Bioorganic & Medicinal Chemistry 18 (2010) 7836–7841

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis, biological evaluation, and molecular docking studies of 2-chloropyridine derivatives possessing 1,3,4-oxadiazole moiety as potential antitumor agents

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ARTICLE INFO

Article history:
Received 19 August 2010
Revised 19 September 2010
Accepted 21 September 2010
Available online 25 September 2010

Keywords:
Synthesis
2-Chloropyridine
1,3,4-Oxadiazole
Molecular docking
Antitumor

ABSTRACT

A series of new 2-chloropyridine derivatives possessing 1,3,4-oxadiazole moiety were synthesized. Antiproliferative assay results indicated that compounds **6o** and **6u** exhibited the most potent activity against gastric cancer cell SGC-7901, which was more potent than the positive control. Especially, compound **6o** exhibited significant telomerase inhibitory activity (IC_{50} = 2.3 ± 0.07 μM), which was comparable to the positive control ethidium bromide. Docking simulation was performed to position compound **6o** into the active site of telomerase (3DU6) to determine the probable binding model.

1. Introduction

Telomerase remains active in the early stages of life maintaining telomere length and the chromosomal integrity of frequently dividing cells. It turns dormant in most somatic cells during adulthood.[1] In cancer cells, however, telomerase gets reactivated and works tirelessly to maintain the short length of telomeres of rapidly dividing cells, leading to their immortality.[2] The essential role of telomerase in cancer and ageing makes it an important target for the development of therapies to treat cancer and other age-associated disorders. Telomere and telomerase are closely related to the occurrence and development of gastric cancer.[3]

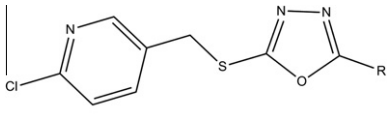
Pyridine derivatives have attracted significant interest in pharmaceutical and agrochemical fields.[4,5] Furthermore, 2-pyridone, a small bioactive molecule, is an important pharmacophore that can form hydrogen bonded structures similar to those encountered with the base-pairing mechanism in DNA and RNA.[6,7] Besides, 1,3,4-oxadiazoles are an important class of heterocyclic compounds. The widespread use of them as a scaffold in medicinal chemistry establishes this moiety as a member of the privileged structures class.[8] They possess a variety of biological activities.[9–13] In particular, a few differently substituted 1,3,4-oxadiazoles have been found to exhibit anticancer activities.[14–16] 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.[17,18]

In view of above mentioned facts and an attempt to achieve new potent antitumor agents with good bioavailability and low toxicity, herein, we describe the synthesis and the SAR of a series of new 2-chloropyridine derivatives possessing 1,3,4-oxadiazole moiety (Fig. 1) as potential antitumor agents. Docking simulations were performed using the X-ray crystallographic structure of the telomerase in complex with an inhibitor to explore the binding modes of these compounds at the active site.

2. Results and discussion

2.1. Chemistry

The synthetic route for the preparation of new 2-chloropyridine derivatives **6a–6u** is outlined in Scheme 1. The synthesis of these



R = Ar and Ar_{Het}

Figure 1. The skeleton of target compounds.

