European Journal of Medicinal Chemistry 216 (2021) 113291

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Design, synthesis, biological evaluation and structure-activity relationship study of quinazolin-4(3*H*)-one derivatives as novel USP7 inhibitors

Peng Li, Ying Liu, Hua Yang*, Hong-Min Liu**

School of Pharmaceutical Sciences, Zhengzhou University, 100 Kexue Avenue, Zhengzhou, Henan, 450001, China

ARTICLE INFO

Article history:
Received 10 November 2020
Received in revised form
10 January 2021
Accepted 20 January 2021
Available online 16 February 2021

Keywords:
USP7
Inhibitors
Quinazolin-4(3*H*)-one
SAR
Gastric cancer
Ubiquitination

ABSTRACT

Recent research has indicated that the abnormal expression of the deubiquitinase USP7 induces tumorigenesis via multiple cell pathways, and in particular, the p53-MDM2-USP7 pathway is well understood. USP7 is emerging as a promising target for cancer therapy. However, there are limited reports on USP7 inhibitors. Here we report design, synthesis and biological evaluation of novel quinazolin-4(3*H*)-one derivatives as potent USP7 inhibitors. Our results indicated that the compounds **C9** and **C19** exhibited the greatest potency against the USP7 catalytic domain, with IC_{50} values of 4.86 μM and 1.537 μM, respectively. Ub-AMC assays further confirmed IC_{50} values of 5.048 μM for **C9** and 0.595 μM for **C19**. MTT assays indicated that gastric cancer MGC-803 cells were more sensitive to these compounds than BGC-823 cells. Flow cytometry analysis revealed that **C9** restricted cancer cell growth at the G0/G1 and S phases and inhibited the proliferation and clone formation of MGC-803 cells. Further biochemical experiments indicated that **C9** decreased the MDM2 protein level and increased the levels of the tumour suppressors p53 and p21 in a dose-dependent manner. Docking studies predicted that solvent exposure of the side chains of **C9** and **C19** would uniquely form hydrogen bonds with Met407 of USP7. Additionally, **C9** exhibited a remarkable anticancer effect in a zebrafish gastric cancer MGC-803 cell model. Our results demonstrated that quinazolin-4(3*H*)-one derivatives were suitable as leads for the development of novel USP7 inhibitors and especially for anti-gastric cancer drugs.

1. Introduction

The process of attaching a ubiquitin chain onto a target protein is called ubiquitination, and this modification plays important roles in controlling the stabilization, function and localization of target proteins [1]. In 2004, the Nobel Prize in chemistry was awarded to two Israeli scientists, Aaron Ciechanover [2] and Avram Hershko [3], and one American scientist, Irwin Rose [4], for the discovery of ubiquitin-mediated protein degradation. Subsequently, the ubiquitin-proteasome pathway has drawn increasing attention, and research has indicated that it is involved in immune diseases [5], mental diseases [6] and tumour genesis [7].

Deubiquitinating enzymes (DUBs) are proteases in the ubiquitin-proteasome pathway which can remove the ubiquitin chain from the ubiquitinated target protein and prevent it from degradation by the 26S proteasome. The human genome encodes nearly 100 DUBs, which can be classified into five types: ubiquitin C-terminal hydrolase (UCH), ubiquitin-specific protease (USP), ovarian tumour domain protease (OTU), Machado-Joseph disease protein domain protease (MJD) and JAB1/MPN/Mov34 metalloenzyme (JAMM) [8]. The de-ubiquitination process is highly regulated and mediates many important cellular processes, such as cell cycle [9], protein degradation [10], gene expression [11] and DNA repair [12]. The ubiquitination and de-ubiquitination processes work together to maintain the balance of all cellular pathways.

USP7 belongs to the USP family and is a papain-like cysteine

Abbreviations: BLI, biolayer interferometry; DMF, *N, N*-dimethylformamide; DMSO, dimethyl sulfoxide; DUB, deubiquitinating enzyme; EA, ethyl acetate; HATU, 2-(7-azabenzotriazol-1-yl)-N,N,N,N-tetramethyluronium hexafluorophosphate; IR, inhibitory rate; MOE, molecular operating environment; MDM2, murine double minute 2; MTD, maximum tolerated dose; TLC, thin-layer chromatography; TMS, tetramethylsilane; TMSOI, trimethylsulfoxonium iodide; TFA, trifluoroacetic acid; TEA, triethylamine; Ub-AMC, ubiquitin-7-amido-4-methylcoumarin; USP7, ubiquitin-specific-processing protease 7.

* Corresponding author.
** Corresponding author.
E-mail addresses: yanghua@zzu.edu.cn (H. Yang), liuhm@zzu.edu.cn (H.-M. Liu).

https://doi.org/10.1016/j.ejmech.2021.113291
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protease [8]. In 2002, the mechanism by which USP7 participates in the ubiquitin-proteasome pathway was illustrated in detail [13,14]. Thereafter, further research indicated that USP7 was involved in tumour formation by affecting multiple cancer routes [15–18]. In particular, the p53-MDM2-USP7 pathway attracted much attention. Both p53 and MDM2 are substrates of USP7, but USP7 shows higher affinity for MDM2 [19]. This leads to the upregulation of MDM2 expression, which promotes the ubiquitination of p53 by serving as E3 ligase [20]. Finally, ubiquitinated p53 is degraded by the 26S proteasome, preventing its anticancer function. The oncogenicity of USP7 makes it a potential target for cancer therapy. The development of USP7 inhibitors has achieved some success. The reported USP7 inhibitors can also be classified into five types based on their molecular skeletons: substituted thiophene derivatives [21–24], acridine derivatives [25], quinazolin-4-one derivatives [26–29], indeno[1,2-*b*]pyrazine derivatives [30,31] and 2-amino-4-ethylpyridine derivatives [32,33]. Additionally, some natural products also exhibit excellent USP7 inhibitory activities [34–37].

Currently, most research concerning USP7 focuses on elucidating its mechanism of action, and limited progress has been made in developing novel USP7 inhibitors. Here, we reported the design, synthesis and biological evaluation of quinazolin-4(3*H*)-one derivatives as novel potent USP7 inhibitors. First, we investigated all published cocrystal structures of the USP7 catalytic domain with small-molecule inhibitors and evaluated their potency and mechanisms. We found abundant co-crystal structure data of quinazolin-4(3*H*)-one derivatives with the USP7 catalytic domain and thus selected this co-crystal structure (PDB ID: 5VSB) and analyzed the binding mode of this substrate [23]. We found that the potency of the substrate (**compound 1**, Fig. 1) against the USP7 catalytic domain was moderate, with an IC_{50} of 12 μM, which was suitable for further development as a lead molecule. Meanwhile, the co-crystal structure indicated that the binding of quinazolin-4(3*H*)-one to the 4-hydroxypiperidine moiety was extremely important for its activity. The C-7 of the quinazolin-4(3*H*)-one ring was open to solvent exposure, whereas the 3-phenylbutanoic acid moiety was buried in the hydrophobic pocket. However, few reports have described the structure-activity relationship (SAR) between these two sites.

In this report, we selected **compound 1** as the lead and control compound and preserved the quinazolin-4(3*H*)-one binding to the 4-hydroxypiperidine moiety as core requirements. The chemical modification of the lead mainly focused on C-7 of the quinazolin-4(3*H*)-one ring and the 3-phenylbutanoic moiety.

2. Results and discussion

2.1. Chemistry

The synthetic procedures of quinazolin-4(3*H*)-one derivatives are summarized in Scheme 1. N-Boc-piperidine-4-one reacted with trimethylsulfoxonium iodide to form the key intermediate **A2**. Then, 2-amino-4-chlorobenzoic acid (**B1**) in formamide was heated at 150 °C for 12 h to form 7-chloroquinazolin-4(3*H*)-one (**B2**). The intermediates **A2** and **B2** were heated at 80 °C in *N, N*-dimethylformamide (DMF) for 12 h to form the intermediate **B3**, whose protecting group Boc was removed with the addition of trifluoroacetic acid (TFA) to form the intermediate **B4**. Finally, **B4** was reacted with different substituted formic acids to form the target products (**B5**–**B31**). Meanwhile, replacement of **B1** with **C1** by the same reaction route led to the formation of the key intermediates **C5** and **C6**, which were further subjected to a Cu-catalyzed Buchwald-Hartwig coupling reaction, leading to the synthesis of the target products (**C7**–**C19**).

2.2. SAR investigations

We applied our optimized screening method based on previous reports [26] to test the lead compound **B5** (Fig. S1). This compound exhibited moderate inhibitory activity against the USP7 catalytic

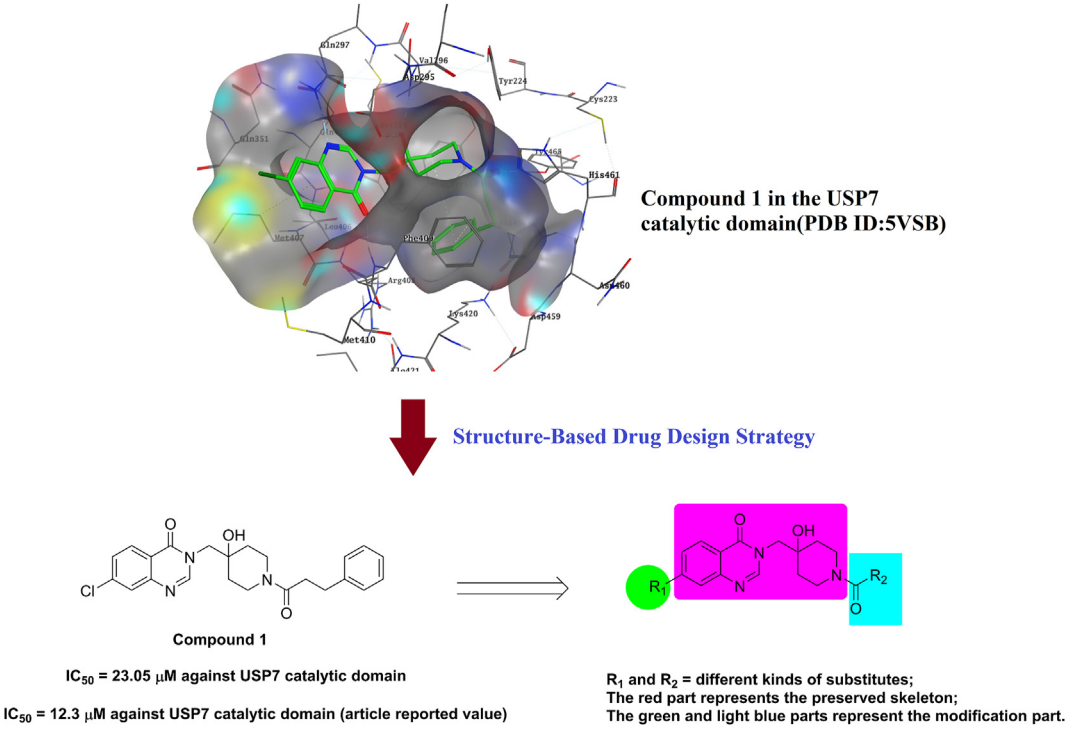
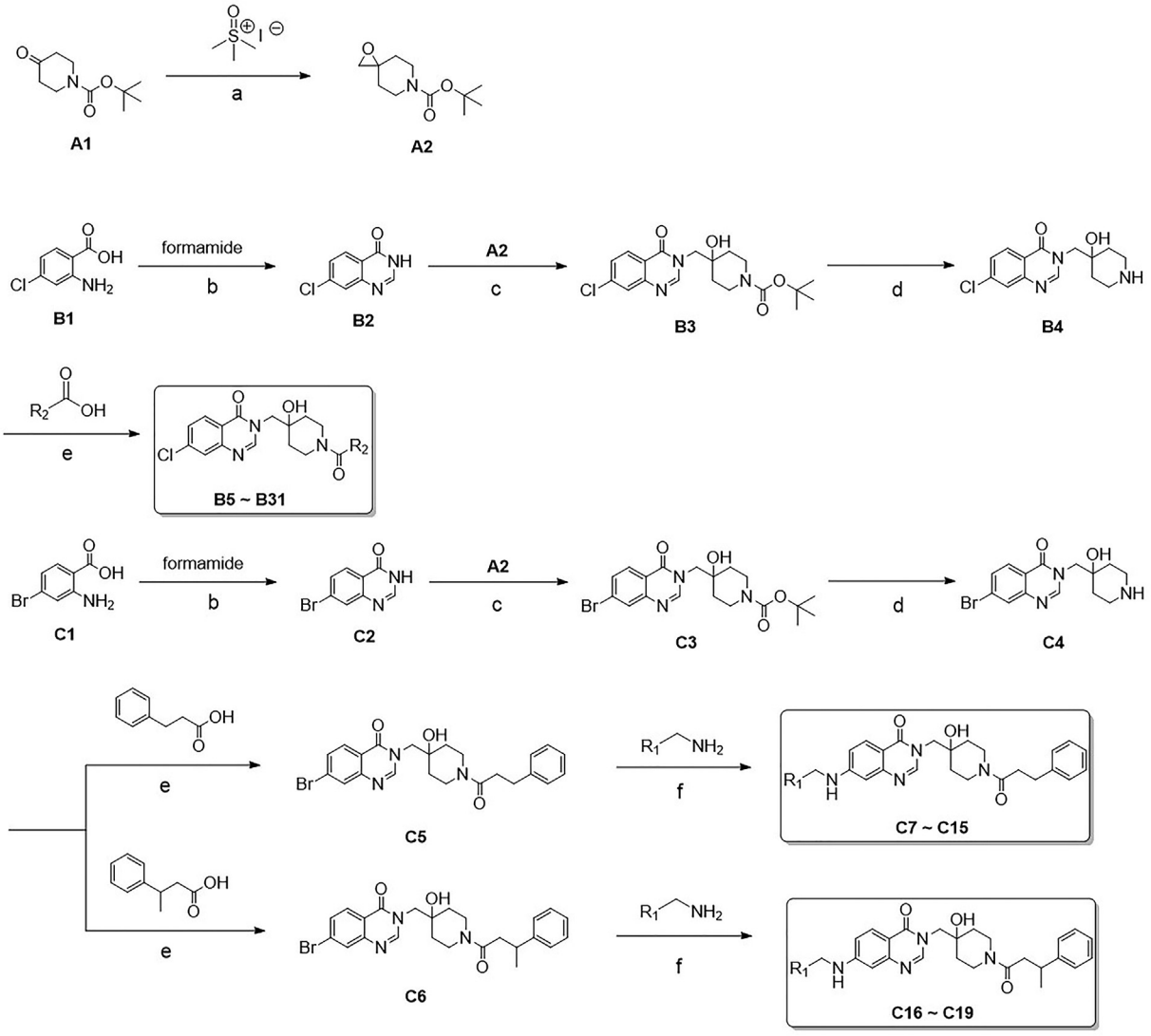


Fig. 1. Design strategy of quinazolin-4(3*H*)-one derivatives as potent USP7 inhibitors.



Scheme 1. Synthesis of compounds **B5**–**B31** and **C7**–**C19**. Reagents and conditions: (a) NaH (60% in mineral oil), dry DMSO, 0 °C, 4 h; (b) 150 °C, 12 h; (c) K_2CO_3, DMF, 80 °C, 12 h; (d) TFA, r.t., 4 h; (e) HATU, TEA, DMF, r.t., 5 h; (f) N_2, 5 mol% CuI, 20 mol% Ligand = (2-hydroxyphenyl)(morpholino)methanone, K_3PO_4, dry DMF, 90 °C, 18 h.

domain, with an IC_{50} of 23.05 μM. Further optimization of the substituent bound at N-1 of the piperidine ring led to the formation of other derivatives, which are summarized in Fig. S1. Introduction of heterocycle to N-1 of the piperidine ring decreased or directly abrogated USP7 inhibitory activity (B7, B8 and B9; IC_{50} > 50 μM; IR <50%, Fig. S1). Hydrophobic groups were favoured (B6, IR = 18.59% vs B14, IR = 42.24%, Fig. S1), whereas strong hydrophilic groups directly abrogated activity (B24, IR = 0.8%, Fig. S1). Interestingly, the C-2 and C-4 of benzoic acid substitutes were preferred to polar groups (B18, IC_{50} = 46.3 μM and B26, IR = 58.34%, Fig. S1), but steric polar groups were not favoured (B12, IR = 4.29%, Fig. S1). The introduction of a Br atom to the benzoic acid substitute remarkably increased USP7 inhibitory activity (B17, IR = 46.53%; B18, IC_{50} = 46.3 μM, Fig. S1). Meanwhile, shortening the alkyl chain or introducing a steric group to N-1 of the piperidine ring abrogated its activity (B29, B30, IC_{50} > 50 μM, Fig. S1). Introducing a heteroatom or steric group to the 3-phenylpropanoic acid moiety decreased USP7 inhibitory activity (B11, IR = 22.71%; B23, IR = 9.83%, Fig. S1). Increasing the rigidity of the 3-phenylpropanoic acid moiety also decreased the inhibitory activity (B27, IR = 32.64%, Fig. S1). Conversely, introducing a racemic methyl group remarkably increased the USP7 inhibitory activity (B13, IC_{50} = 6.788 μM, Fig. S1), whereas introducing a Br atom to C-7 of the quinazolin-4(3*H*)-one ring did not remarkably affect the inhibitory activity (B15, IC_{50} = 27.41 μM, Fig. S1).

The optimization of the quinazolin-4(3*H*)-one moiety at C-7 led to the formation of the derivatives C7–C19, (Fig. S2). Hydrophobic groups were not favoured, and the introduction of hydrophilic groups remarkably increased the USP7 inhibitory activity (**C8**, IC_{50} = 15.31 μM, **C7**, IC_{50} = 9.8 μM and **C9**, IC_{50} = 4.86 μM, Fig. S2). Introduction of a hydrophobic group decreased the inhibitory activity (**C10**, IR = 45.93%, **C11**, IR = 49.91% and **C12**, IR = 49.13%, Fig. S2). Notably, bulky steric groups were not tolerated (**C9**, IC_{50} = 4.86 μM vs **C13**, IC_{50} = 36.95 μM, Fig. S2), and introduction of strong hydrophilic groups decreased the inhibitory activity (**C14**, IC_{50} = 16.42 μM, Fig. S2). Introduction of a racemic methyl group to the 3-phenylpropanoic acid moiety binding N-1 of the piperidine ring led to the formation of **C19**, the most potent compound (IC_{50} = 1.537 μM, Fig. S2), whereas introduction of a lipophilic group at C-7 of the quinazolin-4(3*H*)-one moiety enhanced the lipophilicity of the whole molecule with the help of the racemic methyl group, which led to decreased inhibitory activity (**C15**,

IC_{50} = 5.977 μM vs **C19**, IC_{50} = 1.537 μM, Fig. S2). The SAR analyses of quinazolin-4(3*H*)-one derivatives are summarized in Fig. 2. Finally, we selected the most potent compounds in Fig. S2 and screened them using Ub-AMC assays, and the results of which are summarized in Table S1. These experiments further confirmed the high efficiency of our designed compounds.

