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Graphic Abstract

Design, synthesis and biological evaluation of 4-anilinoquinazoline derivatives as new *c-myc* G-quadruplex ligands

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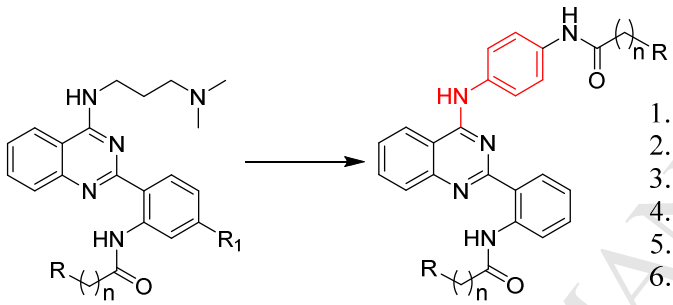

1. Enhanced stabilizing ability
2. Strong binding affinity
3. G-quadruplex selectivity
4. Transcriptional inhibition of *c-myc*
5. Cell proliferation inhibition
6. Cell G0/G1 phase arrest

A series of compounds with an introduction of aniline group into 4-position to previous reported quinazoline derivatives were synthesized and evaluated. These compounds showed good *c-myc* promoter G-quadruplex binding activity and selectivity.

Title page

Design, synthesis and biological evaluation of 4-anilinoquinazoline derivatives as new *c-myc* G-quadruplex ligands

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Abstract

A series of 4-anilinoquinazoline derivatives were designed and synthesized as novel *c-myc* promoter G-quadruplex binding ligands. Subsequent biophysical and biochemical evaluation demonstrated that the introduction of aniline group at 4-position of quinazoline ring and two side chains with terminal amino group improved their binding affinity and stabilizing ability to G-quadruplex DNA. RT-PCR assay and Western blot showed that compound **7a** could down-regulate transcription and expression of *c-myc* gene in Hela cells, which was consistent with the behavior of an effective G-quadruplex ligand targeting *c-myc* oncogene. More importantly, RTCA and colony formation assays indicated that **7a** obviously inhibited Hela cells proliferation, without influence on normal primary cultured mouse mesangial cells. Flow cytometric assays suggested that **7a** induced Hela cells to arrest in G0/G1 phase both in a time-dependent and dose-dependent manner.

Keywords

4-Anilinoquinazoline derivatives; G-quadruplex; *c-myc*; transcriptional regulation; antitumor.

Abbreviations

FRET, fluorescence resonance energy transfer;

T_m, melting temperature;

SPR, surface plasmon resonance;

CD, circular dichroism;

MTT, methyl thiazolyl tetrazolium;

RT-PCR, reverse transcription-polymerase chain reaction;

RTCA, real time cell analysis.

1. Introduction

G-quadruplexes are nucleic acid secondary structures formed from guanine-rich sequences, and comprise a planar arrangement of four guanines (G-quartet, Fig. 1A) stabilized by Hoogsteen hydrogen bonding and monovalent cations [1]. Since G-quadruplex structures are widely located in plenty of important regulatory regions including telomeric DNA, oncogene promoters (such as *c-myc*, *VEGF*, *bcl-2*, *k-RAS*, *h-RAS*, *c-kit*, *HIF* and *HSP90*) and 5′-UTR, it is considered playing a significant role in regulating biological processes such as replication, translation and splicing [2, 3]. The presence of G-quadruplex structures in human cells is now firmly established with antibody [4], which provides the basis for the elucidation of their function in normal and disease states.

c-MYC is a transcription factor whose expression is associated with cell proliferation. Increased levels of *c-myc* expression are observed in 80% of human cancer cells, and its increase promotes tumorigenesis [5]. The nuclear hypersensitivity element III_1 (NHE III_1), a guanine-rich strand of the DNA containing a 27 base pair sequence, which is upstream of *c-myc* promoter, controls 80–90% of the *c-myc* transcription [6]. The NHE III_1 can form intramolecular G-quadruplex structures and functions as a transcriptional repressor [7]. The transcription of *c-myc* can be down-regulated through stabilization of the G-quadruplexes by using specific G-quadruplex binders [8]. A number of *c-myc* G-quadruplex ligands have been reported, including phenanthroimidazole derivatives [9], acridine derivatives [10], prolinamide derivatives [11], platinum(II) complex [12], and showed pronounced antitumor activity.

Inspecting the structures of these G-quadruplex ligands, it is easy to find that most of them are based on a planar aromatic system that interacts with the G-quartet through π–π stacking interaction, with cationic side chains that interact with the negatively charged phosphate backbones in G-quadruplex [13,14]. For these molecules, optimal activity has been achieved through substitution of side chains with terminal amino group,

especially with an amide bond conjugated with the aromatic ring system to maintain planarity on the edge of the G-quartet [15]. Besides, intensive research has suggested that extended aromatic surface always favor ligand-quadruplex binding interactions [16-18]. Neidle and co-workers have increased G-quadruplex binding affinity and biological activity of 3,6-disubstituted acridines by rationally adding an anilino subsituent at the 9-position to give 3,6,9-trisubstituted acridine molecules [19-21], one of which was **BRACO-19** (Fig. 1B).

Quinazoline derivatives, which have a planar chromophore, may interact with G-quartet of G-quadruplex DNA. In our recent study, a few 2,4-disubstituted quinazoline derivatives have been synthesized and found to be telomeric G-quadruplex binders, one of which is compound **LZ-11c** (Fig. 1C) [22]. Subsequently, a new substituted benzene ring has been linked to the *ortho*-position of 2-phenyl group of original quinazoline derivatives through amide bond, like compound **QPB-15e** (Fig. 1C), which have shown stronger binding ability and better selectivity for telomeric G-quadruplex [23]. In order to further develop new *c-myc* G-quadruplex binding ligands, we designed a series of new 4-anilinoquinazoline derivatives, in which aromatic system of the quinazoline is expanded by introducing an anilino group into the 4-position of quinazoline moiety for improving its G-quadruplex binding activity and stability. Unlike rigid aromatic compounds, such unfused aromatic molecules with adaptive structural feature could prevent themselves from intercalating into the duplex DNAs [6]. Based on above consideration, two series of derivatives were designed, including series I and II (Fig. 1D). For series I, we introduced an amide side chain with basic amino terminal at the *para*-position of aniline group (**1a**, **1b**, **2a**, and **2b**), which could be protonated at physiological pH, to establish additional electrostatic interaction with the target. Besides, we also replaced the amide bond with ether bond for the derivatives to uncover their influence on the G-quadruplex recognition (**4a** and **4b**). For Series II, a phenyl group was added at the 2-position of 4-anilinoquinazoline ring (**3a**~**3b**, **5a**~**5f**, **6a**~**6d**, **7a**~**7i**, **8a**, **8b**, and **9a**) to further extend the planarity of unfused aromatic system. In order to investigate the

importance of two side chains on ligand-quadruplex interaction, the second amide side chain was appended to *ortho*-position of 2-phenyl group (**6a**~**6d**, **7a**~**7i**, **8a**, **8b**, and **9a**). Also it should be noted that the intramolecular hydrogen bond is formed between the NH group at the *ortho*-position of 2-phenyl group and the lone pair electrons of nitrogen on pyrimidine ring. Therefore, thirty one 4-anilinoquinazoline derivatives were synthesized. The interactions of synthesized compounds with *c-myc* G-quadruplex DNA were examined through fluorescence resonance energy transfer (FRET), circular dichroism (CD) spectroscopy, surface plasmon resonance (SPR), reverse transcription-polymerase chain reaction (RT-PCR), and Western blot. In addition, effects of these compounds on cell proliferation and cell cycle were also evaluated.

2. Chemistry

The synthetic routes for compounds **1a**~**1b**, **2a**~**2b**, **3a**~**3c** and **4a**~**4b** were shown in Scheme 1. The chlorination of 1*H*, 3*H*-quinazoline-2,4-dione with excess phosphorus oxychloride was carried out to give dichlorinated intermediate **10** [24]. Aniline substitution occurred selectively at C-4 position of **10**, yielding 4-aniline-2-chloroquinazoline **11** [25]. Reaction of **11** with acylchloride gave intermediates **12a** and **12b** [21], followed by treatment of **12a** or **12b** with excess diethylamine, potassium iodide, and potassium carbonate to give target compounds **1a** and **1b**, respectively. In comparison, **12a** or **12b** was treated with diethylamine and potassium carbonate to give target compound **2a** or **2b** as major product. Compounds **3a~3c** were prepared via Suzuki coupling reaction of **2a** or **2b** with appropriate phenylboronic acid [26]. Intermediate **10** was reacted with 4-methoxyaniline to form intermediate **13**, followed by demethylation reaction with BBr_3 to give intermediate **14**, and subsequently by substituting with dibromoalkane to give intermediate **15a** or **15b**. Compounds **4a** and **4b** were obtained through reaction of **15a** or **15b** with diethylamine, respectively.

The synthetic routes for compounds **5a~5f**, **6a~6d**, **7a~7i**, **8a**, **8b**, and **9a** were shown in Scheme 2. The

synthetic method started with the synthesis of acylchloride through the chlorination of 2-methoxybenzoic acid or 2-nitrobenzoic acid, followed with its amidation using anthranilamide to give amide intermediate **16** or **22**, and subsequently, intermediate **17** or **23** was respectively prepared through the oxidative ring closure of **16** or **22** under basic conditions [21]. The chlorination of **17** with excess thionyl chloride was carried out to give intermediate **18** [24]. The chlorination of **23** with excess phosphorus oxychloride and phosphorus pentachloride was carried out to give intermediate **24**. Coupling of 4-methoxyaniline with **18** afforded intermediate **19**, followed with demethylation to give intermediate **20**. Compound **20** was reacted with 1,2-dibromoethane to afford intermediate **21** [27]. Coupling of **24** with 4-nitroaniline afforded intermediate **25**. The reduction of the nitro group with 80% hydrazine hydrate in the presence of 10% Rany-Ni gave amino-substituted intermediate **26**. Reaction of **26** with acylchloride gave intermediates **27a~27d**. Intermediates **21** or **27a~27d** were treated with different alkylamines to give target compounds **5a~5f**, **6a~6d**, **7a~7i**, **8a**, **8b**, and **9a**, respectively.

3. Results and Discussion

3.1 Studies on the stabilization and selectivity of derivatives on c-myc G-quadruplex DNA through FRET

To investigate the stabilizing ability of 4-anilinoquinazoline derivatives on *c-myc* gene promoter G-quadruplex DNA, FRET-melting experiment was employed with promoter G-quadruplex sequence of *c-myc* containing fluorophores at both 5′-end and 3′-end (FPu22T) [28, 29]. An oligonucleotide that formed hairpin duplex DNA structure (F10T) was used as a non-quadruplex control [30]. Quinazoline derivatives **LZ-11c** and **QPB-15e** (Fig. 1C) previously reported by us were used as reference compounds [21, 22]. The results of the FRET-melting experiment were shown in Table 1 and Figure S1, which suggested that these

compounds had a wide range of G-quadruplex stabilization ability. A comparison of the FRET-melting results indicated that some of new synthetic compounds, such as **7a**, **7e**~**7i**, showed better stabilizing ability than reference compounds **LZ-11c** and **QPB-15e**, which indicated that the introduction of anilino group had a significant effect on stabilizing G-quadruplex.

Among Series I compounds, it was found that amino (**1a, 1b**, **4a**, **4b**) substituent at 2-position of quinazoline had obviously higher stabilizing ability for G-quadruplex than chlorine substituent **(2a** and **2b**). In addition, the ΔT_m values of **1a** and **1b** for G-quadruplex stabilization were higher than that of **4a** and **4b**, respectively, which revealed that amide bond at the *para*-position of anilino is better than ether bond to enhance the interaction of ligand with G-quadruplex DNA. This result was consistent with that for **3a**~**3c** over **5a**~**5f** for Series II compounds, indicating the importance of amide bond conjugated with aromatic ring. For Series II compounds, most of the compounds with the second amide side chain in the *ortho*-position of 2-phenyl group, such as **6a**~**6c**, **7a**~**7c**, **7e**~**7i**, **8a**, **8b**, and **9a**, showed stronger stabilizing ability for G-quadruplex, compared to compounds **3a**~**3c**. These results indicated that the introduction of the second alkylamino side chain had a significant effect on the stabilizing ability of these compounds. Analysis of the ΔT_m values of compounds **6a~6d**, **7a~7i**, **8a**, **8b**, and **9a**, gave the following conclusions: (1) Compounds with polyamine side chains (**7f~7i**) could raise the melting temperature of G-quadruplex greatly by about 18.0-26.6 °C. Meanwhile, **7g** and **7i** had higher activity than **7f** and **7h**. Both results indicated that the number and distances apart of positive charges in polyamine side chains could significantly influence their ability of stabilizing G-quadruplex, just as that reported by Savino et al. [31, 32]. (2) With the same terminal bases, compounds **7a**~**7d** with longer side chains (n = 2, with two bonds between carbonyl group and basic *N* terminus) showed stronger stabilizing ability to the G-quadruplex than compounds **6a**~**6d** with shorter side chains (n = 1), while no distinct effect on ΔT_m values was observed with further extension of amide side chain,

as shown for compounds **8a**, **8b**, and **9a** (n = 3 or 4). These results demonstrated the importance of the length of amide side chain for their strong interactions with G-quadruplex. (3) Among the compounds with the same length of side chain and different basic terminal, the least basic compounds (morpholino analogues **6d** and **7d**) had the weakest effect on stabilization of G-quadruplex, which indicated the importance of the basicity of terminal. Besides, the flexibility of amino terminal of compounds might be another influencing factor, for example, diethylamino analogue **7a** with flexible amino terminal showed stronger stabilizing activity for G-quadruplex over pyrrolidino analogue **7b**, *N*-methyl piperazino analogue **7c**, and piperidino analogue **7e**.

