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Original article

New quinazoline derivatives for telomeric G-quadruplex DNA: Effects of an added phenyl group on quadruplex binding ability

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ABSTRACT

To improve the selectivity of indoloquinoline or benzofuroquinoline derivatives, we previously reported several quinazoline derivatives [17]. These compounds could mimic a tetracyclic aromatic system through intramolecular hydrogen bond. Studies showed that these quinazoline derivatives were effective and selective telomeric G-quadruplex ligands. With this encouragement, here we synthesized a series of *N*-(2-(quinazolin-2-yl)phenyl)benzamide (**QPB**) compounds as modified quinazoline derivatives. In this modification, a phenyl group was introduced to the aromatic core. The evaluation results showed that part of **QPB** derivatives had stronger binding ability and better selectivity for telomeric G-quadruplex DNA than **LZ-11**, the most potential compound of reported quinazoline derivatives. Furthermore, telomerase inhibition of **QPB** derivatives and their cellular effects were studied.

1. Introduction

Telomeres are essential chromosomal components protecting the end of eukaryotic chromosomes and maintaining proper replication [1]. These special elements consist of approximately 2–20 kb of TTAGGG duplex repeats and 50–500 bases of single-stranded DNA overhang running 5′–3′ toward the end of chromosome. During the replication in normal cell proliferation, telomeres erode by about 100 bp with each cell division which finally triggering cellular senescence [2,3]. However, in most cancer cells, telomere is protected from shortening by telomerase enzyme [4]. Studies showed that the extreme 3′- ends of telomeres could adopt G-quadruplex structures from stacked tetrads of hydrogen-bonded guanine bases [5–7]. The formation of stable G-quadruplex structures through the interaction with various ligands alters the overhang structure, inhibits catalytic functions of the telomerase enzyme [8–10] and induces the degradation of telomere through a DNA-damage repair pathway and release of one of shelterin proteins from telomeres, e.g. POT1 [11–13]. Such changes lead to a fast induction of tumor cell senescence and apoptosis. Thus, the design of telomeric G-quadruplex binding ligands is a rational and promising strategy for cancer therapy.

Indoloquinoline or benzofuroquinoline derivative **SYUIQ-05** (Fig. 1A) modified from cryptolepine was an effective quadruplex binding ligand developed by our group [14,15]. This polycyclic aromatic compound interacted with telomeric G-quadruplex DNA and showed inhibition of telomerase. However, it also intercalated into duplex DNA to a certain extent [16]. In our recent study, a new G-quadruplex ligand **LZ-11** (Fig. 1B) which shares the quinazoline scaffold was synthesized and its binding properties towards telomeric G-quadruplex DNA were reported [17]. This molecule has an "imitative" tetracyclic aromatic system (like indoloquinoline) formed through intramolecular hydrogen bond, which enables adoption of moderate twisted and co-planar conformations of the aryl groups, and allows the ligand to well stack on the G-quartet. Our experimental results showed that **LZ-11** was a promising selective telomeric G-quadruplex binding ligand with strong discrimination against the duplex DNA. Aiming to develop this kind of G-quadruplex ligands, we tried to modify the main scaffold of the molecule. Recent research suggested that the decreased binding ability of flexible ligands might result from the lessened aromaticity or planarity [18]. Therefore, increasing aromaticity or expanding planarity may be one of feasible modification strategies. Bis-substituted

Abbreviations: CD, circular dichroism; SPR, surface plasmon resonance; TRAP, telomere repeat amplification protocol; MTT, methyl thiazolyl tetrazolium; SA-β-Gal, senescence-associated-galactosidase; TRF, telomeric restriction fragment.

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anthraquinone (AQ) derivatives were proved to be effective G-quadruplex stabilizers and powerful telomerase inhibitors [19–22]. Studies showed that the amide bond direction on side chains could modulate their G-quadruplex recognition and telomerase inhibition [23]. The AQ–NH–CO– arrangement performed invariantly better activity than the AQ–CO–NH– arrangement. Theoretical calculations suggested that the former amide arrangement was co-planar with the aromatic system. These results indicated that amide bond not only served as a space linker, but also expanded the aromatic plane.

On this basis, we modified the original quinazoline derivative by linking a new substituted benzene ring to the 2′ position of phenyl group of original core through amide bond (Fig. 1C). The partial double-bond feature of the amide bond might place the newly added benzene on the same quinazoline plane, which was expected to facilitate the π-π interaction. Meanwhile, the substituted amino side chain at C-10 position was reserved and a second side chain was allocated to *para*-position of the new benzene ring. Therefore, we obtained a series of *N*-(2-(quinazolin-2-yl)phenyl)benzamide (**QPB**) derivatives (Fig. 1C). The interactions of synthesized compounds with telomeric G-quadruplex DNA were examined using circular dichroism spectroscopy (CD) and surface plasmon resonance (SPR). Further studies on their inhibitory effects on telomerase activity were performed using TRAP-LIG, and their cellular effects were evaluated through the measurements of cell senescence and telomere shortening.

2. Chemistry

The synthetic pathway for new quinazoline derivatives is shown in Scheme 1. Compounds **11a**–**11d** and **12a**–**12e** were prepared mainly according to the previously reported procedure [17]. And we used stannous chloride dihydrate as reductant [24,25] instead of hydrazine hydrate to obtain **6a** and **6b**. Compounds **8a** and **8b** were prepared by mixing **6a** and **6b** with 4-nitrobenzoyl chloride in dichloromethane under ice bath condition. The resulting solution was stirred over night at ambient temperature. A second reduction reaction was then performed using stannous chloride dihydrate to give compounds **9a** and **9b**. Reaction of **9a** and **9b** with acylchloride in dichloromethane gave compounds **10a**, **10b** and **10c**. Finally, compounds **13a**–**13d**, **14a**–**14e**, and **15a**–**15e** were obtained through reaction of the compounds **10a**, **10b**, and **10c** with different primary and secondary amines under reflux condition, respectively.

In agreement with our previous report [17], the ^{1}H NMR chemical shifts of acylamide protons, which locate between two phenyl groups, are in the range of δ 13.11–14.62 ppm in $CDCl_3$ or DMSO-d_6. These data indicated the formation of a relatively strong intramolecular H-bonding [26–28]. In order to determine the configuration of the acylamide between the two phenyl groups, we performed 2D NOESY (Fig. S1) using an important intermediate **8a**. Results indicated that the two phenyl groups were in anti position of double bond.

3. Results and discussion

3.1. G-quadruplex–ligand interaction studies with circular dichroism (CD)

To investigate the interaction between the synthesized compounds and telomeric G-quadruplex DNA, CD experiments were carried out. CD spectroscopy is a conventional method for determining the conformation of G-quadruplex structures and the effect of ligand binding on quadruplex structure [29]. Here, we used quindoline derivative **SYUIQ-05** and quinazoline derivative **LZ-11** as reference compounds [15,17]. As shown in Fig. 2, in the presence of 150 mM K^+, the CD spectrum of HTG21 in the absence of any ligand showed a major positive band at 290 nm, a shoulder at around 270 nm, a small positive band at 250 nm, and a minor negative band near 234 nm. This indicated the formation of a mixture of antiparallel and parallel G-quadruplexes, possibly including hybrid-types as well. Upon the addition of different compounds to the above solution, the CD spectra changed differently. Compounds without the second phenyl group (**11a**, **12a** and **LZ-11**) had little conformational change ability to HTG21, while **QPB** derivatives changed the spectra significantly. The positive band at 290 nm dropped, while a new major positive band showed up at 265 nm, together with a major negative band at around 245 nm. The changes revealed that a conformational conversion from antiparallel types to parallel ones was induced in the presence of **QPB** derivatives. The ability to induce conformational conversion varied among different compounds (Table 1). The *N*-methyl piperazino analogues (**13d**, **14d** and **15d**) and 3-dimethylaminopropylamino analogues (**14e** and **15e**) had the most powerful conformational conversion ability. The results above demonstrated that **QPB** derivatives had better inducing ability than the original quinazoline compounds.

We next performed CD melting assay to evaluate the stabilization to G-quadruplex by all synthesized compounds. CD melting assays is a useful method to measure the melting temperature of G-quadruplex [30,31]. In this experiment, CD spectra are recorded every 3 °C when heating. Then CD profiles at a specific wavelength are used for obtaining melting curves. Here, we used CD profiles at 265 nm and 290 nm to calculate the ΔT_m values. A comparison of the CD melting results indicated that the introduction of phenyl group had a significant effect on the stabilizing ability of these compounds. With an addition of a phenyl group on the main structure, compounds **13a**–**13d**, **14a**–**14e** and **15a**–**15e** had a

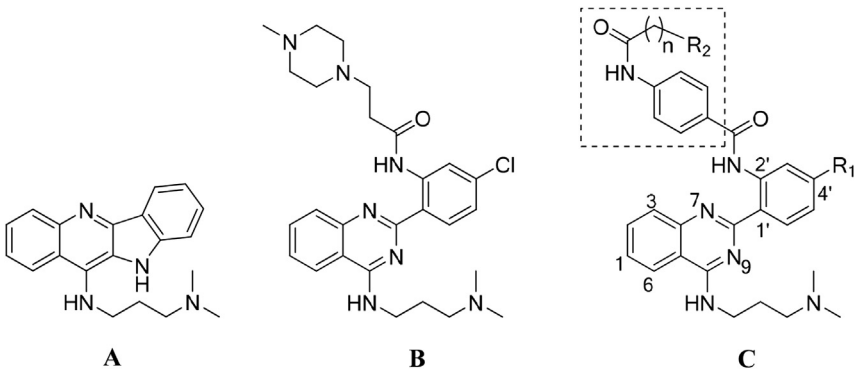
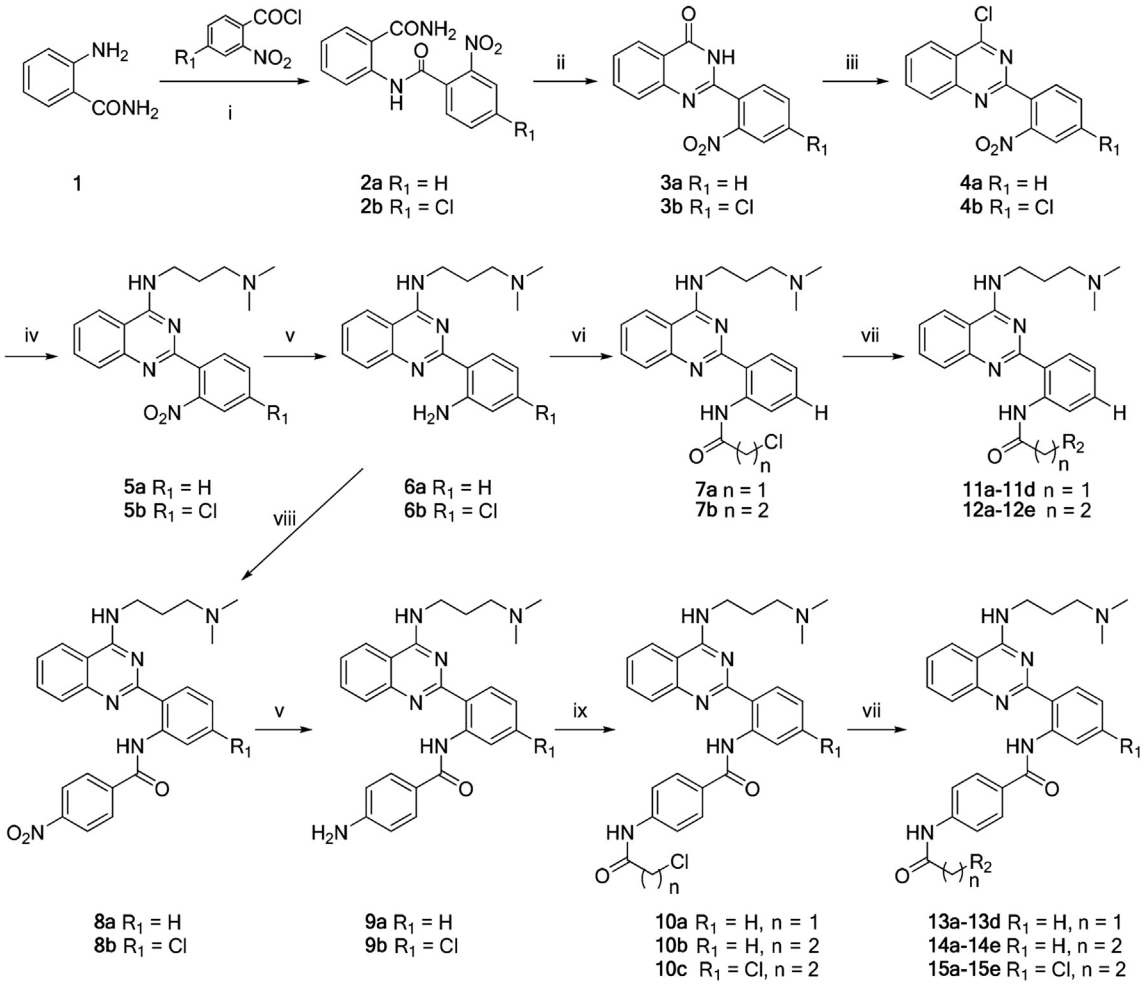
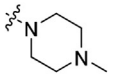
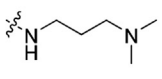