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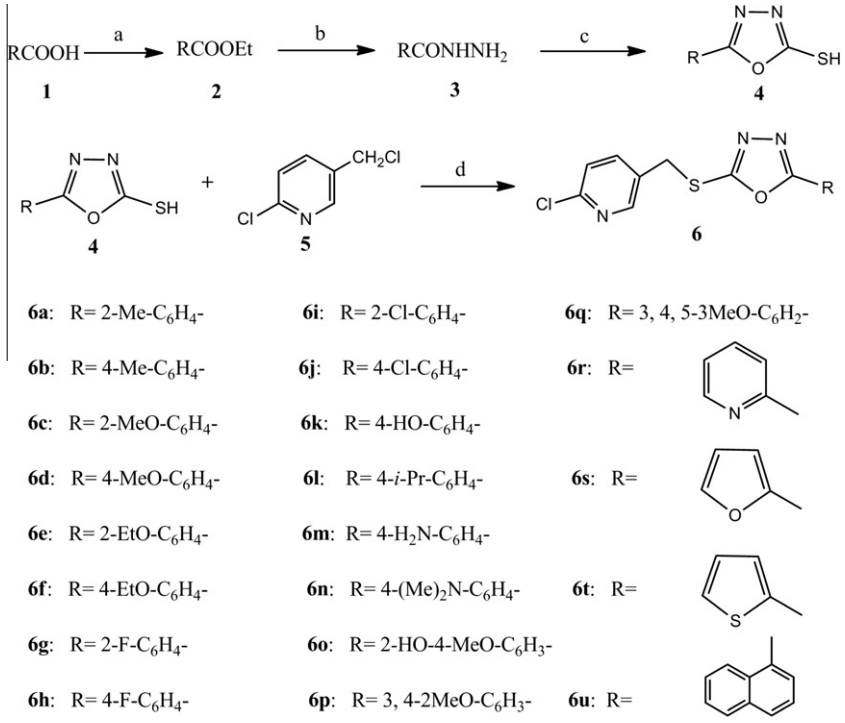
doi:10.1016/j.bmc.2010.09.051



Scheme 1. Synthesis of compounds **6a–6u**. Reagents and conditions: (a) H_2SO_4 (concd), ethanol, reflux, 8–12 h; (b) $NH_2NH_2{\cdot}H_2O$ (85%), ethanol, reflux, 8–12 h; (c) (1) CS_2/KOH, ethanol (95%), reflux, 24 h; (2) HCl, pH 5–6; (d) NaOH, acetonitrile, reflux, 8–24 h.

derivatives started from 5-substituted-2-mercapto-1,3,4-oxadiazoles **4** and 2-chloro-5-(chloromethyl)pyridine **5**.[19] The key intermediates **4** were prepared in three steps. Esterification of the carboxylic acids **1** with ethanol and concentrated sulfuric acid afforded the corresponding esters **2**. The aroyl hydrazides **3** were obtained by reaction of the esters **2** with 85% hydrazine monohydrate in ethanol. Treatment of the hydrazides **3** with carbon disulfide in the presence of KOH and 95% ethanol under reflux gave the key intermediates **4**. The synthesis of compounds **6a–6u** were accomplished by refluxing compounds **4** with 2-chloro-5-(chloromethyl)pyridine **5** in the presence of NaOH in acetonitrile. All the target compounds **6a–6u** were reported for the first time.

All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures. Additionally, the structure of compound **6d** was further confirmed by X-ray diffraction. Its crystal data are presented in Table 1, and Figure 2 gives a perspective view of this compound together with the atomic labeling system.

Table 1
Crystallographical and experimental data for compound **6d**

Compound	**6d**
Formula	$C_{15}H_{12}ClN_3O_2S$
M_r	333.79
Crystal system	Orthorhombic
Space group	*P bca*
a (Å)	12.311(3)
b (Å)	8.123(2)
c (Å)	29.956(6)
α (°)	90
β (°)	90
γ (°)	90
Volume (Å^3)	2995.7(1)
Z	8
D_c (g/cm^3)	1.480
μ (mm^{-1})	0.404
F(0 0 0)	1376
Crystal size (mm^3)	0.28 × 0.22 × 0.16
T (K)	293(2)
θ Range (°)	1.36/25.31
Index range (*h*, *k*, *l*)	−14/14, 0/9, 0/35
Reflections collected/unique	2730/1514
Data/restraints/parameters	1514/0/200
Goodness-of-fit on F^2	1.023
R_1, wR_2 [$I > 2\sigma(I)$][a]	0.0696/0.1613
R_1, wR_2[a]	0.1361/0.1940
$(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$ (e/Å^3)	0.427/−0.530

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w(|F_o|^2]^{1/2}$.