In comparison, it was quite evident that most studied ligands could barely stabilize the hairpin structure formed by F10T, suggesting their poor binding to the duplex DNA. Only **7g** and **7i** showed weak effect on hairpin structure with ΔT_m values of 3.6 and 3.7 °C. The selectivity of these derivatives to G-quadruplex was characterized by using a competitive FRET-melting experiment, and the ability of the ligands to stabilize G-quadruplex was challenged with nonfluorescent duplex DNA ds26 [30]. As shown in Figure 2, in the presence of excess competitor ds26, the thermal stabilization of FPu22T enhanced by some selected compounds was slightly affected, which demonstrated that 4-anilinoquinazoline derivatives could specifically stabilize G-quadruplex without significant effect for duplex DNA.

3.2 Studies on the binding and selectivity of derivatives on c-myc G-quadruplex DNA by using SPR

To investigate the binding affinity and selectivity of the synthesized compounds for *c-myc* G-quadruplex, SPR experiments were carried out quantitatively by using biotinylated *c-myc* G-quadruplex DNA Pu22 and biotinylated duplex DNA attached to a streptavidin-coated sensor chip [33]. The binding constants were determined through equilibrium analysis. As shown in Table 2, the K_D values for most of the derivatives binding to *c-myc* G-quadruplex showed strong binding affinity, ranged from 0.14 to 8.08 μM except **2a**, **3c**,

and **5a**~**5f**. Most of the compounds with two alkylamino side chains (**7a**~**7c**, **7e**, **7f**, **7h**, **7i**, **8a**, **8b**, and **9a**) exhibited strong binding affinity for *c-myc* G-quadruplex among 4-anilinoquinazoline derivatives, with their K_D values lower than 1 μM, while weaker or no obvious binding was found for these compounds to duplex DNA. These results demonstrated that the introduction of two alkylamino side chains onto the scaffold of 4-anilinoquinazoline derivatives had improved binding ability and selectivity for G-quadruplex. In addition, it was found that amide bond at the *para*-position of anilino is better than ether bond to improve the interaction of ligand with G-quadruplex structure, which was in parallel with FRET-melting data. Moreover, SPR experimental data also indicated that the amide side chains of the tested compounds with two bonds between carbonyl group and basic *N* terminus were optimal for their interactions with G-quadruplex. A comparison of the SPR assay results for the reference compound **LZ-11c** (K_D^{Pu22} = 0.49 μM, K_D^{duplex} = 9.18 μM) with some of 4-anilinoquinazoline derivatives, such as **7a**, K_D^{Pu22} = 0.21 μM, K_D^{duplex} > 10 μM, revealed that the introduction of substituted anilino at the 4-position of quinazoline core had beneficial effect on their binding ability and selectivity for *c-myc* G-quadruplex. The results from above FRET and SPR experiments all supported that these 4-anilinoquinazoline derivatives could selectively bind and stabilize G-quadruplexs DNA over duplex DNA, which could act as a new class of highly selective G-quadruplex binding ligands.

3.3 Studies on the binding property of derivatives on G-quadruplex by using CD

CD spectroscopy is an extremely sensitive method for determining the conformation of G-quadruplex structures and the interaction between ligands and G-quadruplex [33]. Oligonucleotide Pu22 (5'-TGAGGGTGGGTAGGGTGGGTAA-3') was used as the *c-myc* sequence. The binding property of some 4-anilinoquinazoline derivatives (**6b**, **7a**, **7b**, **7c**, **7e**, **7f**, and **7h**) to *c-myc* G-quadruplex was further studied with this method. As show in Figure 3A, in the presence of 100 mM KCl, Pu22 showed a positive signal at

about 262 nm and a negative signal at 242 nm, which suggested a typical parallel G-quadruplex structure. Upon addition of compounds to Pu22, no significant change was observed while the peak height was slightly decreased, which indicated that 4-anilinoquinazoline derivatives could still maintain parallel *c-myc* G-quadruplex conformation in the presence of potassium ion.

The CD spectra of Pu22 without any metal cations at room temperature exhibited a negative peak at 240 nm and a positive peak at 260 nm. After the treatment with 4-anilinoquinazoline derivatives, the positive peak at 260 nm and the negative peak at 240 nm were greatly increased (Fig. 3B), which was similar with the CD spectrum of Pu22 in the presence of KCl. The result indicated that 4-anilinoquinazoline derivatives could effectively induce the formation of parallel *c-myc* G-quadruplex conformation in the absence of metal cations and further confirmed a strong interaction between G-quadruplex DAN and our derivatives.

3.4 Studies of the derivatives on antitumor activity by using MTT

To investigation the antitumor activity of the derivatives, MTT assay was employed to evaluate the cytotoxicity of 4-anilinoquinazoline derivatives against cervical cancer cell line Hela, lung adenocarcinoma cell line A549, lymphoma cell lines Raji and CA46, as well as primary cultured mouse mesangial cell line (in which the proliferation does not depend on *c-myc* expression [34]). In the assay, cell viability was determined through reduction of tetrazolium salt to blue formazan based on mitochondrial enzyme activity of succinate dehydrogenase in living cells. As shown in Table 3, although compounds **6b**, **7a**, **7b**, **7c**, **7e**, **7f**, **7h** exhibited strong stabilizing ability and affinity to *c-myc* G-quadruplex, different cytotoxicity occurred among these compounds. Subsequently, we performed cell uptake assay for the compounds, and the result (Fig. S2) showed that cellular uptake of compounds **6b**, **7a**, and **7b** were much higher than that of compounds **7f** and **7h**, which might explain the results that compounds had obviously different cytotoxicity. Based on the above

results, we found that **7a** stabilized *c-myc* G-quadruplex with high ΔT_m value, bound the G-quadruplex with a submicromolar binding constant, and exhibited stronger inhibitory effects on the tumor cells while weaker inhibitory effect on primary cultured mouse mesangial cells. Therefore, compound **7a** may be a good candidate for further cellular studies.

*3.5 Down-regulation of c-myc transcription and expression by **7a** in Hela cells*

RT-PCR was performed to determine the effect of compound **7a** on the transcription of *c-myc* gene. Hela cells were incubated with compound **7a** at 0, 1.25, 2.5, 5, 7.5, 10 μM for 3 h. The total RNA was extracted and reversely transcribed to cDNA. The cDNA was then used as a template for quantitative PCR amplification of the *c-myc* sequence. As shown in Figure 4A, derivative **7a** showed inhibitory activity on the transcription of *c-myc* in a concentration-dependent manner in Hela cells. Next, Western blot was performed to test the effect of compound **7a** on expression of *c-myc*. As shown in Figure 4B, expression level of *c-myc* also decreased upon treatment with compound **7a**. The above results suggested that **7a** might target the G-quadruplex structure in the promoter region of *c-myc* gene and hence down-regulated its expression.

*3.6 Inhibition of cell proliferation by derivative **7a***

Since stabilization of *c-myc* G-quadruplex DNA could influence *c-myc* transcription and inhibit cancer cell growth, to further evaluate such inhibitory effect of compound **7a**, RTCA assays [35] and colony formation assays were carried out. Hela cells (with overexpression of *c-myc*) and primary cultured mouse mesangial cells (the proliferation does not depend on *c-myc* expression) were treated with various concentrations of compound **7a** (0, 1.25, 2.5, 5, 7.5, 10 μM) in RTCA for 80 h, and the cell proliferation results were shown in Figure 5. The compound **7a** showed significant growth arrest on Hela cells, while no

such effect was found on primary cultured mouse mesangial cells.

Colony formation assays showed a more visualized result about cell proliferation inhibition of compound **7a**. Hela cells and primary cultured mouse mesangial cells were treated with various concentrations of **7a** (2, 1, 0.5, 0.25 μM and DMSO control) for 8 days. As shown in Figure 6, 2 μM of **7a** almost completely inhibited Hela cell proliferation, without effect on primary cultured mouse mesangial cells.

*3.7 G0/G1 phase arrest of Hela cells by derivative **7a***

As shown in RTCA and colony formation assays, compound **7a** could obviously inhibit Hela cell proliferation. To explore the mechanism of this inhibition, we performed cell cycle analysis to detect Hela cells treated with **7a** at various concentrations and on different time intervals. Compound **7a** treatment gave an increase in G0/G1 phase from 30.8% to 61.1% in a time-dependent manner (Figure 7A), and an increase from 35.9% to 54.8% in a dose-dependent manner (Fig. 7B) for 48 h measured by using EPICS XL flow cytometer. These results indicated that **7a** could arrest Hela cells in G0/G1 phases.

4. Conclusions

Some unfused aromatic ligands have been known as effective and selective G-quadruplex binding ligands. Based on previous reports for the use of anilino on G-quadruplex ligand design, in the present study, an aniline group was attached to the scaffold of our previously reported quinazoline derivatives to give two series of novel unfused aromatic quinazoline derivatives, including 4-anilinoquinazoline derivatives (Series I) and 4-anilino-2-phenylquinazoline derivatives (Series II). Positively charged side chain has been shown to play an important role in recognizing G-quadruplex, therefore, various positively charged side chains were

incoporated into the molecules. Our FRET and SPR experiments suggested that two positively charged amide side chains showed strong affinity and selectivity to *c-myc* G-quadruplex against the duplex DNA. The introduced two positively charged side chains could participate in electrostatic and H-bonding interactions with the grooves and loops of the G-quadruplex DNA. Further cellular studies showed that compound **7a** significantly down-regulated *c-myc* gene transcription and expression in Hela cells, presumably through the stabilization of *c-myc* G-quadruplex structure. In addition, RTCA and colony formation assays indicated that **7a** significantly inhibited Hela cell proliferation, without influence on normal primary cultured mouse mesangial cells. From flow cytometric assays, we found that **7a** arrested Hela cell cycle in G0/G1 phase in both a time-dependent and a dose-dependent manner. These results showed that the introduction of an anilino group to 2-position of quinazoline core is an efficient approach for obtaining promising *c-myc* promoter G-quadruplex binding ligands. Further studies are needed to investigate the properties of compounds **7a**, e.g. basic experimental ADME test and the inhibitory effect to tumor xenografts growth *in vivo*, and based on these data, more powerful analogs could be designed.

5. Experimental Section

5.1. Synthesis and characterization

All commercial chemicals used as starting materials were analytical grade and utilized without further purification. ^{1}H and ^{13}C NMR spectra were recorded using TMS as the internal standard in DMSO-d_6 or $CDCl_3$ with a Bruker BioSpin GmbH spectrometer at 400 MHz and 100 MHz, respectively; Mass spectra (MS) were recorded on a Shimadzu LCMS-2010A instrument with an ESI or ACPI mass selective detector, and high resolution mass spectra (HRMS) were recorded on Shimadzu LCMS-IT-TOF. Melting points (m.p.) were

determined by using capillary tubes with a MSRS-OptiMelt automated melting point instrument without correction. The purities of synthesized compounds were confirmed to be higher than 95% by using analytical HPLC equipped with a dual pump Shimadzu LC-20AB system with an Ultimate XB-C18 column.

5.1.1 Synthesis of intermediates

The intermediates **10 ~ 27** were prepared following the process shown in Scheme 1 and 2, and the detail of synthesis was described in Supporting Information.

*5.1.2. General procedure A: preparation of **1a** and **1b***

To a stirred suspension of **12a** or **12b** (1 mmol), K_2CO_3 (2 mmol), KI (1 mmol) in DMF (10 mL) was added diethylamine (20 mmol). The solution was stirred under 130 ℃ for 12 h, cooled to room temperature, and diluted with 25 mL water. The reaction mixture was extracted with ethyl acetate (50 mL×2), the combined the organic layers washed six times with water and dried over Na_2SO_4, and then evaporated under vacuum. The crude solid was purified by using chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ elution to afford **1a** and **1b**.

*5.1.2.1. 2-(diethylamino)-N-(4-((2-(diethylamino)quinazolin-4-yl)amino)phenyl)acetamide (**1a**).* Compound **12a** was treated with excess diethylamine according to general procedure A to afford **1a**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 71% yield. m.p. 131.1-133.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.41 (s, 1H), 7.74 (d, J = 8.9 Hz, 2H), 7.66 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 8.9 Hz, 2H), 7.56 – 7.47 (m, 2H), 7.34 (s, 1H), 7.08 (t, J = 8.0 Hz, 1H), 3.71 (q, J = 7.0 Hz, 4H), 3.16 (s, 2H), 2.66 (q, J = 7.1 Hz, 4H), 1.23 (t, J = 7.0 Hz, 6H), 1.11 (t, J =

7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.1, 158.1, 157.3, 153.2, 135.5, 133.2, 132.6, 126.1, 121.4, 120.6, 120.5, 119.8, 110.1, 58.1, 48.9, 41.7, 29.7, 13.6, 12.5. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{24}H_{32}N_6O$) requires m/z 419.2565, found 419.2550.

*5.1.2.2. 3-(diethylamino)-N-(4-((2-(diethylamino)quinazolin-4-yl)amino)phenyl)propanamide (**1b**).* Compound **12b** was treated with excess diethylamine according to general procedure A to afford **1b**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 62% yield. m.p. 135.3-137.4°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.27 (s, 1H), 7.69 (d, *J* = 8.9 Hz, 2H), 7.65 (d, *J* = 8.1 Hz, 1H), 7.53 (d, *J* = 9.0 Hz, 3H), 7.51 – 7.49 (m, 1H), 7.33 (s, 1H), 7.09 – 7.05 (m, 1H), 3.70 (q, *J* = 7.0 Hz, 4H), 2.81 – 2.77 (m, 2H), 2.69 (q, *J* = 7.1 Hz, 4H), 2.55 – 2.49 (m, 2H), 1.22 (t, *J* = 7.0 Hz, 6H), 1.14 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.0, 158.2, 157.4, 153.1, 135.0, 134.4, 132.5, 126.0, 121.6, 120.7, 120.5, 119.9, 110.2, 48.9, 46.0, 41.7, 33.1, 13.5, 11.5. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{25}H_{34}N_6O$) requires m/z 433.2721, found 433.2704.

*5.1.3. General procedure B: preparation of **2a** and **2b***

The mixture of **12a** or **12b** (2 mmol), and K_2CO_3 (4 mmol) in 10 mL DMF was added diethylamine (10 mmol). The solution was stirred at 80 °C for 12 h, cooled to room temperature, and diluted with 25 mL water. The resulting solution was extracted with CH_2Cl_2 (50 mL×2), the combined organic phase washed six times with water and dried over Na_2SO_4, and then evaporated under vacuum. The crude solid was purified by using chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ elution to afford **2a** and **2b**.