Fig. 1. Structures of (A) **SYUIQ-05**, (B) **LZ-11** and (C) **QPB** derivatives.



Compd.	a	b	c	d	e
R_1					

Scheme 1. Synthesis of **QPB** derivatives. Reagent: (i) 2 equiv of TEA, $CHCl_3$, rt, 5 h; (ii) 10% aqueous KOH, EtOH, reflux, 2 h; (iii) *N*,*N*-diethylaniline, $POCl_3$, toluene, reflux, 6 h; (iv) 3-(dimethylamino)-1-propylamine, THF, reflux, 5 h; (v) $SnCl_2{\cdot}2H_2O$, HCl, EtOH, reflux, 1 h; (vi) $Cl(CH_2)_nCOCl$, CH_2Cl_2, rt, 24 h; (vii) 4-nitrobenzoyl chloride, CH_2Cl_2, rt, 24 h; (viii) amine, methanol, reflux, 6 h.

noticeable enhancement of G-quadruplex stabilizing ability. Most **QPB** derivatives showed strong stabilizing ability for telomeric G-quadruplex (ΔT_m values above 20 °C at 265 nm and 8 °C at 290 nm). In some **QPB** compound cases, such as **14e** and **15e**, the melting temperatures were above 94 °C at 265 nm. However, the original quinazoline derivatives **11a**–**11d**, **12a**–**12e** and ***LZ*-11** showed weak effect on G-quadruplex stability with ΔT_m values lower than 15 °C at 265 nm and 7 °C at 290 nm. The lack of additional aromatic ring significantly decreases the stabilizing ability of quinazoline derivatives for G-quadruplex DNA. On the other hand, the increase of T_m values of **QPB** compounds at 290 nm (ΔT_m less than 15 °C) was not as much as those at 265 nm (ΔT_m up to 28 °C). These results indicated that **QPB** derivatives might mainly strengthen the stability of parallel G-quadruplex in buffer solution.

Among the compounds owning the same core structures, the less basic morpholino analogues (**11c**, **12c**, **13c**, **14c** and **15c**) had the weakest effect on the stability of telomeric G-quadruplex. Meanwhile, compounds with a more basic amino terminal (*N*-methylpiperazino analogues, **11d**, **12d**, **13d**, **14d** and **15d**) or a longer amino terminal (3-dimethylaminopropylamino analogues, **12e**, **14e** and **15e**) showed more activity for G-quadruplex DNA. These results demonstrated the importance of length and basicity of amide side chain for strong G-quadruplex interactions. Besides, it should be noted that the chlorine substituent had a strong influence on the stabilizing ability of quinazoline derivatives. Compounds with a chlorine substituent (***LZ*-11** and **15a**–**15e**) showed enhanced activity for G-quadruplex over their counterparts (**12d** and **14a**–**14e**). This might be the result of changes of electric density or interactions of chlorine atom with G-quadruplex backbone. Moreover, a correlation between inducing ability and stabilizing ability of the compounds was observed. Compounds with the most inducing ability (**13d**, **14d**, **14e**, **15d** and**15e**) showed the best

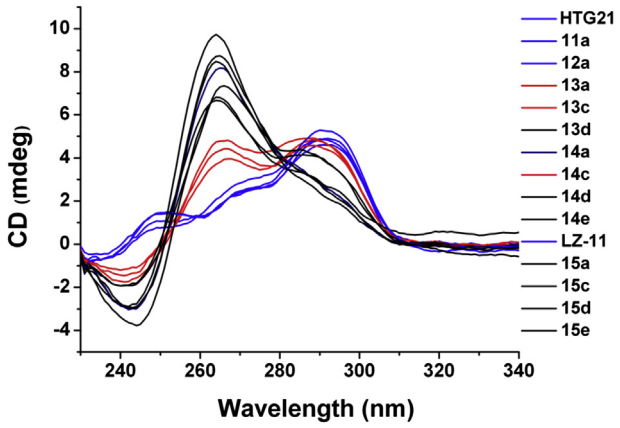


Fig. 2. CD spectra of HTG21 in 10 mM Tris–HCl buffer, pH 7.4, 150 mM KCl. CD spectra of HTG21 (black line), and HTG21 in the presence of 5 equiv of **11a**, **12a**, **13a**, **13c**, **13d**, **14a**, **14c**, **14d**, **14e**, **LZ-11**, **15a**, **15c**, **15d** and **15e**, respectively.

stabilizing ability for G-quadruplex. Together, CD experiment results suggested that **QPB** derivatives had an improved inducing and stabilizing ability for telomeric G-quadruplex DNA.

3.2. Telomeric G-quadruplex binding affinity and selectivity studies with surface plasmon resonance (SPR)

To investigate the binding affinity and selectivity of the synthesized compounds for telomeric G-quadruplex, we performed SPR with biotinylated telomeric G-quadruplex DNA HTG21 and biotinylated duplex DNA attached to a streptavidin-coated sensor chip [32,33]. By simultaneously injecting a range of concentrations of ligands to the immobilized telomeric G-quadruplex DNA, the duplex DNA and the blank reference, SPR sensorgrams were recorded, which were used to building binding plots for calculating equilibrium constants later.

As shown in Table 2, the K_D values of **QPB** compounds binding to telomeric G-quadruplex showed strong binding affinity (K_D from 0.227 to 8.34 μM). Especially for compounds **15a**, **15b**, **15d** and **15e**, the K_D values for G-quadruplex were lower than 1 μM, while no obvious binding was observed for these compounds to duplex DNA even at a at a concentration of 20 μM. Quinazoline compounds **11d** and **12d** had little binding affinity to both telomeric G-quadruplex DNA and duplex DNA, while **12e** bound to both telomeric G-quadruplex and duplex DNA with similar affinity. The data demonstrated that the introduction of a new phenyl group onto the scaffold of quinazoline derivatives gave an improvement of both binding ability and selectivity for G-quadruplex. Consistent with CD melting results, compounds **12e** and **14e** were of the best binding affinity and selectivity for G-quadruplex when compared to their counterparts, and **15c** showed the least binding affinity among the chlorine substituted **QPB** derivatives. Both CD and SPR experimental results indicated that the introduction of phenyl group on the quinazoline derivatives had beneficial effects on their binding ability for telomeric G-quadruplex DNA.

Table 2
Kinetic parameters determined with SPR spectroscopy.

Compound	K_D (M)		Compound	K_D (M)	
	HTG21	Duplex		HTG21	Duplex
11d	–[a]	–[a]	**14e**	1.11×10^{-6}	–[a]
12d	–[a]	–[a]	**15a**	3.02×10^{-7}	–[a]
12e	3.36×10^{-6}	7.90×10^{-6}	**15b**	2.27×10^{-7}	–[a]
13a	8.34×10^{-6}	1.19×10^{-5}	**15c**	3.06×10^{-6}	–[a]
13d	2.75×10^{-6}	3.97×10^{-5}	**15d**	2.76×10^{-7}	–[a]
14a	1.61×10^{-6}	2.20×10^{-5}	**15e**	3.79×10^{-7}	–[a]
14d	1.48×10^{-6}	1.32×10^{-5}	**SYUIQ-05**	2.80×10^{-5}	5.02×10^{-6}

[a] No significant binding was found for addition of up to 20 μM ligand.

3.3. Telomerase inhibition

The ability of the synthesized compounds to inhibit human telomerase activity was examined by TRAP-LIG assay [34]. Considering that the compounds might interfere the PCR step through interacting with the extension products, the ligands were removed after incubation with the enzyme and prior to the amplification step [35]. In the experiments, solutions of derivatives were added to the telomerase reaction mixture containing extract from cracked leukemia cell HL60, and the IC_{50} values of the three chosen

Table 3
Telomerase inhibition by compounds **12e**, **14e** and **15e** in cell-free assay.

	12e	**14e**	**15e**
IC_{50} (μM)	>50	30.9	2.3

Table 1
Inducing G-quadruplex conformational change potential and G-quadruplex stabilization ability obtained from CD experiments in KCl buffer (10 mM Tris–HCl, 60 mM KCl, pH 7.4).

Compound	Inducing ability[a]	ΔT_m/°C[b]		Compound	Inducing ability[a]	ΔT_m/°C[b]	
		265 nm	290 nm			265 nm	290 nm
11a	*	2.1	0.7	**14a**	****	19.6	5.4
11b	*	7.3	2.6	**14b**	***	18.7	4.6
11c	–[c]	0.5	0	**14c**	****	15.3	8.2
11d	–[c]	3.1	0.6	**14d**	*****	21.7	8.2
12a	*	8.2	2.5	**14e**	*****	>28	nd[d]
12b	*	8.7	3.6	**15a**	**	21.9	2.8
12c	–[c]	4.3	0.9	**15b**	****	>28	5.7
12d	**	8	2.1	**15c**	***	21.6	4.7
12e	*	12.4	6.2	**15d**	*****	>28	3.4
13a	****	21.6	11.6	**15e**	*****	>28	14.8
13b	****	21.4	11.7	**LZ-11**	**	14.4	4.4
13c	***	11.9	4.7	**SYUIQ-05**	–[c]	11.2	8
13d	*****	21.8	7.4				

[a] Inducing ability depends on the ratio of the signal values from 265 nm to 290 nm on the CD spectrum.
[b] ΔT_m values were determined using the CD profiles at 265 nm and 290 nm.
[c] No observed conformational change.
[d] Not detected.

