 7.78 (d, J = 7.2 Hz, 1H), 7.73 (dd, J_1 = 8.1 Hz, J_2 = 2.7 Hz, 1H), 7.54-7.61 (m, 1H), 7.29 (dd, J_1 = 9.3 Hz, J_2 = 3.6 Hz, 1H), 7.17-7.24 (m, 1H), 7.11 (m, 1H), 5.27 (s, 2H), 3.30-3.35 (m, 2H), 2.73-2.92 (m, 3H), 1.91-1.96 (m, 2H), 1.69-1.81 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 172.43, 160.98 (d, J_{CF} = 2.3 Hz), 157.51 (d, J_{CF} = 239.9 Hz), 153.04 (d, J_{CF} = 243.7 Hz), 150.41, 137.46, 132.32 (d, J_{CF} = 3.0 Hz), 126.17 (d, J_{CF} = 12.2 Hz), 123.59 (d, J_{CF} = 7.7 Hz), 122.73 (d, J_{CF} = 23.4 Hz), 122.36, 117.54 (d, J_{CF} = 7.6 Hz), 117.32 (d, J_{CF} = 7.5 Hz), 115.71 (d, J_{CF} = 19.9 Hz), 112.84 (d, J_{CF} = 23.9 Hz), 44.84, 42.50, 25.12; HR-MS (ESI): *m/z*, calcd. For $C_{21}H_{21}N_4O_3F_2$ 415.1576 $[M+H]^+$, Found: 415.1570.

5.2.5.18 *N*-(2-Fluoro-5-((7-fluoro-2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl) piperidine-4-carboxamide 2,2,2-trifluoroacetate (**8r**) Following the preparation protocol of Section 5.2.5.1, starting from **7r** (40 mg, 0.08 mmol), the title compound **8r** was obtained as white solid (35 mg, 85.1%); m.p. 232-233.5 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.82 (brs, 1H), 9.81 (s, 1H), 8.04-8.10 (m, 1H), 7.79 (d, J = 6.6 Hz, 1H), 7.10-7.25 (m, 5H), 5.26 (s, 2H), 3.29-3.34 (m, 2H), 2.74-2.94 (m, 3H), 1.91-1.96 (m, 2H), 1.73-1.82 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 172.45, 166.12 (d, J_{CF} = 249.3 Hz), 160.99, 153.10 (d, J_{CF} = 243.9 Hz), 150.69, 142.94 (d, J_{CF} = 12.4 Hz), 132.14 (d, J_{CF} = 2.9 Hz), 130.76 (d, J_{CF} = 11.3 Hz), 126.15 (d, J_{CF} = 12.0 Hz), 123.73 (d, J_{CF} = 7.3 Hz), 122.59, 115.72 (d, J_{CF} = 19.9 Hz), 112.82, 110.52 (d, J_{CF} = 22.8 Hz), 102.28 (d, J_{CF} = 27.9 Hz), 44.72, 42.46, 25.12; HR-MS (ESI): *m/z*, calcd. For $C_{21}H_{21}N_4O_3F_2$ 415.1576 $[M+H]^+$, Found: 415.1568.

5.2.5.19 *N*-(2-Fluoro-5-((8-fluoro-2,4-dioxo-3,4-dihydroquinazolin-1(2*H*)-yl)methyl)phenyl) piperidine-4-carboxamide 2,2,2-trifluoroacetate (**8s**) Following the preparation protocol of Section 5.2.5.1, starting from **7s** (45 mg, 0.09 mmol), the title compound **8s** was obtained as white solid (35 mg, 74.4%); m.p. 159-161 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.93 (s, 1H), 9.78 (s, 1H), 8.68 (brs, 1H), 8.39 (brs, 1H), 7.89-7.92 (m, 1H), 7.72 (m, 1H), 7.52-7.57 (m, 1H), 7.16-7.34 (m, 2H), 6.99 (m, 1H), 5.31 (s, 2H), 3.36-3.39 (m, 2H), 2.74-2.92 (m, 3H), 1.77-1.93 (m, 4H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 172.38, 160.95 (d, J_{CF} = 2.6 Hz), 152.76 (d, J_{CF} = 243.0 Hz), 150.71, 149.09 (d, J_{CF} = 245.2 Hz), 133.87, 129.67 (d, J_{CF} = 6.9 Hz), 125.86 (d, J_{CF} = 12.0 Hz), 123.95, 123.78 (d, J_{CF} = 8.0 Hz), 122.90 (d, J_{CF} = 23.4 Hz), 122.68 (d, J_{CF} = 7.1 Hz), 121.60,

119.07, 115.44 (d, $^{1}J_{CF}$ = 19.7 Hz), 47.93, 47.85, 42.48, 25.09; HR-MS (ESI): *m/z*, calcd. For $C_{21}H_{21}N_4O_3F_2$ 415.1576 $[M+H]^+$, Found: 415.1569.