*5.1.3.1. N-(4-((2-chloroquinazolin-4-yl)amino)phenyl)-2-(diethylamino)acetamide (**2a**).* Compound **12a** was

treated with excess diethylamine according to general procedure B to afford **2a**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 58% yield. m.p. 213.0-215.4°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.56 (s, 1H), 8.04 (d, J = 7.9 Hz, 2H), 7.85 – 7.77 (m, 2H), 7.72 (d, J = 8.9 Hz, 2H), 7.60 (d, J = 8.9 Hz, 2H), 7.56 – 7.52 (m, 1H), 3.22 (s, 2H), 2.70 (d, J = 6.8 Hz, 4H), 1.13 (t, J = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.8, 157.3, 151.4, 134.4, 133.8, 133.7, 128.0, 126.5, 122.9, 121.3, 120.4, 113.6, 58.0, 48.9, 12.4. HRMS (ESI): Calcd for $[M+H]^+$ ($C_{26}H_{35}N_7O$) requires m/z 384.1586, found 384.1588.

*5.1.3.2. N-(4-((2-chloroquinazolin-4-yl)amino)phenyl)-3-(diethylamino)propanamide (**2b**).* Compound **12b** was treated with excess diethylamine according to general procedure B to afford **2b**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 40% yield. m.p. 171.0-172.3°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.21 (s, 1H), 10.17 (s, 1H), 8.55 (d, J = 8.1 Hz, 1H), 7.91 – 7.84 (m, 1H), 7.73 – 7.67 (m, 1H), 7.65 (d, J = 5.9 Hz, 4H), 7.62 (s, 1H), 2.75 (t, J = 6.9 Hz, 2H), 2.55 – 2.50 (m, 4H), 2.43 (t, J = 7.0 Hz, 2H), 0.99 (t, J = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 170.4, 159.4, 156.4, 150.8, 136.2, 133.9, 133.0, 126.8, 126.5, 123.6, 123.4, 119.1, 113.7, 48.4, 46.1, 34.1, 11.8. HRMS (ESI): Calcd for $[M+H]^+$ ($C_{21}H_{24}N_5$ OCl) requires m/z 398.1742, found 398.1741.

*5.1.4. General procedure C: preparation of **3a ~ 3c***

A mixture of **2a** or **2b** (0.3 mmol), appropriate phenylboronic acid (0.45 mmol), Na_2CO_3 (0.9 mmol), and $Pd(PPh_3)_4$ (0.03 mmol) in 10 mL DMF and 2 mL H_2O was purged with nitrogen, then was heated at 100 °C for 12 h under nitrogen protection. The solution was cooled to room temperature, and diluted with 25 mL

water. The resulting solution was extracted with CH_2Cl_2 (50 mL×2), the combined organic phase washed three times with water and dried over Na_2SO_4, and then evaporated under vacuum. The crude solid was purified by using chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ elution to afford **3a** ~ **3c**.

*5.1.4.1. 2-(diethylamino)-N-(4-((2-(4-hydroxyphenyl)quinazolin-4-yl)amino)phenyl)acetamide **(3a)***. Compound **2a** was treated with 4-hydroxyphenylboronic acid according to general procedure C to afford **3a**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 45% yield. m.p. 205.8-207.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.88 (s, 1H), 9.76 (s, 1H), 9.70 (s, 1H), 8.52 (d, *J* = 8.3 Hz, 1H), 8.28 (d, *J* = 8.6 Hz, 2H), 7.90 (d, *J* = 8.9 Hz, 2H), 7.84 – 7.76 (m, 2H), 7.73 (d, *J* = 8.9 Hz, 2H), 7.54 (t, *J* = 7.3 Hz, 1H), 6.87 (d, *J* = 8.6 Hz, 2H), 3.19 (s, 2H), 2.64 (d, *J* = 6.2 Hz, 4H), 1.06 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 170.1, 160.1, 159.7, 158.0, 151.1, 135.4, 134.6, 133.4, 130.1, 129.8, 128.3, 125.6, 123.4, 122.9, 119.9, 115.6, 114.1, 57.9, 48.4, 12.5. HRMS (ESI): Calcd for $[M+H]^+$ ($C_{26}H_{27}N_5O_2$) requires m/z 442.2238, found 442.2221.

*5.1.4.2. N-(4-((2-(2-aminophenyl)quinazolin-4-yl)amino)phenyl)-3-(diethylamino)propanamide **(3b)***. Compound **2b** was treated with 2-aminophenylboronic acid according to general procedure C to afford **3b**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 40% yield. m.p. 120.9-123.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.30 (s, 1H), 8.50 (d, *J* = 7.9 Hz, 1H), 7.94 – 7.86 (m, 2H), 7.77 (m, 3H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.54 – 7.47 (m, 2H), 7.22 (t, *J* = 7.5 Hz, 1H), 6.79 (t, *J* = 7.5 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 6.54 (s, 2H), 2.85 (d, *J* = 5.3 Hz, 2H), 2.75 (dd, *J* = 13.8, 6.8 Hz, 4H), 2.59 (t, *J* = 5.3 Hz, 2H), 1.20 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 170.8, 161.8, 157.5, 150.5, 150.0, 136.0, 134.6, 133.6, 131.4, 131.0, 127.9, 125.9, 123.7, 123.4,

119.5, 118.2, 116.9, 115.1, 113.6, 48.9, 46.6, 34.6, 12.3. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{27}H_{30}N_6O$) requires m/z 453.2408, found 453.2391.

*5.1.4.3. 3-(diethylamino)-N-(4-((2-phenylquinazolin-4-yl)amino)phenyl)propanamide (**3c**)*. Compound **2b** was treated with phenylboronic acid according to general procedure C to afford **3c**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 50% yield. m.p. 152.5-154.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.28 (s, 1H), 8.53 (d, J = 7.0 Hz, 2H), 7.98 (d, J = 8.3 Hz, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.83 (d, J = 8.1 Hz, 2H), 7.78 (t, J = 7.7 Hz, 1H), 7.63 (d, J = 8.1 Hz, 2H), 7.57 (s, 1H), 7.53 – 7.43 (m, 4H), 2.86 – 2.79 (m, 2H), 2.72 (q, J = 7.0 Hz, 4H), 2.60 – 2.51 (m, 2H), 1.17 (t, J = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.1, 160.4, 157.5, 151.0, 138.8, 134.8, 134.5, 132.8, 130.2, 129.1, 128.5, 128.4, 125.9, 122.2, 120.8, 120.2, 114.0, 77.4, 77.1, 76.8, 48.9, 46.0, 33.1, 11.6. HRMS (ESI): Calcd for $[M+H]^+$ ($C_{27}H_{29}N_5O$) requires m/z 440.2445, found 440.2428.

*5.1.5. General procedure D: preparation of **4a** and **4b***

To a stirred suspension of **15a** or **15b** (0.55 mmol) and K_2CO_3 (1.1 mmol) in DMF (10 mL) was added dropwise diethylamine (11 mmol). The mixture was stirred under 110 °C for 12 h, cooled to room temperature, and diluted with 25 mL water. The resulting solution was extracted with CH_2Cl_2 (50 mL×2), the combined organic phase washed six times with water and dried over Na_2SO_4, and then evaporated under vacuum. The crude solid was purified by using chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ elution to afford **4a** and **4b**.

*5.1.5.1. N^4-(4-(2-(diethylamino)ethoxy)phenyl)-N^2,N^2-diethylquinazoline-2,4-diamine (**4a**)*. Compound **15a** was treated with excess diethylamine according to general procedure D to afford **4a**. After column

chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 73% yield. m.p. 149.5-152.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 – 7.57 (m, 3H), 7.52 (m, 2H), 7.13 (s, 1H), 7.07 (dd, *J* = 11.2, 5.0 Hz, 1H), 6.92 (d, *J* = 9.0 Hz, 2H), 4.09 (t, *J* = 6.3 Hz, 2H), 3.69 (q, *J* = 7.0 Hz, 4H), 2.91 (t, *J* = 6.2 Hz, 2H), 2.68 (q, *J* = 7.1 Hz, 4H), 1.20 (t, *J* = 7.0 Hz, 6H), 1.10 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.2, 157.6, 155.2, 153.1, 132.5, 132.2, 126.1, 122.9, 120.5, 120.4, 114.6, 110.0, 77.4, 77.0, 76.7, 66.9, 51.8, 47.9, 41.6, 13.5, 11.8. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{24}H_{33}N_5O$) requires m/z 406.2612, found 406.2597.

*5.1.5.2. N^4-(4-(3-(diethylamino)propoxy)phenyl)-N^2,N^2-diethylquinazoline-2,4-diamine (**4b**).* Compound **15b** was treated with excess diethylamine according to general procedure D to afford **4b**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 70% yield. m.p. 133.0-134.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 – 7.57 (m, 3H), 7.55 – 7.50 (m, 1H), 7.47 (d, *J* = 8.2 Hz, 1H), 7.10 (s, 1H), 7.09 – 7.04 (m, 1H), 6.91 (d, *J* = 8.9 Hz, 2H), 4.03 (t, *J* = 6.3 Hz, 2H), 3.68 (q, *J* = 7.0 Hz, 4H), 2.67 – 2.61 (m, 2H), 2.57 (q, *J* = 7.1 Hz, 4H), 2.00 – 1.90 (m, 2H), 1.20 (t, *J* = 7.0 Hz, 6H), 1.05 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.3, 157.5, 155.4, 153.2, 132.5, 132.1, 126.2, 122.8, 120.4, 120.3, 114.5, 110.0, 66.7, 49.4, 47.0, 41.6, 27.1, 13.6, 11.8. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{25}H_{35}N_5O$) requires m/z 420.2769, found 420.2752.

*5.1.6. General procedure E: preparation of **5a ~ 5f***

To a stirred suspension of **21** (0.3 mmol) and K_2CO_3 (0.6 mmol) in DMF (10 mL) was added dropwise appropriate amine (3 mmol). The mixture was stirred under 100 °C for 12 h, cooled to room temperature, and diluted with 25 mL water. The resulting solution was extracted with CH_2Cl_2 (50 mL×2), the combined organic

phase washed six times with water and dried over Na_2SO_4, and then evaporated under vacuum. The crude solid was purified by using chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ elution to afford **5a ~ 5f**.

*5.1.6.1. 2-(4-((4-(2-(diethylamino)ethoxy)phenyl)amino)quinazolin-2-yl)phenol (**5a**)*. Compound **21** was treated with excess diethylamine according to general procedure E to afford **5a**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 40% yield. m.p. 139.7-141.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.53 (s, 1H), 8.39 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.87 (d, *J* = 8.1 Hz, 1H), 7.83 (d, *J* = 8.3 Hz, 1H), 7.80 – 7.75 (m, 1H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.54 (s, 1H), 7.52 – 7.47 (m, 1H), 7.38 – 7.31 (m, 1H), 7.02 – 6.97 (m, 3H), 6.94 – 6.88 (m, 1H), 4.13 (t, *J* = 6.2 Hz, 2H), 2.94 (t, *J* = 6.1 Hz, 2H), 2.71 (q, *J* = 7.1 Hz, 4H), 1.12 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 161.5, 161.3, 157.2, 156.3, 148.0, 133.4, 132.6, 130.7, 129.5, 127.5, 126.1, 124.2, 120.6, 119.6, 118.6, 117.6, 114.9, 113.3, 66.8, 51.8, 47.9, 11.8. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{26}H_{28}N_4O_2$) requires m/z 427.2139, found 427.2127.

*5.1.6.2. 2-(4-((4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)amino)quinazolin-2-yl)phenol (**5b**)*. Compound **21** was treated with excess pyrrolidine according to general procedure E to afford **5b**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 60% yield. m.p. 183.4-186.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.52 (s, 1H), 8.39 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.84 (d, *J* = 7.5 Hz, 1H), 7.81 – 7.75 (m, 1H), 7.65 (d, *J* = 8.9 Hz, 2H), 7.56 (s, 1H), 7.51–7.49 (m, 1H), 7.35–7.32 (m, 1H), 7.06 – 6.98 (m, 3H), 6.94 – 6.88 (m, 1H), 4.20 (t, *J* = 5.8 Hz, 2H), 2.99 (t, *J* = 5.8 Hz, 2H), 2.73 (s, 4H), 1.88–1.84 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 161.5, 161.3, 157.2, 156.3, 148.0, 133.4, 132.6, 130.8, 129.5, 127.5, 126.1, 124.2, 120.6, 119.6, 118.6, 117.6, 115.0, 113.3, 67.4,

55.2, 54.8, 23.5. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{26}H_{26}N_4O_2$) requires m/z 425.1983, found 425.1969.

*5.1.6.3. 2-(4-((4-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)amino)quinazolin-2-yl)phenol (**5c**).* Compound **21** was treated with excess *N*-methyl piperazine according to general procedure E to afford **5c**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a pale yellow solid in 64% yield. m.p. 142.3-143.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.53 (s, 1H), 8.39 (dd, J = 7.9, 1.5 Hz, 1H), 7.88 (d, J = 8.1 Hz, 1H), 7.83 (d, J = 8.3 Hz, 1H), 7.79– 7.75 (m, 1H), 7.65 (d, J = 8.9 Hz, 2H), 7.57 (s, 1H), 7.53 – 7.47 (m, 1H), 7.38 – 7.32 (m, 1H), 7.00 (d, J = 8.9 Hz, 3H), 6.94 – 6.88 (m, 1H), 4.16 (t, J = 5.8 Hz, 2H), 2.86 (t, J = 5.8 Hz, 2H), 2.69 (s, 4H), 2.55 (s, 4H), 2.34 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 161.4, 161.2, 157.2, 156.1, 147.9, 133.3, 132.5, 131.0, 129.5, 127.3, 126.1, 124.2, 120.8, 119.6, 118.6, 117.6, 114.9, 113.4, 66.2, 57.2, 55.0, 53.5, 46.0. HRMS (ESI): Calcd for $[M+H]^+$ ($C_{27}H_{29}N_5O_2$) requires m/z 456.2394, found 456.2398.