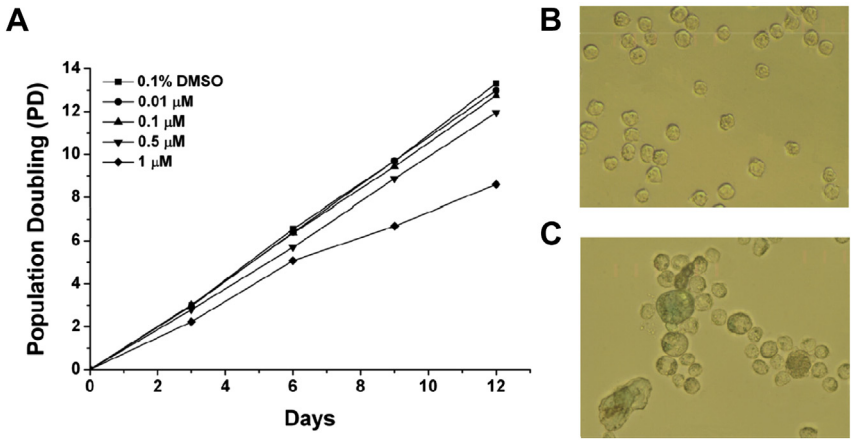


Fig. 3. Senescence induced by compound **15e** on HL60 cells. (A) Long-term incubation of HL60 with compound **15e** at subcytotoxic concentrations. Expression of SA-β-Gal in HL60 cells after treatment with (B) 0.1% DMSO or (C) 1 μM compound **15e** continuously for 12 days.

compounds were obtained as shown in Table 3. Based on CD and SPR results, we chose quinazoline compound **12e** and **QPB** derivatives **14e**, **15e** for telomerase inhibition studies. It was clearly found that the inhibitory effect of chlorine substituted **QPB** ligand **15e** was significantly stronger than none chlorine substituted compound **14e**, while quinazoline derivative **12e** showed little inhibitory ability. Obviously, the telomerase inhibition results had a structure–activity relationship, which was in agreement with CD and SPR results.

3.4. Cell senescence induced by compound *15e*

Based on the study results above, compound **15e** was proved to be one of the most promising ligand as a telomerase inhibitor and telomeric G-quadruplex binding ligand. Thus, the following studies were carried out using this compound. To examine the effect of ligand **15e** on leukemia cell HL60, short-term cell viability was determined by MTT assay first. The results showed that **15e** has a potent inhibitory effect, with an IC_{50} value of 1.7 μM. Thus, subcytotoxic concentrations of **15e** were set at 0.01, 0.1, 0.5 and 1 μM for long-term HL60 cell culture experiments. The inhibitory effect of **15e** to HL60 cells was obviously dose-dependent (Fig. 3A). Treatment of HL60 cells with 1 μM compound **15e** resulted in a significant inhibitory effect. In the presence of even 0.5 μM compound **15e**, a discernible difference was observed between the control and treated cells.

Morphological examination of the cells in long-term studies showed an increased proportion of enlarged and flattened cells with phenotypic characteristics of senescence [36,37]. These flattened cells also stained positively for the senescence-associated β-galactosidase (SA-β-Gal) activity after continuous treatment by compound **15e** (Fig. 3B and C). Results indicated that compound **15e** induced accelerated senescence of HL60 cancer cells.

3.5. Telomere shortening by compound *15e*

It had been reported that treatment of cancer cells with telomeric G-quadruplex ligands could disrupt telomere length maintenance and cause telomeres to erode [38,39]. To investigate whether **15e** could shorten telomeres, the telomere length was evaluated using the telomeric restriction fragment (TRF) length assay. The results showed that the length of telomere shortened about 1.1 kb for HL60 cells after long-term exposure to 1 μM of compound **15e** (Fig. 4). Telomere shortening was also observed after 0.01, 0.1, and 0.5 μM of **15e** treatment. Previous studies indicated that dysfunctional telomere could activate p53 to initiate cellular senescence or apoptosis to suppress tumorigenesis [14]. Therefore, the induction of senescence by compound **15e** might be due to the shortening of telomere length. This result is consistent with the biological activity expected for efficient telomeric G-quadruplex ligand and telomerase inhibitor [40].

4. Conclusions

To enhance the telomeric G-quadruplex binding ability of quinazoline derivatives, we designed and synthesized a series of novel *N*-(2-(quinazolin-2-yl)phenyl)benzamide (**QPB**) derivatives. Based on previous reports of the use of amide bonds on quadruplex ligand design, a new benzene ring was attached to the scaffold of quinazoline derivatives through amide bond. The present studies showed that **QPB** derivatives were more effective then the parental quinazoline compounds. An inducing ability from hybrid type to parallel type G-quadruplex was observed for **QPB** derivatives on CD spectra. And CD melting results indicated that the existence of an additional phenyl group and a chlorine substituent could greatly increase the telomeric G-quadruplex stabilization ability of quinazoline compounds. The same SAR was also found in SPR and TRAP-LIG results. **QPB** derivatives, especially chlorine substituted ones, showed strong binding ability and selectivity for telomeric G-qudruplex DNA. Compound **15e** was found to be a strong telomerase inhibitor. Cellular experiments demonstrated that **15e** could remarkable decrease the population of HL60 cells with a shortening of the telomere length, which might due to its telomerase inhibitory ability. These results proved that the introduction of a phenyl group through amide bond is an effective and efficient approach for obtaining more biologically active telomeric G-quadruplex ligands.

5. Experimental section

5.1. Synthesis and characterization

^1H 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) on Shimadzu LCMS-IT-TOF. Elemental analysis was carried out on an Elementar Vario EL CHNS Elemental Analyzer. Melting points (m.p.) were determined using an SRS-OptiMelt automated

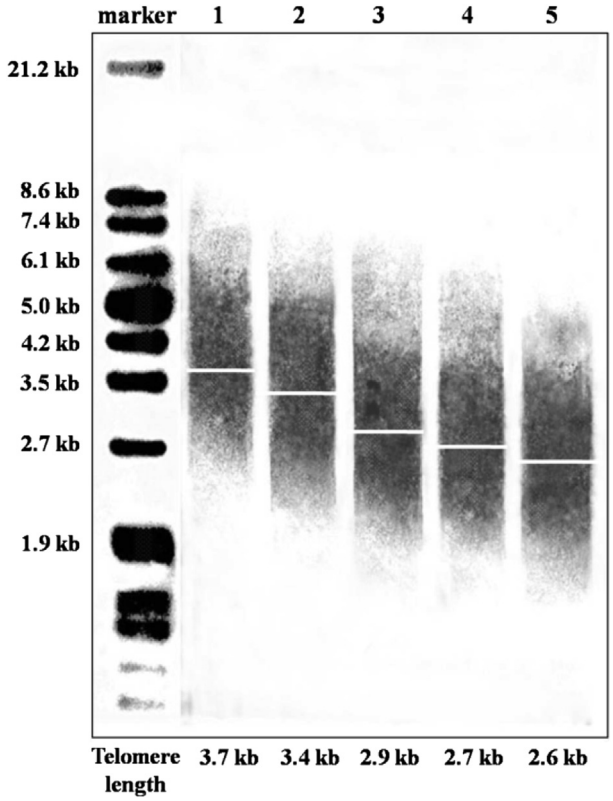


Fig. 4. Effect of quinazoline derivative **15e** on telomere length. TRF analysis of HL60 cells treated with or without compound **15e** for 12 days. Lane 1, 0.1% DMSO; lane 2, 0.01 μM compound **15e**; lane 3, 0.1 μM compound **15e**; lane 4, 0.5 μM compound **15e**; and lane 5, 1 μM compound **15e**.

melting point instrument without correction. Flash column chromatography was performed with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. The purities of synthesized compounds were confirmed to be higher than 95% by analytical HPLC performed with a dual pump Shimadzu LC-20AB system equipped with an Ultimate XB-C18 column and eluted with methanol-water (32 : 68 to 60 : 40) containing 0.1% TFA at a flow rate of 0.6 mL min^{-1}.

*5.1.1. General procedure A: preparation of **2a** and **2b***

The mixture of appropriate nitrobenzoic acid (100 mmol) and thionyl chloride (50 mL) was heated under reflux for 1.5 h at 80 °C. The solution was allowed to cool at room temperature followed with the evaporation of thionyl chloride in vacuo. The resulting wine red solution was added dropwise to a solution of anthranilamide (**1**; 125 mmol) and triethylamine (250 mmol) in chloroform (250 mL), and stirred at room temperature for 5 h. The precipitated solid was collected through filtration, washed with ethanol, and dried. Its recrystallization from ethanol afforded the products **2a** and **2b**.

*5.1.1.1. N-(2-carbamoylphenyl)-2-nitrobenzamide (**2a**).* The compound **1** was treated with 2-nitrobenzoic acid according to general procedure A to afford **2a** as a white solid in 88% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.57 (s, 1H), 8.53 (d, J = 8.2 Hz, 1H), 8.43 (s, 1H), 8.13 (d, J = 8.3 Hz, 1H), 7.95–7.78 (m, 5H), 7.62 (dd, J = 11.4, 4.0 Hz, 1H), 7.29–7.22 (m, 1H); LC–MS m/z: 286 $[M + H]^+$.

*5.1.1.2. N-(2-carbamoylphenyl)-4-chloro-2-nitrobenzamide (**2b**).* The compound **1** was treated with 4-chloro-2-nitrobenzoic acid according to general procedure A to afford **2b** as a white solid in 80% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.56 (s, 1H), 8.44 (d, J = 8.2 Hz, 1H), 8.39 (s, 1H), 8.26 (d, J = 1.6 Hz, 1H), 8.04–7.96 (m, 1H), 7.93–7.85 (m, 2H), 7.81 (s, 1H), 7.59 = 5.6 Hz, 2H) = 7.8 Hz, 1H), 7.24 = 5.6 Hz, 2H) = 7.6 Hz, 1H); LC–MS m/z: 320 $[M + H]^+$.

*5.1.2. General procedure B: preparation of **3a** and **3b***

A mixture of benzamide (88 mmol) in 10% aqueous KOH (100 mL) and EtOH (100 mL) was heated under reflux for 2 h. Ethanol was removed in vacuo, and the aqueous layer was acidified with hydrochloric acid to pH 3 to give a solid residue. The solid was filtered and washed with water, which was purified by using column chromatography, eluting with CH_2Cl_2/MeOH (20:1) to give products **3a** and **3b**.

*5.1.2.1. 2-(2-Nitrophenyl)quinazolin-4(3H)-one (**3a**).* The compound **2a** was treated with KOH according to general procedure B to afford **3a** as a white solid in 98% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.86 (s, 1H), 8.27–8.16 (m, 2H), 7.95–7.81 (m, 4H), 7.67 (d, J = 7.8 Hz, 1H), 7.62–7.56 (m, 1H). LC–MS m/z: 268 $[M + H]^+$.

*5.1.2.2. 2-(4-Chloro-2-nitrophenyl)quinazolin-4(3H)-one (**3b**).* The compound **2b** was treated with KOH according to general procedure B to afford **3b** as a white solid in 91% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.87 (s, 1H), 8.19 (d, J = 2.0 Hz, 1H), 8.05 (d, J = 8.0 Hz, 1H), 7.89 (dd, J = 8.4, 2.0 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.71 = 5.6 Hz, 2H) = 7.8 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.44 = 5.6 Hz, 2H) = 7.7 Hz, 1H); LC–MS m/z: 302 $[M + H]^+$.