2.3. Cellular effects of quinazolin-4(3H)-one derivatives against gastric cancer cells

Most of the USP7 inhibitors that we reviewed exhibited enzymatic activity, but few exhibited cellular activity. The reported USP7 inhibitors were effective in HCT116 cells [18–28], but whether they were effective against other cancer cell lines was unknown. Therefore, we tested our derivatives against two gastric cancer cell lines, MGC-803 and BGC-823. The inhibitory activities of quinazolin-4(3*H*)-one derivatives against these two cell lines are summarized in Table S2. We also appended the inhibitory activity against the catalytic domain of USP7 for ease of understanding.

We found that quinazolin-4(3*H*)-one derivatives exhibited moderate inhibitory activity against the cell lines, especially MGC-803, which was more sensitive to compounds B17, B18 and B19 (Table S2). The most potent compounds against the cell lines and the USP7 catalytic domain were B18 and C8. The most potent compounds against the cell lines were B23 and B25. Interestingly, C9 exhibited high potency against the USP7 catalytic domain but low potency MGC-803 and BGC-823 cells. However, C8, which was ~~the~~ less potent against the USP7 catalytic domain, exhibited moderate inhibitory activity against MGC-803 (IC_{50} = 32.13 μM, Table S2) and BGC-823 cells (IR = 54.01%, Table S2). On comparing the structures of C8 and C9, we found that the inhibitory activity against gastric cancer cells was correlated with the lipophilicity of these compounds. Of note, increasing the hydrophilicity of compounds increases the USP7 inhibitory activity, whereas increasing the lipophilicity of compounds increases the inhibitory activity against gastric cancer cells. Remarkably, the reference compound B5 exhibited no inhibitory activity against either gastric cancer cell line. Flow cytometry and clone formation experiments (Fig. S3 and Fig. S4) also indicated that C9 inhibited the proliferation of MGC-803 cells and induced their death by restricting the cell cycle at the G0/G1 and S phases.

2.4. Biochemical characterization of C9

Cell experiments indicated that quinazolin-4(3*H*)-one derivatives exhibited moderate inhibitory activity against gastric cancer cells. To further illustrate their underlying biochemical mechanism, we used MGC-803 cells and performed Western blot analysis. We used C9 as a target compound, C12 as a negative control compound and B5 as a reference. First, we investigated the effect of C12 and B5 on USP7 expression level in MGC-803 cells, the results of which are summarized as Fig. 3. The reference drug B5 inhibited USP7 activity in MGC-803 cells, whereas C12 had no effect. Interestingly, C12 still exhibited moderate inhibitory activity against MGC-803 and BGC-823 cells, which indicate its inhibitory effect against other cellular proteins. Next, we investigated the inhibitory mechanism of one of the most potent compounds, C9, the results of which are summarized in Fig. 4. The results indicated that USP7 protects MDM2 from degradation and maintains high protein levels of MDM2. In contrast, C9 inhibited USP7 activity and led to a decreased level of MDM2 in a time-dependent manner. Further biochemical exploration of C9 in MGC-803 cells indicated that it blocked the p53-MDM2-USP7 pathway (Fig. 5). The experimental results also revealed that C9 inhibited USP7 activity and led to a decreased level of MDM2. This indirectly led to the upregulation of p53 expression and that of its downstream protein, p21. Meanwhile, biolayer interferometry (BLI) assays also confirmed the high binding affinity of C9 with the USP7 catalytic domain, with a K_D of 7.7×10^{-5} M (Fig. S5). Therefore, C9 was considered a USP7 inhibitor and exerted effects on MGC-803 cells via interference in the p53-MDM2-USP7 pathway.

Additionally, to further prove the efficiency of our designed compounds in the p53-MDM2-USP7 pathway in gastric cancer cells, we tested the effects of C15, C16, C19 against BGC-823 cells (Fig. 6). These compounds also affected the expression levels of USP7-related proteins in a concentration-dependent manner.

2.5. Molecular docking studies

To determine why C19 exhibited the highest USP7 inhibitory activity, we selected C13, C16, C17 and C19 as ligands for molecular modelling research. These compounds had different structures and USP7 inhibitory activities. We used the co-crystal structure (PDB ID: 5VSB) as a model and the ligand B5 (Fig. S1) as a reference. First, we compared the binding mode of the highest score conformation of these compounds, summarized in Fig. S6. The input conformation of the compound was generated in molecular operating environment (MOE) 2014 and further optimized using the 'QuickPrep' function. This optimization result was used as the input conformation. The co-crystal structure of the USP7 catalytic domain was

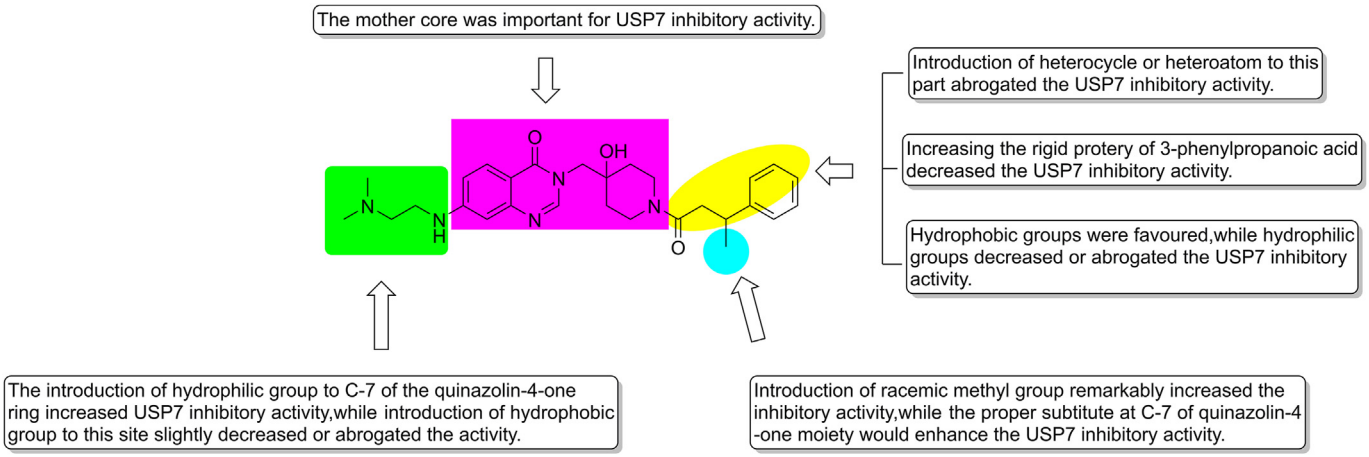


Fig. 2. SAR analysis of quinazolin-4(3*H*)-one derivatives.

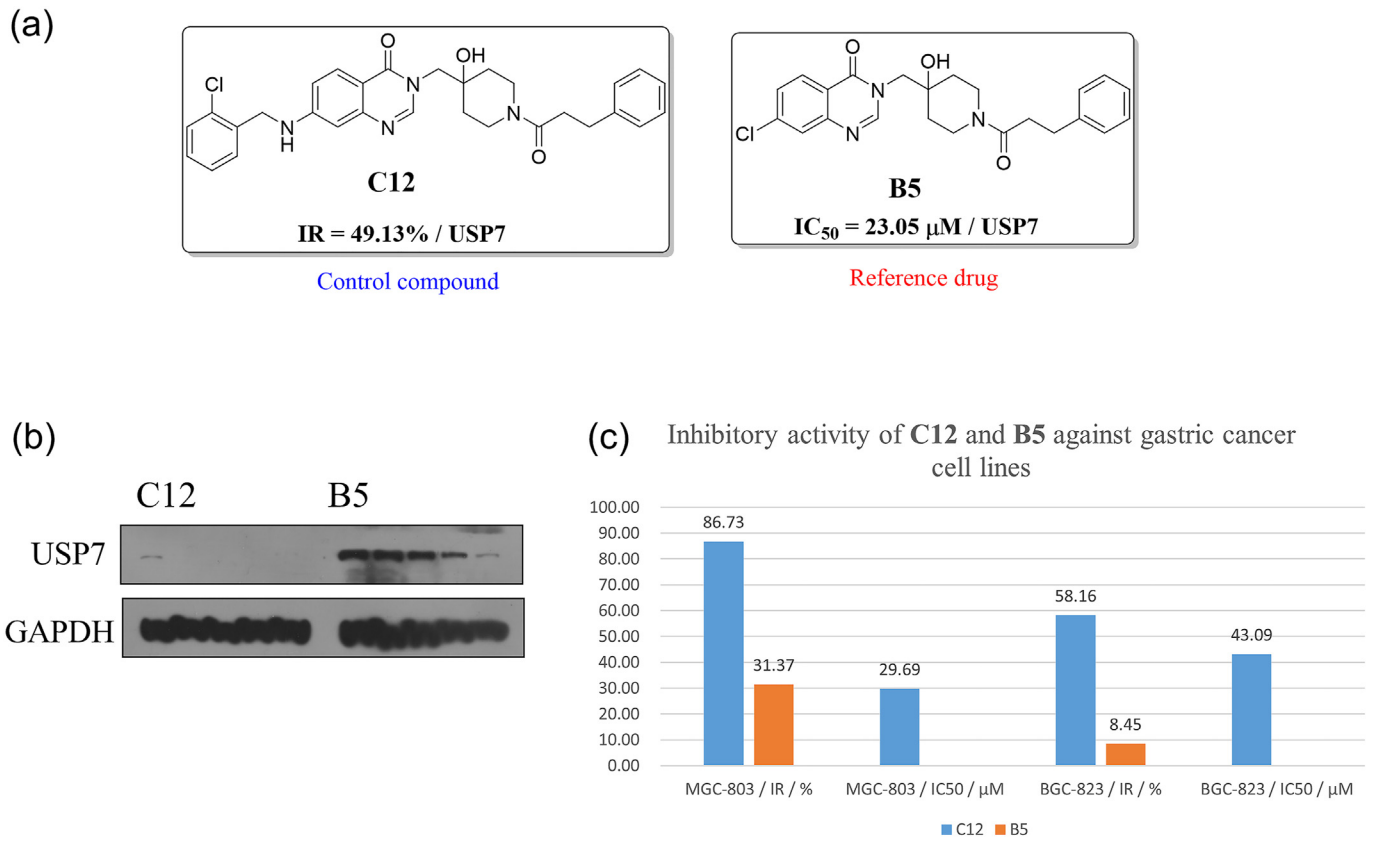


Fig. 3. Analysis of the USP7 inhibitory mechanism against MGC-803 cells. (a) Structures and USP7 inhibitory activity of C12 and B5. (b) Western blot results of cells treated with C12 and B5. (c) Inhibitory activity of C12 and B5 against MGC-803 cells.

also optimized using the 'QuickPrep' function, and the solvent and the same copy of protein were deleted. The optimized result was used as the final model for molecular modelling. We chose the better-overlapped conformation of C16 with the co-crystal ligand as the research target. It was essential to keep the quinazolin-4(3*H*)-one bound to the 4-hydroxypiperidine moiety in the proper conformation for USP7 inhibitory activity. The scores and activities of these compounds are summarized in Table S3. The side chains of C16 and C19 at C-7 of the quinazolin-4(3*H*)-one moiety had the same orientation and solvent exposure contact area; further investigation of the ligand interaction of C16 and C19 revealed the important hydrogen bond interaction with residue Met407 (Fig. 7). Other common important interactions included hydrogen bonds with residues Phe409, Arg408, Gln297, Val296, Asp295 and Tyr465 and the hydrophobic interaction of the 3-phenylbutanoic acid moiety with the binding pocket. Interestingly, no π-π stacking interaction was observed, perhaps due to the special conformation of C19. In particular, the phenyl ring plane of the 3-phenylbutanoic acid moiety was perpendicular to the phenyl ring plane of Phe409. This was common to C16 and C19 (Fig. S7), which was the same as the co-crystal ligand. Additionally, we overlapped C16, C17 and C19 as co-crystal ligands, as shown in Fig. S8. This indicated that the lipophilic properties remarkably affected the location of the side chain at C-7 of the quinazolin-4(3*H*)-one moiety and that both the hydrogen bond interaction and the lipophilicity of the side chain affected the USP7 inhibitory activity.

The racemic methyl group maintained the stability of the special conformation of the ligand, which further enhanced the ligand-receptor interaction. Without the racemic methyl group, the interaction of the side chain at C-7 of the quinazolin-4(3*H*)-one moiety was strong, which further affected the arrangement of the whole molecule in the binding pocket. This was supported by the binding mode of C13 with the co-crystal ligand (Fig. 8).

2.6. *In vivo* anti-tumour effect of compound C9

Currently, studies on USP7 inhibitors mainly focus on cell-level evaluations, and few animal model data are disclosed. Based on the previous evaluation, we evaluated the anti-tumour effect of C9 in a zebrafish gastric cancer MGC-803 cell model. First, we tested the maximum tolerated dose (MTD) to determine the administered concentration (Table S4). The data indicated that C9 did not induce the zebrafish death and toxicity phenotype at 25.0 and 50.0 ng per fish; however, the death rates reached 30.0% and 36.7% when the administered doses were 100 ng per fish and 200 ng per fish, respectively. Subsequently, the MTD of C9 in zebrafish was determined as 50.0 ng per fish. Then, we divided the zebrafish model into three groups: a control group (0 ng per fish), a medium-dose group (16.7 ng per fish, 1/3 MTD) and a high-dose group (50.0 ng per fish, MTD). The drug was injected directly into tumour tissues. The results of the zebrafish model are summarized in Table S5 and Fig. S9. The results indicated that C9 exhibited a remarkable anti-tumour effect *in vivo*.

3. Conclusions

Here we reported several novel quinazolin-4(3*H*)-one derivatives as potent USP7 inhibitors. We analyzed the co-crystal structure (PDB ID: 5VSB) and selected the ligand as a lead compound and reference drug. In combination with structure-based

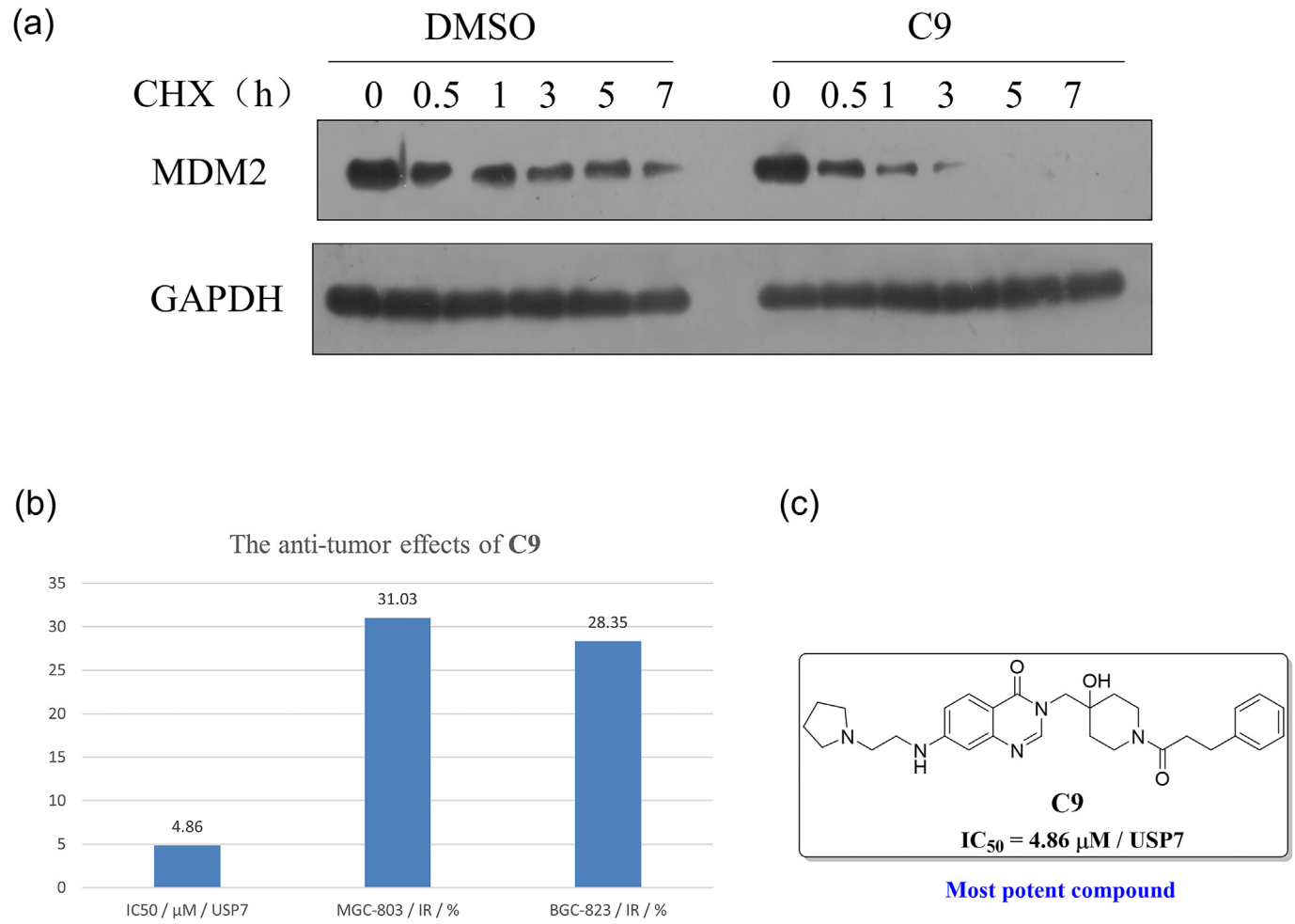


Fig. 4. Effect of C9 on MDM2 protein levels. (a) C9 inhibited USP7 activity and led to the degradation of MDM2. (b) Anti-tumour effects of C9. (c) Structure and USP7 inhibitory activity of C9.