5.3 Computational modeling

Crystal structure of PARP1 in complex with BMN-673(PDB ID: 4PJT) was selected for the molecular modeling. Molecular docking was performed with DOCK 3.5.54.[48] The target compounds were prepared in db format with ZINC protocol.[49] The amino acid residues in binding site were identified within 12 Å of native ligand (BMN673) from the cocrystal structure, and the solvent-accessible molecular surface was calculated with the program DMS using a probe radius of 1.4 Å. Receptor-derived spheres were generated with the program SPHGEN while the ligand-derived spheres were created based on the positions of the heavy atoms of ligands. The PARP1 was kept intact, and the small molecules were subjected to 25 steps of rigid-body minimization process. The obtained ligand conformations were scored on the basis of the total energy, which is the sum of electrostatic and van der Waals interaction energies, corrected by the partial ligand desolvation energy. The refinement of the complex structure in implicit solvent model was performed using the Protein Local Optimization Program (PLOP).[50] In summary, the docked PARP1–ligand complex was submitted to multiscale truncated Newton energy minimization using all-atom OPLS force field and Generalized Born solvent model. The whole binding site defined with residues within 5 Å of the docked ligand was energetically minimized with MM-GB/SA method as described previously.[51, 52]

5.4 Biological evaluation

5.4.1 The assay for PARP1 and PARP2 inhibition

Plasmid pET32a-PARP1 was a gift from Prof. Satoh (Canada). Human recombinant PARP1/2 were expressed and purified as described.[53] The ability of compounds to inhibit PARP1/2 enzyme activity were tested using ELISA method as described.[53] IC_{50} values were calculated using GraphPad Prism 5 software.

5.4.2 The cytotoxicity assay

A2780, Capan-1, MX-1, MDA-MB-231, MDA-MB-453 and MDA-MB-468 were purchased from National Infrastruture of Cell Line Resources, Cells were seeded in a density in 96-well plates (2,000–5,000 cells/well). Cells were treated in their recommended growth media containing increasing concentrations of PARP inhibitors 24 h later. After 72 treatment, cell survival was determined by MTT assay. IC_{50} values were calculated using GraphPad Prism 5 software.

5.4.3 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 5 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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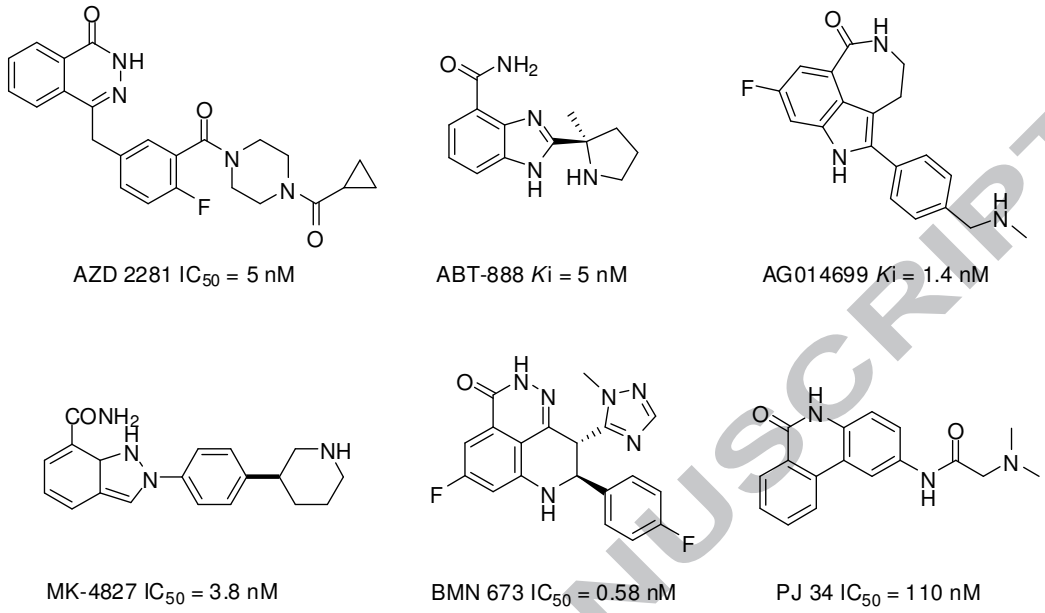


Figure 1 Chemical structures of some known PARP-1 inhibitors.