2.2. Biological activity

All the synthesized 2-chloropyridine derivatives **6a–6u** were evaluated for their ability to antiproliferative activity against gastric cell SGC-7901. The results were summarized in Table 2. A number of 2-chloropyridine derivatives possessing 1,3,4-oxadiazole moiety showed remarkable effects on antiproliferative activity. For compounds **6a–6f**, it could be observed that compounds with electron-donating groups on the *ortho* position of benzene ring (e.g., **6a**, **6c**, **6e**) displayed less inhibitory activity than those with the same groups on the *para* position (e.g., **6b**, **6d**, **6f**). Meanwhile, a comparison of the substitution on benzene ring demonstrated that *para* position-halogen-substituted derivatives (e.g., **6h**, **6j**) have more potent activity than that of *ortho* position-halogen-substituted derivatives (e.g., **6g**, **6i**), and the potency order is Cl < F. Moreover, a free hydroxyl group at the *para* position of C-5 phenyl ring of 1,3,4-oxadiazole ring was detrimental for the activity as evident from the activity of compound **6k**. Compared to the 2-Me analogue **6a**, compound **6l** with a *p*-i-Pr substituent displayed relatively good activity (IC_{50} = 4.32 ± 0.21 μg/mL). Notably, 3,4-dimethoxyl group was well tolerated (**6p**; IC_{50} = 4.48 ± 0.26 μg/mL). Besides, introduction of 4-amino, 4-dimethamino, or 3,4,5-trimethoxyl group to C-5 phenyl ring of 1,3,4-oxadiazole gives compound **6m**, **6n** or **6q** with reduced activity. Furthermore,

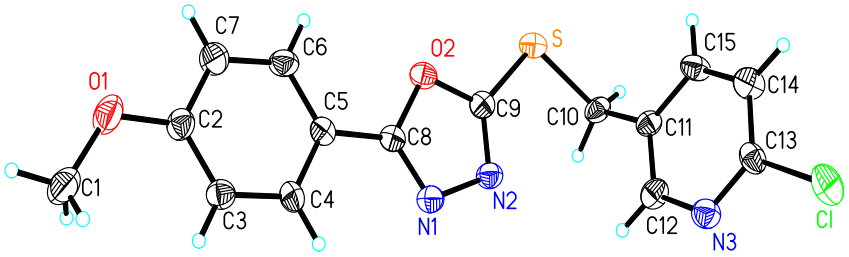


Figure 2. Molecular structure of compound **6d**.

Table 2
Inhibition (IC_{50}) of SGC-7901 cells proliferation by compounds **6a–6u**

Compound	SGC-7901 (IC_{50} ± SD, μg/mL)
6a	8.51 ± 0.56
6b	3.62 ± 0.21
6c	8.20 ± 0.48
6d	3.50 ± 0.13
6e	10.28 ± 0.67
6f	4.97 ± 0.27
6g	9.12 ± 0.55
6h	3.67 ± 0.20
6i	13.45 ± 1.0
6j	8.62 ± 0.63
6k	>20
6l	4.32 ± 0.21
6m	8.48 ± 0.38
6n	8.90 ± 0.41
6o	1.61 ± 0.06
6p	4.48 ± 0.26
6q	10.2 ± 0.75
6r	12.6 ± 0.92
6s	14.3 ± 1.1
6t	15.1 ± 1.3
6u	2.56 ± 0.11
5-Fluorouracil[a]	7.36 ± 0.55

[a] Used as a positive control.

variation of the aromatic ring moiety was also explored. As for compounds **6r–6u**, compared with compound **6o**, compound **6u** with a 1-naphthyl group was compatible with the most potent inhibitory activity. That is to say, compounds **6o** and **6u** exhibited the most potent activity (as for **6o**, IC_{50} = 1.61 ± 0.06 μg/mL; as for **6u**, IC_{50} = 2.56 ± 0.11 μg/mL), which was more potent than the positive control. Subsequently, these two compounds **6o** and **6u** which had the most potent inhibitory activity were selected to be evaluated for their ability to inhibit telomerase by a modified TRAP (telomere repeat amplification protocol). The results (Table 3) indicated that compounds **6o** exhibited significant telomerase inhibitory activity (IC_{50} = 2.3 ± 0.07 μM), which was comparable to the positive control ethidium bromide (IC_{50} = 2.5 ± 0.23 μM).