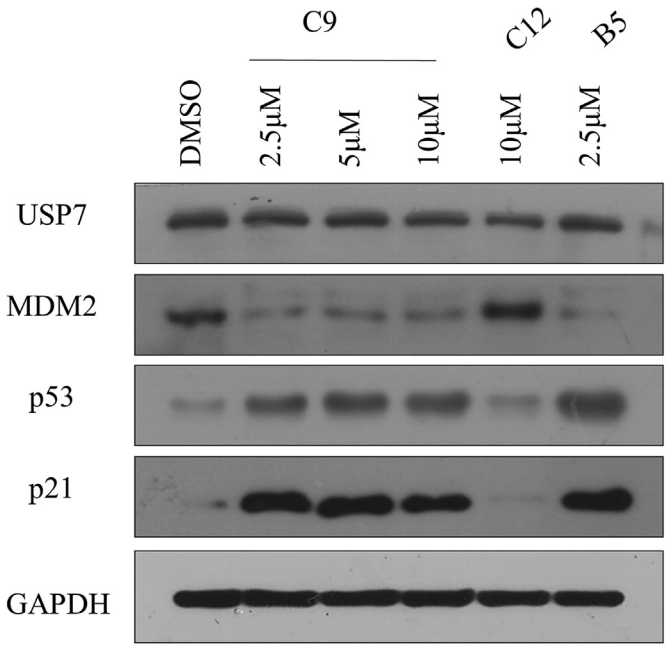


Fig. 5. Effect of C9 on the p53-MDM2-USP7 pathway.

drug design, we designed a series of derivatives. We then utilized the Corey-Chaykovsky epoxidation reaction to synthesize the key intermediate A2 and Cu-catalyzed Buchwald-Hartwig coupling reaction to synthesize the final products. Further anti-tumour evaluation indicated that C9 and C19 exhibited the best inhibitory activity against the catalytic domain of USP7, with IC_{50} values of 4.86 μM and 1.537 μM, respectively, compared with the IC_{50} value of reference drug B5, which was 23.05 μM. This was further confirmed using the Ub-AMC assay, which revealed the IC_{50} for C9 and C19 as 5.048 μM and 0.595 μM, respectively. These derivatives also exhibited moderate inhibitory activity against gastric cancer cells, although MGC-803 cells were more sensitive to these compounds than BGC-823 cells. In particular, B18 (IC_{50} = 30.03 μM for MGC-803; IR = 52.03% for BGC-823; IC_{50} = 46.3 μM for USP7) and C8 (IC_{50} = 32.13 μM for MGC-803; IR = 54.01% for BGC-823; IC_{50} = 15.31 μM for USP7) exhibited better inhibitory activity against both USP7 and gastric cancer cells, whereas the reference drug B5 exhibited no obvious inhibitory activity against gastric cancer cells, with IR < 50% at 50 μM. Furthermore, C9 restricted the cell cycle at the G0/G1 and S phases to inhibit gastric cancer cell proliferation. Western blot using MGC-803 cells indicated that C9 induced the p53-MDM2-USP7 pathway to interfere with cancer progression. C9 inhibited USP7 activity, leading to a decreased level of MDM2; this further increased the levels of the MDM2 downstream proteins p53 and p21. BLI assay also confirmed the high

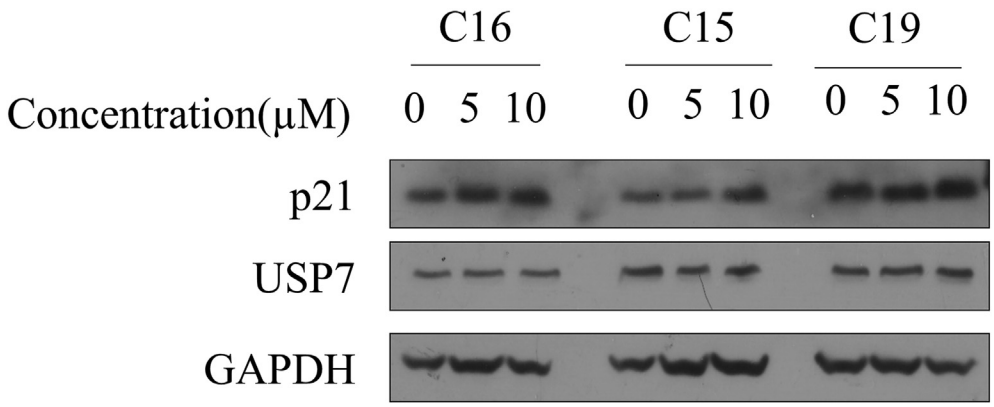


Fig. 6. Effect of C15, C16 and C19 on the p53-MDM2-USP7 pathway in BGC-823 cells.

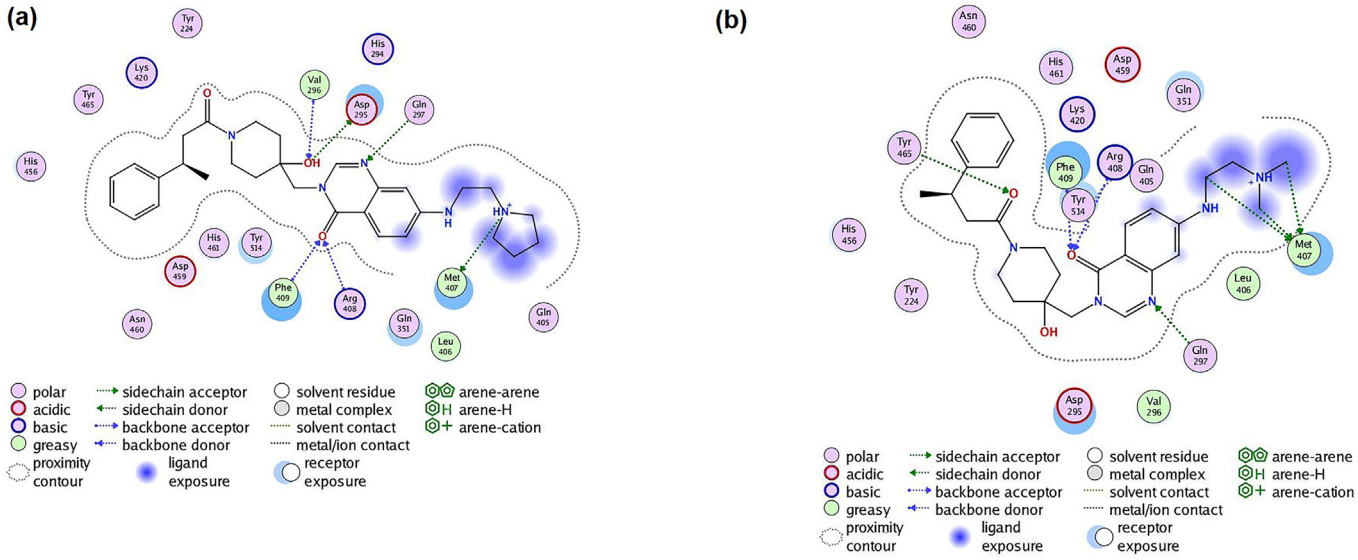


Fig. 7. Ligand interactions of (a) C16 and (b) C19 with the catalytic domain of USP7 (PDB ID: 5VSB).

affinity of C9 for the USP7 catalytic domain. Additionally, docking studies for C9 and C19 predicted that the C-7 side chain of the quinazolin-4(3*H*)-one mother core located at the solvent exposure part of the USP7 catalytic domain forms an important hydrogen bond with Met407, which enhances the ligand-receptor interaction. Finally, C9 exhibited remarkable anti-tumour effects in the zebrafish gastric cancer MGC-803 cell model. These results indicated that quinazolin-4(3*H*)-one derivatives could serve as promising leads for the development of potent USP7 inhibitors and particularly for anti-gastric cancer drugs.

4. Experimental section

4.1. General information

Reagents and solvents were purchased from commercial sources and used directly without further purification. Thin-layer chromatography (TLC) plates were prepared by coating glass plates with silica gel (Qingdao Haiyang Chemical Co., GF254), and ultraviolet light (254 nm), and phosphomolybdic acid were used to visualize the TLC plates. Silica gel (Qingdao Haiyang Chemical Co., 200–300 mesh) was used in the column chromatography procedure. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 400-MHz and 100-MHz spectrometers, respectively. TMS was used as an internal standard in $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6. Chemical shifts were reported as δ ppm values relative to TMS. Proton coupling patterns were described as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), triplet of doublets (td), quartet (q), multiplet (m), and broad (br). High-resolution mass spectra of all derivatives were recorded on a Waters Q-T Micromass spectrometer using electrospray ionization. The melting point (m.p.) was measured on a microscopic melting point apparatus. The purity of compounds was tested by Shimadzu LC-20A HPLC with VWD detector. The HPLC conditions were as follows: Shiseido C18 MG column, 4.6 mm × 250 mm, 5 μm; detection wavelength: 254 nm; flow rate: 1.0 ml/min, eluent: MeOH/H_2O = 40/60 (v/v), and the temperature: 23.5 °C.

4.2. General procedure for the synthesis of A2, B2–B4, B5–B31

4.2.1. tert-Butyl 1-oxa-6-azaspiro[2.5]octane-6-carboxylate (A2)

Trimethylsulfoxonium iodide (3.094 g,14.06 mmol,1.4 eq) was dissolved in 15 ml of dry DMSO in an ice bath, and then NaH (0.562 g, 14.06 mmol,1.4 eq, 60% dispersion in mineral oil) was added in portions. The solution was stirred at 0 °C for 30 min, and then *tert*-butyl 4-oxopiperidine-1-carboxylate (2.0 g, 10.04 mmol,

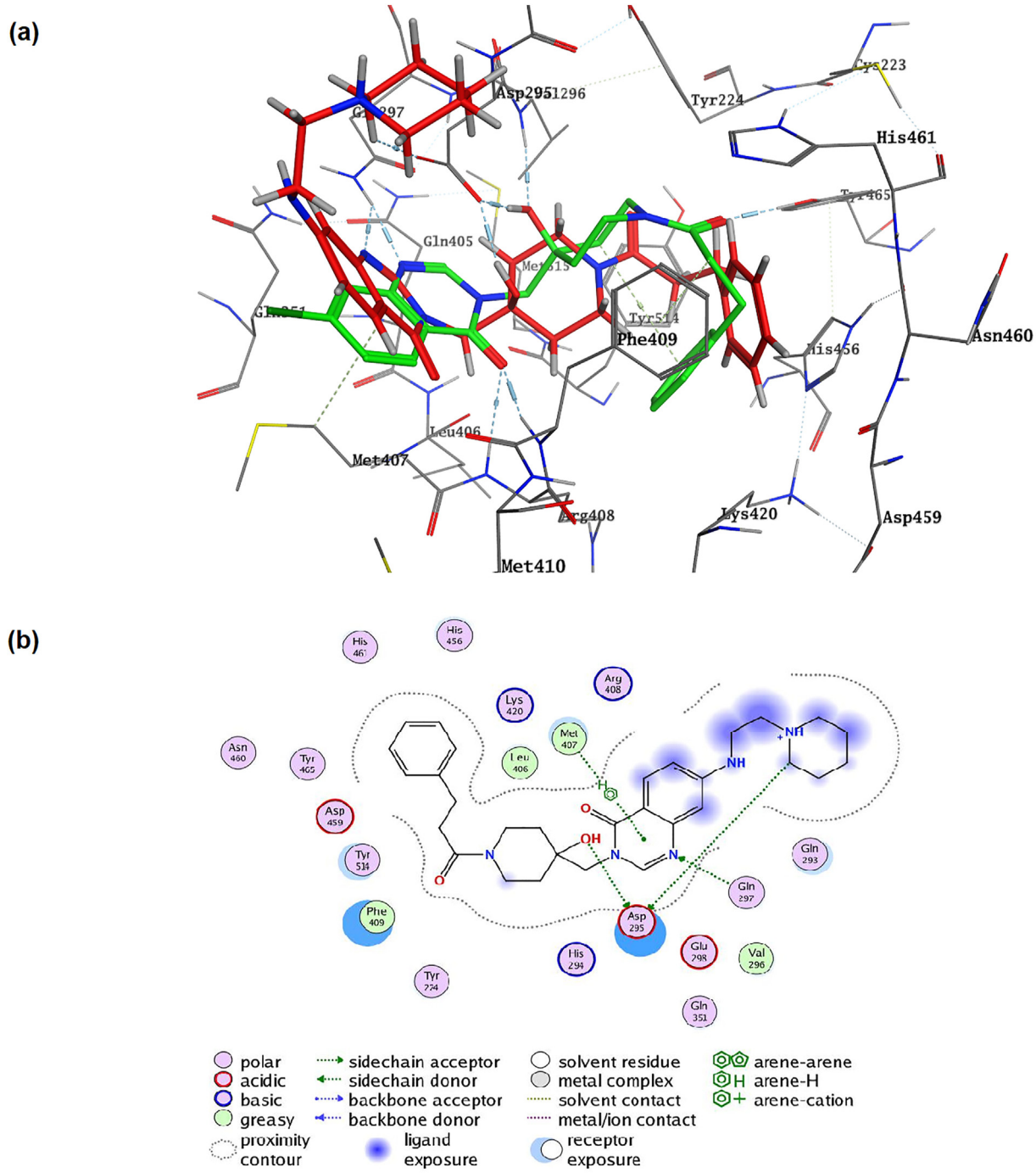


Fig. 8. Binding mode of C13 against the catalytic domain of USP7 (PDB ID: 5VSB). (a) Residues around C13 binding. The red structure represents C13, and the green structure represents the co-crystal ligand. Other structures represent residues of the receptor. (b) The ligand interactions of C13.

1.0 eq) was added. The reaction mixture was stirred at 0 °C for another 4 h. Then 60 ml of water was added to the reaction mixture, and it was extracted with EA (3 × 90 ml). The organic phase was washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield the crude product, which was further purified by flash chromatography, eluting with PE:EA at a ratio of = 10:1 and then 5:1 to yield the pure product A2 (1.285 g, white solid,60% yield). m.p.56.0–56.9 °C. ^{1}H NMR (400 MHz, Chloroform-d) δ 3.72 (m, J = 13.7, 4.9 Hz, 2H), 3.43 (m, J = 13.2, 9.4, 3.7 Hz, 2H), 2.69 (s, 2H), 1.80 (m, J = 13.8, 9.4, 4.5 Hz, 2H), 1.47 (s, 11H). ^{13}C NMR (101 MHz, Chloroform-d) δ14.11, 28.44, 29.69, 53.73, 57.15, 79.73, 154.77. HRMS (ESI) m/z calcd for $C_{11}H_{19}NO_3$ $[M+H]^+$, 214.1365, found: 214.1793.

4.2.2. 7-Chloroquinazolin-4(3H)-one (B2)

2-amino-4-chlorobenzoic acid (1.0 g, 5.83 mmol, 1.0 eq) was suspended in 10 ml of formamide. The mixture was heated at 150 °C for 12 h, cooled to room temperature, and filtered under reduced pressure to yield a crude product, which was washed with ethanol and dried at room temperature overnight to yield the pure product B2 (0.744 g, brown solid, 70.7% yield). m.p.202.7–203.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (s, 1H), 8.18–8.09 (m, 2H),

7.73 (d, J = 2.0 Hz, 1H), 7.56 (dd, J = 8.5, 2.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 121.42, 126.33, 126.98, 127.92, 138.87, 146.89, 149.83, 160.13. HRMS (ESI) m/z calcd for $C_8H_5ClN_2O$ $[M+H]^+$ 181.0090, found: 181.0157.

4.2.3. tert-Butyl 4-((7-chloro-4-oxoquinazolin-3(4H)-yl)methyl)-4-hydroxypiperidine-1-carboxylate (B3)

B2 (2.0 g, 11.07 mmol, 1.0 eq), A2 (2.597 g, 12.18 mmol, 1.1 eq) and anhydrous potassium carbonate (10.821 g, 33.21 mmol, 3.0 eq) in 20 ml of DMF were heated at 80 °C for 12 h. Then, 90 ml of water was added, and it was extracted with EA (3 × 90 ml). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. Next, 10 ml of PE and EA (1:1, v/v) was added to the crude product, followed by stirring at room temperature for 30 min. This was filtered under reduced pressure and dried at room temperature overnight to yield the pure product B3 (3.651 g, white solid, 83.7% yield). m.p.181.1–182.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (s, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.58 (dd, J = 8.6, 2.0 Hz, 1H), 4.93 (s, 1H), 4.00 (s, 2H), 3.66 (d, J = 13.1 Hz, 2H), 3.05 (s, 2H), 1.48 (td, J = 12.4, 10.9, 4.2 Hz, 2H), 1.39 (s, 11H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 28.05, 34.28, 53.77, 69.13, 78.47, 120.32, 126.19, 127.14, 128.42, 138.87, 148.98, 150.39, 153.79, 160.16. HRMS (ESI) m/z calcd for $C_{19}H_{24}ClN_3O_4$ $[M+H]^+$ 394.1455,found: 394.1509.

4.2.4. 7-Chloro-3-((4-hydroxypiperidin-4-yl)methyl)quinazolin-4(3H)-one (B4)

B3 (2.0 g, 5.08 mmol, 1.0 eq) in 6 ml of TFA was stirred at room temperature for 4 h. Next, 20 ml of water was added to the above mixture in an ice bath, and this was basified by the addition of ammonium hydroxide until a pH of 8.0 was attained. This solution was stirred at 0 °C for 30 min and filtered under reduced pressure. The precipitate was dried at room temperature overnight to yield the pure product B4 (1.492 g, white solid, about 100% yield). m.p. 228.5–229.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 1H), 8.17 (d, J = 8.6 Hz, 1H), 8.11 (s, 1H), 7.76 (d, J = 2.1 Hz, 1H), 7.59 (dd, J = 8.7, 2.1 Hz, 1H), 5.38 (s, 1H), 4.06 (s, 2H), 3.18 (d, J = 12.5 Hz, 2H), 3.09–2.93 (m, 2H), 1.87–1.71 (m, 2H), 1.60 (d, J = 14.1 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 31.11, 53.51, 67.49, 120.27, 126.25, 127.24, 128.39, 138.99, 148.96, 150.28, 160.21. HRMS (ESI) m/z calcd for $C_{14}H_{16}ClN_3O_2$ $[M+H]^+$ 294.0931, found: 294.0992.

