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Synthesis, molecular modeling and biological evaluation of 2-aminomethyl-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione quinolone derivatives as novel anticancer agent

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ABSTRACT

A series of quinoline derivatives (**4a**–**4o**) have been synthesized and their biological activities were also evaluated as potential telomerase inhibitors. Bioassay tests demonstrated that most of the compounds exhibited substantial broad-spectrum antitumor activity against the three cancer cell lines (HepG2, SGC-7901 and MCF-7). Moreover, all the title compounds were assayed for telomerase inhibition using the TRAP-PCR-ELISA assay. Compounds **4d** and **4i** displayed the most potent anticancer activities, which were comparable to the positive control. Docking simulation was performed to position compounds **4d** and **4i** into the telomerase structure active site to determine the probable binding model. Compounds **4d** and **4i** with potent inhibitory activity in tumor growth inhibition may be potential anticancer agents.

1. Introduction

Cancer, second cause of mortality in the world, is continuing to be a major health problem in developing as well as undeveloped countries [1]. Although the overall survival rate of cancer patients has substantially increased during the last decades, this is mainly due to early tumor detection [2–4]. In normal human somatic cells, critically short telomeres are suggested to cause irreversible cell growth arrest and cellular senescence [5]. In contrast, most human cancer cells have mechanisms that compensate for telomerase shortening, mainly through the activation of telomerase [6]. Telomerase can allow them to stably maintain their telomeres and grow permanently. The essential role of telomerase in cancer and aging makes it an important target for the development of therapies to treat cancer and other age-associated disorders.

Oxadiazole derivatives play a significant role in various pharmaceutical applications [7–11]. As an important class of heterocyclic compound, 1,3,4-oxadiazoles show a broad spectrum of bioactivities [12–17]. Further, 1,3,4-oxadiazole heterocycles are very good bioisosteres of amides and esters, which can contribute substantially in increasing pharmacological activity by participating in hydrogen bonding interactions with the receptors [18]. In particularly, among these, a few differently substituted 1,3,4-oxadiazoles have exhibited potent antitumor activities [19–21]. Besides, quinoline derivatives have attracted significant interest in pharmaceutical field as anticancer agents [22–25].

Therefore, in this paper, we described the synthesis and the structural relationships (SAR) of a series of novel quinoline analogs possessing 1,3,4-oxadiazole moiety as potential antitumor agents, which were based on molecular modeling and the investigation of SAR between new inhibitors and the X-ray crystallographic structure of the telomerase. To the best of our knowledge, the synthesis and anticancer activities of these compounds have not been reported so far.

2. Results and discussion

2.1. Chemistry

Nineteen quinoline analogs were synthesized to screen for the antitumor activity. All of them were synthesized for the first time. The synthesis of compounds **4a**–**4o** followed the general pathway outlined in Scheme 1. The key compound 5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione **3** was prepared as previously described [26,27]. Firstly, the quinoline-2-carboxylic acid on treatment with methanol containing concentrated H_2SO_4 was refluxed overnight. This step can yield the corresponding ester. Secondly, the ester was treated with hydrazine hydrate in ethanol overnight, refluxing.

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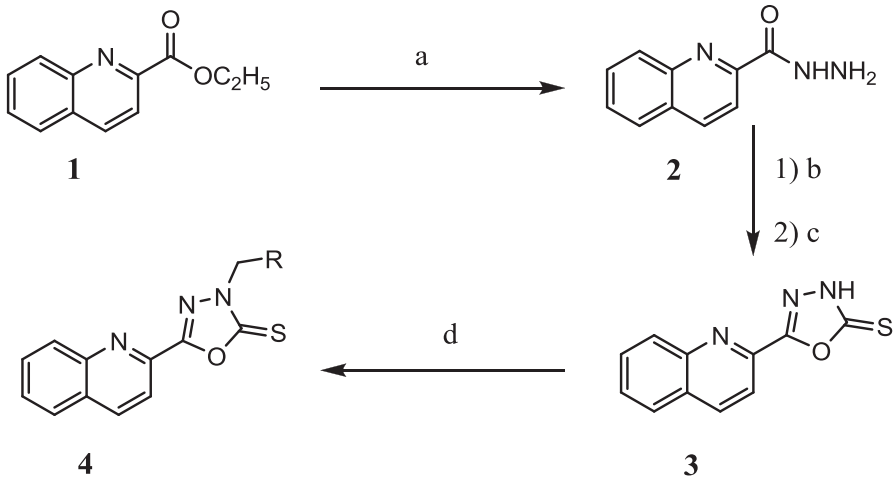