*5.1.3. General procedure C: preparation of **4a** and **4b***

To a mechanically stirred suspension of **3a** or **3b** (80 mmol) in 100 mL toluene was added *N*,*N*-diethylaniline (133 mmol), followed with phosphorus oxychloride (96 mmol). The reaction was heated to 80 °C for 6 h, allowed to cool to room temperature, and then washed with ice water (200 mL). The organic layer was then washed sequentially with 20% NaOH (2 × 100 mL), iced water (200 mL), and brine (200 mL), and dried over anhydrous sodium sulfate. The organic solvent was removed in vacuo, and the residue was purified by using column chromatography with CH_2Cl_2/MeOH (20:1) to give **4a** or **4b** as a colorless powder. The aqueous layers were combined and extracted with dichloromethane. The organic layer was reduced in vacuo to yield a yellow solid, which was also purified by using column chromatography as above.

*5.1.3.1. 4-Chloro-2-(2-nitrophenyl)quinazoline (**4a**).* The compound **3a** was treated with phosphorus oxychloride according to general procedure C to afford **4a** as a pale solid in 70% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.26–8.10 (m, 2H), 7.97–7.82 (m, 4H), 7.69 (d, J = 7.7 Hz, 1H), 7.63–7.58 (m, 1H); LC–MS m/z: 286 $[M + H]^+$.

*5.1.3.2. 4-Chloro-2-(4-chloro-2-nitrophenyl)quinazoline (**4b**).* The compound **3b** was treated with phosphorus oxychloride according to general procedure C to afford **4b** as a pale solid in 61% yield. ^{1}H NMR (400 MHz, DMSO- d_6): δ 8.36–8.31 (m, 1H), 8.26 (d, J = 2.1 Hz, 1H), 8.23–8.17 (m, 2H), 8.10 (d, J = 8.1 Hz, 1H), 8.01–7.92 (m, 2H); LC–MS m/z: 321 $[M + H]^+$.

*5.1.4. General procedure D: preparation of **5a** and **5b***

To a suspension of **4a** or **4b** (56 mmol) in 100 mL THF was slowly added 3-aminopropyldimethylamine (168 mmol). The mixture was heated under reflux for 5 h, and TLC analysis indicated reaction completion. After concentration in vacuo, the resulting residue was dissolved in $CHCl_3$, washed with water and brine, dried over anhydrous $MgSO_4$, and evaporated. The residue was chromatographed on silica gel eluting with CH_2Cl_2/MeOH (20:1) to give **5a** or **5b**.

*5.1.4.1. N^1,N^1-dimethyl-N^3-(2-(2-nitrophenyl)quinazolin-4-yl)propane-1,3-diamine (**5a**).* The compound **4a** was treated with 3-aminopropyldimethylamine according to general procedure D to afford **5a** as a yellow solid in 87% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (s, 1H), 8.11 (dd, J = 7.7, 1.3 Hz, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.67–7.60 (m, 2H), 7.57–7.50 (m, 2H), 7.43 (td, J = 7.8, 1.4 Hz, 1H), 7.38–7.33 (m, 1H), 3.60 (dd, J = 10.4, 5.8 Hz, 2H), 2.50 = 5.6 Hz, 2H) = 5.6 Hz, 2H), 2.29 (s, 6H), 1.76 (dt, J = 11.5, 5.9 Hz, 2H); LC–MS m/z: 352 $[M + H]^+$.

*5.1.4.2. N^1-(2-(4-chloro-2-nitrophenyl)quinazolin-4-yl)-N^3,N^3-dimethylpropane-1,3-diamine (**5b**).* The compound **4b** was treated with 3-aminopropyldimethylamine according to general procedure D to afford **5b** as a yellow solid in 84% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95 (s, 1H), 8.21 (d, J = 8.4 Hz, 1H), 7.84 (d, J = 8.3 Hz, 1H), 7.75–7.66 (m, 2H), 7.62–7.56 (m, 2H), 7.45 = 5.6 Hz, 2H) = 7.3 Hz, 1H), 3.66 (dd, J = 10.4, 5.6 Hz, 2H), 2.63–2.55 (m, 2H), 2.38 (s, 6H), 1.88–1.82 (m, 2H); LC–MS m/z: 386 $[M + H]^+$.

*5.1.5. General procedure E: preparation of **6a**, **6b**, **9a** and **9b***

To a suspension of appropriate nitrobenzene (48 mmol) and stannous chloride dihydrate (144 mmol) in 80 mL ethanol was slowly added 10 M hydrochloric acid (14.4 mL). The mixture was heated under reflux for 5 h, and TLC analysis indicated reaction completion. After concentration in vacuo, the resulting residue was slowly dissolved in sodium hydroxide solution (pH 13–14) with ice water. Then the mixture was quickly extracted with dichloromethane. The organic layer was then dried over anhydrous $MgSO_4$ and evaporated. The residue was chromatographed on silica gel eluting with CH_2Cl_2/MeOH (15:1) to give corresponding aminobenzene product.

*5.1.5.1. N^1-(2-(2-aminophenyl)quinazolin-4-yl)-N^3,N^3-dimethylpropane-1,3-diamine (**6a**).* The compound **5a** was treated with stannous chloride dihydrate according to general procedure E to afford **6a** as a white solid in 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.49 (dd, J = 8.0, 1.6 Hz, 1H), 8.44 (s, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.56 (ddd, J = 8.3, 7.0, 1.3 Hz, 1H), 7.48 (d, J = 8.1 Hz, 1H), 7.26 (ddd, J = 8.1, 7.0, 1.1 Hz, 1H), 7.11 (ddd, J = 8.6, 7.2, 1.7 Hz, 1H), 6.81–6.21 (m, 4H), 3.74 (dd, J = 10.4, 5.9 Hz, 2H), 2.49 = 5.6 Hz, 2H) = 5.6 Hz, 2H), 2.28 (s, 6H), 1.79 (dt, J = 11.6, 5.9 Hz, 2H); LC–MS m/z: 322 $[M + H]^+$.

*5.1.5.2. N^1-(2-(2-amino-4-chlorophenyl)quinazolin-4-yl)-N^3,N^3-dimethylpropane-1,3-diamine (**6b**).* The compound **5b** was treated with stannous chloride dihydrate according to general procedure E to afford **6b** as a white solid in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (s, 1H), 8.51 (d, J = 8.7 Hz, 1H), 7.78–7.73 (m, 1H), 7.70–7.61 (m, 2H), 7.41–7.35 (m, 1H), 6.77 (s, 2H), 6.71 (dt, J = 8.3, 1.9 Hz, 2H), 3.84 (dd, J = 10.5, 5.8 Hz, 2H), 2.69–2.62 (m, 2H), 2.42 (s, 6H), 1.99–1.91 (m, 2H); LC–MS m/z: 356 $[M + H]^+$.

*5.1.5.3. 4-Amino-N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)benzamide (**9a**).* The compound **8a** was treated with stannous chloride dihydrate according to general procedure E to afford **9a** as a white solid in 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.26 (s, 1H), 8.94 (d, J = 8.4 Hz, 1H), 8.81 (s, 1H), 8.76 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 7.9 Hz, 2H), 7.81 (d, J = 8.3 Hz, 1H), 7.74–7.67 (m, 2H), 7.49–7.42 (m, 2H), 7.16 = 5.6 Hz, 2H) = 7.6 Hz, 1H), 6.78 (d, J = 8.1 Hz, 2H), 4.00 (s, 2H), 3.90 (dd, J = 10.3, 5.0 Hz, 2H), 2.69 (t, J = 5.6 Hz, 2H), 2.44 (s, 6H), 1.99–1.93 (m, 2H); LC–MS m/z: 441 $[M + H]^+$.

*5.1.5.4. 4-Amino-N-(5-chloro-2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)benzamide (**9b**).* The compound **8b** was treated with stannous chloride dihydrate according to general procedure E to afford **9b** as a white solid in 74% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.42 (s, 1H), 9.07 (d, J = 1.9 Hz, 1H), 8.96 (s, 1H), 8.73 (d, J = 8.6 Hz, 1H), 8.03 (d, J = 8.4 Hz, 2H), 7.81 (d, J = 8.3 Hz, 1H), 7.72 (t, J = 7.6 Hz, 1H), 7.64 (d, J = 8.2 Hz, 1H), 7.48–7.42 (m, 1H), 7.12 (dd, J = 8.7, 2.0 Hz, 1H), 6.80 (d, J = 8.5 Hz, 2H), 4.05 (s, 2H), 3.88 (dd, J = 10.2, 5.4 Hz, 2H), 2.65 (t, J = 5.6 Hz, 2H), 2.42 (s, 6H), 1.96–1.90 (m, 2H); LC–MS m/z: 476 $[M + H]^+$.

*5.1.6. General procedure F: preparation of **7a**, **7b**, **10a**, **10b** and **10c***

A solution of the appropriate acid halide (9.6 mmol) in CH_2Cl_2 (10 mL) was added to a well-stirred mixture of the amino-substituted compound (8 mmol) in CH_2Cl_2 (50 mL) at 0 °C. The reaction was then allowed to warm up to room temperature, and then stirred overnight. After cooling to 0 °C, the precipitate formed was filtered off, and was further purified by using flash column chromatography with CH_2Cl_2/MeOH (15:1 to 10:1) elution to give the compounds **7a**, **7b**, **10a**, **10b** and **10c**.

*5.1.6.1. 2-Chloro-N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)acetamide (**7a**).* The compound **6a** was treated with chloroacetyl chloride according to general procedure F to afford **7a** as a white solid in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.03 (s, 1H), 8.80 (s, 1H), 8.62–8.57 (m, 2H), 7.87 (dd, J = 8.4, 0.6 Hz, 1H), 7.64 (ddd, J = 8.4, 7.0, 1.3 Hz, 1H), 7.57 (dd, J = 8.1, 0.7 Hz, 1H), 7.39–7.34 (m, 2H), 7.16–7.12 (m, 1H), 4.21 (s, 2H), 3.80 (dd, J = 10.3, 5.8 Hz, 2H), 2.57 (t, J = 5.6 Hz, 2H), 2.33 (s, 6H), 1.88–1.81 (m, 2H); LC–MS m/z: 398 $[M + H]^+$.

*5.1.6.2. 3-Chloro-N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)propanamide (**7b**).* The compound **6a** was treated with 3-chloropropanoyl chloride according to general procedure F to afford **7b** as a white solid in 84% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.20 (s, 1H), 8.95 (s, 1H), 8.74 (d, J = 8.1 Hz, 2H), 7.73 (d, J = 3.9 Hz, 2H), 7.62 (d, J = 8.1 Hz, 1H), 7.46–7.40 (m, 2H), 7.17 (t, J = 7.6 Hz, 1H), 3.97 (t, J = 6.9 Hz, 2H), 3.86 (dd, J = 10.3, 5.5 Hz, 2H), 3.01 (t, J = 6.9 Hz, 2H), 2.63 (t, J = 5.6 Hz, 2H), 2.40 (s, 6H), 1.95–1.88 (m, 2H).; LC–MS m/z: 412 $[M + H]^+$.