2.3. Molecular docking study of compound 6o

In an effort to elucidate the possible mechanism by which the title compounds can induce anticancer activity in the gastric cell SGC-7901 and guide further SAR studies, molecular docking of the potent inhibitor **6o** into ATP binding site of telomerase was performed on the binding model based on the telomerase structure (3DU6.pdb). The binding models of compound **6o** and telomerase are depicted in Figures 3 and 4. In the binding model, compound **6o** is nicely bound to telomerase with its hydroxyl group project toward the amino group of LYS 249, with the hydroxyl group forming a more optimal H-bond (N–H···O: 2.290 Å, 121.1°) interaction, and the hydrogen atom of hydroxyl group of **6o** also forms hydrogen bond (O–H···O: 1.993 Å, 147.7°) with oxygen atom of ASP 344. Meanwhile, the oxygen atom of methoxy group of **6o** forms another hydrogen bond (N–H···O: 2.248 Å, 134.3°) with amino hydrogen of GLY 391. The enzyme assay data and the molecular docking results suggested that compound **6o** was potential inhibitor of telomerase.

Table 3
Telomerase inhibitory activity of compounds **6o** and **6u**

Compound	Telomerase (IC_{50} ± SD, μM)
6o	2.3 ± 0.07
6u	4.2 ± 0.15
Ethidium bromide[a]	2.5 ± 0.23

[a] Used as a positive control.

3. Conclusion

A series of new 2-chloropyridine derivatives possessing 1,3,4-oxadiazole moiety were synthesized. Antiproliferative assay results indicated that compounds **6o** and **6u** exhibited the most potent activity against gastric cancer cell SGC-7901. In particular,, compound **6o** exhibited significant telomerase inhibitory activity (IC_{50} = 2.3 ± 0.07 μM), which was comparable to the positive control ethidium bromide. Docking simulation was performed to position compound **6o** into the active site of telomerase (3DU6) to determine the probable binding model. The enzyme assay data and the molecular docking results suggested that compound **6o** was potential inhibitor of telomerase.

4. Experimental

4.1. Chemistry general

All chemicals and reagents used in 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 DPX500 or 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.

4.2. General procedure for the preparation of target compounds 6a–6u

To a stirred solution of 5-substituted-2-mercapto-1,3,4-oxadiazoles **4** (2 mmol) and sodium hydroxide (2 mmol) in acetonitrile (20 mL) was added dropwise acetonitrile (5 mL) containing 2-chloro-5-(chloromethyl)pyridine **5** (2 mmol, 0.324 g). The