4a R = -NH-C_6H_5
4b R = -NH-C_6H_4 (4-CH_3)
4c R = -NH-C_6H_4 (2-OC_2H_5)
4d R = -NH-C_6H_4 (2-F)
4e R = -NH-C_6H_4 (3-F)
4f R = -NH-C_6H_4 (4-F)
4g R = -NH-C_6H_4 (2-Cl)
4h R = -NH-C_6H_4 (3-Cl)
4i R = -NH-C_6H_4 (4-Cl)
4j R = -NH-C_6H_4 (2-Br)
4k R = -N(CH_3)-C_6H_5
4l R = -N(C_2H_5)-C_6H_5
4m R = -NH-(2-pyridine)
4n R = 1-morpholine
4o R = 1-phenylpiperazine

Scheme 1. General synthesis of compounds **4a**–**4o**. Reagents and conditions: (a) 85% $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 8 h; (b) CS_2/KOH, ethanol (95%), reflux, 24 h; (c) HCl, pH = 5–6; (d) HCHO 40%, different amines.

Thirdly, treatment of the hydrazide **2** with carbon disulfide in the presence of 95% ethanol and potassium hydroxide provided the key intermediate **3**. Then, compounds **4a**–**4o** were obtained by treated with different primary and secondary amines in the presence of formaldehyde in anhydrous ethanol.

All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were full accordance with their depicted structures.

2.2. Biological activity

2.2.1. Cell proliferation assay (MTT method)

All the synthesized derivatives **4a**–**4o** were evaluated for their antiproliferative activity against HepG2 (human hepatoma cells), SGC-7901 (human gastric cancer cells) and MCF-7 (human breast cancer cells) cell lines. The results were shown in Table 1. As evidenced from Table 1, in general our compounds showed comparable activities to 5-Fluorouracil (5-Fu), with the exception of compounds **4d**–**4f**, **4i** and **4l** that showed a greater anticancer activity against one or more of the cell lines used. Most significantly, compounds **4d** and **4i** displayed the most potent anticancer activities.

Structure–activity relationships in these oxadiazole derivatives demonstrated that compounds having halogen atom substituent exhibited high activity, ranging from 0.8 to 26.9 μM, with regard to broad-spectrum antitumor activity. Meanwhile, a comparison of the substitution on the benzene ring was demonstrated as follows: when the compounds were fluorine-substituted derivatives, the potency order was *ortho* > *meta* > *para*, while when the compounds were chlorine-substituted derivatives, the potency order was *para* > *meta* > *ortho*. Interestingly, among the halogen-substituent compounds, the potency order was fluorine > chlorine > bromine.

2.2.2. Telomerase inhibitory assay

The telomerase inhibitory potency of the quinoline derivatives was examined and the results were summarized in Table 2. Most of the tested compounds displayed a potent telomerase inhibitory effect. Among them, compound **4d** and **4i** showed the most potent inhibitory with IC_{50} ranging from 0.8 to 0.9 μM. The results of telomerase inhibitory activity of the tested compounds correlated with the structural relationships (SAR) of their inhibitory effects on the cell proliferation assay. This suggests that the potent inhibitory effects of the synthetic compounds on the cell proliferation assay were causally related to their telomerase inhibitory activities.

2.3. Binding model of compounds into telomerase structure

In an effort to elucidate the possible mechanism by which the title compounds can induce anticancer activity and guide further SAR studies, molecular docking of the potent inhibitors **4d** and **4i**

Table 1
In vitro anticancer activities (IC_{50}, μM) of compounds **4a**–**4o** against human tumor cell lines.

Compounds	IC_{50} (μM)		
	HepG2	SGC-7901	MCF-7
4a	35.6 ± 2.3	41.8 ± 3.0	35.8 ± 2.5
4b	29.8 ± 1.8	32.7 ± 1.7	20.2 ± 1.3
4c	13.7 ± 1.1	31.1 ± 2.0	19.3 ± 0.8
4d	1.2 ± 0.2	8.3 ± 1.6	6.8 ± 0.5
4e	11.3 ± 1.5	12.9 ± 1.3	15.0 ± 0.9
4f	17.3 ± 2.0	18.5 ± 0.8	20.7 ± 1.4
4g	25.7 ± 1.9	26.9 ± 2.3	24.8 ± 2.8
4h	18.1 ± 2.1	17.7 ± 1.6	16.8 ± 2.3
4i	0.8 ± 0.2	7.6 ± 1.0	7.1 ± 0.8
4j	30.7 ± 2.5	45.5 ± 2.9	34.6 ± 1.7
4k	28.1 ± 2.3	40.8 ± 2.5	22.1 ± 1.9
4l	20.6 ± 0.5	22.7 ± 1.7	15.3 ± 1.2
4m	27.6 ± 1.9	40.3 ± 2.8	24.8 ± 2.2
4n	24.9 ± 1.8	39.6 ± 2.7	20.5 ± 1.8
4o	15.2 ± 1.3	34.8 ± 2.5	18.9 ± 1.7
5-Fluorouracil[a]	21.9 ± 1.4	28.5 ± 2.0	17.2 ± 1.5

[a] Used as a positive control.