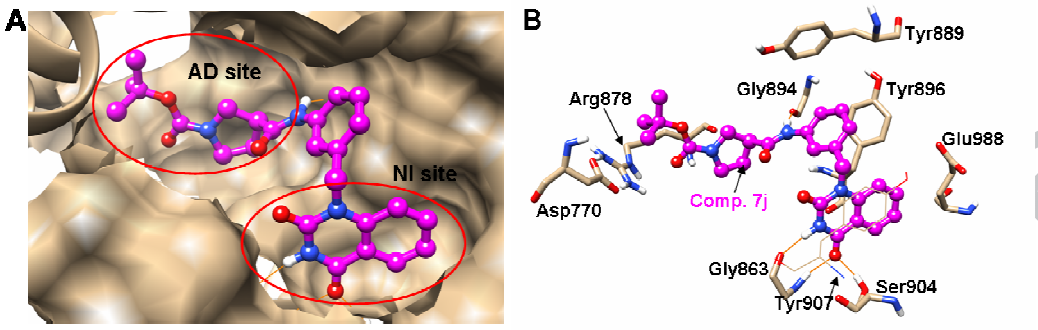
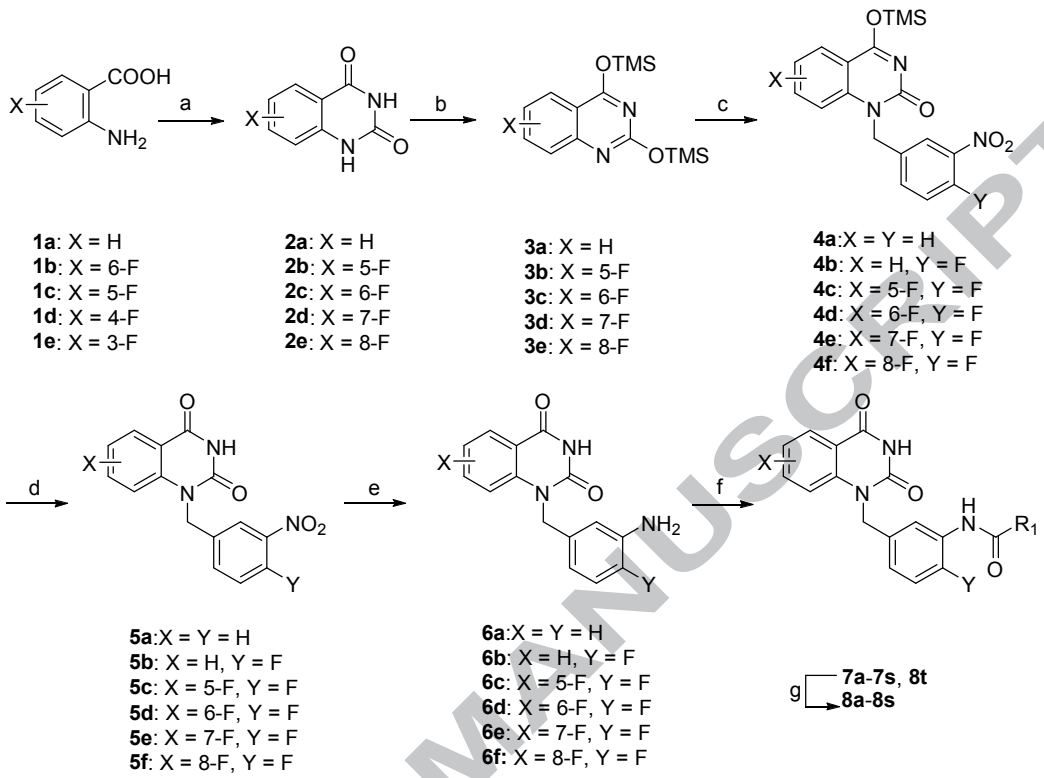
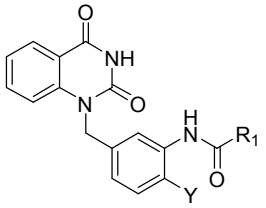


Figure 2 Computational prediction of binding mode for compound **7j**. (A) Predicted binding pose for compound **7j** (ball-and-stick, carbon atoms colored magenta) within the binding pocket of PARP-1 (tan colored surface); (B) The predicted interactions between PARP-1 and compound **7j**, and hydrogen bonds were presented in orange lines. Molecular image was generated with UCSF Chimera.[47]