*5.1.6.4. 2-(4-((4-(2-morpholinoethoxy)phenyl)amino)quinazolin-2-yl)phenol (**5d**).* Compound **21** was treated with excess morpholine according to general procedure E to afford **5d**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 70% yield. m.p. 162.5-164.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.55 (s, 1H), 8.39 (d, J = 6.9 Hz, 1H), 7.85 (d, J = 8.3 Hz, 1H), 7.82 (d, J = 8.3 Hz, 1H), 7.77 (t, J = 7.5 Hz, 1H), 7.65 (d, J = 8.7 Hz, 2H), 7.54 (s, 1H), 7.49 (t, J = 7.4 Hz, 1H), 7.38 – 7.32 (m, 1H), 7.00 (d, J = 8.8 Hz, 3H), 6.91 (t, J = 7.5 Hz, 1H), 4.17 (t, J = 5.6 Hz, 2H), 3.82 – 3.73 (m, 4H), 2.85 (t, J = 5.6 Hz, 2H), 2.63 (d, J = 4.2 Hz, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 161.4, 161.2, 157.1, 156.0, 147.9, 133.4, 132.6, 130.9, 129.5, 127.4, 126.1, 124.1, 120.6, 119.5, 118.6, 117.6, 114.9, 113.3, 66.9, 66.0, 57.7, 54.1. HRMS (ESI): Calcd for $[M+H]^+$ ($C_{26}H_{26}N_4O_3$) requires m/z 443.2078,

found 443.2080.

*5.1.6.5. 2-(4-((4-(2-(piperidin-1-yl)ethoxy)phenyl)amino)quinazolin-2-yl)phenol (**5e**).* Compound **21** was treated with excess piperidine according to general procedure E to afford **5e**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 55% yield. m.p. 131.2-134.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.56 (s, 1H), 8.40 (d, J = 7.9 Hz, 1H), 7.89 (d, J = 8.1 Hz, 1H), 7.85 (d, J = 8.3 Hz, 1H), 7.79 (t, J = 7.6 Hz, 1H), 7.66 (d, J = 8.4 Hz, 2H), 7.57 (s, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.01 (d, J = 8.2 Hz, 3H), 6.92 (t, J = 7.5 Hz, 1H), 4.20 (t, J = 5.7 Hz, 2H), 2.87 (t, J = 6.1 Hz, 2H), 2.61 (s, 4H), 1.72 – 1.62 (m, 4H), 1.49 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 161.3, 161.2, 157.2, 156.0, 147.9, 133.3, 132.5, 130.8, 129.5, 127.3, 126.1, 124.2, 120.8, 119.6, 118.6, 117.5, 114.8, 113.4, 66.0, 57.9, 55.1, 25.7, 24.0. HRMS (ESI): Calcd for $[M+H]^+$ ($C_{27}H_{28}N_4O_2$) requires m/z 441.2285, found 441.2275.

*5.1.6.6. 2-(4-((4-(2-((2-(diethylamino)ethyl)amino)ethoxy)phenyl)amino)quinazolin-2-yl)phenol (**5f**).* Compound **21** was treated with excess *N*,*N*-diethylethylenediamine according to general procedure E to afford **5f**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a pale yellow solid in 40% yield. m.p. 122.9-124.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.60 (s, 1H), 8.39 (d, J = 7.7 Hz, 1H), 7.93 (d, J = 8.1 Hz, 1H), 7.85 – 7.73 (m, 3H), 7.64 (d, J = 8.4 Hz, 2H), 7.49 (t, J = 7.4 Hz, 1H), 7.34 (t, J = 7.5 Hz, 1H), 6.99 (t, J = 9.0 Hz, 3H), 6.91 (t, J = 7.5 Hz, 1H), 4.12 (t, J = 4.8 Hz, 2H), 3.06 (t, J = 4.9 Hz, 2H), 2.81 (t, J = 6.1 Hz, 2H), 2.64 (t, J = 6.1 Hz, 2H), 2.57 (m, 4H), 2.42 (s, 1H), 1.05 (t, J = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 161.4, 161.2, 157.3, 156.2, 148.0, 133.3, 132.5, 130.9, 129.5, 127.3, 126.1, 124.3, 121.0, 119.6, 118.6, 117.6, 114.7, 113.5, 67.5, 52.6, 49.0, 47.5, 47.0, 11.7. HRMS

(ESI): Calcd for $[M+H]^+$ ($C_{28}H_{33}N_5O_2$) requires m/z 472.2707, found 472.2690.

*5.1.7. General procedure F: preparation of **6a~6d**, **7a~7i***

To a stirred suspension of the chloride compound **27a** or **27b** (1 mmol) and K_2CO_3 (2 mmol) in DMF (10 mL) was added dropwise appropriate amine (20 mmol). The mixture was stirred under 80-100 °C for 12 h, cooled to room temperature, and diluted with 25 mL water. The resulting solution was extracted with CH_2Cl_2 (50 mL×2), the combined organic phase washed six times with water and dried over Na_2SO_4, and then evaporated under vacuum. The crude solid was purified by using chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ elution to afford **6a ~ 6d**, **7a ~ 7i**.

*5.1.7.1. 2-(diethylamino)-N-(2-(4-((4-(2-(diethylamino)acetamido)phenyl)amino)quinazolin-2-yl)phenyl)-acetamide (**6a**)*. Compound **27a** was treated with excess diethylamine according to general procedure F to afford **6a**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 60% yield. m.p. 202.7-204.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.29 (s, 1H), 9.40 (s, 1H), 8.71 (d, *J* = 8.2 Hz, 1H), 8.37 (dd, *J* = 7.9, 1.3 Hz, 1H), 8.13 (s, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.90 (d, *J* = 8.3 Hz, 1H), 7.77 (d, *J* = 8.8 Hz, 2H), 7.68 (t, *J* = 7.6 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.40 (t, *J* = 7.1 Hz, 1H), 7.34 (t, *J* = 7.7 Hz, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 3.18 (s, 2H), 3.11 (s, 2H), 2.58 (m, 8H), 1.03 (t, *J* = 7.0 Hz, 6H), 0.88 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.0, 170.4, 160.7, 157.5, 149.6, 138.8, 135.2, 133.7, 132.6, 131.0, 130.6, 128.3, 126.2, 126.0, 123.1, 122.6, 121.6, 121.4, 120.1, 113.7, 58.7, 58.1, 49.1, 48.9, 12.5, 11.7. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{32}H_{39}N_7O_2$) requires m/z 552.3092, found 552.3079.

*5.1.7.2. 2-(pyrrolidin-1-yl)-N-(2-(4-((4-(2-(pyrrolidin-1-yl)acetamido)phenyl)amino)quinazolin-2-yl)phenyl)-acetamide (**6b**).* Compound **27a** was treated with excess pyrrolidine according to general procedure F to afford **6b**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 70% yield. m.p. 248.4-251.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.43 (s, 1H), 9.19 (s, 1H), 8.71 (d, *J* = 8.0 Hz, 1H), 8.42 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.73 (dd, *J* = 12.3, 5.3 Hz, 3H), 7.65 (s, 1H), 7.61 (d, *J* = 8.9 Hz, 2H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.40 – 7.35 (m, 1H), 7.11 (t, *J* = 7.1 Hz, 1H), 3.32 (s, 2H), 3.27 (s, 2H), 2.73 – 2.59 (m, 8H), 1.83 (s, 4H), 1.66 (s, 4H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 169.6, 168.6, 159.9, 157.6, 148.8, 139.0, 134.9, 134.2, 133.0, 130.6, 130.4, 127.4, 126.4, 124.6, 122.9, 122.6, 120.3, 119.5, 113.3, 61.7, 59.5, 54.0, 53.7, 23.5, 23.4. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{32}H_{35}N_7O_2$) requires m/z 548.2779, found 548.2782.

*5.1.7.3. 2-(4-methylpiperazin-1-yl)-N-(2-(4-((4-(2-(4-methylpiperazin-1-yl)acetamido)phenyl)amino)-quinazolin-2-yl)phenyl)acetamide (**6c**).* Compound **27a** was treated with excess *N*-methyl piperazine according to general procedure F to afford **6c**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a pale yellow solid in 68% yield. m.p. 237.5-240.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.09 (s, 1H), 9.23 (s, 1H), 8.77 (d, *J* = 8.3 Hz, 1H), 8.43 (dd, *J* = 7.9, 1.5 Hz, 1H), 8.09 (d, *J* = 8.3 Hz, 1H), 8.06 (d, *J* = 8.2 Hz, 1H), 7.90 (s, 1H), 7.85 (t, *J* = 8.3 Hz, 3H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.50 – 7.43 (m, 1H), 7.24 – 7.17 (m, 1H), 3.24 (s, 2H), 3.20 (s, 2H), 2.71 (s, 4H), 2.58 (s, 8H), 2.37 (s, 3H), 2.32 (s, 4H), 2.13 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.2, 168.5, 160.8, 157.2, 149.5, 138.8, 134.8, 133.9, 133.0, 130.9, 130.8, 129.1, 126.5, 125.4, 123.1, 122.4, 121.1, 121.0, 120.2, 113.5, 64.0, 61.9, 55.2, 54.2, 53.5, 53.3, 46.0, 45.8. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{34}H_{41}N_9O_2$) requires m/z 606.3310, found 606.3290.

*5.1.7.4. 2-morpholino-N-(2-(4-((4-(2-morpholinoacetamido)phenyl)amino)quinazolin-2-yl)phenyl)acetamide (**6d**).* Compound **27a** was treated with excess morpholine according to general procedure F to afford **6d**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (50:1:0.1) elution, the desired product was obtained as a pale yellow solid in 70% yield. m.p. 273.5-275.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.15 (s, 1H), 10.03 (s, 1H), 9.79 (s, 1H), 8.66 (d, *J* = 8.2 Hz, 1H), 8.62 (d, *J* = 8.2 Hz, 1H), 8.34 (d, *J* = 7.8 Hz, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.98 – 7.92 (m, 1H), 7.85 (d, *J* = 8.8 Hz, 2H), 7.73 (d, *J* = 8.8 Hz, 2H), 7.71 – 7.66 (m, 1H), 7.46 (t, *J* = 7.8 Hz, 1H), 7.19 (t, *J* = 7.4 Hz, 1H), 3.71 – 3.62 (m, 4H), 3.41 (s, 4H), 3.18 (s, 2H), 3.16 (s, 2H), 2.54 (s, 4H), 2.41 (s, 4H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 168.5, 167.9, 159.9, 157.6, 148.8, 138.6, 134.8, 134.3, 133.3, 130.6, 130.4, 127.9, 126.5, 124.9, 123.0, 122.9, 122.7, 120.2, 119.6, 113.4, 66.1, 65.5, 64.0, 62.0, 53.2. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{32}H_{35}N_7O_4$) requires m/z 580.2678, found 580.2656.

*5.1.7.5. 3-(diethylamino)-N-(2-(4-((4-(3-(diethylamino)propanamido)phenyl)amino)quinazolin-2-yl)phenyl)-propanamide (**7a**).* Compound **27b** was treated with excess diethylamine according to general procedure F to afford **7a**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (15:1:0.1) elution, the desired product was obtained as a pale yellow solid in 58% yield. m.p. 150.3-152.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.60 (s, 1H), 11.39 (s, 1H), 8.70 (d, *J* = 7.7 Hz, 1H), 8.56 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.05 (d, *J* = 8.0 Hz, 1H), 7.96 (s, 1H), 7.86 (d, *J* = 7.6 Hz, 1H), 7.82 – 7.76 (m, 1H), 7.74 (d, *J* = 8.9 Hz, 2H), 7.59 (d, *J* = 8.9 Hz, 2H), 7.55 – 7.49 (m, 1H), 7.45 – 7.38 (m, 1H), 7.17 – 7.09 (m, 1H), 3.02 – 2.95 (m, 2H), 2.83 – 2.77 (m, 2H), 2.70 (q, *J* = 7.1 Hz, 4H), 2.64 – 2.57 (m, 6H), 2.56 – 2.52 (m, 2H), 1.16 (t, *J* = 7.1 Hz, 6H), 1.05 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.3, 171.0, 160.9, 157.2, 148.9, 140.0, 135.0, 134.3, 133.1, 131.1, 130.1, 127.7, 126.3, 123.6, 122.9, 122.7, 121.8, 120.5, 120.3, 113.6, 48.9, 48.9, 46.9, 46.0, 36.3, 33.1, 11.7, 11.5. HRMS

(ESI): Calcd for $[M-H]^-$ ($C_{34}H_{43}N_7O_2$) requires m/z 580.3405, found 580.3386.

*5.1.7.6. 3-(pyrrolidin-1-yl)-N-(2-(4-((4-(3-(pyrrolidin-1-yl)propanamido)phenyl)amino)quinazolin-2-yl)-phenyl)propanamide (**7b**).* Compound **27b** was treated with excess pyrrolidine according to general procedure F to afford **7b**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (15:1:0.1) elution, the desired product was obtained as a pale yellow solid in 64% yield. m.p. 198.6-202.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.60 (s, 1H), 11.30 (s, 1H), 8.74 – 8.68 (m, 1H), 8.54 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.09 (s, 1H), 8.07 (d, *J* = 8.1 Hz, 1H), 7.86 – 7.81 (m, 1H), 7.79 – 7.74 (m, 1H), 7.73 (d, *J* = 8.9 Hz, 2H), 7.53 (d, *J* = 8.9 Hz, 2H), 7.51 – 7.47 (m, 1H), 7.43 – 7.38 (m, 1H), 7.15 – 7.10 (m, 1H), 2.94 (dd, *J* = 9.4, 5.7 Hz, 2H), 2.90 – 2.84 (m, 2H), 2.68 (dd, *J* = 10.4, 4.6 Hz, 6H), 2.57 (t, *J* = 5.9 Hz, 6H), 1.91 (s, 4H), 1.79 – 1.71 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.2, 170.6, 160.8, 157.2, 148.9, 140.0, 135.1, 134.4, 133.1, 131.1, 130.8, 127.7, 126.3, 123.6, 122.9, 122.7, 121.73, 120.5, 120.4, 113.6, 54.1, 53.2, 52.1, 51.4, 38.2, 34.7, 23.7, 23.5. HRMS (ESI): Calcd for $[M+H]^+$ ($C_{34}H_{39}N_7O_2$) requires m/z 578.3238, found 578.3265.