Table 2
Telomerase inhibitory activity of compounds **4a**–**4o**.

Compounds	Telomerase inhibitation IC_{50} (μM) ± SD
4a	12.8 ± 0.5
4b	7.3 ± 0.3
4c	2.8 ± 0.2
4d	0.8 ± 0.1
4e	1.1 ± 0.3
4f	3.1 ± 0.8
4g	5.5 ± 0.7
4h	3.0 ± 0.1
4i	0.9 ± 0.0
4j	10.8 ± 0.5
4k	9.3 ± 0.2
4l	5.1 ± 0.4
4m	3.1 ± 0.4
4n	2.9 ± 0.3
4o	4.8 ± 0.1
Staurosporine[a]	8.3 ± 0.8

[a] Used as a positive control.

into the binding site of telomerase were performed on the binding model based on the telomerase complex structure (3DU6, pdb). Binding models of compounds **4d** and **4i** and telomerase were depicted in Figs. 1 and 2. In the binding model, compound **4d** is nicely bound to the telomerase with its nitrogen atom of the quinoline ring project toward the amino hydrogen of ARG 194, with the hydroxyl group forming a more optimal H-bond interaction. Besides, both the fluorine atom of the benzene ring with amino hydrogen of LYS 189 and the nitrogen atom of NH with an oxygen atom of ASP 343 have optimal H-bond interactions. Compound **4i** is nicely bound to the telomerase with its nitrogen atom of oxadiazole ring project toward the amino hydrogen of GLN 308, with the hydroxyl group forming a more optimal H-bond interaction. Besides, oxygen atom of oxadiazole ring project toward the amino hydrogen of ARG 194, with the hydroxyl group forming a more optimal H-bond interaction as well. Meanwhile, π-cation interaction was shown as orange line (in the web version).

3. Conclusion

A series of 5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione quinoline derivatives have been synthesized and evaluated for their antitumor activities against HepG2, SGC-7901 and MCF-7 cell lines. Preliminary results showed that most of the compounds displayed enhanced inhibitory activities. Of all the studied compounds, compounds having fluoro substituent at *ortho* position (**4d**) and having chloro substituent at the *para* position (**4i**) of anilines displayed the most potent anticancer activities. Docking simulation was performed to position compounds **4d** and **4i** into the active site of telomerase (3DU6) to determine the probable binding model. Antiproliferative and enzyme assay results suggested that compounds **4d** and **4i** were potential antitumor agents. Considering the results mentioned above, it could be concluded that some quinoline derivatives are good candidates for antitumor agents screening and research. The template quinoline with 1,3,4-oxadiazole moiety was suitable to reconstruct and design for development of more potential therapeutic drugs against cancer. Further research would be ongoing for the synthesis and discovery of more enhanced quinoline derivatives with outstanding anticancer activity and low toxicity and side-effects.

4. Experimental

4.1. Chemistry

All chemicals and reagents used in the current study were of analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points (uncorrected) were determined on a XT4MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, and ^{1}H NMR spectra were collected on a Bruker DPX300 spectrometer at room temperature with TMS and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument, and were within ±0.4% of the theoretical values.

4.1.1. General procedure for the preparation of target compounds ***4a*–*4o***

Formaldehyde (40%, 1.5 mmol) was added to a stirred solution of 5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3*H*)-thione **3** (1.5 mmol) in anhydrous ethanol (20 mL). Anhydrous ethanol (5 mL) containing the appropriate amine (1.5 mmol) was added dropwise to the reaction mixture stirred for 10–15 h and then left overnight at room temperature. The resulting solid was collected and washed with cold ethanol, dried and crystallized from anhydrous ethanol to get the desired compounds **4a**–**4o**.

*4.1.1.1. 3-((Phenylamino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4a**).* White crystals, yield: 72%, mp: 153–155 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.27 (brs, 1H), 5.66 (s, 2H), 6.85 (t,