Scheme 1. Reagents and conditions: (a) urea, 150 °C, 6 h; (b) HMDS, conc.H_2SO_4, toluene, reflux, 2 h; (c) 1-(bromomethyl)-3-nitrobenzene or 1-(bromomethyl)-4-flouro-3-nitrobenzene, 130 °C, 3 h; (d) MeOH, dioxane, 70 °C, 15 min; (e) 10% Pd-C, H_2, THF, MeOH, 2 h; (f) R_1COOH, HATU, HOBt, Et_3N, DMF, overnight or R_1COOH, EDCI, HOBt, Et_3N, DMF, overnight or R_1COOH, HBTU, HOBt, DIEA, DMF, overnight; (g) TFA, DCM, 5 h.

Table 1. Chemical structures and PARP-1 inhibitory activities of compounds **7a**–**7g**, **8a**-**8g** and **8t**.[a]



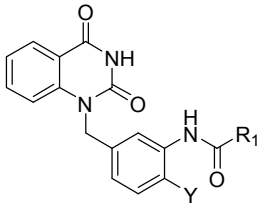
Compd.	Y	R_1	IC_{50}/nM [b]
7a	H	-CH_2NHBoc	921
7b	H	-$CH_2N(CH_3)Boc$	5650
7c	H	(*S*)-$CH[CH(CH_3)_2]NHBoc$	34100
7d	H	(*R*)-*N*-Boc-pyrrolidin-2-yl	NA[c]
7e	F	(*R*)-*N*-Boc-pyrrolidin-2-yl	577
7f	H	(*S*-*N*-Boc-pyrrolidin-2-yl	NA
7g	F	(*S*)-*N*-Boc-pyrrolidin-2-yl	1072
8a	H	-CH_2NH_2	171
8b	H	-CH_2NHCH_3	444
8c	H	(*S*)-$CH[CH(CH_3)_2]NH_2$	5290
8d	H	(*R*)-pyrrolidin-2-yl	502
8e	F	(*R*)-pyrrolidin-2-yl	48.2
8f	H	(*S*)-pyrrolidin-2-yl	3050
8g	F	(*S*)-pyrrolidin-2-yl	244
8t	H	-$CH_2N(CH_3)_2$	4440

[a] IC_{50} for ABT-888 was 5.18 nM. IC_{50} for AZD-2281 was 2.09 nM.

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

[c] NA: not active

Table 2. Chemical structures and PARP-1 inhibitory activities of compounds **7h**-**7o**, **8h**-**8o.**[a]

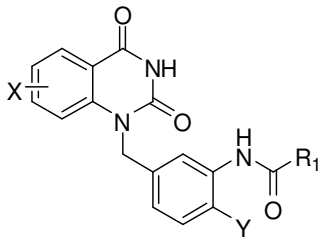


Compd.	Y	R_1	IC_{50}/nM[b]
7h	H	(*R*)-*N*-Boc-pyrrolidin-3-yl	96.1
7i	F	(*R*)-*N*-Boc-pyrrolidin-3-yl	23
7j	H	(*S*)-*N*-Boc-pyrrolidin-3-yl	24.1
7k	F	(*S*)-*N*-Boc-pyrrolidin-3-yl	22.3
7l	H	*N*-Boc-piperidin-3-yl	819
7m	F	*N*-Boc-piperidin-3-yl	14.7
8h	H	(*R*)- pyrrolidin-3-yl	41.5
8i	F	(*R*)- pyrrolidin-3-yl	11.9
8j	H	(*S*)- pyrrolidin-3-yl	24.3
8k	F	(*S*)- pyrrolidin-3-yl	23.6
8l	H	piperidin-3-yl	554
8m	F	piperidin-3-yl	44.2

[a] IC_{50} for ABT-888 was 5.18 nM. IC_{50} for AZD-2281 was 2.09 nM.

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

Table 3. Chemical structures and PARP-1 inhibitory activities of compounds **7n**-**7s**, **8n**-**8s.**[a]



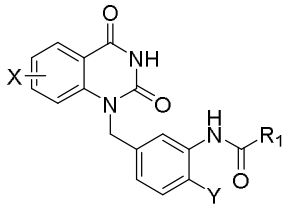
Compd.	X	Y	R_1	IC_{50}/nM[b]
7n	H	H	*N*-Boc-piperidin-4-yl	NA[c]
7o	H	F	*N*-Boc-piperidin-4-yl	30.9
7p	5-F	F	*N*-Boc-piperidin-4-yl	9.51
7q	6-F	F	*N*-Boc-piperidin-4-yl	74.8
7r	7-F	F	*N*-Boc-piperidin-4-yl	466
7s	8-F	F	*N*-Boc-piperidin-4-yl	448
8n	H	H	piperidin-4-yl	478
8o	H	F	piperidin-4-yl	23.1
8p	5-F	F	piperidin-4-yl	30.4
8q	6-F	F	piperidin-4-yl	40
8r	7-F	F	piperidin-4-yl	234
8s	8-F	F	piperidin-4-yl	29.1

[a] IC_{50} for ABT-888 was 5.18 nM. IC_{50} for AZD-2281 was 2.09 nM.