*5.1.7.7. 3-(4-methylpiperazin-1-yl)-N-(2-(4-((4-(3-(4-methylpiperazin-1-yl)propanamido)phenyl)amino)-quinazolin-2-yl)phenyl)propanamide (**7c**).* Compound **27b** was treated with excess *N*-methyl piperazine according to general procedure F to afford **7c**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (15:1:0.1) elution, the desired product was obtained as a pale yellow solid in 63% yield. m.p. 210.6-210.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.47 (s, 1H), 11.08 (s, 1H), 8.70 (d, *J* = 8.3 Hz, 1H), 8.56 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 7.89 – 7.84 (m, 1H), 7.83 (t, *J* = 7.0 Hz, 2H), 7.75 (d, *J* = 8.9 Hz, 2H), 7.63 (d, *J* = 8.9 Hz, 2H), 7.59 – 7.54 (m, 1H), 7.46 – 7.41 (m, 1H), 7.18 – 7.12 (m, 1H), 2.88 (t, *J* = 7.4 Hz, 2H), 2.79 – 2.75 (m, 2H), 2.72 – 2.51 (m, 16H), 2.45 (s, 4H), 2.37 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ

170.8, 170.6, 160.8, 157.2, 149.0, 139.9, 135.0, 134.4, 133.2, 131.1, 130.8, 127.7, 126.3, 123.7, 123.0, 122.7, 121.7, 120.6, 120.3, 113.6, 55.3, 54.9, 54.2, 53.6, 52.8, 52.3, 46.0, 45.9, 36.2, 32.6. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{36}H_{45}N_9O_2$) requires m/z 634.3623, found 634.3594.

*5.1.7.8. 3-morpholino-N-(2-(4-((4-(3-morpholinopropanamido)phenyl)amino)quinazolin-2-yl)phenyl)-propanamide (**7d**)*. Compound **27b** was treated with excess morpholine according to general procedure F to afford **7d**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (15:1:0.1) elution, the desired product was obtained as a pale yellow solid in 62% yield. m.p. 239.0-242.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.22 (s, 1H), 10.09 (s, 1H), 10.06 (s, 1H), 8.58 (d, J = 8.1 Hz, 1H), 8.54 (dd, J = 8.3, 0.9 Hz, 1H), 8.44 (dd, J = 8.0, 1.6 Hz, 1H), 7.96 – 7.86 (m, 2H), 7.76 (d, J = 9.0 Hz, 2H), 7.72 – 7.64 (m, 3H), 7.48 – 7.41 (m, 1H), 7.20 – 7.14 (m, 1H), 3.63 – 3.57 (m, 4H), 3.46 – 3.40 (m, 4H), 2.68 – 2.59 (m, 4H), 2.53 – 2.50 (m, 2H), 2.43 (s, 6H), 2.36 – 2.30 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 169.9, 169.9, 160.0, 157.4, 148.5, 139.5, 135.8, 133.6, 133.6, 130.8, 130.4, 127.2, 126.5, 123.5, 123.4, 123.1, 122.4, 120.1, 119.2, 113.4, 66.2, 66.0, 54.2, 53.1, 53.0, 35.4, 33.9. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{34}H_{39}N_7O_4$) requires m/z 608.2991, found 608.2967.

*5.1.7.9. 3-(piperidin-1-yl)-N-(2-(4-((4-(3-(piperidin-1-yl)propanamido)phenyl)amino)quinazolin-2-yl)phenyl)-propanamide (**7e**)*. Compound **27b** was treated with excess piperidine according to general procedure F to afford **7e**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (15:1:0.1) elution, the desired product was obtained as a pale yellow solid in 60% yield. m.p. 211.9-214.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.53 (s, 1H), 11.48 (s, 1H), 8.71 (d, J = 8.0 Hz, 1H), 8.56 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.88 (d, J = 8.4 Hz, 1H), 7.85 (s, 1H), 7.84 – 7.78 (m, 1H), 7.73 (d, J = 8.2 Hz, 2H), 7.62 (d, J = 8.1 Hz, 2H), 7.54 (t, J =

7.3 Hz, 1H), 7.43 (t, *J* = 7.6 Hz, 1H), 7.15 (t, *J* = 7.4 Hz, 1H), 2.83 (t, *J* = 7.2 Hz, 2H), 2.73 – 2.67 (m, 2H), 2.67 – 2.62 (m, 2H), 2.56 (d, *J* = 5.2 Hz, 6H), 2.44 (s, 4H), 1.72 (d, *J* = 4.3 Hz, 4H), 1.60 – 1.50 (m, 6H), 1.40 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.0, 170.8, 161.0, 157.1, 149.0, 140.1, 135.5, 133.9, 133.2, 131.3, 130.9, 128.1, 126.4, 123.6, 122.9, 122.7, 121.2, 120.6, 120.2, 113.5, 55.0, 54.4, 54.3, 53.7, 36.3, 32.5, 26.3, 25.9, 24.3, 24.2. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{36}H_{43}N_7O_2$) requires m/z 604.3405, found 604.3388.

5.1.7.10.

*3-((2-(diethylamino)ethyl)amino)-N-(2-(4-((4-(3-((2-(diethylamino)ethyl)amino)propanamido)-phenyl)-amino)quinazolin-2-yl)phenyl)propanamide (**7f**)*. Compound **27b** was treated with excess *N*,*N*-diethylethylenediamine according to general procedure F to afford **7f**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a yellow solid in 30% yield. m.p. 140.0-143.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.52 (s, 1H), 11.01 (s, 1H), 8.66 (d, *J* = 8.1 Hz, 1H), 8.55 (d, *J* = 7.9 Hz, 1H), 8.07 (s, 1H), 8.03 (d, *J* = 8.1 Hz, 1H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.79 – 7.73 (m, 1H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.61 (d, *J* = 8.8 Hz, 2H), 7.49 (t, *J* = 7.3 Hz, 1H), 7.41 (t, *J* = 7.1 Hz, 1H), 7.13 (t, *J* = 7.2 Hz, 1H), 3.02 (t, *J* = 6.0 Hz, 4H), 2.82 – 2.71 (m, 4H), 2.66 – 2.47 (m, 16H), 1.04 (t, *J* = 7.1 Hz, 6H), 0.98 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.4, 170.6, 160.6, 157.1, 149.0, 139.9, 135.2, 134.2, 133.1, 131.1, 130.8, 127.8, 126.3, 123.6, 123.0, 122.7, 121.6, 120.7, 120.5, 113.5, 52.3, 47.3, 47.1, 47.0, 46.7, 45.7, 45.3, 37.9, 36.0, 11.7, 11.5. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{38}H_{53}N_9O_2$) requires m/z 666.4249, found 666.4273.

*5.1.7.11. 3-((3-(diethylamino)propyl)amino)-N-(2-(4-((4-(3-((3-(diethylamino)propyl)amino)propanamido)-phenyl)amino)quinazolin-2-yl)phenyl)propanamide (**7g**)*. Compound **27b** was treated with excess

N,*N*-diethyl-1,3-propanediamine according to general procedure F to afford **7g**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a yellow solid in 35% yield. m.p. 143.5-146.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.53 (s, 1H), 10.96 (s, 1H), 8.67 (d, *J* = 8.3 Hz, 1H), 8.56 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.04 (d, *J* = 8.2 Hz, 1H), 8.00 (s, 1H), 7.86 – 7.82 (m, 1H), 7.77 (t, *J* = 7.6 Hz, 1H), 7.71 (d, *J* = 8.9 Hz, 2H), 7.62 (d, *J* = 8.9 Hz, 2H), 7.54 – 7.49 (m, 1H), 7.45 – 7.39 (m, 1H), 7.16 – 7.12 (m, 1H), 2.99 (dd, *J* = 8.0, 4.7 Hz, 4H), 2.78 (t, *J* = 6.7 Hz, 2H), 2.69 (t, *J* = 6.9 Hz, 2H), 2.61 – 2.42 (m, 16H), 1.79 – 1.71 (m, 2H), 1.65 (m, 2H), 1.07 – 1.00 (t, *J* = 7.1 Hz, 6H), 0.98 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.4, 170.7, 160.6, 157.2, 148.9, 139.8, 135.1, 134.3, 133.1, 131.0, 130.8, 127.7, 126.3, 123.6, 123.0, 122.7, 121.8, 120.5, 120.5, 113.5, 51.5, 51.0, 48.6, 48.3, 46.8, 46.7, 45.7, 45.4, 37.8, 36.0, 27.2, 26.8, 11.5, 11.4. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{40}H_{57}N_9O_2$) requires m/z 694.4562, found 694.4581.

*5.1.7.12. 3-((2-(dimethylamino)ethyl)amino)-N-(2-(4-((4-(3-((2-(dimethylamino)ethyl)amino)propanamido)-phenyl)amino)quinazolin-2-yl)phenyl)propanamide (**7h**)*. Compound **27b** was treated with excess *N*,*N*-dimethylethylenediamine according to general procedure F to afford **7h**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a yellow solid in 32% yield. m.p. 140.1-143.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.40 (s, 1H), 10.76 (s, 1H), 8.55 (d, *J* = 7.8 Hz, 1H), 8.45 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.24 (s, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.70 (d, *J* = 7.4 Hz, 1H), 7.67 – 7.62 (m, 1H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.41 – 7.36 (m, 1H), 7.34 – 7.29 (m, 1H), 7.07 – 7.01 (m, 1H), 2.94 (t, *J* = 6.2 Hz, 4H), 2.70 (m, 4H), 2.52 – 2.47 (m, 2H), 2.46 – 2.40 (m, 4H), 2.35 (t, *J* = 6.2 Hz, 2H), 2.19 (s, 6H), 2.13 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.3, 170.5, 160.4, 157.2, 148.9, 139.7, 135.0, 134.4, 133.0, 131.0, 130.8, 127.6, 126.2, 123.7, 123.1, 122.7, 122.0, 120.7, 120.4, 113.5, 58.6, 58.5, 47.0, 46.4, 45.6, 45.4, 45.4, 45.3, 37.5, 36.0. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{34}H_{45}N_9O_2$) requires m/z

610.3623, found 610.3637.

5.1.7.13.

*3-((3-(dimethylamino)propyl)amino)-N-(2-(4-((4-(3-((3-(dimethylamino)propyl)amino)propan-amido)-phenyl)amino)quinazolin-2-yl)phenyl)propanamide (**7i**).* Compound **27b** was treated with excess *N*,*N*-dimethyl-1,3-propanediamine according to general procedure F to afford **7i**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a yellow solid in 36% yield. m.p. 143.5-146.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.49 (s, 1H), 10.80 (s, 1H), 8.59 (d, *J* = 7.9 Hz, 1H), 8.46 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.21 (s, 1H), 8.01 (d, *J* = 8.2 Hz, 1H), 7.72 – 7.68 (m, 1H), 7.66 (d, *J* = 7.0 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 2H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.40 – 7.35 (m, 1H), 7.35 – 7.30 (m, 1H), 7.06 – 7.01 (m, 1H), 2.91 (dd, *J* = 10.0, 5.6 Hz, 4H), 2.69 (t, *J* = 6.8 Hz, 2H), 2.60 (t, *J* = 7.1 Hz, 2H), 2.48 (m, 4H), 2.31 (t, *J* = 7.0 Hz, 2H), 2.22 – 2.18 (m, 2H), 2.15 (s, 6H), 2.09 (s, 6H), 1.71 – 1.63 (m, 2H), 1.56 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.3, 170.7, 160.5, 157.2, 148.8, 139.8, 135.0, 134.4, 133.0, 131.0, 130.7, 127.5, 126.2, 123.6, 123.1, 122.7, 122.0, 120.5, 120.4, 113.5, 57.9, 57.8, 48.2, 47.7, 45.7, 45.4, 45.3, 37.9, 36.0, 29.6, 27.7, 27.6. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{36}H_{49}N_9O_2$) requires m/z 638.3936, found 638.3940.

*5.1.8. General procedure G: preparation of **8a**, **8b** and **9a***

To a stirred suspension of the chloride compounds **27c** or **27d** (1 mmol), K_2CO_3 (2 mmol) and KI (1 mmol) in DMF (10 mL) was added dropwise appropriate amine (20 mmol). The mixture was stirred under 110-130 °C for 12 h, cooled to room temperature, and diluted with 25 mL water. The resulting solution was extracted with CH_2Cl_2 (50 mL×2), the combined organic phase washed six times with water and dried over

Na_2SO_4, and then evaporated under vacuum. The crude solid was purified by using chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ elution to afford **8a**, **8b** and **9a**.

*5.1.8.1. 4-(diethylamino)-N-(2-(4-((4-(4-(diethylamino)butanamido)phenyl)amino)quinazolin-2-yl)phenyl)-butanamide (**8a**)*. Compound **27c** was treated with excess diethylamine according to general procedure G to afford **8a**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (10:1:0.1) elution, the desired product was obtained as a pale yellow solid in 32% yield. m.p. 161.7-163.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.44 (s, 1H), 10.32 (s, 1H), 8.70 (d, *J* = 8.3 Hz, 1H), 8.56 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.05 (d, *J* = 8.3 Hz, 1H), 7.95 (s, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.84 – 7.78 (m, 1H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 8.8 Hz, 2H), 7.55 (t, *J* = 7.6 Hz, 1H), 7.46 – 7.39 (m, 1H), 7.12 (t, *J* = 7.6 Hz, 1H), 2.66 (dd, *J* = 14.4, 7.2 Hz, 4H), 2.63 – 2.59 (m, 2H), 2.58 – 2.52 (m, 8H), 2.35 (t, *J* = 7.3 Hz, 2H), 1.94 – 1.87 (m, 4H), 1.09 (t, *J* = 7.1 Hz, 6H), 1.01 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.3, 171.9, 160.7, 157.2, 149.1, 140.0, 135.1, 134.4, 133.2, 131.1, 130.8, 127.8, 126.4, 123.7, 123.1, 122.6, 121.9, 121.1, 120.5, 113.6, 52.8, 52.2, 46.7, 37.0, 36.3, 22.8, 11.5, 11.1. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{36}H_{47}N_7O_2$) requires m/z 608.3718, found 608.3694.