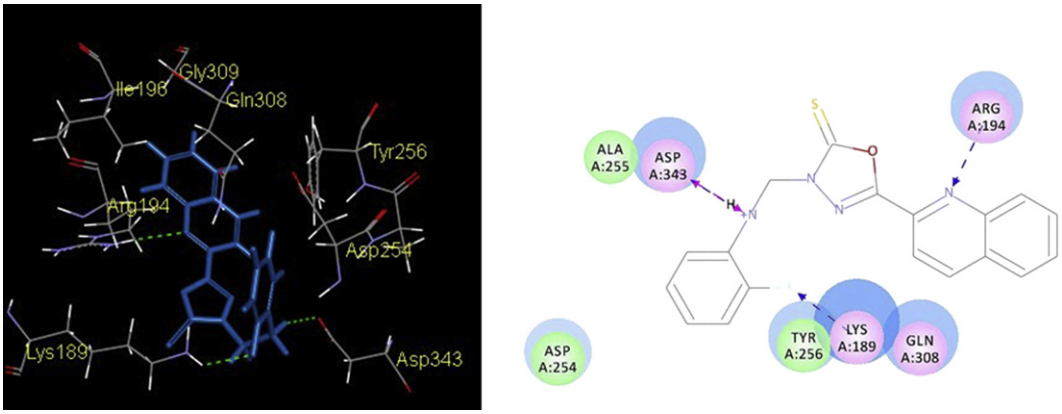

Fig. 1. Molecular docking modeling of compound **4d** with telomerase: for clarity, only interacting residues are displayed. Left: 3D model of the interaction between compound **4d** and the telomerase binding site. Right: 2D model of the interaction between compound **4d** and the telomerase binding site.

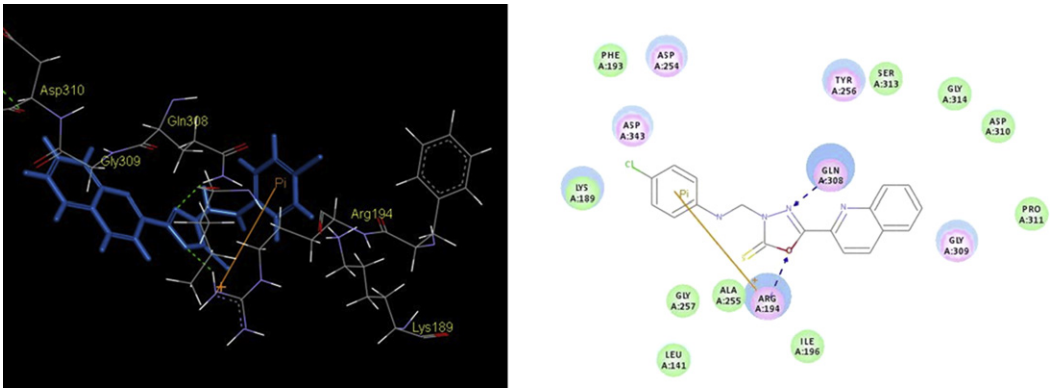

Fig. 2. Molecular docking modeling of compound **4i** with telomerase: for clarity, only interacting residues are displayed. Left: 3D model of the interaction between compound **4i** and the telomerase binding site. The π-cation interactions are shown as orange lines. Right: 2D model of the interaction between compound **4i** and the telomerase binding site. The π-cation interactions are shown as orange line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

J = 7.5 Hz, 1H), 6.98 (d, J = 7.5 Hz, 2H), 7.25 (t, J = 7.5 Hz, 2H), 7.69 (t, J = 7.5 Hz, 1H), 7.84 (t, J = 7.5 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H), 8.29 (d, J = 8.5 Hz, 1H), 8.35 (d, J = 8.5 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 177.4, 150.3, 150.2, 149.6, 148.3, 137.4, 134.5, 129.6, 129.5, 129.3, 127.7, 127.4, 126.9, 123.2, 118.7, 118.3, 112.9, 60.3. MS (ESI): 334.11, 228.33, 170.15, 128.23, 106.07, 76.11. ($C_{18}H_{15}N_4OS$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{14}N_4OS$: C, 64.65; H, 4.22; N, 16.75; Found: C, 64.58; H, 4.16; N, 16.84.

*4.1.1.2. 5-(Quinolin-2-yl)-3-((p-tolylamino)methyl)-1,3,4-oxadiazole-2(3H)-thione (**4b**).* Yellow crystals, yield: 75%, mp: 170–172 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.24 (s, 3H), 5.17 (brs, 1H), 5.64 (d, J = 7.0 Hz, 2H), 6.89 (d, J = 8.0 Hz, 2H), 7.05 (d, J = 8.0 Hz, 2H), 7.68 (t, J = 7.5 Hz, 1H), 7.84 (t, J = 7.5 Hz, 2H), 7.90 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.5 Hz, 1H), 8.29 (d, J = 8.5 Hz, 1H), 8.34 (d, J = 8.5 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 178.5, 149.6, 149.3, 149.1, 147.4, 137.6, 135.5, 130.6, 129.2, 128.4, 127.7, 127.3, 125.9, 124.2, 119.2, 117.3, 113.4, 59.3, 22.3. MS (ESI): 348.22, 228.17, 170.22, 128.09, 120.17, 76.21. ($C_{19}H_{17}N_4OS$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{16}N_4OS$: C, 65.50; H, 4.63; N, 16.08; Found: C, 65.61; H, 4.62; N, 16.01.