*5.1.6.3. 4-(2-Chloroacetamido)-N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)benzamide (**10a**).* The compound **9a** was treated with chloroacetyl chloride according to general procedure F to afford **10a** as a pale solid in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.47 (s, 1H), 9.50 (s, 1H), 8.94 (d, J = 8.2 Hz, 1H), 8.84 (s, 1H), 8.77 (d, J = 7.9 Hz, 1H), 8.17 (d, J = 8.5 Hz, 2H), 7.81–7.72 (m, 4H), 7.65 (d, J = 8.0 Hz, 1H), 7.56–7.40 (m, 2H), 7.19 (t, J = 7.3 Hz, 1H), 3.87 (dd, J = 10.1, 5.3 Hz, 2H), 3.13 (s, 2H), 2.67–2.62 (m, 2H), 2.41 (s, 6H), 1.95–1.89 (m, 2H); LC–MS m/z: 518 $[M + H]^+$.

*5.1.6.4. 4-(3-Chloropropanamido)-N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)benzamide (**10b**).* The compound **9a** was treated with 3-chloropropanoyl chloride according to general procedure F to afford **10b** as a pale solid in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.45 (s, 1H), 11.15 (s, 1H), 8.94 (d, J = 8.3 Hz, 1H), 8.89 (s, 1H), 8.77 (d, J = 8.0 Hz, 1H), 8.14 (d, J = 8.6 Hz, 2H), 7.81 (d, J = 8.2 Hz, 1H), 7.73 (dt, J = 7.0, 2.6 Hz, 3H), 7.60 (d, J = 7.8 Hz, 1H), 7.49 (t, J = 7.0 Hz, 1H), 7.43 (t, J = 7.1 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 3.94 (t, J = 5.6 Hz, 2H), 3.88 (dd, J = 10.1, 5.4 Hz, 2H), 3.03 (t, J = 5.6 Hz, 2H), 2.63 (t, J = 5.6 Hz, 2H), 2.40 (s, 6H), 1.93–1.88 (m, 2H); LC–MS m/z: 532 $[M + H]^+$.

*5.1.6.5. N-(5-chloro-2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)-4-(3-chloropropanamido)benzamide (**10c**).* The compound **9b** was treated with 3-chloropropanoyl chloride according to general procedure F to afford **10c** as a pale solid in 84% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.49 (s, 1H), 10.75 (s, 1H), 8.98 (t, J = 5.3 Hz, 1H), 8.89 (d, J = 2.2 Hz, 1H), 8.75 (d, J = 8.7 Hz,

1H), 8.44 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 8.7 Hz, 2H), 7.92 (d, J = 8.7 Hz, 2H), 7.84 (t, J = 7.7 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H), 7.58 (t, J = 7.4 Hz, 1H), 7.29 (dd, J = 8.7, 2.2 Hz, 1H), 3.93 (d, J = 6.1 Hz, 2H), 3.74 (d, J = 5.7 Hz, 2H), 3.06 (t, J = 7.0 Hz, 2H), 2.95 (t, J = 6.2 Hz, 2H), 2.66 (s, 6H), 2.15–2.08 (m, 2H); LC–MS m/z: 566 $[M + H]^+$.

*5.1.7. General procedure G: preparation of **8a** and **8b***

The mixture of 4-nitrobenzoic acid (52.5 mmol) and thionyl chloride (50 mL) was heated under reflux for 1.5 h at 80 °C. The solution was allowed to cool at room temperature followed with the evaporation of thionyl chloride in vacuo. The resulting wine red solution was added dropwise to a solution of **6a** or **6b** (**1**; 125 mmol) in dichloromethane (150 mL) at 0 °C, then stirred at room temperature for 5 h. The precipitated solid was collected through filtration, washed with ethanol, and dried. Its recrystallization from ethanol afforded the products **8a** or **8b**.

*5.1.7.1. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl) phenyl)-4-nitrobenzamide (**8a**).* The compound **6a** was treated with 4-nitrobenzoic acid according to general procedure G to afford **8a** as a pale solid in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.89 (s, 1H), 9.05 (s, 1H), 8.92 (d, J = 7.9 Hz, 1H), 8.81 (d, J = 7.5 Hz, 1H), 8.40 (d, J = 7.9 Hz, 2H), 8.32 (d, J = 7.7 Hz, 2H), 7.74 (t, J = 8.0 Hz, 1H), 7.67 (d, J = 5.9 Hz, 1H), 7.62 (d, J = 8.1 Hz, 1H), 7.56–7.42 (m, 3H), 3.90 (dd, J = 8.9, 5.3 Hz, 2H), 2.68 (t, J = 5.6 Hz, 2H), 2.44 (s, 6H), 1.99–1.90 (m, 2H); LC–MS m/z: 471 $[M + H]^+$.

*5.1.7.2. N-(5-chloro-2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)-4-nitrobenzamide (**8b**).* The compound **6b** was treated with 4-nitrobenzoic acid according to general procedure G to afford **8b** as a pale solid in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.89 (s, 1H), 9.06 (t, J = 4.4 Hz, 1H), 8.92 (d, J = 8.2 Hz, 1H), 8.81 (dd, J = 8.0, 1.4 Hz, 1H), 8.40 (d, J = 8.7 Hz, 2H), 8.32 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 6.7 Hz, 2H), 7.54–7.49 (m, 1H), 7.46 (t, J = 7.6 Hz, 1H), 3.89 (dd, J = 10.2, 5.6 Hz, 2H), 2.65 (t, J = 5.6 Hz, 2H), 2.42 (s, 6H), 1.95–1.89 (m, 2H); LC–MS m/z: 505 $[M + H]^+$.

*5.1.8. General procedure H: preparation of **11a–11d**, **12a–12e**, **13a–13d**, **14a–14e** and **15a–15e***

To a stirred refluxing suspension of the chloride compounds **7a**, **7b**, **10a**, **10b** and **10c** (0.5 mmol) in MeOH (15 mL) was added dropwise appropriate secondary amine (2.0 mL) in EtOH (5 mL). The mixture was stirred under reflux for 6 h, cooled to room temperature, and diluted with distilled water. The resulting solution was filtered, washed with ether and water, and then evaporated under vacuum. The crude solid was purified by using chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ elution to afford **11a–11d**, **12a–12e**, **13a–13d**, **14a–14e** and **15a–15e.**

*5.1.8.1. 2-(Diethylamino)-N-(2-(4-((3-(dimethylamino)propyl) amino)quinazolin-2-yl)phenyl)acetamide (**11a**).* The compound **7a** was treated with excess diethylamine according to general procedure H to afford **11a**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a white solid in 76% yield. m.p. 128–129 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.35 (s, 1H), 8.75 (t, J = 6.0 Hz, 2H), 8.51 (dd, J = 7.9, 1.6 Hz, 1H), 7.95–7.86 (m, 1H), 7.77–7.65 (m, 2H), 7.43 (ddd, J = 8.6, 6.5, 2.1 Hz, 2H), 7.17 (t, J = 8.1 Hz, 1H), 3.89 (dd, J = 10.7, 5.6 Hz, 2H), 3.28 (s, 2H), 2.76–2.58 (m, 6H), 2.45 (s, 6H), 1.97 (dt, J = 11.4, 5.9 Hz, 2H), 0.97 (t, J = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.77, 161.31, 159.56, 149.05, 138.82, 131.99, 130.87, 130.38, 128.05, 126.43, 125.58, 122.78, 121.26, 121.06, 113.77, 59.63, 58.77, 49.04, 45.47, 42.31, 24.78, 11.63; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{25}H_{34}N_6O$) requires m/z 433.2716, found 433.2709.

*5.1.8.2. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl) phenyl)-2-(pyrrolidin-1-yl)acetamide (**11b**).* The compound **7a** was treated with excess pyrrolidine according to general procedure H to afford **11b**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a white solid in 74% yield. m.p. 147–149 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.58 (s, 1H), 8.78 (d, J = 8.3 Hz, 2H), 8.56 (dd, J = 7.9, 1.5 Hz, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.76–7.66 (m, 2H), 7.48–7.39 (m, 2H), 7.16 (t, J = 7.1 Hz, 1H), 3.90 (dd, J = 10.6, 5.5 Hz, 2H), 3.42 (s, 2H), 2.79–2.70 (m, 2H), 2.67 (t, J = 5.8 Hz, 4H), 2.48 (s, 6H), 2.03–1.97 (m, 2H), 1.74–1.65 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.30, 161.25, 159.46, 148.89, 139.22, 131.93, 130.70, 130.56, 128.04, 125.70, 125.66, 122.69, 121.06, 121.02, 113.73, 62.21, 59.68, 54.52, 45.47, 42.39, 24.70, 23.92; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{25}H_{32}N_6O$) requires m/z 431.2559, found 431.2550.

*5.1.8.3. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl) phenyl)-2-morpholinoacetamide (**11c**).* The compound **7a** was treated with excess morpholine according to general procedure H to afford **11c**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a white solid in 78% yield. m.p. 144–145 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.31 (s, 1H), 8.84 (s, 1H), 8.66 (d, J = 8.2 Hz, 1H), 8.46 (dd, J = 7.9, 1.5 Hz, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.42–7.33 (m, 2H), 7.11 (t, J = 7.1 Hz, 1H), 3.80 (dd, J = 10.4, 5.6 Hz, 2H), 3.54–3.47 (m, 4H), 3.23 (s, 2H), 2.59–2.55 (m, 2H), 2.53–2.46 (m, 4H), 2.34 (s, 6H), 1.87–1.80 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 168.73, 161.34, 159.51, 148.69, 138.87, 132.30, 130.74, 130.69, 128.08, 125.87, 125.69, 122.92, 121.21, 120.95, 113.80, 66.34, 64.79, 59.67, 53.78, 45.47, 42.47, 24.62; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{25}H_{32}N_6O_2$) requires m/z 447.2508, found 447.2500.

*5.1.8.4. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl) phenyl)-2-(4-methylpiperazin-1-yl)acetamide (**11d**).* The compound **7a** was treated with excess *N*-methyl piperazine according to general procedure H to afford **11d**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a white solid in 76% yield. m.p. 152–154 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.11 (s, 1H), 8.83 (s, 1H), 8.65 (d, J = 8.3 Hz, 1H), 8.41 (d, J = 9.1 Hz, 1H), 7.89 (d, J = 8.3 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.58 (d, J = 8.1 Hz, 1H), 7.43–7.31 (m, 2H), 7.10 (t, J = 7.6 Hz, 1H), 3.80 (dd, J = 10.4, 5.5 Hz, 2H), 3.22 (s, 2H), 2.72–2.40 (m, 6H), 2.34 (s, 6H), 2.28–2.12 (m, 4H), 2.02 (s, 3H), 1.86 (dt, J = 7.1, 3.4 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 168.11,160.39,158.51, 147.83, 137.69, 131.31, 129.58, 127.60, 124.78, 121.90, 120.08, 120.04, 63.18, 58.68, 53.17, 52.36, 44.79, 44.42, 41.46, 23.46; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{26}H_{35}N_7O$) requires m/z 460.2825, found 460.2814.