*5.1.8.2. 4-(pyrrolidin-1-yl)-N-(2-(4-((4-(4-(pyrrolidin-1-yl)butanamido)phenyl)amino)quinazolin-2-yl)phenyl)-butanamide (**8b**)*. Compound **27c** was treated with excess pyrrolidine according to general procedure G to afford **8b**. After column chromatography with CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ (10:1:0.1) elution, the desired product was obtained as a pale yellow solid in 42% yield. m.p. 211.9-212.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.30 (s, 1H), 10.07 (s, 1H), 9.98 (s, 1H), 8.63 – 8.55 (m, 2H), 8.46 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.95 – 7.90 (m, 1H), 7.90 – 7.85 (m, 1H), 7.75 – 7.64 (m, 5H), 7.48 – 7.41 (m, 1H), 7.19 – 7.12 (m, 1H), 2.43 (dd, *J* = 9.8, 4.3 Hz, 6H), 2.40 – 2.31 (m, 8H), 2.27 (t, *J* = 7.2 Hz, 2H), 1.81 – 1.74 (m, 4H), 1.71 – 1.63 (m, 4H), 1.61 – 1.53

(m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 171.6, 171.5, 160.5, 157.9, 149.1, 140.2, 136.5, 134.1, 133.9, 131.4, 130.9, 127.7, 127.0, 124.0, 123.7, 123.6, 122.7, 120.4, 119.6, 113.8, 55.6, 55.3, 54.0, 53.9, 36.0, 34.9, 25.0, 24.9, 23.6, 23.5. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{36}H_{43}N_7O_2$) requires m/z 604.3405, found 604.3406.

*5.1.8.3. 5-(diethylamino)-N-(2-(4-((4-(5-(diethylamino)pentanamido)phenyl)amino)quinazolin-2-yl)phenyl)-pentanamide (**9a**)*. Compound **27d** was treated with excess diethylamine according to general procedure G to afford **9a**. After column chromatography with CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ (10:1:0.1) elution, the desired product was obtained as a pale yellow solid in 34% yield. m.p. 209.6-211.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.32 (s, 1H), 8.68 (d, *J* = 8.3 Hz, 1H), 8.52 (d, *J* = 8.0 Hz, 1H), 8.16 (s, 1H), 8.00 (d, *J* = 8.2 Hz, 1H), 7.90 (s, 1H), 7.81 (q, *J* = 8.2 Hz, 2H), 7.69 (d, *J* = 8.2 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.52 (t, *J* = 7.2 Hz, 1H), 7.41 (t, *J* = 7.7 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 2.58 – 2.42 (m, 14H), 2.31 (t, *J* = 7.3 Hz, 2H), 1.81 – 1.70 (m, 4H), 1.60 – 1.49(m, 4H), 1.08 – 0.96 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.1, 171.9, 160.7, 157.0, 149.2, 139.8, 134.7, 134.4, 133.2, 131.1, 130.8, 127.8, 126.4, 123.8, 122.9, 122.7, 121.5, 121.1, 120.6, 113.4, 52.7, 52.3, 46.7, 46.7, 38.3, 37.2, 26.7, 26.5, 23.9, 23.8, 11.3. HRMS (ESI): Calcd for $[M-H]^-$ ($C_{38}H_{51}N_7O_2$) requires m/z 636.4031, found 636.4005.

5.2. Materials

All oligomers/primers used in this study were purchased from Invitrogen (China). Stock solutions of all the derivatives (10 mM) were made using DMSO. Further dilutions to working concentrations were made with double-distilled deionized water.

5.3. FRET assay

FRET assay was carried out on a real-time PCR apparatus following previously published procedures. The fluorescently labeled oligonucleotides FPu22T: 5′-FAM-TGAGGGTGGGTAGGGTGGGTAA-TAMRA-3′ and F10T: 5′-FAM-dTATAGCTATA-HEG-TATAGCTATA-TAMRA-3′ (donor fluorophore FAM is 6-carboxyfluorescein; acceptor fluorophore TAMRA is 6-carboxytetramethylrhodamine; HEG linker is [(-CH_2-CH_2-O-)$_6$]) were used as the FRET probes. Fluorescence melting curves were determined with a Roche LightCycler 2 realtime PCR machine, using a total reaction volume of 20 μL, with 0.4 μM of labeled oligonucleotide in Tris-HCl buffer (10 mM, pH 7.4) containing 10 mM or 60 mM KCl. Fluorescence readings with excitation at 470 nm and detection at 530 nm were taken at intervals of 1 °C over the range 37-99 °C, with a constant temperature being maintained for 30 s prior to each reading to ensure a stable value. The melting of the G-quadruplex was monitored alone or in the presence of various concentrations of compounds and/or double-stranded competitor ds26. Final analysis of the data was carried out using Origin9.0 (OriginLab Corp.).

5.4. Surface plasmon resonance

SPR measurements were performed on a ProteOn XPR36 Protein Interaction Array system (Bio-Rad Laboratories, Hercules, CA) using a Neutravidin-coated GLH sensor chip. In a typical experiment, biotinylated duplex DNA and biotinylated Pu22 (5′-TGAGGGTGGGTAGGGTGGGTAA-3′) were folded in filtered and degassed running buffer (50 mM Tris-HCl, 150 mM KCl, pH 7.4, 0.005% Tween20). The DNA samples were then captured (~1000 RU) in flow cells 1 and 2, leaving the third flow cell as a blank. Ligand solutions (at 0.0625, 0.125, 0.25, 0.3125, 0.5, 0.625, 1, 1.25, 2, 2.5, 5, and 10 μM) were prepared with running buffer by serial dilutions from stock solutions. Six concentrations were injected simultaneously at a flow rate of 25 mL min^{-1} for 240 s of association phase, followed with 240 s of dissociation phase at 25 °C. The GLH

sensor chip was regenerated with short injection of 50 mM NaOH between consecutive measurements. The final graphs were obtained by subtracting blank sensorgrams from the duplex or quadruplex sensorgrams. Data were analyzed with ProteOn manager software, using the Langmuir model for fitting kinetic data.

5.5. CD measurements

The oligomer Pu22 (5′-TGAGGGTGGGTAGGGTGGGTAA-3′) at a final concentration of 3 μM was resuspended in Tris-HCl buffer (10 mM, pH 7.2) containing the derivatives to be tested. The samples were heated to 95 °C for 5 min, then gradually cooled to room temperature, and incubated for at least 6 h. The CD spectra were recorded on Chirascan (AppliedPhotophysics) spectrophotometer. A quartz cuvette with 1 mm path length was used for the spectra recorded over a wavelength range of 230-330 at 1 nm bandwidth, 1 nm step size, and 0.5 s per point. The CD spectra were obtained by taking the average of two scans made from 230 to 330 nm. Final analysis of the data was carried out using Origin 9.0 (OriginLab Corp.).

5.6. Cell culture

The human cervical cancer cell line Hela, human lung adenocarcinoma cell line A549, human lymphoma cell lines Raji and CA46 were purchased from China Center for Type Culture Collection in Wuhan. The primary cultured mouse mesangial cell line was separated and purified from the laboratory of Pei-Qing Liu. The cell lines were maintained in RPMI-1640 or DMEM medium supplemented with 10% fetal calf serum at 37 °C in a humidified atmosphere with 5% CO_2.

5.7. MTT assay

Hela cervical cancer cell line, A549 lung adenocarcinoma cell line, Raji and CA46 lymphoma cell lines

and primary cultured mouse mesangial cell line were seeded on 96-well plates (5.0×10^3/well) and exposed to various concentrations of compounds. After 48 h of treatment at 37 °C in a humidified atmosphere of 5% CO_2, and 20 mL of 2.5 mg/mL methyl thiazolyl tetrazolium (MTT) solution was added to each well and further incubated for 4 h. The cells in each well were then treated with dimethyl sulfoxide (DMSO) (200 mL for each well) and the optical density (OD) was recorded at 570 nm. All drug doses were parallel tested in triplicate, and the cytotoxicity was evaluated based on the percentage of cell survival in a dose-dependent manner regard to the negative control. The final IC_{50} values were calculated by using the Graph Pad Prism 5.

5.8. Uptake Assay

Hela cervical cancer cells were plated in 6-well plate (1×10^5 cells/well) and 10 μM of different compounds were added. After 48 h of treatment at 37 °C in a humidified atmosphere of 5% CO_2, cells were collected and counted for every well and then detected with ultraviolet absorption at 340 nm. The uptake results were calculated by using the Graph Pad Prism 5.

5.9. RNA extraction and RT-PCR

Hela cervical cancer cells were plated in 6-well plate (2×10^5 cells/well) and **7a** was added at final concentration of 10, 7.5, 5, 2.5, 1.25 μM or with DMSO as control. After incubation for 3 h, cells were harvested, and the RNA was extracted according to the manufacturer's instructions. Total RNA was used as a template for reverse transcription using the following protocol: each 25 μL reaction contained 5 μL 5 × M-MLV buffer, 1.25 μL 2.5mM dNTP, 1 μL 100 pM oligo dT18 primer, 1 μL M-MLV reverse transcriptase, 0.625 μL 40U/μL RNase inhibitor, DEPC treated water (DEPC H_2O), and 2 μg of total RNA. Briefly, RNA and oligo dT18 primer were incubated at 70 °C for 10 min, and then immediately placed on ice. Next, the

other components were added, and incubated at 42 °C for 1 h, and then at 70 °C for 15 min. Finally, the reacted solution was stored at -20 °C. Both *c-myc* and *β-actin* were amplified by using a real-time PCR apparatus (Roche Light Cycler 2), and the PCR products were analyzed with electrophoresis on 1.5% agarose gel at 120 V for 40 min.

5.10. Western blot

Hela cells harvested from each well of the culture plates were lysed in 100 μL of extraction buffer. The suspension was centrifuged at 12,000 rpm at 4 °C for 10 min, and then quantitated by using BCA. Total protein lysates (20 μg) were electrophoresed on 12% SDS−PAGE and transferred to a nitrocellulose membrane at 100 V for 1.5 h. The membrane was blocked for 1 h with 5% solution Bovine Serum Albumin in TBS, 0.05% Tween at room temperature. Western blotting was performed using anti-*c-myc*, anti-*GAPDH* primary antibody and horseradish-peroxidase-conjugated anti-mouse or anti-rabbit secondary antibody. The protein bands were visualized using chemiluminescence substrate.

5.11. RTCA

Hela cervical cancer cells and primary cultured mouse mesangial cells were seeded on E-Plate 16 PET culturing about 20 h before compound treatment. Cells were treated with various concentrations of **7a** or DMSO control. The cells were sampled every minute for 15 min. The data were obtained by using the Graph Pad Prism 5.

5.12. Colony formation assay

Hela cervical cancer cells and primary cultured mouse mesangial cells were seeded on 6-well plates

(500/well) and exposed to **7a** at final concentrations of 2, 1, 0.5, 0.25 μM and DMSO control treatment at 37 °C in a humidified atmosphere of 5% CO_2. DMEM was replaced and different concentrations of **7a** were added every 4 days. Cells were fixed with methyl alcohol and dyed with crystal violet after cultured 8 days. The pictures were taken by using cell imager.

5.13. Cell cycle arrest assay

The HeLa cells were incubated in 10% FBS-supplemented culture medium with compound **7a** at 1.25, 2.5, 5 μM for 48 h at 37 °C and 5% CO_2. After treatment, cells were collected and fixed with ice-cold 70% ethanol at -20 °C overnight. Fixed cells were re-suspended in 0.5 mL of PBS containing 50 μg/mL propidium iodide and 100 μg/mL RNase A. The cell cycle distribution was analyzed by using EPICS XL flow cytometer and calculated by using EXPO 32 software.

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Figure and Scheme Captions

Figure 1. Structures of (A) G-quartet, (B) 3,6-disubstituted acridine and **BRACO-19**, (C) 2,4-disubstituted quinazoline derivatives **LZ-11c** and **QPB-15e**, and (D) new 4-anilinoquinazoline derivatives (series I and II).

Figure 2. Competitive FRET results for 4-anilinoquinazoline derivatives without and with 10-fold (4 μM), 50-fold (20 μM) or 100-fold (40 μM) excess of duplex DNA competitor (ds26). The concentration of FPu22T was 0.4 μM.

Figure 3. CD spectra of Pu22 (3 μM) in 10 mM Tris-HCl buffer, pH 7.4. CD spectra of Pu22 (black line), and Pu22 in the presence of 2 equiv. of **6b**, **7a**, **7b**, **7c**, **7e**, **7f**, and **7h**, respectively. (A) with 100 mM KCl; (B) without KCl.

Figure 4. (A) RT-PCR was used to determine the transcription of *c-myc* in Hela cells treated with various concentrations of **7a** (0, 1.25, 2.5, 5, 7.5 and 10 μM) for 3 h. *β-actin* gene was used as internal control. (B) Western blot was used to determine the expression level of *c-myc* in Hela cells treated with various concentrations of **7a** (0, 1.25, 2.5, 5, 7.5, and 10 μM) for 6 h. *GAPDH* protein was used as intracellular house-keeping protein.

Figure 5. The effect of compound **7a** on cell proliferation. (A) Hela cells and (B) primary cultured mouse mesangial cells were measured by using RTCA assay.

Figure 6. The difference inhibition effect of cell proliferation between (A) Hela cells and (B) primary cultured mouse mesangial cells after treatment with compound **7a** by using Colony Formation assays.

Figure 7. The induction of G0/G1 phase arrest in Hela cells by compound **7a** was measured by using cell cycle analysis in time-dependent (A) and dose-dependent (B) mode.