*4.1.1.3. 3-(((2-Ethoxyphenyl)amino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4c**).* Light yellow crystals, yield: 80%, mp: 184–185 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.25 (t, J = 7.1 Hz, 3H), 4.12 (q, J = 7.1 Hz, 2H), 5.68 (s, 2H), 5.85 (s, 1H), 6.75–6.79 (m, 2H), 6.87–6.91 (m, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.65 (t, J = 7.0 Hz, 1H), 7.81 (t, J = 7.0 Hz, 1H), 7.88 (d, J = 8.5 Hz, 1H), 8.01 (d, J = 8.5 Hz, 1H), 8.26 (d, J = 8.4 Hz, 1H), 8.32 (d, J = 8.5 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 175.4, 154.2, 152.2, 150.6, 149.3, 139.5, 137.4, 130.8, 130.3, 129.5, 128.8, 127.6, 125.4, 122.8, 121.9, 118.3, 111.4, 61.2. MS (ESI): 378.39, 349.41, 228.31, 128.10, 121.20, 76.45. ($C_{20}H_{19}N_4O_2S$, $[M + H]^+$). Anal. Calcd for $C_{20}H_{18}N_4O_2S$: C, 63.47; H, 4.79; N, 14.80; Found: C, 63.40; H, 4.80; N, 14.88.

*4.1.1.4. 3-(((2-Fluorophenyl)amino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4d**).* Light yellow powder, yield: 71%, mp: 182–183 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.48 (brs, 1H), 5.69 (d, J = 7.5 Hz, 2H), 6.76–6.81 (m, 1H), 7.00–7.04 (m, 1H), 7.07 (t, J = 8.0 Hz, 1H), 7.29–7.31 (m, 1H), 7.69 (t, J = 8.0 Hz, 1H), 7.84 (t, J = 8.0 Hz, 1H), 7.91 (d, J = 8.5 Hz, 1H), 8.04 (d, J = 8.5 Hz, 1H), 8.29 (d, J = 8.5 Hz, 1H), 8.35 (d, J = 8.5 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 176.4, 163.3, 148.2, 147.9, 137.1, 135.4, 133.5, 128.1, 127.3, 127.0, 125.7, 125.1, 123.9, 123.2, 116.4, 115.3, 114.2, 58.8. MS (ESI): 352.19, 228.29, 170.27, 128.07, 124.11, 76.26. ($C_{18}H_{14}FN_4OS$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{13}FN_4OS$: C, 61.35; H, 3.72; N, 15.90; Found: C, 61.45; H, 3.77; N, 15.81.

*4.1.1.5. 3-(((3-Fluorophenyl)amino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4e**).* White powder, yield: 73%, mp: 191–193 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.31 (t, J = 8.0 Hz, 1H), 5.60 (d, J = 8.0 Hz, 2H), 6.52 (t, J = 7.3 Hz, 1H), 6.68–6.72 (m, 2H), 7.12–7.20 (m, 1H), 7.66 (t, J = 7.3 Hz, 1H), 7.82 (t, J = 7.3 Hz, 1H), 7.89 (d, J = 8.5 Hz, 1H), 8.02 (d, J = 8.6 Hz, 1H), 8.27 (d, J = 8.4 Hz, 1H), 8.34 (d, J = 8.6 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 175.4, 163.7, 152.2, 150.6, 149.3, 136.6, 135.4, 129.8, 129.2, 128.0, 127.7, 127.2, 126.9, 116.2, 115.9, 111.7, 108.8, 60.3. MS (ESI): 352.11, 228.31, 170.22, 128.24, 124.26, 76.12. ($C_{18}H_{14}FN_4OS$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{13}FN_4OS$: C, 61.35; H, 3.72; N, 15.90; Found: C, 61.46; H, 3.70; N, 15.83.

*4.1.1.6. 3-(((4-Fluorophenyl)amino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4f**).* White powder, yield: 70%, mp: 177–179 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.19 (brs, 1H), 5.62 (s, 2H), 6.90–6.97 (m, 4H), 7.69 (t, J = 7.0 Hz, 1H), 7.83 (t, J = 7.0 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H), 8.29 (d, J = 8.5 Hz, 1H), 8.36 (d, J = 8.5 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 176.2, 155.8, 152.3, 149.6, 149.1, 137.4, 134.5, 129.6, 129.5, 129.3, 128.7, 127.4, 124.8, 124.4, 116.9, 116.3, 115.9, 61.4. MS (ESI): 352.28, 228.18, 170.15, 128.09, 124.21, 76.08. ($C_{18}H_{14}FN_4OS$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{13}FN_4OS$: C, 61.35; H, 3.72; N, 15.90; Found: C, 61.22; H, 3.78; N, 15.92.