4.2.5. 7-Chloro-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)quinazolin-4(3H)-one (B5)

3-phenylpropanoic acid (0.278 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and triethylamine (TEA) (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added. The mixture was then stirred at room temperature for 10 min. B4 (0.5, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added to the above mixture. The reaction mixture was stirred at room temperature for 5 h. Next, 50 ml of water was added, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was purified by flash column chromatography, eluting with PE:EA at a ratio of = 2:1 and then 1:2, to yield the pure product B5 (0.419 g, colorless oil, 80% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (s, 1H), 8.16 (d, J = 8.7 Hz, 1H), 7.74 (s, 1H), 7.57 (d, J = 8.7 Hz, 1H), 7.25 (d, J = 9.3 Hz, 4H), 7.16 (t, J = 7.4 Hz, 1H), 4.98 (s, 1H), 4.09 (d, J = 13.0 Hz, 1H), 3.99 (s, 2H), 3.63 (d, J = 13.6 Hz, 1H), 3.23 (t, J = 12.2 Hz, 1H), 2.92 (t, J = 11.6 Hz, 1H), 2.81 (t, J = 7.8 Hz, 2H), 2.71–2.57 (m, 2H), 2.51 (s, 1H), 1.43 (d, J = 15.6 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 30.87, 33.87, 34.20, 34.89, 36.94, 38.20, 40.80, 53.79, 69.26, 120.32, 125.76, 126.19, 127.10, 128.14, 128.34, 128.39, 138.87, 141.42, 148.97, 150.32, 160.12, 169.56. HRMS (ESI) m/z calcd for $C_{23}H_{24}ClN_3O_3$ $[M+H]^+$, 426.1506, found: 426.1568. LC t_R: 1.971 min, purity 54.74%.

4.2.6. 3-((1-benzoyl-4-hydroxypiperidin-4-yl)methyl)-7-chloroquinazolin-4(3H)-one (B6)

Benzoic acid (0.226 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added. The reaction mixture was stirred at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added to the above mixture, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product, which was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product. The crude product was further purified by flash column chromatography, eluting with PE:EA at a ratio of = 2:1 and then 1:2 to yield the pure product B6 (0.415 g, colorless oil, 85% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.57 (dd, J = 8.5, 2.1 Hz, 1H), 7.47–7.42 (m, 3H), 7.39 (dd, J = 6.7, 3.0 Hz, 2H), 5.05 (s, 1H), 4.21 (s, 1H), 4.04 (s, 2H), 3.27 (s, 1H), 3.15 (s, 1H), 1.63 (s, 2H), 1.54 (s, 1H), 1.36 (d, J = 13.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.90, 160.18, 150.38, 148.98, 138.87, 136.30, 129.27, 128.41, 128.34, 127.12, 126.62, 126.19, 120.34, 69.40, 53.85. HRMS (ESI) m/z calcd for $C_{21}H_{20}ClN_3O_3$ $[M+H]^+$, m/z: 398.1193, found: 398.1262.

4.2.7. 7-Chloro-3-((4-hydroxy-1-(1H-pyrrole-2-carbonyl) piperidin-4-yl)methyl)quinazolin-4(3H)-one (B7)

1*H*-pyrrole-2-carboxylic acid (0.206 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added. The mixture was stirred at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added to the above mixture, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of = 2:1 and then 1:2 to yield the pure product B7 (0.214 g, brown solid, 45% yield). m.p.116.8–116.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (s, 1H), 8.32 (s, 1H), 8.17 (d, J = 8.6 Hz, 1H), 7.76 (d, J = 2.2 Hz, 1H), 7.58 (dd, J = 8.6, 2.1 Hz, 1H), 6.87 (s, 1H), 6.46 (s, 1H), 6.10 (q, J = 2.8 Hz, 1H), 5.04 (s, 1H), 4.12 (d, J = 13.3 Hz, 2H), 4.04 (s, 2H), 3.30 (s, 2H), 1.66–1.54 (m, 2H), 1.48 (d, J = 13.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.39, 160.18, 150.39, 148.99, 138.89, 128.44, 127.15, 126.21, 124.37, 120.82, 120.34, 111.34, 108.22, 69.50, 53.81, 34.75. HRMS (ESI) m/z calcd for $C_{19}H_{19}ClN_4O_3$ $[M+H]^+$, m/z: 387.1146, found: 387.1208.

4.2.8. 7-Chloro-3-((4-hydroxy-1-picolinoylpiperidin-4-yl)methyl) quinazolin-4(3H)-one (B8)

Picolinic acid (0.228 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g,2.46 mmol,2.0 eq) were added. The mixture was stirred at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Next, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was

washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, and left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of = 2:1 and then 1:2 to yield the pure product B8 (0.152 g, brown solid, 31% yield). m.p.124.3–124.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (d, J = 4.8 Hz, 1H), 8.31 (s, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.98–7.88 (m, 1H), 7.75 (d, J = 2.2 Hz, 1H), 7.61–7.52 (m, 2H), 7.47 (dd, J = 7.5, 5.1 Hz, 1H), 5.06 (s, 1H), 4.23 (d, J = 13.0 Hz, 1H), 4.09 (d, J = 13.7 Hz, 1H), 4.01 (d, J = 13.7 Hz, 1H), 3.48 (d, J = 13.6 Hz, 1H), 3.32–3.05 (m, 2H), 1.72–1.51 (m, 3H), 1.37 (d, J = 13.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.59, 160.17, 154.35, 150.37, 148.97, 148.37, 138.89, 137.28, 128.42, 127.15, 126.20, 124.37, 122.77, 120.32, 69.39, 53.83, 42.40, 37.40, 34.90, 34.24. HRMS (ESI) m/z calcd for $C_{20}H_{19}ClN_4O_3$ $[M+H]^+$, m/z: 399.1146, found: 399.1223.

4.2.9. 7-Chloro-3-((4-hydroxy-1-nicotinoylpiperidin-4-yl)methyl) quinazolin-4(3H)-one (B9)

Nicotinic acid (0.228 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added to the above mixture, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, and left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of = 2:1 and then 1:2 to yield the pure product B9 (0.196 g, brown solid, 40% yield). m.p.145.5–145.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67–8.59 (m, 2H), 8.30 (s, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.58 (dd, J = 8.6, 2.1 Hz, 1H), 7.48 (dd, J = 7.7, 5.0 Hz, 1H), 5.06 (s, 1H), 4.23 (d, J = 12.8 Hz, 1H), 4.04 (s, 2H), 3.32 (s, 1H), 3.14 (t, J = 12.1 Hz, 1H), 1.66 (d, J = 12.4 Hz, 2H), 1.53 (d, J = 13.6 Hz, 1H), 1.38 (d, J = 13.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.61, 160.18, 150.38, 150.23, 148.98, 147.38, 138.87, 134.52, 132.07, 128.42, 127.13, 126.19, 123.48, 120.35, 69.35, 53.87. HRMS (ESI) m/z calcd for $C_{20}H_{19}ClN_4O_3$ [M+H], 399.1146, found: 399.1218.

4.2.10. 7-Chloro-3-((1-(3,4-dimethoxybenzoyl)-4-hydroxypiperidin-4-yl)methyl)quinazolin-4(3H)-one (B10)

3,4-dimethoxybenzoic acid (0.337 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g,2.46 mmol,2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF, and added, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. It was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of = 2:1 and then 1:2, to yield the pure product B10 (0.366 g, white solid, 65% yield). m.p.250.4–250.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (s, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.58 (dd, J = 8.6, 2.1 Hz, 1H), 6.97 (d, J = 8.8 Hz, 3H), 5.02 (s, 1H), 4.04 (s, 2H), 3.78 (d, J = 4.6 Hz, 6H), 3.55 (s, 1H), 3.21 (s, 3H), 1.68–1.56 (m, 2H), 1.44 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.89, 160.18, 150.38, 149.63, 148.98, 148.37, 138.87, 128.41, 128.36, 127.13, 126.19, 120.34, 119.57, 111.07, 110.83, 69.45, 55.53, 55.50, 53.86. HRMS (ESI) m/z calcd for $C_{23}H_{24}ClN_3O_5$ $[M+H]^+$, 458.1404, found: 458.1477.

4.2.11. 7-Chloro-3-((4-hydroxy-1-(2-phenoxyacetyl)piperidin-4-yl) methyl)quinazolin-4(3H)-one (B11)

Phenol (1.0 g, 10.63 mmol, 1.0 eq), ethyl 2-bromoacetate (2.664 g, 15.95 mmol, 1.5 eq) and potassium carbonate (2.938 g, 21.26 mmol, 2.0 eq) were dissolved in 10 ml of DMF and stirred at room temperature for 4 h. Then, 60 ml of water was added, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 10 ml of methanol, and potassium hydroxide (2.385 g, 42.52 mmol, 4.0 eq) and water (5 ml) were added. This was heated at 80 °C for 4 h. The solvent was removed under reduced pressure, and 20 ml of water was added. The solution was acidified using 1 M HCl until the pH reached approximately 3.0 and then extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$.The solvent was removed under reduced pressure to yield 2-phenoxyacetic acid (1.051 g, colorless oil, 65% yield), which was used directly in the next step.

2-phenoxyacetic acid (0.281 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 s to yield the pure product B11 (0.447 g, colorless oil, 85% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H), 8.17 (d, J = 8.6 Hz, 1H), 7.76 (d, J = 2.1 Hz, 1H), 7.58 (dd, J = 8.7, 2.1 Hz, 1H), 7.27 (t, J = 7.8 Hz, 2H), 6.91 (d, J = 8.4 Hz, 3H), 5.05 (s, 1H), 4.86–4.73 (m, 2H), 4.02 (q, J = 13.9 Hz, 3H), 3.64 (d, J = 13.6 Hz, 1H), 3.30 (t, J = 12.4 Hz, 1H), 2.99 (t, J = 11.7 Hz, 1H), 1.66 (t, J = 11.1 Hz, 1H), 1.46 (q, J = 10.6, 8.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.55, 160.16, 158.03, 150.37, 148.98, 138.90, 129.30, 128.42, 127.17, 126.22, 120.73, 120.33, 114.51, 69.26, 65.87, 53.78, 37.18, 34.80, 34.16. HRMS (ESI) m/z calcd for $C_{22}H_{22}ClN_3O_4$ $[M+Na]^+$, 450.1299, found: 450.1184.

4.2.12. 7-Chloro-3-((4-hydroxy-1-(2-nitrobenzoyl)piperidin-4-yl) methyl)quinazolin-4(3H)-one (B12)

2-nitrobenzoic acid (0.309 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF, and added, followed by stirring at room temperature for 5 h. Next, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product, and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and 1:2 to yield the pure product B12 (0.121 g, white solid, 22.2% yield).

m.p.162.1–162.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (s, 1H), 8.17 (dd, J = 14.2, 8.4 Hz, 2H), 7.84 (t, J = 7.7 Hz, 1H), 7.75 (d, J = 2.0 Hz, 1H), 7.69 (t, J = 7.9 Hz, 1H), 7.58 (dd, J = 8.6, 2.1 Hz, 2H), 5.08 (s, 1H), 4.21 (s, 1H), 4.07 (d, J = 13.6 Hz, 1H), 4.01 (d, J = 13.8 Hz, 1H), 3.21 (s, 2H), 3.20–3.13 (m, 1H), 1.67 (s, 1H), 1.56 (d, J = 13.6 Hz, 2H), 1.34 (d, J = 13.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.06, 160.17, 150.37, 148.98, 138.90, 134.80, 132.68, 130.08, 128.41, 127.90, 127.16, 126.21, 124.67, 120.32, 69.36, 53.80, 36.91, 34.18, 33.77. HRMS (ESI) m/z calcd for $C_{21}H_{19}ClN_4O_5$ [M+Na]$^+$, 465.1044, found: 465.0940.

4.2.13. 7-Chloro-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)quinazolin-4(3H)-one (B13)

3-phenylbutanoic acid (0.304 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g,2.46 mmol,2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Next, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product, and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of = 2:1 first and then 1:2 to yield the pure product B13 (0.271 g, white solid, 50% yield). m.p.157.2–157.4 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.28 (d, J = 15.3 Hz, 1H), 8.16 (d, J = 8.5 Hz, 1H), 7.81–7.73 (m, 1H), 7.59 (dd, J = 8.5, 2.1 Hz, 1H), 7.33–7.21 (m, 5H), 7.16 (m, J = 8.7, 6.2, 2.5 Hz, 1H), 4.95 (d, J = 7.2 Hz, 1H), 4.13–4.00 (m, 1H), 4.00–3.87 (m, 1H), 3.66 (t, J = 14.1 Hz, 1H), 3.27–3.11 (m, 2H), 2.98–2.81 (m, 1H), 2.67–2.56 (m, 2H), 1.48–1.28 (m, 3H), 1.26–1.13 (m, 5H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.25, 22.35, 22.55, 34.67, 34.81, 35.38, 35.51, 36.39, 36.49, 36.71, 37.39, 38.72, 40.70, 41.42, 41.53, 44.05, 54.28, 69.71, 69.77, 120.85, 126.41, 126.45, 126.73, 127.18, 127.36, 127.40, 127.68, 128.67, 128.70, 128.74, 128.94, 139.41, 147.06, 147.18, 149.50, 150.89, 160.62, 165.08, 169.60, 173.38. HRMS (ESI) m/z calcd for $C_{24}H_{26}ClN_3O_3$, [M+Na]+, m/z: 462.1663, found: 462.1562.

4.2.14. 3-((1-(3-bromobenzoyl)-4-hydroxypiperidin-4-yl)methyl)-7-chloroquinazolin-4(3H)-one (B14)

3-bromobenzoic acid (0.372 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product, and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 first and then 1:2 to yield the pure product B14 (0.381 g, colorless oil, 65% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (s, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.70–7.53 (m, 3H), 7.40 (d, J = 4.9 Hz, 2H), 5.03 (s, 1H), 4.18 (d, J = 12.1 Hz, 1H), 4.03 (s, 2H), 3.27 (s, 2H), 3.15 (d, J = 26.0 Hz, 1H), 1.65 (d, J = 12.8 Hz, 2H), 1.50 (d, J = 12.7 Hz, 1H), 1.37 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.14, 160.18, 150.40, 148.99, 138.87, 138.65, 132.09, 130.63, 129.29, 128.44, 127.14, 126.20, 125.55, 121.66, 120.35, 69.37, 53.85, 43.02, 34.67. HRMS (ESI) m/z calcd for $C_{21}H_{19}BrClN_3O_3$ [M+2 + H]$^+$, 478.0298, found: 478.0350.

4.2.15. 7-Bromo-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)quinazolin-4(3H)-one (B15)

The synthetic procedures for B15 were the same as those for C5.

Compound B15, colorless oil, 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 8.08 (d, J = 8.7 Hz, 1H), 7.90 (s, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.31–7.13 (m, 5H), 4.96 (s, 1H), 4.09 (d, J = 12.9 Hz, 1H), 3.98 (s, 1H), 3.63 (d, J = 13.6 Hz, 1H), 3.22 (t, J = 12.4 Hz, 1H), 2.97–2.87 (m, 1H), 2.81 (t, J = 7.8 Hz, 2H), 2.69 (d, J = 3.4 Hz, 1H), 2.66–2.57 (m, 2H), 1.41 (q, J = 15.3, 13.6 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.57, 160.25, 150.27, 149.03, 141.43, 129.89, 129.29, 128.39, 128.35, 128.19, 128.15, 127.82, 125.76, 120.62, 69.26, 53.83, 40.80, 38.20, 36.94, 34.89, 34.21, 33.86, 30.86. HRMS (ESI) m/z calcd for $C_{23}H_{24}BrN_3O_3$ [M+H]$^+$, 470.1001, found: 470.1061.

4.2.16. 7-Chloro-3-((1-(4,5-dimethoxy-2-nitrobenzoyl)-4-hydroxypiperidin-4-yl)methyl) quinazolin-4(3H)-one (B16)

4,5-dimethoxy-2-nitrobenzoic acid (0.420 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 first and then 1:2 to yield the pure product B16 (0.340 g, white solid, 55% yield). m.p.138.4–138.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (s, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.58 (dd, J = 8.6, 2.1 Hz, 1H), 6.60 (s, 1H), 6.36 (s, 1H), 4.97 (s, 1H), 4.02 (s, 2H), 3.82–3.73 (m, 2H), 3.70 (s, 3H), 3.63 (s, 3H), 3.19 (t, J = 11.8 Hz, 2H), 1.68–1.56 (m, 2H), 1.42 (d, J = 13.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.73, 160.17, 150.85, 150.41, 148.99, 141.32, 139.54, 138.86, 128.43, 127.13, 126.19, 120.35, 112.83, 110.05, 100.18, 69.47, 56.41, 55.14, 53.81, 34.61. HRMS (ESI) m/z calcd for $C_{23}H_{23}ClN_4O_7$ [M+H]$^+$, 503.1255, found: 503.1535.

4.2.17. 3-((1-(2-amino-4-chlorobenzoyl)-4-hydroxypiperidin-4-yl) methyl)-7-chloroquinazolin-4(3H)-one (B17)

2-amino-4-chlorobenzoic acid (0.317 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF, and added, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 first and then 1:2 to yield the pure product B17 (0.147 g, brown solid, 26.7% yield). m.p.132.1–132.2 °C. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.22 (d, J = 8.6 Hz, 1H), 8.09 (s, 1H), 7.71 (d, J = 2.1 Hz, 1H), 7.48 (dd, J = 8.5, 2.0 Hz, 1H), 6.97 (d, J = 8.1 Hz, 1H), 6.75–6.63 (m, 2H), 4.09 (s, 4H), 3.49 (s, 1H), 3.32 (d, J = 12.9 Hz, 3H), 1.65 (d, J = 15.4 Hz, 4H), 1.32–1.21 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-d) δ 169.27, 162.23, 148.98, 148.23, 146.94, 141.12, 136.43, 128.99, 128.38, 128.30, 127.09, 120.11, 117.71, 117.55, 116.40, 70.55, 56.29. HRMS (ESI) m/z calcd for $C_{21}H_{20}Cl_2N_4O_3$ [M+H]$^+$, 447.0912, found: 447.0978.