*5.1.8.5. 3-(Diethylamino)-N-(2-(4-((3-(dimethylamino)propyl) amino)quinazolin-2-yl)phenyl)propanamide (**12a**).* The compound **7b** was treated with excess diethylamine according to general procedure H to afford **12a**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a white solid in 79% yield. m.p. 112–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.94 (s, 1H), 8.93 (s, 1H), 8.77–8.69 (m, 2H), 7.80 (d, J = 7.9 Hz, 1H), 7.73 (t, J = 7.6 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.47–7.40 (m, 2H), 7.15 (t, J = 8.2 Hz, 1H), 3.89 (dd, J = 10.4, 5.7 Hz, 2H), 3.10–3.01 (m, 2H), 2.81–2.73 (m, 2H), 2.71–2.62 (m, 6H), 2.42 (s, 6H), 1.97–1.91 (m, 2H), 1.09 (t, J = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) : δ 170.87, 161.40, 159.22, 148.27, 140.25, 132.50, 131.01, 130.67, 127.47, 125.78, 123.74, 122.36, 121.21, 120.35, 113.80, 59.68, 49.02, 46.98, 45.46, 42.50, 36.63, 24.62, 11.85; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{26}H_{36}N_6O$) requires m/z 447.2872, found 447.2864.

*5.1.8.6. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl) phenyl)-3-(pyrrolidin-1-yl)propanamide (**12b**).* The compound **7b** was treated with excess pyrrolidine according to general procedure H to afford **12b**. After column chromatography with $CHCl_3$/MeOH/$NH_3\cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a white solid in 74% yield. m.p. 156–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.99 (s, 1H), 8.95 (s, 1H), 8.72 (d, J = 8.1 Hz, 2H), 7.84 (d, J = 8.3 Hz, 1H), 7.74 (t, J = 7.3 Hz, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.43 (dt, J = 8.3, 4.3 Hz, 2H), 7.15 (t, J = 7.6 Hz, 1H), 3.90 (dd, J = 10.3, 5.6 Hz, 2H), 3.09 (t, J = 7.5 Hz, 2H), 2.89 (t, J = 7.5 Hz, 2H), 2.73 (t, J = 6.4 Hz, 4H), 2.68–2.63 (m, 2H), 2.43 (s, 6H), 1.94 (dt, J = 11.3, 5.8 Hz, 2H), 1.84 (dt, J = 6.7, 3.4 Hz, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.44, 161.38, 159.20, 148.25, 140.30, 132.50, 130.97, 130.68, 127.51, 125.75, 123.72, 122.31, 121.18, 120.36, 113.80, 59.85, 54.15, 52.25, 45.50, 42.67, 38.54, 24.59, 23.53; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{26}H_{34}N_6O$) requires m/z 445.2716, found 445.2705.

*5.1.8.7. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl) phenyl)-3-morpholinopropanamide (**12c**).* The compound **7b** was treated with excess morpholine according to general procedure H to afford **12c**. After column chromatography with $CHCl_3$/MeOH/$NH_3\cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a white solid in 75% yield. m.p. 151–153 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.91 (s, 1H), 8.92 (s, 1H), 8.71 (t, J = 7.0 Hz, 2H), 7.79–7.68 (m, 3H), 7.49–7.41 (m, 2H), 7.15 (t, J = 7.6 Hz, 1H), 3.91 (dd, J = 10.5, 5.3 Hz, 2H), 3.69–3.61 (m, 4H), 2.90 (t, J = 7.2 Hz, 2H), 2.79–2.69 (m, 4H), 2.57–2.50 (m, 4H), 2.47 (s, 6H), 2.02–1.96 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.29, 161.40, 159.21, 148.21, 140.20, 132.52, 131.02, 130.72, 127.32, 125.79, 123.75, 122.42, 121.29, 120.37, 113.82, 66.92, 59.83, 54.78, 53.54, 45.50, 42.66, 36.42, 24.58; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{26}H_{34}N_6O_2$) requires m/z 461.2665, found 461.2655.

*5.1.8.8. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl) phenyl)-3-(4-methylpiperazin-1-yl)propanamide (**12d**).* The compound **7b** was treated with excess N-methyl piperazine according to general procedure H to afford **12d**. After column chromatography with $CHCl_3$/MeOH/$NH_3\cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a white solid in 74% yield. m.p. 153–155 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.87 (s, 1H), 8.91 (s, 1H), 8.71 (t, J = 8.7 Hz, 2H), 7.82–7.65 (m, 3H), 7.49–7.40 (m, 2H), 7.15 (t, J = 7.3 Hz, 1H), 3.90 (dd, J = 10.5, 5.5 Hz, 2H), 2.93 (t, J = 7.4 Hz, 2H), 2.76 (t, J = 7.4 Hz, 2H), 2.72–2.68 (m, 2H), 2.67–2.51 (m, 4H), 2.50–2.37 (m, 10H), 2.27 (s, 3H), 2.01–1.94 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.39, 161.43, 159.22, 148.28, 140.22, 132.53, 131.01, 130.67, 127.47, 125.77, 123.77, 122.36, 121.19, 120.39, 113.82, 59.92, 55.09, 54.34, 52.99, 46.00, 45.51, 42.74, 36.47, 24.55; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{27}H_{37}N_7O$) requires m/z 474.2981, found 474.2970.

*5.1.8.9. 3-((3-(Dimethylamino)propyl)amino)-N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)propanamide (**12e**).* The compound **7b** was treated with excess 3-(dimethylamino)-1-propylamine according to general procedure H to afford **12e**. After column chromatography with $CHCl_3$/MeOH/$NH_3\cdot H_2O$ (25:1:0.1) elution, the desired product was obtained as a white solid in 73% yield. m.p. 124–126 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.01 (s, 1H), 8.91 (s, 1H), 8.72 (t, J = 6.8 Hz, 2H), 7.80–7.70 (m, 2H), 7.62 (d, J = 8.1 Hz, 1H), 7.47–7.40 (m, 2H), 7.16 (t, J = 7.6 Hz, 1H), 3.88 (dd, J = 9.9, 4.8 Hz, 2H), 3.10 (t, J = 6.0 Hz, 2H), 2.84 (t, J = 6.2 Hz, 2H), 2.77 (t, J = 6.8 Hz, 2H), 2.64 (t, J = 4.8 Hz, 2H), 2.40 (s, 6H), 2.34 (t, J = 7.2 Hz, 2H), 2.20 (s, 6H), 1.95–1.90 (m, 2H), 1.73–1.67 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.35, 161.28, 159.22, 148.22, 140.09, 132.60, 131.02, 130.69, 127.62, 127.59, 125.83, 122.50, 121.18, 120.28, 113.78, 59.79, 58.09, 48.55, 45.56, 45.46, 45.35, 42.63, 37.74, 27.03, 24.52; HRMS (ESI): Calcd for $[M + H]^+$ ($C_{27}H_{39}N_7O$) requires m/z 478.3289, found 478.3288.

*5.1.8.10. 4-(2-(Diethylamino)acetamido)-N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)benzamide (**13a**).* The compound **10a** was treated with excess diethylamine according to general procedure H to afford **13a**. After column chromatography with $CHCl_3$/MeOH/$NH_3\cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 78% yield. m.p. 160–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.36 (s, 1H), 9.53 (s, 1H), 8.86 (d, J = 8.3 Hz, 1H), 8.69 (d, J = 8.0 Hz, 2H), 8.09 (d, J = 8.5 Hz, 2H), 7.73–7.67 (m, 3H), 7.65 (d, J = 7.6 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.41 (t, J = 7.3 Hz, 1H), 7.35 (t, J = 7.4 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 3.80 (dd, J = 10.3, 5.2 Hz, 2H), 3.12 (s, 2H), 2.60 (m, 6H), 2.34 (s, 6H), 1.88–1.83 (m, 2H), 1.05 (t, J = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.47, 164.74, 160.41, 158.23, 147.22, 139.57, 139.52, 131.71, 130.99, 130.02, 129.87, 128.00, 125.98, 124.80, 123.30, 121.50, 120.33, 119.41, 117.69, 112.74, 58.42, 57.17, 47.87, 44.27, 41.17, 23.47, 11.41; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{32}H_{39}N_7O_2$) requires m/z 552.3092, found 552.3115.

*5.1.8.11. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl) phenyl)-4-(2-(pyrrolidin-1-yl)acetamido)benzamide (**13b**).* The compound **10a** was treated with excess pyrrolidine according to general procedure H to afford **13b**. After column chromatography with $CHCl_3$/MeOH/$NH_3\cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 72% yield. m.p. 174–176 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.48 (s, 1H), 9.34 (s, 1H), 8.96 (d, J = 8.4 Hz, 1H), 8.88 (s, 1H), 8.78 (d, J = 7.9 Hz, 1H), 8.18 (d, J = 8.5 Hz, 2H), 7.83–7.75 (m, 4H), 7.66 (d, J = 8.0 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.45 (t, J = 7.4 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 3.89 (dd, J = 10.1, 5.3 Hz, 2H), 3.35 (s, 2H), 2.75 (t, J = 6.3 Hz, 4H), 2.66 (t, J = 6 Hz, 2H), 2.43 (s, 6H), 1.96–1.90 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 168.52, 164.71, 160.34, 158.15, 147.14, 139.67, 139.51, 131.65, 130.93, 129.95, 129.90, 127.91, 125.87, 124.74, 123.27, 121.47, 120.28, 119.34, 117.88, 112.71, 58.87, 58.47, 53.62, 44.33, 41.25, 23.55, 23.08; HRMS (ESI): Calcd for $[M + H]^+$ ($C_{32}H_{37}N_7O_2$) requires m/z 552.3082, found 552.3082.

*5.1.8.12. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl) phenyl)-4-(2-morpholinoacetamido)benzamide (**13c**).* The compound **10a** was treated with excess morpholine according to general procedure H to afford **13c**. After column chromatography with $CHCl_3$/MeOH/$NH_3\cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 79% yield. m.p. 188–189 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.41 (s, 1H), 9.19 (s, 1H), 8.87 (d, J = 8.3 Hz, 1H), 8.80 (s, 1H), 8.70 (d, J = 7.9 Hz, 1H), 8.10 (d, J = 8.3 Hz, 2H), 7.73–7.65 (m, 4H), 7.57 (d, J = 8.0 Hz, 1H), 7.42 (t, J = 7.5 Hz, 1H), 7.36 (t, J = 8 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 3.81 (dd, J = 10.4, 5.9 Hz, 2H), 3.74 (t, J = 6 Hz, 4H), 3.13 (s, 2H), 2.62–2.55 (m, 6H), 2.34 (s, 6H), 1.87–1.83 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.24, 164.65, 160.50, 158.27, 147.26, 139.50, 139.32, 131.73, 131.31, 130.09, 129.89, 128.03, 126.01, 124.81, 123.32, 121.57, 120.30, 119.45, 117.86, 112.79, 66.03, 61.57, 58.64, 52.83, 44.39, 41.41, 23.49; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{32}H_{37}N_7O_3$) requires m/z 566.2885, found 566.2871.