*4.1.1.7. 3-(((2-Chlorophenyl)amino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4g**).* White powder, yield: 77%, mp: 174–175 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.72 (d, J = 7.5 Hz, 2H), 5.83 (t, J = 7.5 Hz, 1H), 6.78 (t, J = 7.0 Hz, 1H), 7.21–7.32 (m, 3H), 7.69 (t, J = 7.5 Hz, 1H), 7.84 (t, J = 7.5 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 8.5 Hz, 1H), 8.29 (d, J = 8.5 Hz, 1H), 8.36 (d, J = 8.5 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 177.2, 153.3, 149.6, 148.4, 145.3, 136.4, 131.7, 131.4, 131.1, 129.5, 129.2, 127.9, 124.6, 123.2, 120.2, 119.3, 115.7, 58.5. MS (ESI): 368.68, 228.45, 170.32, 140.50, 128.12, 76.16. ($C_{18}H_{14}ClN_4OS$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{13}ClN_4OS$: C, 58.61; H, 3.55; N, 15.19; Found: C, 58.75; H, 3.55; N, 15.10.

*4.1.1.8. 3-(((3-Chlorophenyl)amino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4h**).* Light yellow powder, yield: 70%, mp: 171–173 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.28 (brs, 1H), 5.60 (d, J = 7.3 Hz, 2H), 6.78–6.86 (m, 2H), 6.97 (t, J = 2.0 Hz, 1H), 7.13 (t, J = 8.1 Hz, 1H), 7.66 (t, J = 7.0 Hz, 1H), 7.82 (t, J = 7.0 Hz, 1H), 7.88 (d, J = 8.2 Hz, 1H), 8.01 (d, J = 8.6 Hz, 1H), 8.26 (d, J = 8.6 Hz, 1H), 8.34 (d, J = 8.6 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 177.3, 152.2, 150.6, 149.5, 149.3, 136.4, 135.4, 133.1, 130.8, 130.4, 129.6, 129.3, 128.9, 122.8, 121.1, 117.5, 115.7, 60.4. MS (ESI): 368.74, 228.25, 170.18,

140.50, 128.29, 76.24. ($C_{18}H_{14}ClN_4OS$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{13}ClN_4OS$: C, 58.61; H, 3.55; N, 15.19; Found: C, 58.55; H, 3.49; N, 15.28.

*4.1.1.9. 3-(((4-Chlorophenyl)amino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4i**).* Light yellow crystals, yield: 75%, mp: 205–208 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.25 (brs, 1H), 5.60 (d, J = 7.5 Hz, 2H), 6.89 (d, J = 8.9 Hz, 2H), 7.17 (d, J = 8.9 Hz, 2H), 7.66 (t, J = 8.0 Hz, 1H), 7.82 (t, J = 8.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 8.6 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.33 (d, J = 8.6 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 173.8, 149.4, 148.6, 147.3, 116.2, 134.4, 133.4, 128.8, 128.3, 127.6, 126.9, 125.8, 124.7, 123.3, 123.5, 122.8, 113.7, 58.3. MS (ESI): 368.58, 228.13, 170.22, 140.48, 128.32, 76.11. ($C_{18}H_{14}ClN_4OS$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{13}ClN_4OS$: C, 58.61; H, 3.55; N, 15.19; Found: C, 58.73; H, 3.51; N, 15.09.

*4.1.1.10. 3-(((2-Bromophenyl)amino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4j**).* White powder, yield: 80%, mp: 188–191 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.71 (d, J = 8.0 Hz, 2H), 5.82 (t, J = 8.0 Hz, 1H), 6.72 (t, J = 7.0 Hz, 1H), 7.25–7.31 (m, 2H), 7.47 (d, J = 7.0 Hz, 1H), 7.69 (t, J = 8.0 Hz, 1H), 7.85 (t, J = 8.0 Hz, 1H), 7.91 (d, J = 8.5 Hz, 1H), 8.04 (d, J = 8.5 Hz, 1H), 8.29 (d, J = 8.5 Hz, 1H), 8.36 (d, J = 8.5 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 174.3, 150.3, 147.6, 146.3, 135.7, 135.3, 134.3, 132.7, 128.5, 127.7, 127.4, 126.9, 125.9, 125.4, 125.1, 124.9, 114.7, 56.9. MS (ESI): 413.18, 228.12, 185.17, 170.21, 128.30, 76.31. ($C_{18}H_{14}BrN_4OS$, $[M + H]^+$). Anal. Calcd for $C_{18}H_{13}BrN_4OS$: C, 52.31; H, 3.17; N, 13.56; Found: C, 52.19; H, 3.21; N, 13.65.