Scheme 1. Synthetic routes of Series I (**1a**, **1b**, **2a**, **2b**, **4a**, and **4b**) and II (**3a**~**3c**) compounds. Reagents and conditions: (i) $POCl_3$, *N*,*N*-dimethylaniline, 110 °C, 24 h, 56%; (ii) *p*-phenylenediamine, AcONa, THF/H_2O, rt, 70 min, 80%; (iii) $Cl(CH_2)_nCOCl$, K_2CO_3, CH_2Cl_2, rt, 12 h; (iv) diethylamine, K_2CO_3, KI, DMF, 130 °C, 12 h, 62-71%; (v) diethylamine, K_2CO_3, DMF, 80 °C, 12 h, 40-58%; (vi) phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DMF/H_2O, 100 °C, 16 h, 40-50%; (vii) 4-methoxyaniline, NaOAc, THF/H_2O, 65 °C, 1 h, 95%; (viii) BBr_3, CH_2Cl_2, -40 °C, 30min, rt, 2.5h, 99%; (ix) $Br(CH_2)_nBr$, $CsCO_3$, CH_3CN, reflux, 12 h, 55-60%; (x) diethylamine, K_2CO_3, DMF, 110 °C, 12 h, 70-80%.

Scheme 2. Synthetic routes of Series II (**5a**~**5f**, **6a**~**6d**, **7a**~**7i**, **8a**, **8b**, **9a**) compounds. Reagents and conditions: (i) Et_3N, CH_2Cl_2, room temperature, 5-12 h, 85%; (ii) 10% aqueous KOH, EtOH, reflux, 2 h, 90-95%; (iii) $SOCl_2$, DMF, reflux, 4 h, 78%; (iv) 4-methoxyaniline, K_2CO_3, DMF, 90 °C, 5 h, 60%; (v) 33% HBr/AcOH, reflux, 10 h, 80%; (vi) $Br(CH_2)_2Br$, tetrabutylammonium bromide, NaOH, 2-butanone/H_2O, reflux, 10 h, 60%; (vii) alkylamines, K_2CO_3, DMF, 100 °C, 12 h, 40-70%; (viii) $POCl_3$, PCl_5, reflux, 24 h, 80%; (ix) 4-nitroaniline, K_2CO_3, DMF, 130°C, 12 h, 85%; (x) Raney-Ni, N_2H_4-H_2O, THF, room temperature, 20 h, 90%; (xi) $Cl(CH_2)_nCOCl$, K_2CO_3, CH_2Cl_2, room temperature, 12 h, 85%.

Table 1. Stabilization temperatures (ΔT_m) obtained from FRET-melting assay[a]

Compd.	ΔT_m (°C)		Compd.	ΔT_m (°C)	
	FPu22T	F10T		FPu22T	F10T
1a	10.0 ± 0.4	0.4 ± 0.1	**6c**	12.5 ± 0.9	0.1 ± 0.0
1b	12.2 ± 1.0	0.2 ± 0.2	**6d**	0.2 ± 0.2	0.2 ± 0.1
2a	0.1 ± 0.1	0.1 ± 0.0	**7a**	19.6 ± 0.6	0.8 ± 0.2
2b	1.0 ± 0.3	0.1 ± 0.1	**7b**	14.2 ± 0.2	0.9 ± 0.1
3a	7.2 ± 0.5	0.7 ± 0.2	**7c**	13.2 ± 0.2	0.3 ± 0.2
3b	5.3 ± 0.2	0.1 ± 0.1	**7d**	4.8 ± 0.6	0.3 ± 0.2
3c	4.6 ± 0.7	0.2 ± 0.1	**7e**	17.9 ± 0.7	1.4 ± 0.2
4a	7.1 ± 0.6	0.4 ± 0.1	**7f**	18.0 ± 1.1	0.6 ± 0.2
4b	8.2 ± 0.4	0.2 ± 0.1	**7g**	26.6 ± 0.2	3.6 ± 0.4
5a	0.4 ± 0.1	0.1 ± 0.0	**7h**	19.4 ± 0.2	0.8 ± 0.2
5b	1.6 ± 0.4	0.2 ± 0.1	**7i**	21.8 ± 0.9	3.7 ± 0.3
5c	0.4 ± 0.3	0.1 ± 0.1	**8a**	13.6 ± 1.0	0.9 ± 0.1
5d	0.7 ± 0.5	0.1 ± 0.0	**8b**	12.6 ± 1.0	0.8 ± 0.2
5e	1.4 ± 0.6	0.2 ± 0.1	**9a**	13.1 ± 0.1	0.4 ± 0.1
5f	0.4 ± 0.1	0.2 ± 0.0	**LZ-11c**	11.4 ± 0.7	3.7 ± 0.3
6a	8.8 ± 1.0	0.3 ± 0.1	**QPB-15e**	16.6 ± 1.0	0.6 ± 0.3
6b	13.3 ± 0.8	0.6 ± 0.2			

[a] $\Delta T_m = T_m$ (DNA + ligand) – T_m (DNA). The concentrations of FPu22T and F10T were 0.4 μM, and the concentrations of compounds were 2.0 μM.

Table 2. Equilibrium binding constants (K_D) determined with SPR assay

Compd.	K_D (μM)			Compd.	K_D (μM)		
	Pu22	Duplex	K_D^{Duplex}/K_D^{Pu22}		Pu22	Duplex	K_D^{Duplex}/K_D^{Pu22}
1a	3.34	> 10 [b]	> 3.0	**6b**	0.16	> 10 [b]	> 62.5
1b	1.64	> 10 [b]	> 6.1	**6c**	0.61	– [a]	NA
2a	– [a]	– [a]	NA	**6d**	8.08	– [a]	NA
2b	5.52	– [a]	NA	**7a**	0.21	> 10 [b]	> 47.6
3a	6.30	– [a]	NA	**7b**	0.20	> 10 [b]	> 50
3b	4.68	– [a]	NA	**7c**	0.40	– [a]	NA
3c	> 10 [b]	– [a]	NA	**7d**	1.33	– [a]	NA
4a	2.89	– [a]	NA	**7e**	0.22	> 10 [b]	> 45.4
4b	2.46	– [a]	NA	**7f**	0.21	– [a]	NA
5a	> 10 [b]	– [a]	NA	**7g**	1.60	6.21	3.9
5b	– [a]	– [a]	NA	**7h**	0.14	– [a]	NA
5c	> 10 [b]	– [a]	NA	**7i**	0.57	5.89	10.3
5d	– [a]	– [a]	NA	**8a**	0.54	> 10 [b]	> 18.5
5e	> 10 [b]	– [a]	NA	**8b**	0.55	> 10 [b]	> 18.2
5f	> 10 [b]	– [a]	NA	**9a**	0.52	> 10 [b]	> 19.2
6a	3.94	– [a]	NA	**LZ-11c**	0.49	9.18	18.7

[a] No significant binding was found for addition of up to 10 μM ligand.

[b] The compounds showed weak binding ability to the DNA.

NA, Not available.

Table 3. IC_{50} value[a] (μM) of compounds against tumor cells and the primary cultured mouse mesangial cell

compouds	Hela (μM)	A549 (μM)	Raji (μM)	CA46 (μM)	mesangial cells (μM)
1a	21.00	20.81	13.54	4.22	10.22
1b	6.33	6.08	5.06	10.31	6.41
2a	＞50	＞50	＞50	33.23	＞50
2b	＞50	45.25	＞50	31.34	＞50
3b	21.03	27	12.94	13.28	12.88
3c	7.17	9.07	14.48	3.29	5.71
3a	8.25	17.56	8.45	3.62	6.2
4a	17.36	5	20.32	20.45	9.83
4b	2.3	4.84	5.74	12.2	8.08
6a	29.78	＞50	6.49	3.14	2.72
6b	2.87	18.73	1.86	1.76	16.52
6c	28.67	21.64	17.92	12.31	38.218
6d	＞50	＞50	12.01	2.14	＞50
7a	4.44	5.88	3.1	6.48	13.11
7b	6.97	26.63	4	7.24	23.33
7c	20.09	＞50	27.84	19.52	＞50
7d	＞50	＞50	＞50	＞50	＞50
7e	7.37	7.54	1.26	4.54	6.25
7f	＞50	＞50	＞50	＞50	＞50
7g	＞50	＞50	＞50	＞50	＞50
7h	＞50	＞50	＞50	28.2	＞50
7i	＞50	＞50	＞50	＞50	＞50
8a	32.48	24.51	27.3	20.95	＞50
8b	22.44	27.24	31.6	13.69	＞50
9a	12.71	＞50	12.61	5.69	＞50

[a] MTT assay was carried out (see Experimental Section**).** IC_{50}, the tested compounds' concentration resulting in 50% mortality of cells.

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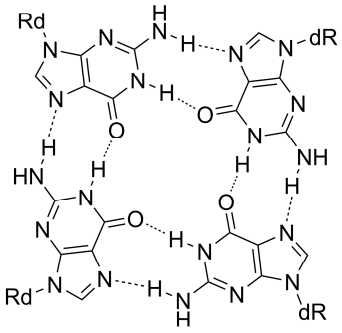

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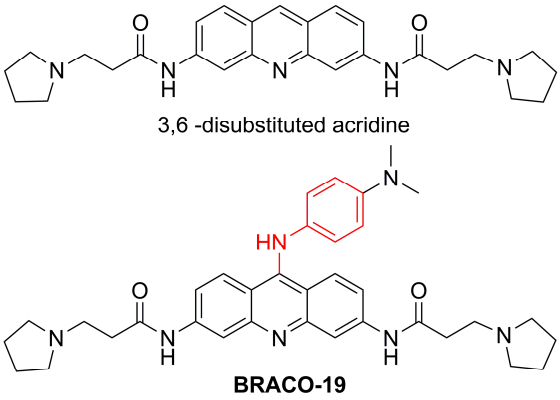

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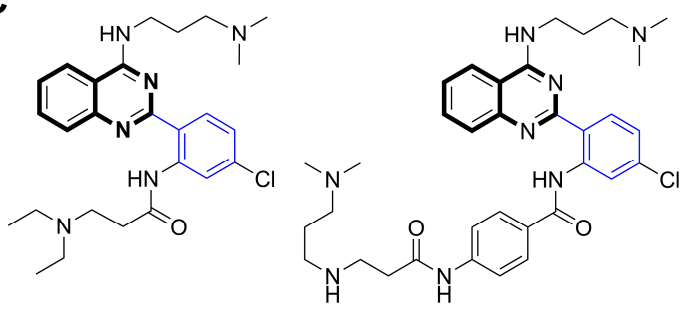

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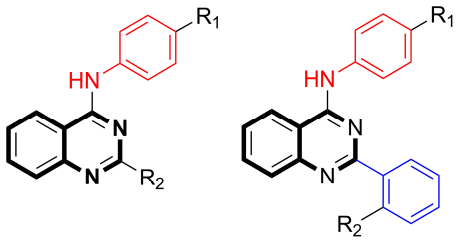