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

[c]NA: not active

Table 4. Chemical structures and inhibitory activities against PARP-1 and PARP-2 of selected compounds.



Compd.	X	Y	R_1	PARP-1 IC_{50}/nM[a]	PARP-2 IC_{50}/nM[b]
7j	H	H	(*S*)-*N*-Boc-pyrrolidin-3-yl	24.1	61.4
7k	H	F	(*S*)-*N*-Boc-pyrrolidin-3-yl	22.3	1.47
7p	5-F	F	*N*-Boc-piperidin-4-yl	9.51	82.5
7q	6-F	F	*N*-Boc-piperidin-4-yl	74.8	371
8e	H	F	(*R*)-pyrrolidin-2-yl	48.2	14.2
8k	H	F	(*S*)-pyrrolidin-3-yl	23.6	30.8
8p	5-F	F	piperidin-4-yl	30.4	74.4
8q	6-F	F	piperidin-4-yl	40	121

[a] Concentration for 50% inhibition in PARP-1 enzyme assay (IC_{50}); IC_{50} for ABT-888 was 5.18 nM. IC_{50} for AZD-2281 was 2.09 nM.

[b] Concentration for 50% inhibition in PARP-2 enzyme assay (IC_{50}); IC_{50} for ABT-888 was 2.89 nM. IC_{50} for AZD-2281 was 2.26 nM.

Table 5. Cytotoxicities and potentiation effects (PF_{50}) of selected compounds on MX-1 cells.

Compd	IC_{50} (μM)[a]	PF_{50}[b]	Compd	IC_{50} (μM)[a]	PF_{50}[b]
7h	12.52	1.04	**7p**	14.19	1.36
7i	27.29	1.32	**7q**	15.41	1.20
7j	4.59	3.77	**8e**	38.25	3.47
7m	14.00	2.01	**8k**	25.88	ND[c]
7o	16.65	1.41	**8m**	44.83	1.54
ABT-888	50.37	6.72			

[a]Cytotoxicity (IC_{50}): The concentration required to reduce cell proliferation and growth by 50% in single-agent cytotoxicity assay.

[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 5 μM.

[c]ND: not determined

Table 6. Cellular activities of compound **7j** in various cells with or without deficient HR DNA repair gene.

Cell Line	Cell Type	Deficient gene	IC_{50} (μM)[a]	
			7j	AZD-2281
MX-1	Breast Cancer	BRCA1/2	4.59	8.26
Capan-1	Pancreatic Cancer	BRCA2	49.19	83.46
MDA-MB-468	Breast Cancer	PTEN	5.36	43.57
MDA-MB-453	Breast Cancer	PIK3CA	9.1	16.3
A2780	Ovarian Cancer	–[b]	4.56	7.07
MDA-MB-231	Breast Cancer	–[b]	61.39	>100

[a]Cytotoxicity (IC_{50}): The concentration required to reduce cell profliferation and growth by 50% in single-agent cytotoxicity assay.

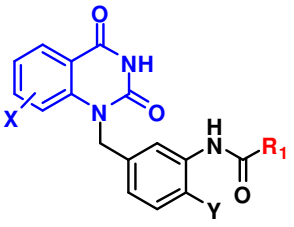
[b]The cells without deficient HR DNA repair gene.

Graphical Abstract:

Discovery of 1-substituted benzyl-quinazoline-2,4(1*H*,3*H*)-dione derivatives as novel poly(ADP-ribose)polymerase-1 inhibitors

Haiping Yao, Ming Ji, Zhixiang Zhu, Jie zhou, Xiaoguang Chen, Bailing Xu

A series of novel quinazoline-2,4(1*H*,3*H*)-dione derivatives were synthesized and evaluated as inhibitors of poly(ADP-ribose)polymerase-1 (PARP-1).



X, Y = H or F

R_1 = a range of amino acid fragments

IC_{50}: 9.51 – 5290 nM