4.2.18. 3-((1-(2-amino-4-bromobenzoyl)-4-hydroxypiperidin-4-yl) methyl)-7-chloroquinazolin-4(3H)-one (B18)

2-amino-4-bromobenzoic acid (0.4 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. Compound B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product, and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 first and then 1:2 to yield the pure product B18 (0.150 g, brown solid, 24.8% yield). m.p.133.8–134.0 °C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.23 (d, J = 8.5 Hz, 1H), 8.08 (s, 1H), 7.73 (s, 1H), 7.49 (d, J = 8.6 Hz, 1H), 6.92 (d, J = 8.1 Hz, 1H), 6.88 (s, 1H), 6.83 (d, J = 8.2 Hz, 1H), 4.11 (s, 3H), 3.35 (s, 3H), 1.65 (s, 5H), 1.26 (t, J = 7.1 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 169.32, 162.35, 148.97, 148.12, 147.10, 129.13, 128.40, 127.14, 124.67, 120.44, 120.11, 119.38, 118.16, 70.60. HRMS (ESI) m/z calcd for $C_{21}H_{20}BrClN_4O_3$ $[M+2+H]^+$, 493.0407, found: 493.0450.

4.2.19. 7-Chloro-3-((1-(2,3-dichlorobenzoyl)-4-hydroxypiperidin-4-yl)methyl)quinazolin-4(3H)-one (B19)

2,3-dichlorobenzoic acid (0.353 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for another 5 h. Next, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 first and then 1:2 to yield the pure product B19 (0.1 g, brown solid, 17.4% yield). m.p.226.3–227.0 °C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.22 (dd, J = 8.5, 6.3 Hz, 1H), 8.09 (d, J = 13.4 Hz, 1H), 7.80–7.69 (m, 1H), 7.48 (d, J = 8.2 Hz, 2H), 7.33–7.10 (m, 3H), 4.52 (dd, J = 39.2, 13.6 Hz, 1H), 4.28–3.94 (m, 2H), 3.49–3.18 (m, 3H), 1.74 (d, J = 4.7 Hz, 3H), 1.54 (d, J = 12.6 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 162.50, 162.14, 148.27, 148.07, 141.20, 133.76, 130.94, 130.85, 128.38, 128.31, 128.16, 127.14, 125.62, 120.11, 70.52, 70.42, 56.73, 56.02, 42.86, 42.24, 37.28, 35.89, 35.46, 34.99. HRMS (ESI) m/z calcd for $C_{21}H_{18}BrCl_3N_3O_3$ $[M+Na]^+$, 488.0414, found: 488.0296.

4.2.20. 7-Chloro-3-((4-hydroxy-1-(2-(2-nitrophenyl)acetyl) piperidin-4-yl)methyl)quinazolin-4(3H)-one (B20)

2-(2-nitrophenyl)acetic acid (0.335 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF, and added, followed by stirring at room temperature for 5 h. Next, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 first and then 1:2 to yield the pure product B20 (0.253 g, white solid, 45% yield). m.p.122.0–122.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 1H), 8.17 (d, J = 8.5 Hz, 1H), 8.04 (dd, J = 8.1, 1.3 Hz, 1H), 7.76 (d, J = 2.1 Hz, 1H), 7.67 (td, J = 7.5, 1.4 Hz, 1H), 7.62–7.49 (m, 2H), 7.46 (dd, J = 7.7, 1.5 Hz, 1H), 5.05 (s, 1H), 4.18 (d, J = 16.6 Hz, 1H), 4.11 (d, J = 15.0 Hz, 2H), 4.00 (td, J = 10.5, 8.6, 4.4 Hz, 2H), 3.80 (dd, J = 14.2, 3.8 Hz, 1H), 3.39 (td, J = 11.1, 5.7 Hz, 1H), 2.99 (m, J = 13.9, 10.6, 4.0 Hz, 1H), 1.69 (td, J = 12.4, 11.3, 4.2 Hz, 1H), 1.54–1.39 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.86, 160.17, 150.37, 149.14, 149.00, 138.90, 133.47, 133.33, 131.92, 128.42, 128.02, 127.16, 126.22, 124.46, 120.33, 69.31, 53.80, 40.99, 37.87, 37.37, 34.86, 34.39. HRMS (ESI) m/z calcd for $C_{22}H_{21}ClN_4O_5$ $[M+Na]^+$, 479.1200, found: 479.1081.

4.2.21. 7-Chloro-3-((4-hydroxy-1-(5-methoxy-2-nitrobenzoyl) piperidin-4-yl)methyl)quinazolin-4(3H)-one (B21)

5-methoxy-2-nitrobenzoic acid (0.365 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF, and added, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 first and then 1:2 to yield the pure product B21 (0.120 g, brown solid, 20.6% yield). m.p.139.5–139.7 °C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.20 (q, J = 8.8, 8.3 Hz, 2H), 8.04 (s, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.47 (t, J = 8.7 Hz, 1H), 7.04–6.91 (m, 1H), 6.80 (d, J = 13.8 Hz, 1H), 4.65 (d, J = 13.3 Hz, 1H), 4.29 (dd, J = 22.0, 14.2 Hz, 1H), 4.19–4.07 (m, 1H), 3.92 (d, J = 10.6 Hz, 3H), 3.56–3.39 (m, 1H), 3.29 (p, J = 12.4 Hz, 2H), 1.95–1.56 (m, 4H), 1.46 (d, J = 13.1 Hz, 1H), 1.26 (s, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.42, 147.99, 141.22, 138.03, 135.49, 128.35, 127.52, 127.18, 114.76, 112.72, 70.64, 70.52, 57.52, 56.27, 43.04, 37.29, 35.27, 34.48. HRMS (ESI) m/z calcd for $C_{22}H_{21}ClN_4O_6$ $[M+Na]^+$, 495.1150, found: 495.1026.

4.2.22. 3-((1-(2-(4-(benzyloxy)phenyl)acetyl)-4-hydroxypiperidin-4-yl)methyl)-7-chloroquina-zolin-4(3H)-one (B22)

2-(4-(benzyloxy)phenyl)acetic acid (0.448 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF, and added, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 first and then 1:2 to yield the pure product B21 (0.135 g, brown solid, 21.2% yield). m.p.222.6–223.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 8.15 (d, J = 8.6 Hz, 1H), 7.75 (s, 1H), 7.61–7.54 (m, 1H), 7.39 (m, J = 27.0, 19.3, 7.4 Hz, 6H), 7.13 (d, J = 8.1 Hz, 2H), 6.93 (d, J = 8.1 Hz, 2H), 5.08 (s, 2H), 4.96 (s, 1H), 4.02 (d, J = 13.7 Hz, 2H), 3.93 (d, J = 13.7 Hz, 1H), 3.70 (d, J = 14.4 Hz, 1H), 3.68–3.55 (m, 2H), 3.25 (t, J = 11.7 Hz, 1H), 2.97 (t, J = 11.5 Hz, 1H),

1.47−1.43 (m, 1H), 1.41 (s, 2H), 1.33 (d, J = 14.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.82, 160.13, 156.85, 150.37, 138.89, 137.17, 129.81, 128.42, 128.38, 128.09, 127.73, 127.60, 127.16, 126.21, 120.32, 114.60, 69.22, 69.13, 53.68, 41.36, 38.69, 37.08, 34.89, 34.25. HRMS (ESI) m/z calcd for $C_{29}H_{28}ClN_3O_4$ $[M+H]^+$, 518.1768, found: 518.1827.

4.2.23. 7-Chloro-3-((4-hydroxy-1-(2-(naphthalen-1-yloxy)acetyl) piperidin-4-yl)methyl) quinaz-olin-4(3H)-one (B23)

The key intermediate 2-(naphthalen-1-yloxy)acetic acid was synthesized according to previously reported procedures [38].

2-(naphthalen-1-yloxy)acetic acid (0.374 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF, and added, followed by stirring at room temperature for 5 h. Next, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 first and then 1:2 to yield the pure product B23 (0.5 g, white solid, 85% yield). m.p.212.5−213.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (s, 1H), 8.25−8.19 (m, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.91−7.84 (m, 1H), 7.76 (d, J = 2.1 Hz, 1H), 7.59 (dd, J = 8.6, 2.1 Hz, 1H), 7.53 (dq, J = 5.9, 3.5, 2.0 Hz, 2H), 7.48 (d, J = 8.5 Hz, 1H), 7.39 (t, J = 8.0 Hz, 1H), 6.91 (d, J = 7.7 Hz, 1H), 5.06−4.95 (m, 3H), 4.08 (d, J = 13.3 Hz, 2H), 3.99 (d, J = 13.7 Hz, 1H), 3.74 (d, J = 13.5 Hz, 1H), 3.41−3.30 (m, 1H), 3.03 (t, J = 11.8 Hz, 1H), 1.69 (t, J = 12.0 Hz, 1H), 1.54 (s, 1H), 1.46 (d, J = 13.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.37, 160.16, 153.43, 150.38, 148.99, 138.90, 133.99, 128.43, 127.40, 127.18, 126.41, 126.22, 125.98, 125.30, 124.85, 121.56, 120.35, 120.20, 105.55, 69.28, 66.39, 53.78, 37.24, 34.86, 34.19. HRMS (ESI) m/z calcd for $C_{26}H_{24}ClN_3O_4$ $[M+Na]^+$, 500.1455, found: 500.1348.

4.2.24. 7-Chloro-3-((4-hydroxy-1-tosylpiperidin-4-yl)methyl) quinazolin-4(3H)-one (B24)

B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 4 ml of DMF, and 4-methyl benzenesulfonyl chloride (0.281 g, 1.48 mmol, 1.2 eq) was added, followed by stirring at room temperature for 4 h. Next, 50 ml of water was added, followed by stirring for 10 min. This was filtered under reduced pressure and dried at room temperature overnight to yield the pure product B24 (0.141 g, white solid, 46.2% yield). m.p.210.4−210.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H), 8.15 (d, J = 8.6 Hz, 1H), 7.74 (d, J = 2.2 Hz, 1H), 7.65−7.54 (m, 3H), 7.44 (d, J = 7.9 Hz, 2H), 4.80 (s, 1H), 3.95 (s, 2H), 3.37 (d, J = 11.5 Hz, 2H), 2.40 (s, 3H), 1.71−1.59 (m, 2H), 1.47 (d, J = 13.4 Hz, 2H), 1.32−1.21 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.12, 150.26, 148.94, 143.43, 138.90, 132.67, 129.77, 128.44, 127.42, 127.18, 126.20, 120.31, 68.09, 53.78, 41.57, 33.63, 20.97. HRMS (ESI) m/z calcd for $C_{21}H_{22}ClN_3O_4S$ $[M+Na]^+$, 470.1020, found: 470.0900.

4.2.25. 7-Chloro-3-((4-hydroxy-1-(4-hydroxybenzoyl)piperidin-4-yl)methyl)quinazolin-4(3H)-one (B25)

4-hydroxybenzoic acid (0.256 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product B25 (0.148 g, white solid, 29.1% yield). m.p.203.8−204.0 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.29 (m, J = 25.5, 23.4, 14.7, 5.8 Hz, 4H), 8.17 (m, J = 7.9, 4.1, 2.0 Hz, 1H), 8.09−7.92 (m, 2H), 7.85−7.70 (m, 1H), 7.48 (td, J = 8.7, 5.4, 2.4 Hz, 1H), 7.44−7.36 (m, 1H), 7.02−6.89 (m, 1H), 5.16−4.97 (m, 1H), 4.31−3.95 (m, 2H), 3.11 (d, J = 4.7 Hz, 6H), 1.77−1.34 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 38.72, 40.66, 54.38, 69.92, 116.20, 118.64, 119.31, 120.88, 122.51, 122.71, 123.23, 126.72, 127.66, 128.78, 128.97, 131.45, 132.18, 132.94, 133.44, 139.40, 150.92, 160.72. HRMS(ESI) m/z calcd for $C_{21}H_{20}ClN_3O_4$ $[M+H]^+$, 414.1142, found: 414.1233.

4.2.26. 7-Chloro-3-((4-hydroxy-1-(4-nitrobenzoyl)piperidin-4-yl) methyl)quinazolin-4(3H)-one (B26)

4-nitrobenzoic acid (0.309 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Next, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 first and then 1:2 to yield the pure product B26 (0.136 g, white solid, 25% yield). m.p.256.1−256.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, J = 7.3 Hz, 3H), 8.15 (d, J = 8.4 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.68 (d, J = 8.2 Hz, 2H), 7.58 (dd, J = 8.5, 2.1 Hz, 1H), 5.06 (s, 1H), 4.23 (d, J = 13.0 Hz, 1H), 4.04 (s, 2H), 3.31−3.24 (m, 2H), 3.15 (t, J = 12.2 Hz, 1H), 1.67 (m, J = 22.9, 12.3, 10.9 Hz, 2H), 1.53 (d, J = 13.7 Hz, 1H), 1.35 (d, J = 13.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.93, 160.18, 150.38, 148.99, 147.63, 142.68, 138.88, 128.41, 127.99, 127.15, 126.21, 123.74, 120.35, 69.34, 53.84, 42.89, 37.34, 34.62, 33.94. HRMS (ESI) m/z calcd for $C_{21}H_{19}ClN_4O_5$ $[M+H]^+$, 443.1044, found: 443.1105.

4.2.27. 7-Chloro-3-((1-cinnamoyl-4-hydroxypiperidin-4-yl) methyl)quinazolin-4(3H)-one (B27)

B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 4 ml of DMF, and cinnamoyl chloride (0.247 g, 1.48 mmol, 1.2 eq) was added, followed by stirring at room temperature for 4 h. Then, 50 ml of water was added to it, and it was stirred for 10 min. The mixture was filtered under reduced pressure and dried at room temperature overnight to yield the pure product B27 (0.135 g, white solid, 26% yield). m.p.209.4−209.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.76 (d, J = 2.1 Hz, 1H), 7.74−7.67 (m, 2H), 7.59 (dd, J = 8.6, 2.1 Hz, 1H), 7.47 (d, J = 15.4 Hz, 1H), 7.44−7.32 (m, 3H), 7.28 (d, J = 15.4 Hz, 1H), 5.03 (s, 1H), 4.17 (d, J = 12.8 Hz, 1H), 4.13−3.94 (m, 3H), 3.39 (t, J = 12.3 Hz, 1H), 3.07 (t, J = 11.6 Hz, 1H), 1.59 (s, 1H), 1.54 (d, J = 12.0 Hz, 1H), 1.47 (d, J = 12.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.27, 160.17, 150.39, 149.00, 141.26, 138.90, 135.18, 129.39, 128.68, 128.40, 127.95, 127.17, 126.23, 120.34, 118.44, 69.40, 53.84, 41.06, 37.67, 35.47, 34.43. HRMS (ESI) m/z calcd for $C_{23}H_{22}ClN_3O_3$ $[M+H]^+$, 424.1350, found: 424.1416.

4.2.28. 7-Chloro-3-((1-(2-(4-fluorophenyl)acetyl)-4-hydroxypiperidin-4-yl)methyl)quinazolin-4(3H)-one (B28)

2-(4-fluorophenyl)acetic acid (0.285 g, 1.85 mmol, 1.5 eq) was

dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 first and then 1:2 to yield the pure product B28 (0.125 g, white solid, 42.7% yield). m.p.202.3–202.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.58 (dd, J = 8.6, 2.1 Hz, 1H), 7.29–7.20 (m, 2H), 7.16–7.05 (m, 2H), 4.98 (s, 1H), 4.08–3.99 (m, 2H), 3.94 (d, J = 13.7 Hz, 1H), 3.76–3.63 (m, 3H), 3.27 (m, J = 13.9, 10.9, 3.2 Hz, 1H), 2.97 (m, J = 13.5, 10.4, 3.9 Hz, 1H), 1.43 (q, J = 8.1, 4.3 Hz, 3H), 1.35 (d, J = 13.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.47, 160.13, 150.36, 148.97, 138.89, 132.23, 132.20, 130.83, 130.75, 128.41, 127.16, 126.21, 120.31, 114.97, 114.76, 69.21, 53.70, 41.28, 38.48, 37.14, 34.88, 34.23. HRMS (ESI) m/z calcd for $C_{22}H_{21}ClFN_3O_3$ $[M+H]^+$, 430.1255, found: 430.1322.

4.2.29. 7-Chloro-3-((4-hydroxy-1-(2-(naphthalen-1-yl)acetyl) piperidin-4-yl)methyl)quinazolin-4(3H)-one (B29)

2-(naphthalen-1-yl)acetic acid (0.344 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Then, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product B29 (0.180 g, white solid, 31.7% yield). m.p.223.4–223.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (s, 1H), 8.16 (d, J = 8.5 Hz, 1H), 7.99–7.87 (m, 2H), 7.81 (d, J = 8.2 Hz, 1H), 7.78–7.70 (m, 1H), 7.58 (dd, J = 8.6, 2.1 Hz, 1H), 7.55–7.46 (m, 2H), 7.43 (dd, J = 8.2, 7.0 Hz, 1H), 7.41–7.29 (m, 1H), 5.02 (s, 1H), 4.16 (s, 2H), 4.07 (td, J = 13.1, 7.5 Hz, 2H), 3.98 (d, J = 13.8 Hz, 1H), 3.91–3.76 (m, 1H), 3.42–3.26 (m, 1H), 3.09–2.98 (m, 1H), 1.64–1.50 (m, 1H), 1.46 (s, 2H), 1.45–1.38 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.53, 160.15, 150.38, 148.99, 138.90, 133.27, 132.75, 132.02, 128.43, 128.30, 127.16, 126.94, 126.91, 126.22, 125.85, 125.55, 125.39, 124.22, 120.33, 69.27, 53.75, 41.36, 37.18, 37.15, 34.95, 34.32. HRMS (ESI) m/z calcd for $C_{26}H_{24}ClN_3O_3$ $[M+H]^+$, 462.1506, found: 462.1570.