*5.1.8.13. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl) phenyl)-4-(2-(4-methylpiperazin-1-yl)acetamido)benzamide (**13d**).* The compound **10a** was treated with excess *N*-methylpiperazine according to general procedure H to afford **13d**. After column chromatography with $CHCl_3$/MeOH/$NH_3\cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 71% yield. m.p. 183–184 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.41 (s, 1H), 9.28 (s, 1H), 8.87 (d, J = 8.3 Hz, 1H), 8.75 (s, 1H), 8.70 (d, J = 7.9 Hz, 1H), 8.10 (d, J = 8.4 Hz, 2H), 7.73–7.67 (m, 4H), 7.62 (d, J = 8.2 Hz, 1H), 7.42 (t, J = 7.8 Hz, 1H), 7.36 (t, J = 7.4 Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H), 3.80 (dd, J = 9.4, 5.3 Hz,2H), 3.12 (s, 2H), 2.65–2.57 (m, 6H), 2.51–2.44 (m, 4H), 2.35 (s, 6H), 2.09 (s, 3H), 1.90–1.84 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.67, 164.70, 160.42, 158.26, 147.22, 139.50, 139.47,

131.73, 131.14, 130.04, 129.89, 128.00, 125.96, 124.81, 123.31, 121.55, 120.37, 119.42, 117.82, 112.77, 60.95, 58.23, 54.20, 52.44, 44.94, 44.21, 41.06, 23.52; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{33}H_{40}N_8O_2$) requires *m*/*z* 579.3201, found 579.3181.

*5.1.8.14. 4-(3-(Diethylamino)propanamido)-N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)benzamide (**14a**).* The compound **10b** was treated with excess diethylamine according to general procedure H to afford **14a**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 79% yield. m.p. 155–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.37 (s, 1H), 11.55 (s, 1H), 8.88 (d, J = 8.3 Hz, 1H), 8.70 (d, J = 7.2 Hz, 2H), 8.06 (d, J = 8.2 Hz, 2H), 7.74 (d, J = 8.6 Hz, 1H), 7.64 (d, J = 7.9 Hz, 4H), 7.43–7.36 (m, 2H), 7.11 (t, J = 7.7 Hz, 1H), 3.82 (dd, J = 9.3, 5.8 Hz, 2H), 2.75 (t, J = 5.5 Hz, 2H), 2.68–2.61 (m, 6H), 2.49 (t, J = 5.5 Hz, 2H), 2.38 (s, 6H), 1.92–1.87 (m, 2H), 1.11 (t, J = 7.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.20, 164.95, 160.47, 158.30, 147.31, 140.79, 139.63, 131.66, 130.51, 130.07, 129.84, 127.98, 126.12, 124.81, 123.26, 121.44, 120.36, 119.43, 117.83, 112.78, 58.17, 47.90, 44.98, 44.14, 40.99, 32.24, 23.49, 10.51; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{33}H_{41}N_7O_2$) requires *m*/*z* 566.3249, found 566.3225.

*5.1.8.15. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)-4-(3-(pyrrolidin-1-yl)propanamido)benzamide (**14b**).* The compound **10b** was treated with excess pyrrolidine according to general procedure H to afford **14b**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 70% yield. m.p. 175–176 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.46 (s, 1H), 11.61 (s, 1H), 8.96 (dd, J = 8.3, 0.7 Hz, 1H), 8.89 (t, J = 4.4 Hz, 1H), 8.79 (dd, J = 8.0, 1.5 Hz, 1H), 8.15 (d, J = 8.6 Hz, 2H), 7.82 (d, J = 8.2 Hz, 1H), 7.76–7.71 (m, 1H), 7.68 (d, J = 8.6 Hz, 2H), 7.62 (d, J = 7.9 Hz, 1H), 7.52–7.47 (m, 1H), 7.46–7.42 (m, 1H), 7.22–7.17 (m, 1H), 3.89 (dd, J = 10.2, 5.7 Hz, 2H), 2.91 (t, J = 5.8 Hz, 2H), 2.73 (t, J = 6.4 Hz, 4H), 2.64 (t, J = 5.5 Hz, 2H), 2.59 (t, J = 5.8 Hz, 2H), 2.41 (s, 6H), 1.98–1.88 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.15, 164.90, 160.56, 158.28, 147.35, 140.87, 139.63, 131.66, 130.52, 130.09, 129.86, 127.96, 126.20, 124.77, 123.30, 121.44, 120.17, 119.44, 117.99, 112.80, 58.86, 52.11, 50.24, 44.49, 41.64, 33.75, 23.56, 22.76; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{33}H_{39}N_7O_2$) requires *m*/*z* 564.3092, found 564.3067.

*5.1.8.16. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)-4-(3-morpholinopropanamido)benzamide (**14c**).* The compound **10b** was treated with excess morpholine according to general procedure H to afford **14c**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 73% yield. m.p. 199–201 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.41 (s, 1H), 11.01 (s, 1H), 8.87 (d, J = 8.3 Hz, 1H), 8.80 (s, 1H), 8.70 (d, J = 7.8 Hz, 1H), 8.08 (d, J = 8.5 Hz, 2H), 7.73 (d, J = 8.2 Hz, 1H), 7.67–7.62 (m, 3H), 7.58 (d, J = 8.1 Hz, 1H), 7.42 (t, J = 7.8 Hz, 1H), 7.36 (t, J = 7.4 Hz, 1H), 7.12 (t, J = 7.3 Hz, 1H), 3.84–3.77 (m, 6H), 2.71 (t, J = 5.6 Hz, 2H), 2.64–2.56 (m, 6H), 2.53 (t, J = 5.8 Hz, 2H), 2.35 (s, 6H), 1.89–1.84 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.54, 164.79, 160.44, 158.21, 147.20, 140.59, 139.54, 131.58, 130.67, 130.05, 129.89, 128.01, 125.97, 124.79, 123.26, 121.51, 120.26, 119.35, 117.87, 112.77, 66.05, 58.67, 53.06, 51.80, 44.42, 41.46, 31.44, 23.54; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{33}H_{39}N_7O_3$) requires *m*/*z* 580.3042, found 580.3027.

*5.1.8.17. N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)-4-(3-(4-methylpiperazin-1-yl)propanamido)benzamide (**14d**).* The compound **10b** was treated with excess N-methyl piperazine according to general procedure H to afford **14d**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 71% yield. m.p. 164–166 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.32 (s, 1H), 10.49 (s, 1H), 8.80 (d, J = 8.1 Hz, 1H), 8.75–8.66 (m, 2H), 8.28 (d, J = 7.8 Hz, 1H), 8.05 (d, J = 8.0 Hz, 2H), 7.86–7.79 (m, 3H), 7.73 (d, J = 8.1 Hz, 1H), 7.57 (t, J = 7.4 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.24 (t, J = 7.2 Hz, 1H), 3.70 (dd, J = 11.4, 6.4 Hz, 2H), 2.67 (t, J = 6.4 Hz, 2H), 2.56–2.50 (m, 4H), 2.40–2.31 (m, 6H), 2.19 (s, 6H), 2.16 (s, 3H), 1.93–1.83 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.78, 164.88, 160.39, 158.44, 147.32, 140.71, 139.57, 131.76, 130.62, 130.12, 129.80, 128.01, 126.03, 124.91, 123.29, 121.50, 120.76, 119.50, 117.94, 112.82, 56.81, 54.23, 52.54, 51.16, 44.89, 43.43, 39.77, 31.64, 23.31; HRMS (ESI): Calcd for $[M - H]^-$ ($C_{34}H_{42}N_8O_3$) requires *m*/*z* 593.3358, found 593.3359.

*5.1.8.18. 4-(3-((3-(Dimethylamino)propyl)amino)propanamido)-N-(2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)benzamide (**14e**).* The compound **10b** was treated with excess 3-(dimethylamino)-1-propylamine according to general procedure H to afford **14e**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 73% yield. m.p. 108–109 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.38 (s, 1H), 11.08 (s, 1H), 8.87 (d, J = 8.3 Hz, 1H), 8.82 (t, J = 3.9 Hz, 1H), 8.71 (dd, J = 8.0, 1.5 Hz, 1H), 8.07 (d, J = 8.6 Hz, 2H), 7.74 (d, J = 8.3 Hz, 1H), 7.68–7.65 (m, 3H), 7.53 (d, J = 7.8 Hz, 1H), 7.44–7.39 (m, 1H), 7.38–7.33 (m, 1H), 7.14–7.10 (m, 1H), 3.81 (dd, J = 10.1, 5.4 Hz, 2H), 2.96 (t, J = 5.6 Hz, 2H), 2.74 (t, J = 6.7 Hz, 2H), 2.56 (t, J = 5.5 Hz, 2H), 2.49 (t, J = 5.6 Hz, 2H), 2.37–2.31 (m, 8H), 2.17 (s, 6H), 1.86–1.81 (m, 2H), 1.73–1.69 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.40, 165.94, 161.53, 159.27, 148.32, 141.73, 140.59, 132.74, 131.54, 131.09, 130.89, 128.93, 127.14, 125.79, 124.34, 122.49, 121.19, 120.46, 119.06, 113.80, 59.82, 58.19, 47.87, 45.57, 45.52, 45.34, 42.60, 36.05, 27.71, 24.59; HRMS (ESI): Calcd for $[M + 2H]^{2+}$ ($C_{34}H_{44}N_8O_2$) requires *m*/*z* 299.1866, found 299.1864.

*5.1.8.19. N-(5-chloro-2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)-4-(3-(diethylamino)propanamido)benzamide (**15a**).* The compound **10c** was treated with excess diethylamine according to general procedure H to afford **15a**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 54% yield. m.p. 173–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.59 (s, 1H), 11.67 (s, 1H), 9.07 (d, J = 1.9 Hz, 1H), 8.91 (s, 1H), 8.71 (d, J = 8.6 Hz, 1H), 8.13 (d, J = 8.6 Hz, 2H), 7.80 (d, J = 8.0 Hz, 1H), 7.75–7.70 (m, 4H), 7.45 (t, J = 7.5 Hz, 1H), 7.13 (dd, J = 8.6, 2.0 Hz, 1H), 3.89 (dd, J = 10.1, 5.0 Hz, 2H), 2.83 (t, J = 5.8 Hz, 2H), 2.75–2.70 (m, 6H), 2.57 (t, J = 5.8 Hz, 2H), 2.48 (s, 6H), 2.01–1.97 (m, 2H), 1.18 (t, J = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.16, 165.06, 159.76, 158.28, 147.18, 140.96, 140.56, 135.95, 131.89, 130.85, 130.10, 128.03, 126.06, 125.09, 121.48, 121.41, 120.56, 119.20, 117.88, 112.83, 47.94, 45.03, 43.99, 38.18, 32.24, 28.68, 23.16, 10.49; HRMS (ESI): Calcd for $[M + 2H]^{2+}$ ($C_{33}H_{40}ClN_7O_2$) requires *m*/*z* 301.6539, found 301.6547.

*5.1.8.20. N-(5-Chloro-2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)-4-(3-(pyrrolidin-1-yl)propanamido)benzamide (**15b**).* The compound **10c** was treated with excess pyrrolidine according to general procedure H to afford **15b**. After column chromatography with $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 53% yield. m.p. 195–196 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.60 (s, 1H), 11.66 (s, 1H), 9.06 (s, 1H), 8.97 (s, 1H), 8.72 (d, J = 8.7 Hz, 1H), 8.13 (d, J = 8.1 Hz, 2H), 7.79 (d, J = 8.1 Hz, 1H), 7.75–7.66 (m, 3H), 7.60 (d, J = 7.5 Hz, 1H), 7.43 (t, J = 7.2 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 3.86 (dd, J = 9.3, 4.9 Hz, 2H), 2.90 (t, J = 5.0 Hz, 2H), 2.73 (t, J = 5.6 Hz, 4H), 2.65–2.57 (m, 4H), 2.40 (s, 6H), 1.97–1.89 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.20, 165.03, 159.85, 158.22, 147.17, 141.09, 140.52, 135.91, 131.80,

130.92, 130.07, 128.01, 126.11, 124.94, 121.49, 121.44, 120.22, 119.16, 118.02, 112.86, 58.88, 52.10, 50.23, 44.47, 41.70, 33.71, 23.40, 22.75; HRMS (ESI): Calcd for $[M+2H]^{2+}$ ($C_{33}H_{38}ClN_7O_2$) requires *m*/*z* 300.6461, found 300.6455.