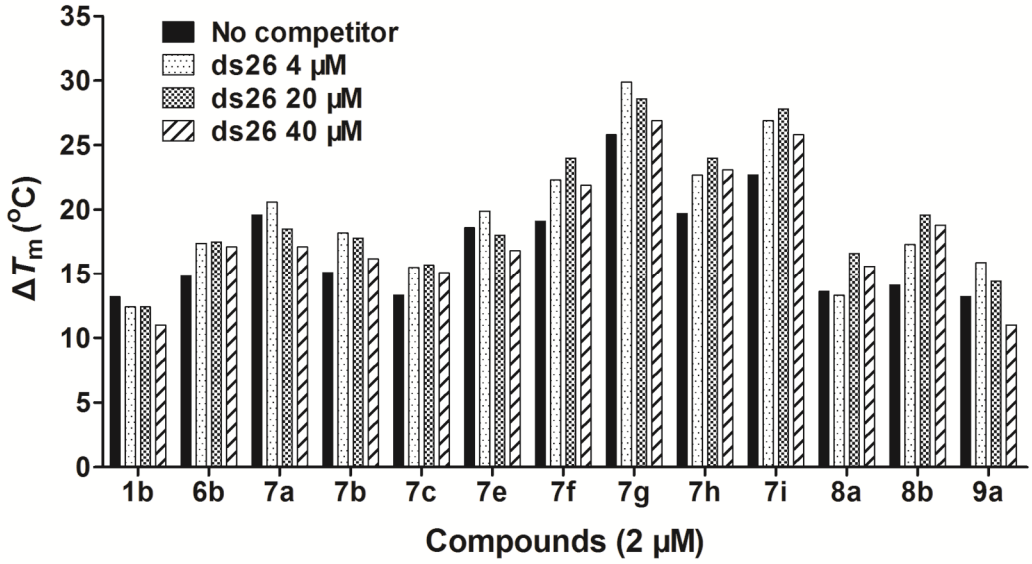

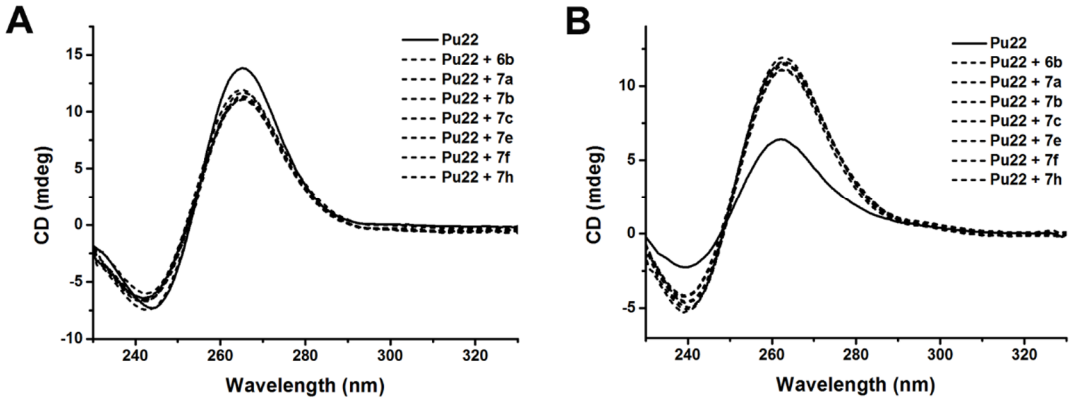

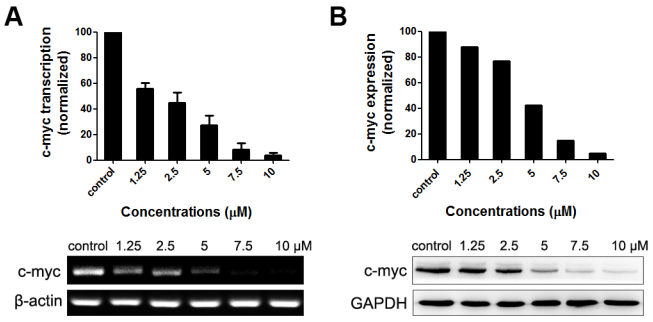

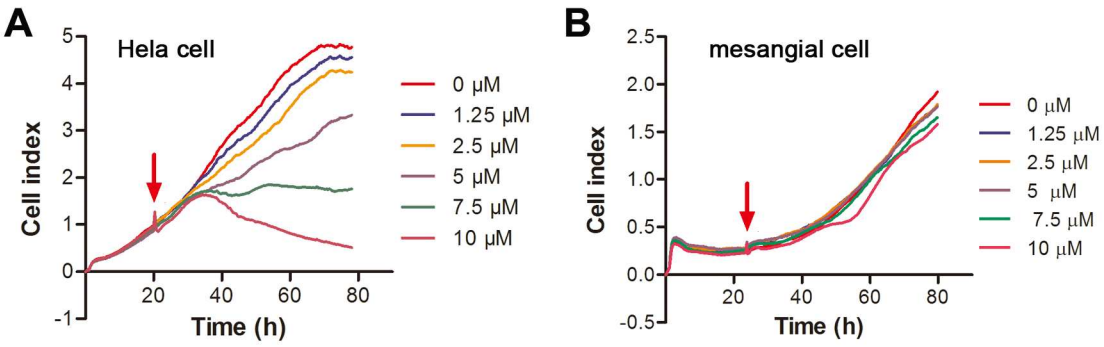

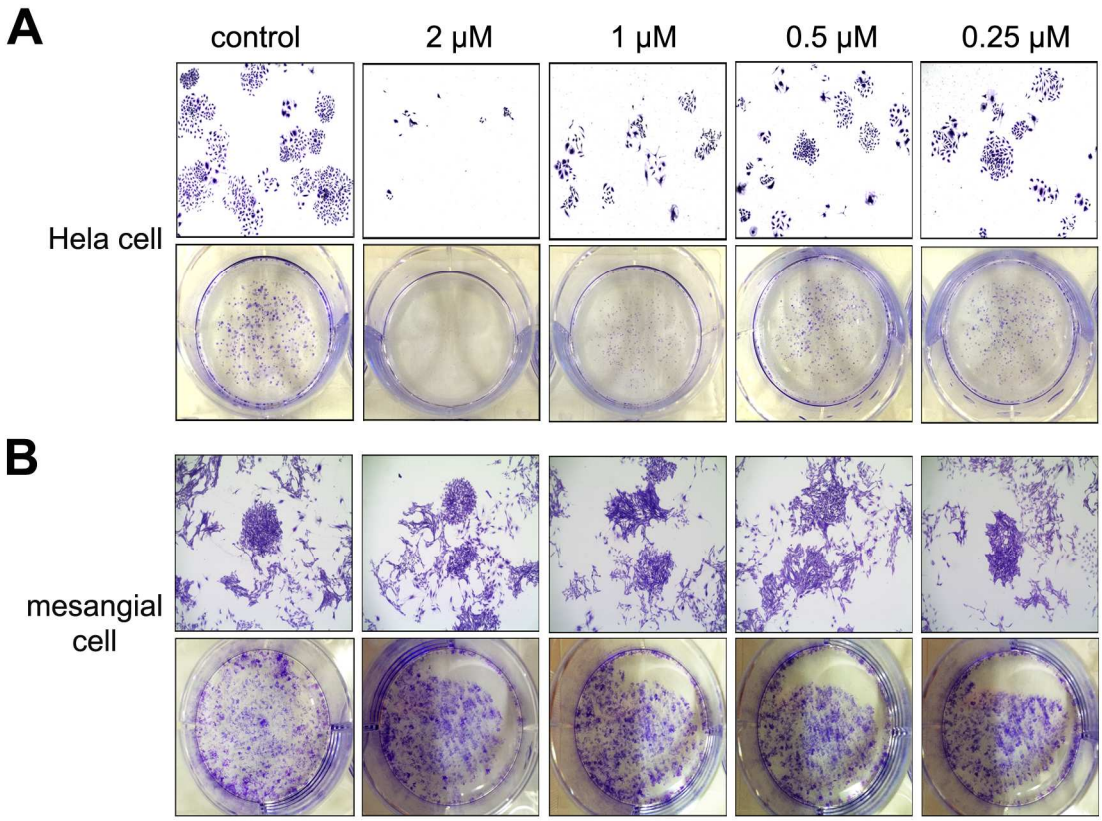

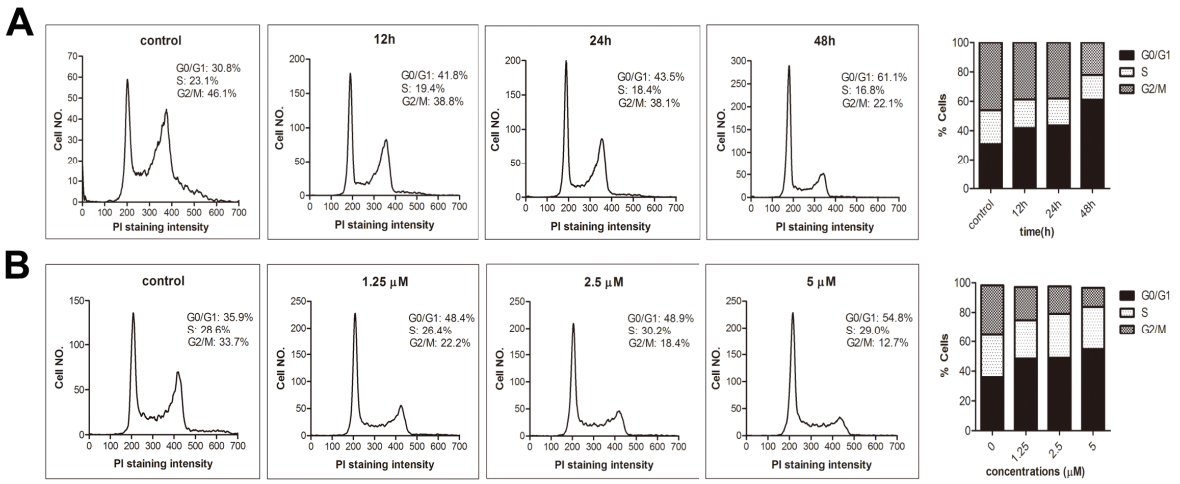

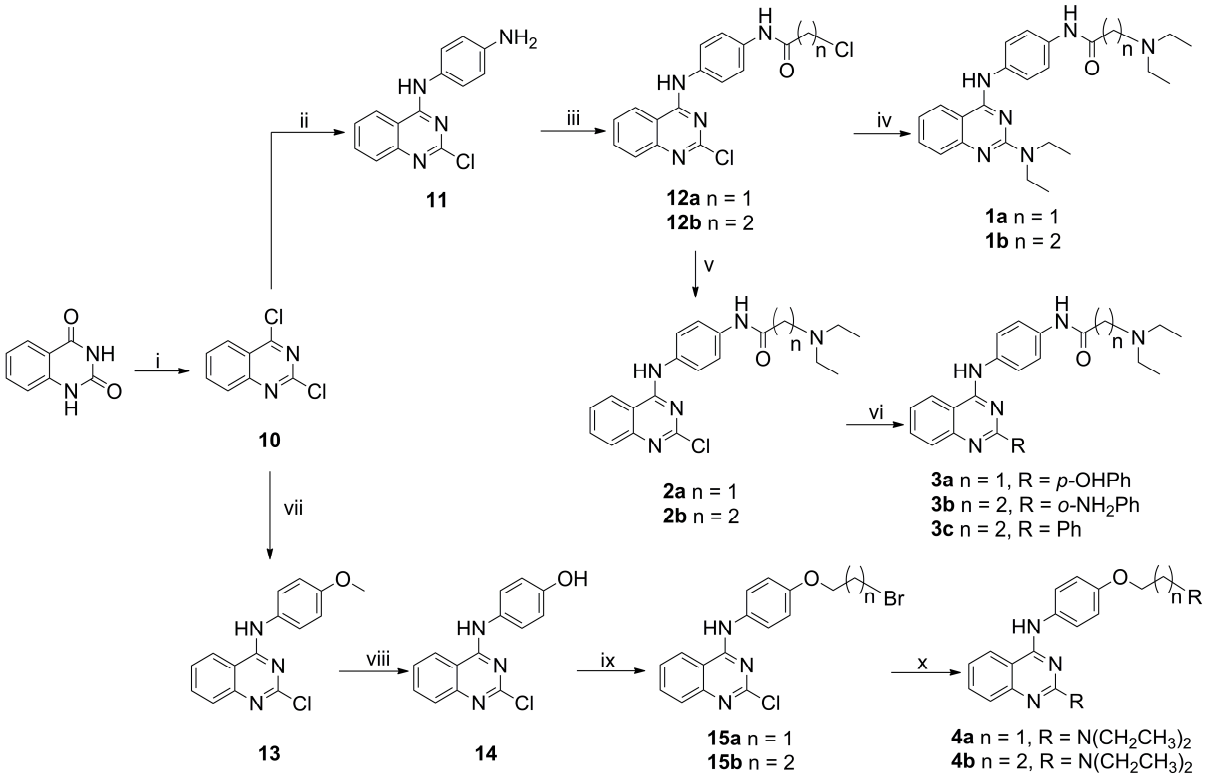

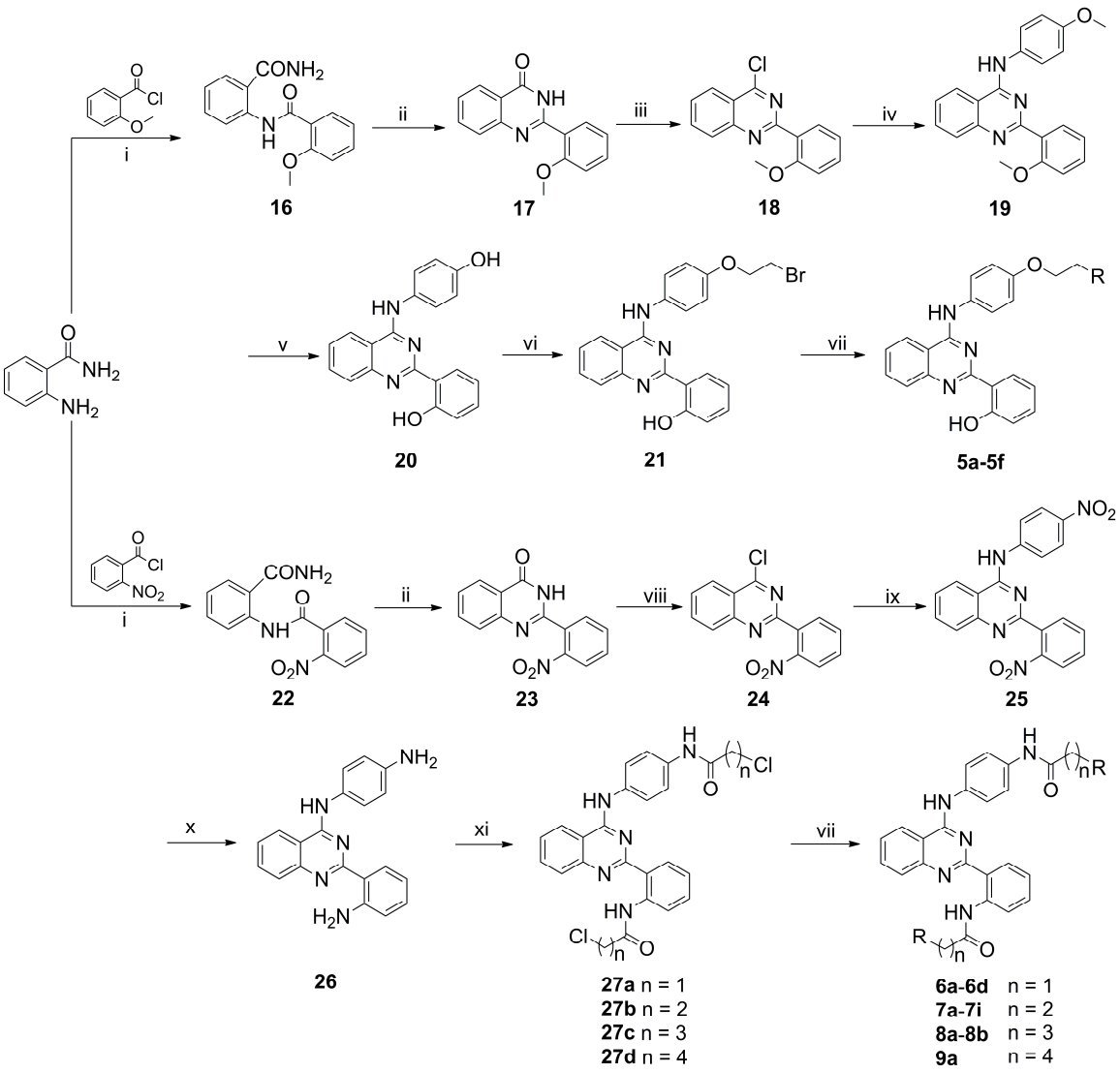

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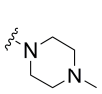
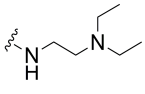
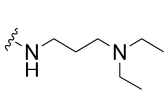
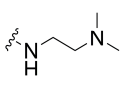
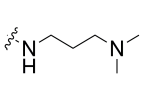
R									

Research highlights

► 31 new 4-anilinoquinazoline derivatives were synthesized as G-quadruplex binding ligands.

► Some of compounds showed a improved interacting ability to *c-myc* G-quadruplex DNA.

► **7a** was the best compound as out of the synthesized compounds.

► **7a** significantly down-regulated *c-myc* gene transcription and expression in Hela cells.