4.2.30. 7-Chloro-3-((4-hydroxy-1-(2-(4-(trifluoromethyl)phenyl) acetyl)piperidin-4-yl)methyl) quinazolin-4(3H)-one (B30)

2-(4-(trifluoromethyl)phenyl)acetic acid (0.378 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Next, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and 1:2 to yield the pure product B30 (0.266 g, white solid, 45% yield). m.p.222.3–222.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (s, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.66 (d, J = 8.1 Hz, 2H), 7.58 (dd, J = 8.6, 2.1 Hz, 1H), 7.44 (d, J = 8.0 Hz, 2H), 5.00 (s, 1H), 4.11–4.02 (m, 2H), 3.95 (d, J = 13.7 Hz, 1H), 3.83 (d, J = 3.3 Hz, 2H), 3.79–3.70 (m, 1H), 3.30 (m, J = 14.0, 11.1, 3.1 Hz, 1H), 2.99 (m, J = 13.3, 10.7, 3.7 Hz, 1H), 1.58–1.35 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.93, 160.14, 150.36, 148.97, 141.13, 138.89, 130.01, 128.40, 127.15, 126.21, 124.97, 124.93, 124.89, 124.85, 120.31, 69.22, 53.71, 41.27, 37.19, 34.88, 34.22. HRMS (ESI) m/z calcd for $C_{23}H_{21}ClF_3N_3O_3$ $[M+H]^+$, 480.1224, found: 480.1287.

4.2.31. 3-((1-(4-(benzyloxy)benzoyl)-4-hydroxypiperidin-4-yl) methyl)-7-chloroquinazolin-4(3H)-one (B31)

The key intermediate 4-(benzyloxy)benzoic acid was synthesized according to previously reported procedures [39].

4-(benzyloxy)benzoic acid (0.422 g, 1.85 mmol, 1.5 eq) was dissolved in 2 ml of DMF, and then TEA (0.249 g, 2.46 mmol, 2.0 eq) and HATU (0.935 g, 2.46 mmol, 2.0 eq) were added, followed by stirring at room temperature for 10 min. B4 (0.5 g, 1.23 mmol, 1.0 eq) was dissolved in 2 ml of DMF and added, followed by stirring at room temperature for 5 h. Next, 50 ml of water was added to the mixture, and it was extracted with EA (3 × 60 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to yield crude product. This was dissolved in 5 ml of EA, left at room temperature overnight, filtered to remove the by-product and concentrated under reduced pressure to yield crude product, which was further purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and 1:2 to yield the pure product B31 (0.403 g, white solid, 65% yield). m.p.132.2–132.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.57 (dd, J = 8.6, 2.1 Hz, 1H), 7.50–7.29 (m, 7H), 7.06 (d, J = 8.2 Hz, 2H), 5.14 (s, 2H), 5.03 (s, 1H), 4.04 (s, 3H), 3.33–3.07 (m, 2H), 2.70 (d, J = 2.3 Hz, 1H), 1.61 (d, J = 12.6 Hz, 2H), 1.45 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.86, 160.18, 159.15, 150.36, 148.98, 138.88, 136.76, 128.75, 128.43, 127.88, 127.70, 127.12, 126.19, 120.34, 114.40, 69.43, 69.30, 53.87. HRMS (ESI) m/z calcd for $C_{28}H_{26}ClN_3O_4$ $[M+H]^+$, 504.1612, found: 504.1674.

4.3. General procedure for the synthesis of C2–C19

4.3.1. General procedure for the synthesis of C2–C6

The procedures for the synthesis of C2–C6 were identical to those for B2–B5; therefore, we simply state the results of corresponding synthesis reactions here.

Compound C2, brown solid, 89% yield. m.p.263.8–264.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (s, 1H), 8.15 (d, J = 2.1 Hz, 1H), 8.04 (dd, J = 8.5, 2.1 Hz, 1H), 7.91–7.85 (m, 1H), 7.69 (dt, J = 8.5, 1.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.27, 149.88, 146.84, 129.73, 129.41, 127.91, 127.83, 121.71. HRMS (ESI) m/z calcd for $C_8H_5BrN_2O$ $[M+H]^+$, 224.9585, found: 224.9649.

Compound C3, white solid, 90% yield. m.p.191.9–192.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 8.16–8.01 (m, 1H), 7.89 (dd, J = 6.6, 1.9 Hz, 1H), 7.70 (m, J = 9.4, 7.5, 2.0 Hz, 1H), 4.93 (s, 1H), 4.00 (s, 2H), 3.66 (d, J = 12.8 Hz, 2H), 3.05 (s, 2H), 1.49 (m, J = 15.3, 11.3, 4.4 Hz, 2H), 1.39 (s, 11H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.29, 153.79, 150.31, 149.03, 146.82, 129.89, 129.75, 129.45, 129.28, 128.40, 127.92, 127.82, 120.60, 78.47, 69.12, 53.79, 28.05. HRMS (ESI) m/z calcd for $C_{19}H_{24}BrN_3O_4$ $[M+H]^+$, 438.0950, found: 438.1004.

Compound C4, white solid, 90% yield. m.p.239.1–240.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.31 (s, 1H), 8.08 (d, J = 8.5 Hz, 1H), 7.92 (s, 1H), 7.72 (d, J = 8.6 Hz, 1H), 5.38 (s, 1H), 4.06 (s, 2H), 3.18 (d, J = 12.6 Hz, 2H), 3.03 (t, J = 12.2 Hz, 2H), 1.85–1.73 (m, 2H), 1.60 (d, J = 14.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.34, 150.21, 149.01, 130.01, 129.34, 128.37, 127.94, 120.56, 67.48, 53.53, 31.10. HRMS (ESI) m/z calcd for $C_{14}H_{16}BrN_3O_2$ $[M+H]^+$, 338.0426, found: 338.0484.

Compound C5, colorless oil, 85% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 8.08 (d, J = 8.7 Hz, 1H), 7.90 (s, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.31–7.13 (m, 5H), 4.96 (s, 1H), 4.09 (d, J = 12.9 Hz, 1H), 3.98 (s, 1H), 3.63 (d, J = 13.6 Hz, 1H), 3.22 (t, J = 12.4 Hz, 1H), 2.97–2.87 (m, 1H), 2.81 (t, J = 7.8 Hz, 2H), 2.69 (d, J = 3.4 Hz, 2H), 2.66–2.57 (m, 2H), 1.41 (q, J = 15.3, 13.6 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.57, 160.25, 150.27, 149.03, 141.43, 129.89, 129.29, 128.39, 128.35, 128.19, 128.15, 127.82, 125.76, 120.62, 69.26, 53.83, 40.80, 38.20, 36.94, 34.89, 34.21, 33.86, 30.86. HRMS (ESI) m/z calcd for $C_{23}H_{24}BrN_3O_3$ $[M+H]^+$, 470.1001, found:470.1061.

Compound C6, white solid, 87% yield. m.p.182.4–183.6 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.27 (d, J = 15.4 Hz, 1H), 8.08 (d, J = 8.5 Hz, 1H), 7.98–7.84 (m, 1H), 7.71 (dd, J = 8.5, 2.0 Hz, 1H), 7.26 (dd, J = 8.2, 6.0 Hz, 4H), 7.15 (m, J = 8.5, 6.1, 2.5 Hz, 1H), 4.94 (d, J = 7.1 Hz, 1H), 4.04 (m, J = 13.2, 4.4, 4.0 Hz, 1H), 3.94 (d, J = 30.3 Hz, 2H), 3.65 (m, J = 14.2, 4.3 Hz, 1H), 3.28–3.07 (m, 2H), 2.95–2.82 (m, 1H), 2.68–2.55 (m, 1H), 1.48–1.26 (m, 3H), 1.20 (dd, J = 6.9, 2.4 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.35, 22.55, 34.68, 34.82, 35.38, 35.51, 36.48, 36.70, 37.39, 40.54, 40.71, 41.42, 41.53, 54.32, 69.71, 69.76, 121.13, 121.20, 126.40, 126.45, 127.36, 127.40, 128.35, 128.66, 128.70, 128.92, 129.34, 129.81, 130.43, 135.12, 140.11, 147.06, 147.18, 149.55, 150.79, 150.82, 151.60, 160.74, 160.80, 169.61. HRMS (ESI) m/z calcd for $C_{24}H_{26}BrN_3O_3$ $[M+H]^+$, 484.1158, found: 484.1208.

4.3.2. 7-((furan-2-ylmethyl)amino)-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl) quinazolin-4(3H)-one (C7)

C5 (0.2 g, 0.43 mmol, 1.0 eq), furan-2-ylmethanamine (0.062 g, 0.64 mmol, 1.5 eqv), cuprous iodide (0.004 g, 0.022 mmol, 0.05 eq),(2-hydroxyphenyl)(morpholino)methanone (0.018 g, 0.086 mmol, 0.2 eq) and tripotassium phosphate (0.183 g, 0.86 mmol, 2.0 eq) were mixed in a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C7 (0.044 g, brown solid, 21.3% yield). m.p.197.5–197.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (d, J = 10.3 Hz, 0H), 8.08 (d, J = 8.4 Hz, 1H), 7.82 (d, J = 8.8 Hz, 1H), 7.74–7.65 (m, 0H), 7.59 (s, 1H), 7.30–7.10 (m, 5H), 6.86 (dd, J = 8.8, 2.2 Hz, 1H), 6.66 (d, J = 2.3 Hz, 1H), 6.48 (s, 0H), 6.42–6.37 (m, 1H), 6.34 (d, J = 3.2 Hz, 1H), 4.90 (s, 1H), 4.37 (d, J = 5.7 Hz, 1H), 4.05 (d, J = 12.7 Hz, 1H), 4.02–3.92 (m, 1H), 3.89 (d, J = 6.8 Hz, 1H), 3.62 (d, J = 13.0 Hz, 1H), 3.21 (t, J = 12.2 Hz, 1H), 2.92 (t, J = 11.2 Hz, 1H), 2.79 (t, J = 7.9 Hz, 2H), 2.60 (q, J = 8.9, 8.5 Hz, 2H), 1.38 (m, J = 14.0, 12.4 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.58, 169.55, 160.22, 153.13, 152.25, 150.07, 148.82, 142.22, 141.44, 128.36, 128.16, 127.17, 125.78, 114.60, 110.63, 110.39, 107.29, 104.59, 69.27, 53.25, 40.83, 36.98, 34.90, 34.24, 33.86, 30.86. HRMS (ESI) m/z calcd for $C_{28}H_{30}N_4O_4$ $[M+H]^+$, 487.2267, found: 487.2327.

4.3.3. 3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl) methyl)-7-(phenethylamino) quinazolin-4(3H)-one (C8)

C5 (0.2 g, 0.43 mmol, 1.0 eq), 2-phenylethan-1-amine (0.078 g, 0.64 mmol, 1.5 eqv), cuprous iodide (0.004 g, 0.022 mmol, 0.05 eq), (2-hydroxyphenyl)(morpholino)methanone (0.018 g, 0.086 mmol, 0.2 eq) and tripotassium phosphate (0.183 g, 0.86 mmol, 2.0 eq) were added to a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and then it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C8 (0.080 g, brown oil, 36.9% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.82 (d, J = 8.8 Hz, 1H), 7.36–7.15 (m, 9H), 7.15 (d, J = 7.3 Hz, 1H), 6.81 (dd, J = 8.8, 2.2 Hz, 1H), 6.76 (t, J = 5.4 Hz, 1H), 6.59 (d, J = 2.4 Hz, 1H), 4.93 (d, J = 16.0 Hz, 1H), 4.06 (d, J = 12.7 Hz, 1H), 4.01–3.92 (m, 1H), 3.90 (d, J = 6.9 Hz, 1H), 3.62 (d, J = 13.9 Hz, 1H), 3.38 (d, J = 6.8 Hz, 1H), 3.22 (t, J = 12.4 Hz, 1H), 2.89 (q, J = 9.7, 7.7 Hz, 3H), 2.80 (t, J = 7.8 Hz, 2H), 2.75–2.53 (m, 2H), 1.40 (td, J = 19.8, 6.9 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.58, 169.55, 160.23, 153.43, 150.25, 148.82, 141.44, 139.47, 128.69, 128.36, 128.32, 128.16, 127.28, 126.10, 125.77, 114.38, 110.20, 104.01, 69.28, 53.84, 53.24, 43.94, 40.85, 36.99, 34.91, 34.38, 34.26, 33.86, 30.86. HRMS (ESI) m/z calcd for $C_{31}H_{34}N_4O_3$ $[M+H]^+$, 511.2631, found: 511.2695.

4.3.4. 3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl) methyl)-7-((2-(pyrrolidin-1-yl)ethyl) amino)quinazolin-4(3H)-one (C9)

C5 (0.2 g, 0.43 mmol, 1.0 eq), 2-(pyrrolidin-1-yl)ethan-1-amine (0.073 g, 0.64 mmol, 1.5 eqv), cuprous iodide (0.004 g,0.022 mmol,0.05 eq), (2-hydroxyphenyl)(morpholino) methanone (0.018 g, 0.086 mmol, 0.2 eq) and tripotassium phosphate (0.183 g, 0.86 mmol, 2.0 eq) were added to a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C9 (0.119 g, brown solid, 55.6% yield). m.p.105.8–106.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.81 (d, J = 8.8 Hz, 1H), 7.30–7.12 (m, 5H), 6.82 (d, J = 8.9 Hz, 1H), 6.62 (d, J = 5.7 Hz, 1H), 6.57 (s, 1H), 4.94 (s, 1H), 4.06 (d, J = 13.0 Hz, 1H), 3.97–3.84 (m, 2H), 3.70–3.58 (m, 5H), 3.24 (m, J = 24.2, 5.7, 5.1 Hz, 3H), 2.99–2.87 (m, 1H), 2.80 (t, J = 7.7 Hz, 2H), 2.73–2.61 (m, 2H), 2.60 (d, J = 8.5 Hz, 1H), 2.57–2.45 (m, 2H), 1.91 (s, 0H), 1.71 (d, J = 5.8 Hz, 4H), 1.38 (m, J = 18.1, 13.3, 9.2 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.56, 153.48, 150.23, 148.81, 141.43, 128.35, 128.16, 127.21, 125.77, 114.46, 110.17, 103.91, 69.28, 53.98, 53.60, 53.20, 41.34, 40.86, 37.00, 34.91, 34.24, 33.86, 30.86, 23.06. HRMS (ESI) m/z calcd for $C_{29}H_{37}N_5O_3$ $[M+H]^+$, 504.2896, found: 504.2959. LC t_R: 4.09 min, purity 100%.

4.3.5. 3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl) methyl)-7-((2-methoxybenzyl) amino) quinazolin-4(3H)-one (C10)

C5 (0.2 g, 0.43 mmol, 1.0 eq), (2-methoxyphenyl)methanamine (0.088 g, 0.64 mmol, 1.5 eqv), cuprous iodide (0.004 g, 0.022 mmol, 0.05 eq), (2-hydroxyphenyl)(morpholino)methanone (0.018 g,

0.086 mmol, 0.2 eq) and tripotassium phosphate (0.183 g, 0.86 mmol, 2.0 eq) were added to a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and then it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C10 (0.119 g, brown solid, 55.6% yield). m.p.103.9–104.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11–8.01 (m, 1H),7.81 (d, J = 8.8 Hz, 1H),7.25 (d, J = 10.0 Hz, 6H), 7.22–7.09 (m, 2H), 7.03 (d, J = 8.2 Hz, 1H), 6.92–6.81 (m, 1H), 6.46 (s, 1H), 4.88 (s, 1H), 4.33 (d, J = 5.8 Hz, 1H), 4.05 (d, J = 13.1 Hz, 1H), 3.93 (dd, J = 37.5, 5.2 Hz, 2H), 3.86 (s, 2H), 3.61 (d, J = 11.9 Hz, 1H), 3.21 (t, J = 12.3 Hz, 1H), 2.97–2.85 (m, 1H), 2.84–2.71 (m, 2H), 2.71–2.52 (m, 3H), 1.46–1.29 (m, 5H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.54, 160.20, 156.93, 153.53, 150.30, 150.13, 148.79, 141.44, 129.92, 129.30, 128.35, 128.16, 128.12, 127.68, 127.20, 126.17, 125.77, 120.17, 114.54, 110.67, 110.32, 104.25, 69.26, 55.37, 53.83, 53.24, 40.84, 36.97, 34.90, 34.24, 33.86, 30.86. HRMS (ESI) m/z calcd for $C_{31}H_{34}N_4O_4$ $[M+H]^+$, 527.2580, found: 527.2649.

4.3.6. 7-((4-chlorobenzyl)amino)-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl) quinazolin-4(3H)-one (C11)

C5 (0.2 g, 0.43 mmol, 1.0 eq), (4-chlorophenyl)methanamine (0.091 g, 0.64 mmol, 1.5 eqv), cuprous iodide (0.004 g, 0.022 mmol, 0.05 eq),(2-hydroxyphenyl)(morpholino)methanone (0.018 g, 0.086 mmol, 0.2 eq) and tripotassium phosphate (0.183 g, 0.86 mmol, 2.0 eq) were added to a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and the tube was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C11 (0.042 g, brown solid, 18.4% yield). m.p.95.9–96.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11–8.02 (m, 1H), 7.82 (d, J = 8.8 Hz, 1H), 7.39 (s, 2H), 7.35–7.19 (m, 2H), 7.24 (s, 3H), 7.16 (d, J = 6.9 Hz, 1H), 6.85 (dd, J = 8.9, 2.2 Hz, 1H), 6.49 (d, J = 2.3 Hz, 1H), 4.92 (d, J = 29.9 Hz, 1H), 4.40 (d, J = 5.9 Hz, 1H), 4.12–3.82 (m, 3H), 3.60 (d, J = 11.9 Hz, 1H), 3.23 (d, J = 12.6 Hz, 1H), 2.92 (dd, J = 16.1, 6.4 Hz, 1H), 2.84–2.67 (m, 3H), 2.64–2.54 (m, 3H), 1.37 (d, J = 15.7 Hz, 5H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.54, 160.19, 153.21, 150.08, 148.82, 141.44, 138.30, 131.31, 129.31, 128.91, 128.35, 128.16, 127.25, 125.77, 114.71, 110.58, 104.62, 69.26, 53.83, 53.24, 45.14, 40.83, 36.97, 34.90, 34.24, 33.86, 30.86. HRMS (ESI) m/z calcd for $C_{30}H_{31}ClN_4O_3$ $[M+H]^+$, 531.2085, found: 531.2157.