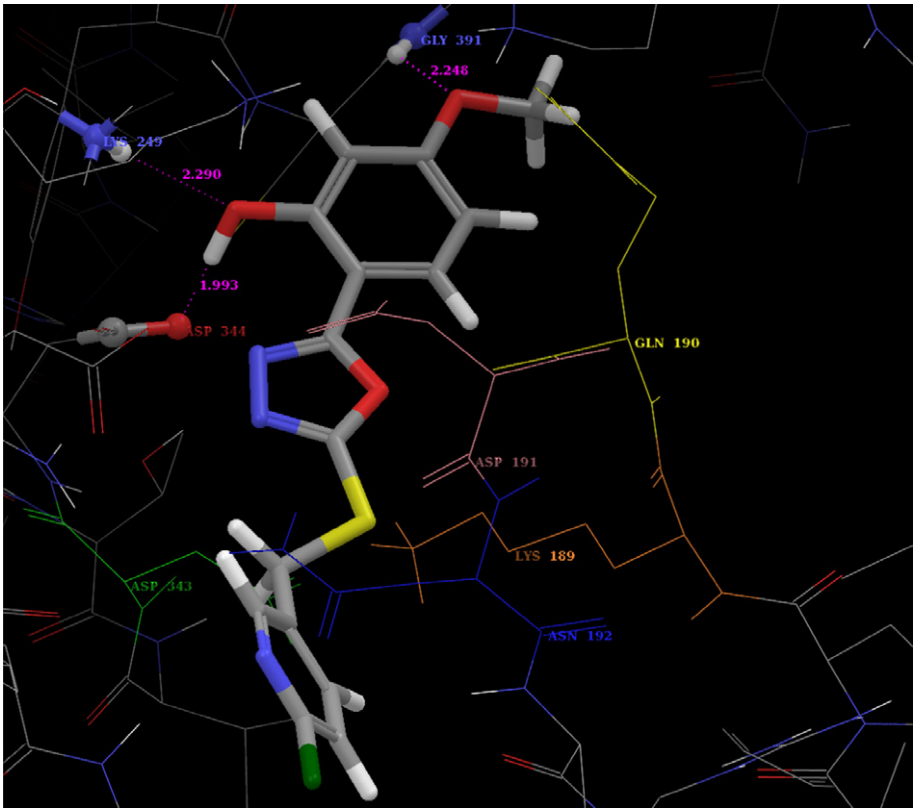


Figure 3. Molecular docking modeling of compound **6o** with telomerase: compound **6o** is nicely bound to the telomerase with its hydroxyl group project toward the amino group of LYS 249, with the hydroxyl group forming a more optimal H-bond (N–H···O: 2.290 Å, 121.1°) interaction, and the hydrogen atom of hydroxyl group of **6o** also forms hydrogen bond (O–H···O: 1.993 Å, 147.7°) with oxygen atom of ASP 344. Meanwhile, the oxygen atom of methoxy group of **6o** forms another hydrogen bond (N–H···O: 2.248 Å, 134.3°) with amino hydrogen of GLY 391.

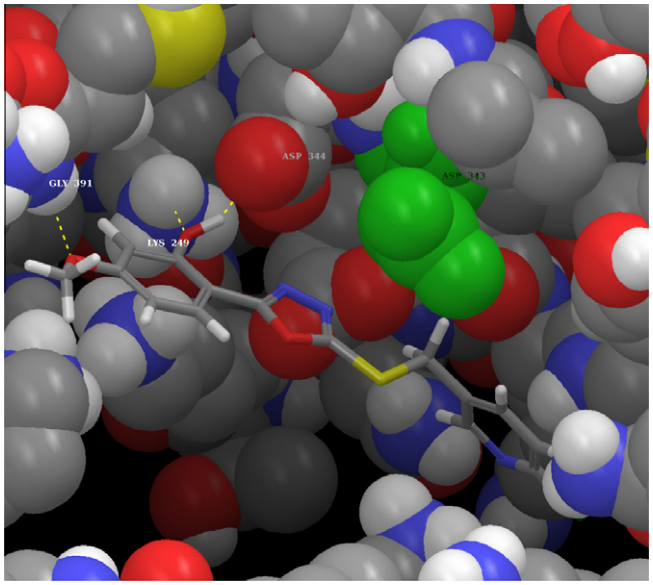


Figure 4. 3D model of the interaction between compound **6o** and telomerase binding site. Telomerase is represented by molecular surface. Compound **6o** is depicted by sticks and balls.

resulting mixture was heated under reflux for 8–24 h and the reaction was monitored by TLC. Afterwards the solution was cooled to room temperature and the organic solvent was removed in vacuo. The residue was dissolved in ethyl acetate and the organic layer was washed with water and saturated brine, respectively. Then the organic phase was dried over anhydrous Na_2SO_4, filtered, and removed in vacuo. The purification of the residue by recrystallization from acetonitrile yielded the desired compounds **6a–6u**.