*5.1.8.21. N-(5-Chloro-2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)-4-(3-morpholinopropanamido)benzamide (**15c**).* The compound **10c** was treated with excess morpholine according to general procedure H to afford **15c**. After column chromatography with $CHCl_3/MeOH/NH_3\cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 54% yield. m.p. 212–214 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.62 (s, 1H), 11.13 (s, 1H), 9.07 (d, J = 2.1 Hz, 1H), 8.99 (t, J = 3.6 Hz, 1H), 8.73 (d, J = 8.6 Hz, 1H), 8.15 (d, J = 8.6 Hz, 2H), 7.79 (d, J = 7.8 Hz, 1H), 7.74–7.70 (m, 3H), 7.64 (d, J = 8.2 Hz, 1H), 7.47–7.41 (m, 1H), 7.14 (dd, J = 8.6, 2.2 Hz, 1H), 3.89–3.85 (m, 6H), 2.82–2.78 (m, 2H), 2.71–2.64 (m, 6H), 2.61 (t, J = 5.8 Hz, 2H), 2.43 (s, 6H), 1.97–1.90 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.53, 164.91, 159.85, 158.24, 147.15, 140.75, 140.49, 136.63, 135.95, 131.78, 130.92, 130.40, 128.11, 126.02, 125.01, 121.55, 121.43, 120.35, 119.17, 117.91, 66.09, 58.67, 53.11, 51.82, 44.36, 41.46, 31.34, 23.33; HRMS (ESI): Calcd for $[M+2H]^{2+}$ ($C_{33}H_{38}ClN_7O_3$) requires *m*/*z* 308.6435, found 308.6431.

*5.1.8.22. N-(5-Chloro-2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)-4-(3-(4-methylpiperazin-1-yl)propanamido)benzamide (**15d**).* The compound **10c** was treated with excess *N*-methyl piperazine according to general procedure H to afford **15d**. After column chromatography with $CHCl_3/MeOH/NH_3\cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 60% yield. m.p. 182–183 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.60 (s, 1H), 11.35 (s, 1H), 9.07 (d, J = 2.2 Hz, 1H), 8.92 (t, J = 4.0 Hz, 1H), 8.71 (d, J = 8.6 Hz, 1H), 8.15 (d, J = 8.6 Hz, 2H), 7.80 (d, J = 7.8 Hz, 1H), 7.76–7.71 (m, 4H), 7.48–7.43 (m, 1H), 7.14 (dd, J = 8.6, 2.2 Hz, 1H), 3.90 (dd, J = 10.6, 5.4 Hz, 2H), 2.88–2.52 (m, 14H), 2.49 (s, 6H), 2.39 (s, 3H), 2.02–1.98 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.83, 164.96, 159.81, 158.20, 147.13, 140.93, 140.48, 135.90, 131.75, 130.93, 130.20, 128.06, 126.03, 124.93, 121.51, 121.42, 120.29, 119.13, 117.93, 112.87, 58.72, 54.38, 52.55, 51.30, 45.04, 44.42, 41.54, 31.59, 23.39; HRMS (ESI): Calcd for $[M+2H]^{2+}$ ($C_{34}H_{41}ClN_8O_2$) requires *m*/*z* 315.1593, found 315.1583.

*5.1.8.23. N-(5-chloro-2-(4-((3-(dimethylamino)propyl)amino)quinazolin-2-yl)phenyl)-4-(3-((3-(dimethylamino)propyl)amino)propanamido)benzamide (**15e**).* The compound **10c** was treated with excess 3-(dimethylamino)-1-propylamine according to general procedure H to afford **15e**. After column chromatography with $CHCl_3/MeOH/NH_3\cdot H_2O$ (5:1:0.1) elution, the desired product was obtained as a white solid in 56% yield. m.p. 127–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.58 (s, 1H), 11.16 (s, 1H), 9.04 (d, J = 1.9 Hz, 1H), 8.91 (s, 1H), 8.70 (d, J = 8.6 Hz, 1H), 8.11 (d, J = 8.5 Hz, 2H), 7.75–7.68 (m, 4H), 7.58 (d, J = 8.1 Hz, 1H), 7.40 (t, J = 7.2 Hz, 1H), 7.11 (dd, J = 8.6, 1.9 Hz, 1H), 3.82 (dd, J = 9.6, 5.2 Hz, 2H), 3.03 (t, J = 5.6 Hz, 2H), 2.80 (t, J = 6.6 Hz, 2H), 2.61 (t, J = 5.2, 2H), 2.56 (t, J = 5.6 Hz, 2H), 2.43–2.37 (m, 8H), 2.23 (s, 6H), 1.91–1.86 (m, 2H), 1.81–1.74 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.39, 166.00, 160.73, 159.15, 148.08, 141.93, 141.49, 136.79, 132.79, 131.94, 131.08, 128.96, 126.97, 125.91, 122.45, 122.42, 121.20, 120.09, 119.07, 113.83, 59.83, 58.20, 47.90, 45.54, 45.49, 45.32, 42.66, 36.01, 27.63, 24.46; HRMS (ESI): Calcd for $[M+2H]^{2+}$ ($C_{34}H_{43}ClN_8O_2$) requires *m*/*z* 316.1672, found 316.1683.

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 (10%) or double-distilled deionized water. Further dilutions to working concentrations were made with double-distilled deionized water.

5.3. CD measurements

The CD spectra were recorded on Chirascan (Applied Photophysics) spectrophotometer. A quartz cuvette with 1 mm path length was used for the spectra recorded over a wavelength range of 230–320 at 1 nm bandwidth, 1 nm step size, and 0.5 s per point. The oligomer HTG21 (5′-d(GGG[TTAGGG]$_3$)-3′) was diluted from stock to the required concentration (5 μM) in 10 mM Tris–HCl buffer, pH 7.4, 150 mM KCl, and then annealed by heating at 95 °C in the presence of 25 μM compound for 5 min, gradually cooled to room temperature, and incubated at 4 °C overnight. CD melting experiments were carried out by recording the CD values at 265 or 290 nm every 3 °C in a temperature range of 25–94 °C at a heating rate of 1 °C/min in buffer containing 60 mM KCl. Final analysis of the data was carried out using Origin 8.0 (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 HTG21 (5′-d(GGG[TTAGGG]$_3$)-3′) were folded in filtered and degassed running buffer (50 mM Tris–HCl, 100 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 0.015625, 0.025, 0.03125, 0.05, 0.0625, 0.1, 0.125, 0.15625, 0.2, 0.25, 0.3125, 0.4, 0.5, 0.625, 0.8, 1, 1.25, 2, 2.5, 5, 10, and 20 μM) were prepared with running buffer by serial dilutions from stock solutions. Six concentrations were injected simultaneously at a flow rate of 50 μL min^{-1} for 400 s (or 30 μL min^{-1} for 666 s, 30 μL min^{-1} for 800 s) of association phase, followed with 400 s (or 666 s, 800 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 are analyzed with ProteOn manager software, using the Langmuir model for fitting kinetic data.

5.5. TRAP-LIG assay

The ability of synthesized compounds to inhibit telomerase in a cell-free system was assessed with the TRAP-LIG assay following previously published procedures [36]. Protein extracts from exponentially growing HL60 leukemia cells were used. Briefly, 0.1 μg of TS forward primer (5′-AATCCGTC-GAGCAGAGTT-3′) was elongated by telomerase (500 ng protein extract) in TRAP buffer (20 mM Tris–HCl [pH 8.3], 68 mM KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, and 0.05% Tween 20) containing 125 μM dNTPs and 0.05 μg BSA. The mix was added to tubes containing freshly prepared ligand at various concentrations and to a negative control containing no ligand. The initial elongation step was carried out for 20 min at 30 °C, followed by 94 °C for 5 min and a final maintenance of the mixture at 20 °C. To purify the elongated product and to remove the bound ligands, the QIA quick nucleotide purification kit (Qiagen) was used according to the manufacturer's instructions. The purified extended samples were then subject to PCR amplification. For this, a second PCR master mix was prepared consisting of 1 μM ACX reverse primer (5′-GCGCGG[CTTACC]$_3$CTAACC-3′), 0.1 μg TS forward primer (5′-AATCCGTCGAGCAGAGTT-3′), TRAP buffer, 5 μg BSA, 0.5 mM dNTPs, and 2 units of Taq polymerase. A 10 μL aliquot of the master

mix was added to the purified telomerase extended samples and amplified for 35 cycles of 94 °C for 30 s, of 61 °C for 1 min, and of 72 °C for 1 min. Samples were separated on a 16% PAGE and visualized with silver-stained. Tel IC_{50} values were then calculated from the optical density quantitated from the AlphaEaseFC software.

5.6. Short-term cell viability

HL60 leukemia cell line was seeded on 96-well plates (1.0×10^3 well^{-1}) and exposed to various concentrations of derivatives. After 48 h of treatment at 37 °C in a humidified atmosphere of 5% CO_2, 10 μL of 5 mg/mL^{-1} 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 μL for each well), and the optical density (OD) was recorded at 570 nm. All drug doses were parallel tested in triplicate, and the IC_{50} values were derived from the mean OD values of the triplicate tests versus drug concentration curves.

5.7. Long-term cell culture experiments

Long-term proliferation experiments were carried out using the HL60 leukemia cell line. Cells (1.0×10^5) were grown in 6-well plates and exposed to a subcytotoxic concentration of a ligand or an equivalent volume of 0.1% DMSO every 3 days. The cells in control and drug-exposed flasks were counted and flasks reseeded with 1.0×10^5 cells. The remaining cells were collected and used for the measurements described below. This process was continued for 12 days.

5.8. SA-β-Gal assay

After the long-term incubation, the growth medium was aspirated and the cells were fixed in 2% formaldehyde/0.2% glutaraldehyde for 15 min at room temperature. The fixing solution was removed, and the cells were gently washed twice with PBS and then stained using the β-Gal stain solution containing 1 mg/mL of 5-bromo-4-chloro-3-indolyl-β-D-galactoside, followed by incubation overnight at 37 °C. The staining solution was removed, and the cells were washed three times with PBS. The cells were viewed under an optical microscope and photographed.

5.9. Telomere length assay

Cells were incubated with the ligand for 12 days. To measure the telomere length, genomic DNA was digested with Hinf1/Rsa1 restriction enzymes. The digested DNA fragments were separated on 0.8% agarose gel, transferred to a nylon membrane, and the transferred DNA fixed on the wet blotting membrane by baking the membrane at 120 °C for 20 min. The membrane was hybridized with a DIG-labeled hybridization probe for telomeric repeats and incubated with anti-DIG-alkaline phosphatase. TRF was performed with chemiluminescence detection.

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Appendix A. Supplementary data

The supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejmech.2013.01.051.

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