*4.1.1.11. 3-((Methyl(phenyl)amino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4k**).* White crystals, yield: 81%, mp: 158–159 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.34 (s, 3H), 5.77 (s, 2H), 6.86 (t, J = 7.3 Hz, 1H), 7.09 (d, J = 7.3 Hz, 2H), 7.28–7.33 (m, 2H), 7.65 (t, J = 7.0 Hz, 1H), 7.80 (t, J = 7.0 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 8.6 Hz, 1H), 8.24 (d, J = 8.4 Hz, 1H), 8.29 (d, J = 8.4 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 175.3, 159.8, 151.2, 149.6, 148.3, 136.4, 135.4, 132.7, 131.5, 131.0, 129.9, 129.4, 128.6, 128.1, 116.8, 116.0, 115.7, 72.1, 36.2. MS (ESI): 348.28, 228.23, 170.17, 128.34, 120.16, 76.22. ($C_{19}H_{17}N_4OS$, $[M + H]^+$). Anal. Calcd for $C_{19}H_{16}N_4OS$: C, 65.50; H, 4.63; N, 16.08; Found: C, 65.38; H, 4.58; N, 16.12.

*4.1.1.12. 3-((Ethyl(phenyl)amino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4l**).* Light yellow powder, yield: 76%, mp: 157–158 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32 (t, J = 7.1 Hz, 3H), 3.79 (q, J = 7.1 Hz, 2H), 5.74 (s, 2H), 6.85 (t, J = 7.3 Hz, 1H), 7.10 (d, J = 8.0 Hz, 2H), 7.27–7.33 (m, 2H), 7.65 (t, J = 7.0 Hz, 1H), 7.81 (t, J = 7.0 Hz, 1H), 7.87 (d, J = 8.2 Hz, 1H), 8.01 (d, J = 8.6 Hz, 1H), 8.23–8.31 (m, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 176.3, 160.9, 152.2, 150.6, 149.3, 137.4, 136.6, 131.9, 131.5, 131.1, 130.8, 130.2, 129.6, 128.7, 121.4, 120.8, 116.7, 70.8, 48.6, 14.9. MS (ESI): 362, 39, 228.18, 170.26, 134.29, 128.44, 76.21. ($C_{20}H_{19}N_4OS$, $[M + H]^+$). Anal. Calcd for $C_{20}H_{18}N_4OS$: C, 66.28; H, 5.01; N, 15.46; Found: C, 66.41; H, 4.98; N, 15.55.

*4.1.1.13. 3-((Pyridin-2-ylamino)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4m**).* Yellow powder, yield: 79%, mp: 154–155 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.80–5.87 (m, 3H), 6.71–6.81 (m, 2H), 7.51 (t, J = 8.0 Hz, 1H), 7.65 (t, J = 7.0 Hz, 1H), 7.81 (t, J = 7.1 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 8.6 Hz, 1H), 8.18 (d, J = 5.0 Hz, 1H), 8.26 (d, J = 8.6 Hz, 1H), 8.32 (d, J = 8.6 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 176.1, 173.2, 152.3, 149.4, 148.2, 144.5, 140.0, 137.1, 135.7, 131.5, 130.7, 130.2, 128.9, 117.0, 114.7, 111.6, 57.1. MS (ESI): 335.18, 228.06, 170.11, 128.14, 107.24, 77.06. ($C_{17}H_{14}N_5OS$, $[M + H]^+$). Anal. Calcd for $C_{17}H_{13}N_5OS$: C, 60.88; H, 3.91; N, 20.88; Found: C, 61.02; H, 3.90; N, 20.82.

*4.1.1.14. 3-(Morpholinomethyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4n**).* White crystals, yield: 85%, mp: 175–176 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.94 (t, J = 4.5 Hz, 4H), 3.75 (t, J = 4.5 Hz, 4H), 5.18 (s, 2H), 7.70 (t, J = 7.5 Hz, 1H), 7.85 (t, J = 7.5 Hz, 1H), 7.93 (d, J = 7.5 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 8.30 (d, J = 8.0 Hz, 1H), 8.38 (d, J = 8.0 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 177.7, 159.8, 150.6, 148.5, 136.4, 135.1, 130.8, 130.1, 129.4, 128.9, 115.6, 79.0, 67.4, 67.1, 50.7, 50.3. MS (ESI): 328.19, 228.15, 170.09, 128.23, 100.04, 44.26. ($C_{16}H_{17}N_4O_2S$, $[M + H]^+)^+$. Anal. Calcd for $C_{16}H_{16}N_4O_2S$: C, 58.52; H, 4.91; N, 17.06; Found: C, 58.36; H, 4.89; N, 17.12.