4.2.1. 2-Chloro-5-[[(5-(*o*-tolyl)-1,3,4-oxadiazol-2-yl)thio]methyl] pyridine (6a)

White solid, yield 65.8%, mp: 80–81 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.55 (s, 3H), 4.59 (s, 2H), 7.38–7.43 (m, 2H), 7.46–7.52 (m, 2H), 7.81 (d, J = 7.9 Hz, 1H), 7.98 (d, J = 8.2 Hz, 1H), 8.51 (s, 1H). MS (ESI): 318.0 ($C_{15}H_{13}ClN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{12}ClN_3OS$: C, 56.69; H, 3.81; N, 13.22. Found: C, 56.66; H, 3.78; N, 13.26.

4.2.2. 2-Chloro-5-[[(5-(*p*-tolyl)-1,3,4-oxadiazol-2-yl)thio]methyl] pyridine (6b)

Light yellow crystal, yield 65.1%, mp: 148–149 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.40 (s, 3H), 4.60 (s, 2H), 7.41 (d, J = 8.0 Hz, 2H), 7.53 (d, J = 9.0 Hz, 1H), 7.84 (d, J = 8.0 Hz, 2H), 8.00 (d, J = 8.5 Hz, 1H), 8.52 (s, 1H). MS (ESI): 318.0 ($C_{15}H_{13}ClN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{12}ClN_3OS$: C, 56.69; H, 3.81; N, 13.22. Found: C, 56.76; H, 3.75; N, 13.28.

4.2.3. 2-Chloro-5-[[(5-(*o*-methoxyphenyl)-1,3,4-oxadiazol-2-yl) thio] methyl]pyridine (6c)

Light yellow crystal, yield 52.7%, mp: 73–74 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.88 (s, 3H), 4.57 (s, 2H), 7.11 (t, J = 7.3 Hz, 1H), 7.26 (d, J = 8.4 Hz, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.60 (t, J = 7.3 Hz, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.98 (d, J = 8.3 Hz, 1H), 8.51 (s, 1H). MS (ESI): 334.0 ($C_{15}H_{13}ClN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{12}ClN_3O_2S$: C, 53.97; H, 3.62; N, 12.59. Found: C, 53.91; H, 3.55; N, 12.65.

4.2.4. 2-Chloro-5-[[(5-(*p*-methoxyphenyl)-1,3,4-oxadiazol-2-yl) thio]methyl]pyridine (6d)

Light yellow crystal, yield 53.9%, mp: 120–121 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.85 (s, 3H), 4.58 (s, 2H), 7.13 (d, J = 8.8 Hz, 2H), 7.51 (d, J = 8.2 Hz, 1H), 7.88 (d, J = 8.8 Hz, 2H), 7.98 (d, J = 8.2 Hz, 1H), 8.50 (s, 1H). MS (ESI): 334.0 ($C_{15}H_{13}ClN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{12}ClN_3O_2S$: C, 53.97; H, 3.62; N, 12.59. Found: C, 53.88; H, 3.53; N, 12.67.

4.2.5. 2-Chloro-5-[[(5-(*o*-ethoxyphenyl)-1,3,4-oxadiazol-2-yl) thio]methyl]pyridine (6e)

Brown crystal, yield 52.2%, mp: 87–88 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.33 (t, J = 6.5 Hz, 3H), 4.17 (q, J = 7.0 Hz, 2H), 4.58 (s, 2H), 7.11 (t, J = 8.0 Hz, 1H), 7.25 (d, J = 8.5 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.57–7.60 (m, 1H), 7.78 (d, J = 7.5 Hz, 1H), 7.98 (d, J = 8.5 Hz, 1H), 8.50 (s, 1H). MS (ESI): 348.0 ($C_{16}H_{15}ClN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{14}ClN_3O_2S$: C, 55.25; H, 4.06; N, 12.08. Found: C, 55.33; H, 4.01; N, 12.11.

4.2.6. 2-Chloro-5-[[(5-(*p*-ethoxyphenyl)-1,3,4-oxadiazol-2-yl) thio]methyl]pyridine (6f)

White solid, yield 57.1%, mp: 117–119 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.36 (t, J = 7.0 Hz, 3H), 4.13 (q, J = 6.5 Hz, 2H), 4.59 (s, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 9.0 Hz, 2H), 8.00 (d, J = 8.5 Hz, 1H), 8.52 (s, 1H). MS (ESI): 348.0 ($C_{16}H_{15}ClN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{14}ClN_3O_2S$: C, 55.25; H, 4.06; N, 12.08. Found: C, 55.29; H, 4.05; N, 12.11.