4.3.7. 7-((2-chlorobenzyl)amino)-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl) quinazolin-4(3H)-one (C12)

C5 (0.2 g, 0.43 mmol, 1.0 eq), (2-chlorophenyl)methanamine (0.091 g, 0.64 mmol, 1.5 eqv), cuprous iodide (0.004 g, 0.022 mmol, 0.05 eq), (2-hydroxyphenyl)(morpholino)methanone (0.018 g, 0.086 mmol, 0.2 eq) and tripotassium phosphate (0.183 g, 0.86 mmol, 2.0 eq) were added to a 20-ml glass tube, and evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C12 (0.036 g, brown solid, 15.8% yield). m.p.113.1–113.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11–7.97 (m, 1H), 7.84 (d, J = 8.9 Hz, 1H), 7.52–7.44 (m, 1H), 7.43–7.09 (m, 8H), 6.86 (t, J = 8.7 Hz, 1H), 6.48 (d, J = 22.8 Hz, 1H), 4.92 (d, J = 27.8 Hz, 1H), 4.43 (dd, J = 25.2, 5.8 Hz, 1H), 4.11–3.80 (m, 3H), 3.71–3.55 (m, 1H), 3.23 (d, J = 12.4 Hz, 1H), 3.02–2.86 (m, 1H), 2.79 (d, J = 8.0 Hz, 2H), 2.59 (q, J = 9.1, 8.3 Hz, 2H), 1.54–1.14 (m, 5H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.58, 169.55, 160.18, 153.13, 150.12, 148.90, 141.44, 135.89, 132.38, 129.36, 128.75, 128.35, 128.16, 127.34, 127.25, 125.77, 114.59, 104.41, 69.26, 53.26, 43.79, 40.82, 36.97, 34.90, 34.24, 33.86, 30.86. HRMS (ESI) m/z calcd for $C_{30}H_{31}ClN_4O_3$ $[M+H]^+$, 531.2085, found: 531.2145.

4.3.8. 3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl) methyl)-7-((2-(piperidin-1-yl)ethyl) amino)quinazolin-4(3H)-one (C13)

C5 (0.2 g,0.43 mmol, 1.0 eq), 2-(piperidin-1-yl)ethan-1-amine (0.091 g, 0.64 mmol, 1.5 eqv), cuprous iodide (0.004 g, 0.022 mmol, 0.05 eq), (2-hydroxyphenyl)(morpholino)methanone (0.018 g, 0.086 mmol, 0.2 eq) and tripotassium phosphate (0.183 g, 0.86 mmol, 2.0 eq) were added to a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C13 (0.119 g, brown solid, 55.6% yield). m.p.139.0–140.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.80 (d, J = 8.9 Hz, 1H), 7.32–7.21 (m, 3H), 7.16 (t, J = 7.2 Hz, 1H), 6.81 (d, J = 8.9 Hz, 1H), 6.59–6.45 (m, 1H), 4.05 (d, J = 12.9 Hz, 1H), 3.99–3.83 (m, 2H), 3.62 (d, J = 14.1 Hz, 2H), 3.52 (d, J = 5.7 Hz, 1H), 3.22 (q, J = 7.5 Hz, 3H), 2.91 (d, J = 12.7 Hz, 1H), 2.79 (t, J = 7.7 Hz, 2H), 2.61 (p, J = 8.0 Hz, 1H), 2.39 (s, 2H), 2.31 (d, J = 7.8 Hz, 1H), 1.87 (s, 2H), 1.50 (h, J = 8.4, 7.1 Hz, 3H), 1.36 (m, J = 10.3, 8.0 Hz, 5H), 1.28–1.11 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.56, 160.23, 153.58, 150.24, 148.81, 141.44, 128.35, 128.16, 127.19, 125.77, 110.12, 69.27, 57.07, 54.17, 53.21, 40.85, 34.91, 34.26, 33.86, 30.86, 25.52, 24.02. HRMS (ESI) m/z calcd for $C_{30}H_{39}N_5O_3$ $[M+H]^+$, 518.3053, found: 518.3119.

4.3.9. 3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl) methyl)-7-((2-hydroxyethyl) amino) quinazolin-4(3H)-one (C14)

C5 (0.2 g, 0.43 mmol, 1.0 eq), 2-aminoethan-1-ol (0.079 g, 0.64 mmol, 1.5 eqv), cuprous iodide (0.004 g, 0.022 mmol, 0.05 eq), (2-hydroxyphenyl)(morpholino)methanone (0.018 g, 0.086 mmol, 0.2 eq) and tripotassium phosphate (0.183 g, 0.86 mmol, 2.0 eq) were added to a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C14 (0.119 g, brown solid, 55.6% yield). m.p.151.7–151.9 °C. ^{1}H NMR (600 MHz, DMSO-d_6)

δ 8.07 (s, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.33–7.21 (m, 4H), 7.21–7.10 (m, 1H), 6.82 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.65 (t, *J* = 5.6 Hz, 1H), 6.57 (d, *J* = 2.3 Hz, 1H), 4.91 (s, 1H), 4.78 (t, *J* = 5.5 Hz, 1H), 4.13–4.01 (m, 1H), 3.90 (q, *J* = 13.8 Hz, 2H), 3.70–3.54 (m, 3H), 3.29–3.15 (m, 3H), 2.93 (m, *J* = 13.2, 10.4, 3.9 Hz, 1H), 2.80 (t, *J* = 8.1 Hz, 2H), 2.69–2.55 (m, 2H), 1.52–1.30 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 14.56, 31.36, 34.37, 34.76, 35.41, 37.50, 41.36, 45.57, 53.73, 59.75, 69.79, 104.40, 110.58, 126.29, 127.69, 128.68, 128.88, 141.96, 149.30, 150.76, 154.28, 160.74, 170.08. HRMS (ESI) *m*/*z* Calcd for $C_{25}H_{30}N_4O_4$ $[M+H]^+$, 451.2267, found: 451.2348.

4.3.10. 7-((2-(dimethylamino)ethyl)amino)-3-((4-hydroxy-1-(3-phenylpropanoyl)piperidin-4-yl)methyl)quinazolin-4(3H)-one (C15)

C5 (0.2 g, 0.43 mmol, 1.0 eq), *N*,*N*-dimethylethane-1,2-diamine (0.057 g, 0.64 mmol, 1.5 eqv), cuprous iodide (0.004 g, 0.022 mmol, 0.05 eq), (2-hydroxyphenyl)(morpholino)methanone (0.018 g, 0.086 mmol, 0.2 eq) and tripotassium phosphate (0.183 g, 0.86 mmol, 2.0 eq) were added to a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C15 (0.069 g, light yellow oil, 34% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.32–7.20 (m, 4H), 7.19–7.11 (m, 1H), 6.83 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.60–6.46 (m, 2H), 5.02 (s, 1H), 4.06 (m, *J* = 13.0, 4.5 Hz, 1H), 3.90 (q, *J* = 13.8 Hz, 2H), 3.63 (m, *J* = 13.8, 8.8, 5.1 Hz, 1H), 3.29–3.17 (m, 3H), 3.12 (s, 1H), 2.93 (m, *J* = 13.4, 10.3, 4.2 Hz, 1H), 2.80 (t, *J* = 8.1 Hz, 2H), 2.70–2.56 (m, 2H), 2.46 (t, *J* = 6.6 Hz, 2H), 2.19 (s, 5H), 1.54–1.28 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ31.37, 34.37, 34.76, 35.41, 37.50, 40.93, 41.36, 45.75, 53.69, 57.94, 69.76, 104.38, 110.63, 114.98, 126.29, 127.68, 128.68, 128.87, 141.96, 149.34, 150.76, 154.08, 160.74, 170.08. HRMS(ESI) *m*/*z* Calcd for $C_{27}H_{35}N_5O_3$ $[M+H]^+$, 478.2740, found: 478.2814. LC t_R: 4.605 min, purity 99.23%.

4.3.11. 3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl)-7-((2-(pyrrolidin-1-yl)ethyl) amino)quinazolin-4(3H)-one (C16)

C6 (0.2 g, 0.47 mmol, 1.0 eq), 2-(pyrrolidin-1-yl)ethan-1-amine (0.081 g, 0.71 mmol, 1.5 eqv), cuprous iodide (0.005 g, 0.024 mmol, 0.05 eq), (2-hydroxyphenyl)(morpholino)methanone (0.019 g, 0.094 mmol, 0.2 eq) and tripotassium phosphate (0.2 g, 0.94 mmol, 2.0 eq) were added to a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C16 (0.080 g, brown solid, 36.9% yield). m.p.173.5–173.7 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.06 (d, *J* = 16.2 Hz, 1H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.29–7.22 (m, 4H), 7.15 (td, *J* = 6.1, 3.1 Hz, 1H), 6.82 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.59 (m, *J* = 5.5, 1.7 Hz, 1H), 6.56 (d, *J* = 2.3 Hz, 1H), 4.90 (d, *J* = 8.3 Hz, 1H), 4.06–3.94 (m, 2H), 3.86 (d, *J* = 20.7 Hz, 2H), 3.69–3.61 (m, 1H), 3.21 (m, *J* = 42.7, 7.7, 7.0 Hz, 4H), 2.89 (m, *J* = 10.2, 9.3, 5.5 Hz, 1H), 2.66–2.55 (m, 4H), 2.55–2.47 (m, 3H), 1.70 (m, *J* = 6.6, 3.2 Hz, 4H), 1.50 (m, *J* = 13.4, 11.2, 4.4 Hz, 1H), 1.38 (m, *J* = 15.2, 4.3 Hz, 1H), 1.31 (m, *J* = 18.7, 15.3, 10.0, 4.1 Hz, 2H), 1.20 (dd, *J* = 6.9, 2.7 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.37, 22.55, 23.61, 34.73, 34.88, 35.41, 35.52, 36.50, 36.68, 37.45, 40.69, 40.73, 41.49, 41.59, 42.11, 53.71, 54.17, 54.66, 69.74, 69.79, 104.37, 110.60, 110.63, 126.39, 126.44, 127.37, 127.71, 128.67, 128.70, 147.06, 147.18, 149.31, 150.76, 154.06, 160.71, 160.75, 169.55, 169.57. HRMS (ESI) *m*/*z* Calcd for $C_{30}H_{39}N_5O_3$ $[M+H]^+$, 518.3053, found: 518.3120. LC t_R: 4.853 min, purity 97.84%.

4.3.12. 3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl) methyl)-7-(phenethylamino) quinazolin-4(3H)-one (C17)

C6 (0.2 g, 0.47 mmol, 1.0 eq), 2-phenylethan-1-amine (0.086 g, 0.71 mmol, 1.5 eqv), cuprous iodide (0.005 g, 0.024 mmol, 0.05 eq), (2-hydroxyphenyl)(morpholino)methanone (0.019 g, 0.094 mmol, 0.2 eq) and tripotassium phosphate (0.2 g, 0.94 mmol, 2.0 eq) were added to a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C17 (0.080 g, brown solid, 36.9% yield). m.p.167.3–167.9 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.07 (d, *J* = 16.2 Hz, 1H), 7.82 (d, *J* = 8.7 Hz, 1H), 7.39–7.18 (m, 8H), 7.15 (m, *J* = 11.2, 6.0, 2.6 Hz, 1H), 6.88–6.72 (m, 2H), 6.59 (d, *J* = 2.2 Hz, 1H), 4.91 (d, *J* = 8.3 Hz, 1H), 4.08–3.92 (m, 1H), 3.86 (d, *J* = 21.3 Hz, 1H), 3.65 (td, *J* = 13.6, 6.9 Hz, 1H), 3.38 (dt, *J* = 7.9, 5.9 Hz, 2H), 3.28–3.09 (m, 2H), 2.89 (t, *J* = 7.4 Hz, 3H), 2.70–2.55 (m, 2H), 2.54–2.48 (m, 3H), 1.44–1.25 (m, 3H), 1.20 (dd, *J* = 7.0, 2.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ22.37, 22.55, 34.73, 34.88, 35.40, 35.52, 36.50, 36.69, 37.45, 40.69, 40.73, 41.49, 41.59, 44.46, 53.71, 69.74, 69.79, 104.53, 110.70, 110.73, 126.39, 126.44, 126.62, 127.37, 127.78, 128.67, 128.70, 128.73, 128.83, 129.21, 139.99, 147.06, 147.19, 149.33, 150.77, 153.94, 160.72, 160.76, 169.58. HRMS(ESI) *m*/*z* Calcd for $C_{32}H_{36}N_4O_3$ $[M+H]^+$, 525.2787, found: 525.2868.

4.3.13. 7-((furan-2-ylmethyl)amino)-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl)methyl) quinazolin-4(3H)-one (C18)

C6 (0.2 g, 0.47 mmol, 1.0 eq), furan-2-ylmethanamine (0.069 g, 0.71 mmol, 1.5 eqv), cuprous iodide (0.005 g, 0.024 mmol, 0.05 eq), (2-hydroxyphenyl)(morpholino)methanone (0.019 g, 0.094 mmol, 0.2 eq) and tripotassium phosphate (0.2 g, 0.94 mmol, 2.0 eq) were added to a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C17 (0.107 g, brown solid, 45% yield). m.p.103.1–104.0 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.06 (d, *J* = 16.2 Hz, 1H), 7.87–7.79 (m, 1H), 7.60 (dd, *J* = 2.0, 0.8 Hz, 1H), 7.34–7.22 (m, 4H), 7.15 (m, *J* = 12.0, 5.7, 2.2 Hz, 2H), 6.86 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.66 (d, *J* = 2.3 Hz, 1H), 6.40 (dd, *J* = 3.3, 1.8 Hz, 1H), 6.35 (dd, *J* = 3.2, 0.9 Hz, 1H), 4.96–4.83 (m, 1H), 4.38 (d, *J* = 5.9 Hz, 2H), 4.11–3.90 (m, 2H), 3.85 (d, *J* = 21.2 Hz, 1H), 3.64 (t, *J* = 13.7 Hz, 1H), 3.28–3.08 (m, 2H), 2.88 (m, *J* = 10.4, 3.9 Hz, 1H), 2.66–2.55 (m, 1H), 1.44–1.24 (m, 3H), 1.23–1.14 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 14.56, 17.69, 22.37, 22.55, 30.58, 34.71, 34.86, 35.40, 35.51, 36.49, 36.69, 37.44, 40.68, 40.72, 41.48, 41.58, 48.95, 53.73, 69.73, 69.78,

105.10, 107.80, 110.90, 111.13, 111.16, 115.11, 126.39, 126.45, 127.37, 127.68, 128.67, 128.70, 142.74, 147.05, 147.18, 149.32, 150.59, 152.77, 153.65, 160.71, 160.75, 169.56, 169.58. HRMS (ESI) *m/z* Calcd for $C_{29}H_{32}N_4O_4$ $[M+H]^+$, 501.2424, found: 501.2491.

4.3.14. 7-((2-(dimethylamino)ethyl)amino)-3-((4-hydroxy-1-(3-phenylbutanoyl)piperidin-4-yl) methyl)quinazolin-4(3H)-one (C19)

C6 (0.2 g, 0.47 mmol, 1.0 eq), *N,N*-dimethylethane-1,2-diamine (0.063 g, 0.71 mmol, 1.5 eqv), cuprous iodide (0.005 g, 0.024 mmol, 0.05 eq), (2-hydroxyphenyl)(morpholino)methanone (0.019 g, 0.094 mmol, 0.2 eq) and tripotassium phosphate (0.2 g, 0.94 mmol, 2.0 eq) were added to a 20-ml glass tube, which was evacuated and backfilled with nitrogen three times. Then, 0.5 ml of dry DMF was added, and it was evacuated and backfilled with nitrogen three additional times. The reaction mixture was heated at 90 °C for 18 h and cooled to room temperature, and 20 ml of water was added. It was extracted with EA (3 × 60 ml) and washed with brine. The organic phase was dried over anhydrous $MgSO_4$ and removed under reduced pressure to yield crude product, which was purified by flash column chromatography, eluting with PE:EA at a ratio of 2:1 and then 1:2 to yield the pure product C19 (0.117 g, white solid, 58% yield). m.p.108.7–109.0 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.07 (d, J = 16.3 Hz, 1H), 7.81 (d, J = 8.8 Hz, 1H), 7.29–7.22 (m, 4H), 7.15 (td, J = 6.2, 3.1 Hz, 1H), 6.83 (dd, J = 8.9, 2.3 Hz, 1H), 6.56 (d, J = 2.3 Hz, 1H), 6.51 (td, J = 5.3, 1.8 Hz, 1H), 4.96 (s, 1H), 4.05–3.94 (m, 2H), 3.86 (d, J = 21.5 Hz, 2H), 3.68–3.61 (m, 1H), 3.27–3.11 (m, 4H), 2.92–2.85 (m, 1H), 2.65–2.55 (m, 2H), 2.54–2.43 (m, 2H), 2.19 (s, 5H), 1.49 (m, J = 13.4, 11.2, 4.3 Hz, 1H), 1.41–1.34 (m, 1H), 1.36–1.31 (m, 1H), 1.28 (dd, J = 17.2, 3.1 Hz, 1H), 1.20 (dd, J = 7.0, 2.6 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 22.37, 22.55, 34.73, 34.87, 35.40, 35.52, 36.50, 36.69, 37.45, 40.69, 40.73, 40.93, 41.49, 41.59, 45.74, 53.69, 57.95, 69.72, 69.77, 104.40, 110.62, 110.65, 114.98, 126.39, 126.44, 127.37, 127.68, 128.67, 128.70, 147.05, 147.18, 149.32, 150.75, 154.07, 160.72, 160.76, 169.56, 169.58. HRMS(ESI) *m/z* Calcd for $C_{28}H_{37}N_5O_3$ $[M+H]^+$, 492.2896, found: 492.2960. LC t_R: 4.786 min, purity 97.26%.

4.4. Biochemistry investigation

4.4.1. USP7-UbA10 inhibition assay

The recombinant USP7 catalytic domain and UbA10 substrate were provided by the School of Pharmaceutical Sciences, Zhengzhou University. The Ub-A10 substrate was tagged with GST and biotin, and these tags could be recognized by Streptavidin-d2 and anti-GST-Eu cryptate. At the start of the USP7-UbA10 inhibition assay, the tags of the substrates were removed by USP7, and the reduction in TR-FRET signal was measured using a microplate reader (PerkinElmer, New Britain, PA, USA). Briefly, the tested compounds were dissolved in DMSO to prepare a 2.5 mM stock solution, which was diluted with DMSO to various concentrations. At the initiation of the assay, 7.8 μl of buffer (50 mM HEPES, pH 7.5, 0.01% Triton X-100), 1 μl of recombinant USP7 catalytic domain, and 0.2 μl of stock solution were added to a 384-well plate. The plates were incubated at 25 °C for 10 min. Then, 1 μl of Ub-A10 substrate was added to the wells, and the plate was incubated at 37 °C for 75 min. Finally, 10 μl of tested reagents were added to the wells, and the plate was incubated at 25 °C for 1 h. The fluorescence intensity was recorded using a microplate reader (excitation = 615 nm, emission = 665 nm). IC_{50} values were calculated by regression analysis using IBM SPSS software.