*4.1.1.15. 3-((4-Phenylpiperazin-1-yl)methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (**4o**).* White powder, yield: 88%, mp: 177–178 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.08 (t, J = 4.2 Hz, 4H), 3.22 (t, J = 4.2 Hz, 4H), 5.24 (s, 2H), 6.82–6.92 (m, 3H), 7.22–7.24 (m, 2H), 7.67 (t, J = 7.1 Hz, 1H), 7.82 (t, J = 7.1 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 8.06 (d, J = 8.6 Hz, 1H), 8.28 (d, J = 8.6 Hz, 1H), 8.35 (d, J = 8.6 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 176.3, 152.6, 151.7, 149.7, 147.3, 134.4, 133.3, 131.8, 131.4, 129.7, 129.1, 128.9, 126.9, 121.3, 117.5, 117.1, 114.7, 70.1, 55.3, 54.9, 49.5, 49.1. MS (ESI): 403.28, 228.21, 175.19, 170.18, 128.20, 84.11, 76.23. ($C_{22}H_{22}N_5OS$, $[M + H]^+$). Anal. Calcd for $C_{22}H_{21}N_5OS$: C, 65.49; H, 5.25; N, 17.36; Found: C, 65.62; H, 5.22; N, 17.28.

4.2. Cell proliferation assay

The *in vitro* anticancer activities of the prepared compounds **4a**–**4o** against HepG2, SGC-7901 and MCF-7 cell lines were evaluated as described in the literature [28] with some modifications. Target tumor cells were grown to log phase in *RPMI 1640* medium supplemented with 10% fetal bovine serum. After reaching a dilution of 3×10^4 cells mL^{-1} with the medium, 100 μL of the obtained cell suspension was added to each well of 96-well culture plates. Subsequently, incubation was performed at 37 °C in 5% CO_2 atmosphere for 48 h before the cytotoxicity assessment. Tested samples at pre-set concentrations were added to 6 wells with 5-fluorouracil being employed as a positive reference. After 72 h exposure period, 25 μL of PBS containing 2.5 mg mL^{-1} of MTT was added to each well. After 4 h, the medium was replaced by 150 μL DMSO to dissolve the purple formazan crystals produced. The absorbance at 570 nm of each well was measured with an ELISA plate reader. The data represented the mean of three independent experiments in triplicate and were expressed as means ± SD. The IC_{50} value was defined as the concentration at which 50% of the cells could survive.

4.3. Telomerase inhibitory assay

Fifteen oxadiazole derivatives containing quinoline moiety were tested in a search for small molecule inhibitors of telomerase using the TRAP-PCR-ELISA assay. In detail, the HepG2 cells were firstly maintained in DMEM medium (GIBCO, New York, USA) supplemented with 10% fetal bovine serum (GIBCO, New York, USA), streptomycin (0.1 mg/mL) and 420 penicillin (100 IU/mL) at 37 °C in a humidified atmosphere containing 5% CO_2. After trypsinization, 5×10^4 cultured cells in logarithmic growth were seeded into T25 flasks (Corning, New York, USA) and cultured to allow for adherence. The cells were then incubated with Staurosporine (Santa Cruz, Santa Cruz, USA) and the drugs with a series of concentration as 60, 20, 6.67, 2.22, 0.74, 0.25 and 0.082 μg/mL, respectively. After 48 h treatment, the cells were harvested by cell scraper orderly following by washed once with PBS. The cells were lysed in 150 μL RIPA cell lysis buffer (Santa Cruz, Santa Cruz, USA), and incubated on ice for 30 min. The cellular supernatants were obtained via centrifugation 430 at 12,000 *g* for 20 min at 4 °C and stored at −80 °C.

The TRAP-PCR-ELISA assay was performed using a telomerase detection kit (Roche, Basel, Switzerland) according to the

manufacturer's protocol. In brief, 2 μL of cell extracts were mixed with 48 μL TRAP reaction mixtures. PCR was then initiated at 94 °C, 120 s for pre-denaturation and performed using 35 cycles each consisting of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 90 s. Then 20 μL of PCR products were hybridized to a digoxigenin (DIG)-labeled telomeric repeat specific detection probe. And the PCR products were immobilized via the biotin-labeled primer to a streptavidin-coated 440 microtiter plate subsequently. The immobilized DNA fragment was detected with a peroxidase-conjugated anti-DIG antibody and visualized following addition of the stop regent. The microtitre plate was assessed on TECAN Infinite M 200 microplate reader (Männedorf, Switzerland) at a wavelength of 570 nm, and the final value was presented as mean ± SD.

4.4. Experimental protocol of docking study

Automated docking studies were carried out using Discovery Studio (version 3.1) as implemented through the graphical user interface DS-CDocker protocol.

The three-dimensional structures of the aforementioned compounds were constructed using Chem. 3D ultra 11.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2009)], then they were energetically minimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10. The Gasteiger–Hückel charges of ligands were assigned. The crystal structures of telomerase (PDB code: 3DU6) complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). All bound water and ligands were eliminated from the protein and the polar hydrogens and the Kollman-united charges were added to the proteins.

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