4.4.2. Ub-AMC assay

In vitro enzymatic inhibition was determined using the USP7 inhibitor screening assay kit (BPS Bioscience, catalog #79256) according to the manufacturer's instructions. Briefly, the compounds were dissolved in DMSO to prepare the 60 mM stock solution. These were diluted 10-fold in 1X buffer to prepare working solutions. DTT was added to 1X buffer to prepare 6.5 μM DTT 1X buffer. Then, the working solution and vehicle control (1X buffer) were added to the 96-well plates. The USP7 enzyme was diluted in 1X buffer and added to the above plate, which was incubated at 25 °C for 40 min. The Ub-AMC substrate was diluted 800-fold in 1X buffer and added to the plate, followed by incubation at 25 °C for 30 min. The fluorescence signal (excitation = 360 nm, emission = 460 nm) was recorded using a microplate reader (PerkinElmer). IC_{50} values were calculated by regression analysis using GraphPad Prism software.

4.4.3. BLI assay

BLI assays were performed using Octet RE96E (fortebio). Recombinant USP7 catalytic domain (208–560) was provided by DetaiBio (Nanjing, China). Briefly, 200 μl of the USP7 catalytic domain and 1.73 μl of biotin reagent (molar ratio 1:1) were incubated at 30 °C for 2 h. Then, excess biotin was removed by gravity column chromatography, and the residue was diluted to 400 μl with PBS and immobilized onto SSA biosensors. The test compounds were dissolved in DMSO to prepare 4.96 mM stock solutions, which were diluted to 496 μM with PBST (pH 7.4) and then further diluted twice with 10% DMSO and PBST to six different concentrations for further tests. Finally, the test compound stock solutions were added to the sample plate containing the biosensor. Regression analysis and calculation of K_D were performed using ForteBio data analysis software.

4.5. Inhibitory activity experiments

4.5.1. Cellular level inhibitory assay (MTT assay)

The gastric cancer cell lines MGC-803 and BGC-823 were provide by the School of Pharmaceutical Sciences, Zhengzhou University. MTT and other materials/reagents were purchased from commercial vendors. Briefly, the tested compounds were dissolved in DMSO and diluted to 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 μM. The test compounds (three parallel replicates) and 20 μl of MTT solution were placed in a 96-well plate containing the cells, followed by incubation at 37 °C for 4 h. Then, the culture medium was removed, and 200 μl of DMSO was added. Optical density was recorded using a microplate reader at 490 nm. IC_{50} values were calculated using IBM SPSS software.

4.5.2. Clone formation assay

MGC-803 cells were provided by Hunter Biotech (Nanjing, China). Briefly, cells in logarithmic phase were digested with trypsin-EDTA to prepare a single-cell suspension. This suspension was centrifuged, and the clear solution was removed. Cells were seeded in a 6-well plate in a density of 200 cells/3 ml per well. Plates were incubated at 37 °C and 5% CO_2 for 12 h. Then, the cells were treated with 3 ml of culture medium containing the indicated concentrations of C9, and the control group was treated with an equal amount of DMSO. The cells were then incubated for 7 days. Finally, the culture medium was removed, and the cells were washed with PBS and fixed with 4% paraformaldehyde. Next, 1.5 ml of 0.1% crystal violet solution was added to each well, and plates were incubated for 15 min. The cells were washed with PBS three times.

4.5.3. Flow cytometry assay

Cell apoptosis was determined using the Annexin V PI kit (Invitrogen, California, USA), and the cell cycle was analyzed using the cell cycle kit (BD biosciences, catalog #550825, NY, USA) according to the manufacturer's instructions.

MGC-803 cells were treated with various concentrations of test compounds and incubated for 72 h. The cells were harvested and washed with PBS at 4 °C. Then, the cells were suspended in 1X Annexin (Invitrogen, California, USA), and the cell density was adjusted to 1×10^6 cells/ml. Then, 5 μl of Alexa-Fluor 488 Annexin V and 1 μl of 100 μg/ml PI were added per 100 μl of cell solution, followed by incubation at room temperature for 15 min. Cell apoptosis assays were performed using flow cytometry (Beckmann Coulter).

MGC-803 cells were treated using the same method as described above. Cells were harvested and washed with PBS at 4 °C, then suspended in 125 μl of trypsin buffer and incubated at room temperature for 10 min. Next, 100 μl of trypsin inhibitor and RNase buffer were added, and cells were incubated at room temperature for 10 min. Then, 100 μl of PI solution was added, and cells were incubated in an ice bath in the dark for 10 min. Finally, the cell cycle was analyzed using flow cytometry (Beckmann Coulter).

4.6. Molecular docking studies

All molecular docking studies were performed with Molecular Operating Environment software (MOE 2014). The co-crystal structure of the small molecule with the USP7 catalytic domain (PDB ID: 5VSB) was downloaded from the RCSB protein database (www.rcsb.org) [40]. The repeat units of the protein were deleted. Before further operations, all solvent molecules were deleted. Structure correction and protonation of the receptor protein were performed using the 'QuickPrep' function. The chemical structures for the docking process were generated using 'Builder' module and were further optimized using 'QuickPrep' function. We used the co-crystal ligand site as the docking site. 'Triangle Matcher' was used as the placement method, and the conformation was scored by 'London dG' with 60 poses. The refinement method was 'Rigid Receptor', and the conformation was scored by 'GBVI/WSA dG' with 30 poses. 'Amber 10:EHT force field' was used for energy minimization. Other parameters were not changed from default settings. The highest score conformation of the docking result was used unless stated otherwise.

4.7. In vivo anti-tumour effect evaluation

Wild AB-type zebrafish and MGC-803 cells were provided by Hunter Biotech (Nanjing, China). CM-DiI-marked MGC-803 cells were seeded into 2 dpf normal wild AB-type zebrafish vitellicles by microinjection; the number of cancer cells injected to construct the animal tumour model was 200 per fish. The fish were kept at 35 °C until 3 dpf. If the tumour tissue of the zebrafish grew well, they were selected and transferred to 6-well plates randomly. Thirty fish were maintained per well. This assay was performed with three experimental groups: control group (0 ng per fish), middle-dose group (16.7 ng per fish) and high-dose group (50.0 ng per fish). Compounds were administered via intravenous injection into the tumour tissue. After the indicated operations, fish were kept at 35 °C until 5 dpf. Finally, 10 fish per group were chosen randomly to take images under a fluorescence microscope.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by National Natural Science Foundation of China (Project No. 81773562 for H.-M.L) National Key Research Program of Proteins (No. 2016YFA0501800, for H.-M. L.); Key Research Program of Henan Province (No. 161100310100, for H.-M. L.).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113291.

References

[1] A. Hershko, A. Ciechanover, The ubiquitin system, Annu. Rev. Biochem. 67 (1998) 425–479, https://doi.org/10.1146/annurev.biochem.67.1.425.
[2] A. Ciechanover, The ubiquitin proteolytic system-from bench to the bedside, Chin. Bull. Life Sci. 22 (2010) 213–215, https://doi.org/10.13376/j.cbls/2010.03.002.
[3] A. Hershko, The role of ubiquitin system in health and disease, Sci. Technol. Rev. 34 (2016) 144–145, doi:CNKI:SUN:KJDB.0.2016-23-027.
[4] A.L. Haas, J.V.B. Warms, I.A. Rose, Ubiquitin adenylate:structure and role in ubiquitin activation, Biochemistry 22 (1983) 4388–4394, https://doi.org/10.1021/bi00288a007.
[5] D.G. Martin, M.D. Zamudio, J.A. Varela, Ubiquitination system and autoimmunity:The bridge towards the modulation of the immune response, Autoimmun. Rev. 7 (2008) 284–290, https://doi.org/10.1016/j.autrev.2007.11.026.
[6] W. Dauer, S. Przedborski, Parkinson's disease:mechanisms and models, Neuron 39 (2003) 889–909, https://doi.org/10.1016/S0896-6273(03)00568-3.
[7] J. Seo, M.W. Kim, K.H. Bae, S.C. Lee, J. Song, E.W. Lee, The roles of ubiquitination in extrinsic cell death pathways and its implications for therapeutics, Biochem. Pharmacol. 162 (2019) 21–40, https://doi.org/10.1016/j.bcp.2018.11.012.
[8] F.E.R. Turcu, K.H. Ventii, K.D. Wilkinson, Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes, Annu. Rev. Biochem. 78 (2009) 363–397, https://doi.org/10.1146/annurev.biochem.78.082307.091526.
[9] L. Song, M. Rape, Reverse the curse=the role of deubiquitination in cell cycle control, Curr. Opin. Cell Biol. 20 (2008) 156–163, https://doi.org/10.1016/j.ceb.2008.01.012.
[10] A. Guterman, M.H. Glikman, Deubiquitinating enzymes are in(trinsic to proteasome function), Curr. Protein Pept. Sci. 5 (2004) 201–210, https://doi.org/10.2174/1389203043379756.
[11] J.A. Daniel, P.A. Grant, Multi-tasking on chromatin with the SAGA coactivator complexes, Mutat. Res. 618 (2007) 135–148, https://doi.org/10.1016/j.mrfmmm.2006.09.008.
[12] R.D. Kennedy, A.D. Andrea, The Fanconi Anemia/BRCA pathway:new faces in the crowd, Genes Dev. 19 (2005) 2925–2940, https://doi.org/10.1101/gad.1370505.
[13] M.Y. Li, D. Chen, A. Shiloh, J.Y. Luo, A.Y. Nikolaev, J. Qin, W. Gu, Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization, Nature 416 (2002) 648–653, https://doi.org/10.1038/nature737.
[14] M.Y. Li, C.L. Brooks, N. Kon, W. Gu, A dynamic role of HAUSP in the p53-Mdm2 pathway, Mol. Cell 13 (2004) 879–886, https://doi.org/10.1016/S1097-2765(04)00157-1.
[15] M.S. Song, L. Salmena, A. Carracedo, A. Egia, F.L. Coco, J.T. Feldstein, P.P. Pandolfi, The dequbiquitinylation and localization of PTEN are regulated by a HAUSP-PML network, Nature 455 (2008) 813–817, https://doi.org/10.1038/nature07290.
[16] A.V.D. Horst, A.M.M.V. Smits, A.B. Brenkman, M.H. Triest, N.V.D. Broek, F. Colland, et al., FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP, Nat. Cell Biol. 8 (2006) 1064–1073, https://doi.org/10.1038/ncb1469.
[17] J.T. Lee, W. Gu, The multiple levels of regulation by p53 ubiquitination, Cell Death Differ. 17 (2010) 86–92, https://doi.org/10.1038/cdd.2009.77.
[18] J.A.V.D. Knaap, B.R.P. Kumar, Y.M. Moshkin, K. Langenberg, J. Krijgsveld, A.J.R. Heck, et al., GMP synthetase stimulates Histone H2B debiuitylation by the Epigenetic Silencer USP7, Mol. Cell 17 (2005) 695–707, https://doi.org/10.1016/j.molcel.2005.02.013.
[19] C.L. Brooks, M. Li, M. Hu, Y. Shi, W. Gu, The p53-Mdm2-HAUSP complex is involved in p53 stabilization by HAUSP, Oncogene 26 (2007) 7262–7266, https://doi.org/10.1038/sj.onc.1210531.
[20] A. Pozhidaeva, I. Bezsonova, USP7:Structure,substrate specificity, and inhibition, DNA Repair 76 (2019) 30–39, https://doi.org/10.1016/j.dnarep.2019.02.005.
[21] D. Chauhan, Z. Tian, B. Nicholson, K.G.S. Kumar, B. zhou, R. Carrasco, et al., A small mole molecule inhibitor of ubiquitin-specific protease-7 induces apoptosis in multiple myeloma cells and overcomes Bortezomib resistance, Canc. Cell 22 (2012) 345–358, https://doi.org/10.1016/j.ccr.2012.08.007.
[22] M. Altun, H.B. Kramer, L.I. Willems, J.L. McDermott, C.A. Leach, S.J. Goldenberg, et al., Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes, Chem. Biol. 18 (2011) 1401–1412, https://doi.org/10.1016/j.chembiol.2011.08.018.
[23] J. Weinstock, J. Wu, P. Cao, W.D. Kingsbury, J.L. McDermott, M.P. Kodrasov, et

al., Selective dual inhibitors of the cancer-related deubiquitylating proteases USP7 and USP47, ACS Med. Chem. Lett. 3 (2012) 789–792, https://doi.org/10.1021/ml200276j.
[24] C. Chen, J.M. Song, J.Z. Wang, C. Xu, C.P. Chen, W. Gu, et al., Synthesis and biological evaluation of thiazole derivatives as novel USP7 inhibitors, Bioorg. Med. Chem. Lett 27 (2017) 845–849, https://doi.org/10.1016/j.bmcl.2017.01.018.
[25] C. Reverdy, S. Conrath, R. Lopez, C. Planquette, C. Atmanene, V. Collura, et al., Discovery of specific inhibitors of Human USP7/HAUSP deubiquitinating enzyme, Chem. Biol. 19 (2012) 467–477, https://doi.org/10.1016/j.chembiol.2012.02.007.
[26] I. Lamberto, X.X. Liu, H.S. Seo, N.J. Schauer, R.E. Lacob, W.Y. Hu, et al., Structure-guided development of a potent and selective non-covalent active-site inhibitor of USP7, Cell Chem. Biol. 24 (2017) 1490–1500, https://doi.org/10.1016/j.chembiol.2017.09.003.
[27] G. Gavory, C.R.O. Dowd, M.D. Helm, J. Flasz, E. Arkoudis, A. Dossang, et al., Discovery and characterization of highly potent and selective allosteric USP7 inhibitors, Nat. Chem. Biol. 14 (2018) 118–125, https://doi.org/10.1038/nchembio.2528.
[28] C.R.O. Dowd, M.D. Helm, J.S.S. Rountree, J.T. Flasz, E. Arkoudis, H. Miel, et al., Identification and structure-guided development of pyrimidinone based USP7 inhibitors, ACS Med. Chem. Lett. 9 (2018) 238–243, https://doi.org/10.1021/acsmedchemlett.7b00512.
[29] A.P. Turnbull, S. Ioannidis, W.W. Krajewski, A.P. Fernandez, C. Heride, A.C.L. Martin, et al., Molecular basis of USP7 inhibition by selective small-molecule inhibitor, Nature 550 (2017) 481–486, https://doi.org/10.1038/nature24451.
[30] M. Colombo, S. Vallese, I. Peretto, X. Jacq, J.C. Rain, F. Colland, P. Guedat, Synthesis and biological evaluation of 9-oxo-9H-indeno[1,2-b]pyrazine-2,3-dicarbonitrile analogues as potential inhibitors of deubiquitinating enzymes, ChemMedChem 5 (2010) 552–558, https://doi.org/10.1002/cmdc.200900409.
[31] F. Colland, Jacq X. Formstecher, C. Reverdy, C. Planquette, S. Conrath, et al., Small-molecule inhibitor of USP7/HAUSP7 ubiquitin protease stabilizes and activates p53 in cells, Mol. Canc. Therapeut. 8 (2009) 2286–2295, https://doi.org/10.1158/1535-7163.MCT-09-0097.
[32] L. Kategaya, P.D. Lello, L. Rouge, R. Pastor, K.R. Clark, J. Drummond, et al., USP7 small-molecule inhibitors interfere with ubiquitin binding, Nature 550 (2017) 534–538, https://doi.org/10.1038/nature24006.
[33] P.D. Lello, R. Pastor, J.M. Murray, R.A. Blake, F. Cohen, T.D. Crawford, et al., Discovery of small-molecule inhibitors of ubiquitin specific protease 7(USP7) using integrated NMR and in silico techniques, J. Med. Chem. 60 (2017) 10056–10070, https://doi.org/10.1021/acs.jmedchem.7b01293.
[34] M. Yamaguchi, M. Miyazaki, M.P. Kodrasov, H. Rotinsulu, F. Losung, R.E.P. Mangindaan, et al., Spongiacidin C, a pyrrole alkaloid from the marine sponge Stylissa massa,functions as USP7 inhibitor, Bioorg. Med. Chem. Lett 23 (2013) 3884–3886, https://doi.org/10.1016/j.bmcl.2013.04.066.
[35] N. Tanokashira, S. Kukita, H. Kato, T. Nehira, E.D. Angkouw, R.E.P. Mangindaan, et al., Petroquinones:trimeric and dimeric xestoquinone derivatives isolated from the marine sponge Petrosia alfiani, Tetrahedron 72 (2016) 5530–5540, https://doi.org/10.1016/j.tet.2016.07.045.
[36] A.H. Afifi, I. Kagiyama, A.H.E. Desoky, H. Kato, R.E.P. Mangindaan, N.J.D. Voogd, et al., Sulawesins A-C, furanosesterterpene tetronic acids that inhibit USP7, from a Psammocinia sp.marine sponge, J. Nat. Prod. 80 (2017) 2045–2050, https://doi.org/10.1021/acs.jnatprod.7b00184.
[37] B. Jing, M. Liu, L. Yang, H.Y. Cai, J.B. Chen, Z.X. Li, et al., Characterization of naturally occurring pentacyclic triterpenes as novel inhibitors of deubiquitinating protease USP7 with anticancer activity in vitro, Acta Pharmacol. Sin. 39 (2018) 492–498, https://doi.org/10.1038/aps.2017.119.
[38] A. Basak, T. Mahato, K. Ghosh, A. Nag, Studies on PLE catalysed hydrolysis: dependence on substituent flexibility, Biotechnol. Lett. 17 (1995) 1099–1100, https://doi.org/10.1007/BF00143109.
[39] H.W. Li, M. Li, R.Y. Xu, S.X. Wang, Y.M. Zhang, L.H. Zhang, et al., Synthesis,-structure activity relationship and in vitro anti-influenza virus activity of novel polyphenol-pentacyclic triterpene conjugates, Eur. J. Med. Chem. 163 (2019) 560–568, https://doi.org/10.1016/j.ejmech.2018.12.006.
[40] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, et al., The protein data bank, Nucleic Acids Res. 28 (2000) 235–242.