4.2.7. 2-Chloro-5-[[(5-(*o*-fluorophenyl)-1,3,4-oxadiazol-2-yl) thio]methyl]pyridine (6g)

White crystal, yield 62.8%, mp: 109–110 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.60 (s, 2H), 7.40–7.53 (m, 3H), 7.65–7.73 (m, 1H), 7.95–8.01 (m, 2H), 8.52 (s, 1H). MS (ESI): 322.0 ($C_{14}H_{10}ClFN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{14}H_9ClFN_3OS$: C, 52.26; H, 2.82; N, 13.06. Found: C, 52.21; H, 2.73; N, 13.12.

4.2.8. 2-Chloro-5-[[(5-(*p*-fluorophenyl)-1,3,4-oxadiazol-2-yl) thio]methyl]pyridine (6h)

Light yellow crystal, yield 56.2%, mp: 132–133 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.61 (s, 2H), 7.45 (t, J = 8.5 Hz, 2H), 7.53 (d, J = 8.0 Hz, 1H), 7.99–8.04 (m, 3H), 8.53 (s, 1H). MS (ESI): 322.0 ($C_{14}H_{10}ClFN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{14}H_9ClFN_3OS$: C, 52.26; H, 2.82; N, 13.06. Found: C, 52.33; H, 2.77; N, 13. 01.

4.2.9. 2-Chloro-5-[[(5-(*o*-chlorophenyl)-1,3,4-oxadiazol-2-yl) thio]methyl]pyridine (6i)

White crystal, yield 58.4%, mp: 79–80 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.61 (s, 2H), 7.51–7.58 (m, 2H), 7.61–7.72 (m, 2H), 7.94 (d, J = 7.7 Hz, 1H), 7.99 (d, J = 8.2 Hz, 1H), 8.52 (s, 1H). MS (ESI): 338.0 ($C_{14}H_{10}Cl_2N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{14}H_9Cl_2N_3OS$: C, 49.72; H, 2.68; N, 12.42. Found: C, 49.62; H, 2.60; N, 12.45.

4.2.10. 2-Chloro-5-[[(5-(*p*-chlorophenyl)-1,3,4-oxadiazol-2-yl) thio]methyl]pyridine (6j)

White solid, yield 61.3%, mp: 147–148 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.61 (s, 2H), 7.52 (d, J = 8.2 Hz, 2H), 7.67 (d, J = 8.4 Hz, 2H), 7.95–8.00 (m, 2H), 8.52 (s, 1H). MS (ESI): 338.0 ($C_{14}H_{10}Cl_2N_3OS$, $[M+H]^+$). Anal. Calcd for $C_{14}H_9Cl_2N_3OS$: C, 49.72; H, 2.68; N, 12.42. Found: C, 49.83; H, 2.61; N, 12.38.

4.2.11. 2-Chloro-5-[[(5-(*p*-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) thio]methyl]pyridine (6k)

Yellow crystal, yield 67.5%, mp: 198–200 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.58 (s, 2H), 6.93 (d, J = 9.0 Hz, 2H), 7.53 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 8.5 Hz, 2H), 7.98 (d, J = 8.5 Hz, 1H), 8.50 (s, 1H), 10.35 (s, 1H). MS (ESI): 320.0 ($C_{14}H_{11}ClN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{10}ClN_3O_2S$: C, 52.59; H, 3.15; N, 13.14. Found: C, 52.66; H, 3.18; N, 13.17.

4.2.12. 2-Chloro-5-[[(5-(*p*-isopropylphenyl)-1,3,4-oxadiazol-2-yl)thio]methyl]pyridine (6l)

Yellow solid, yield 51.4%, mp: 195–197 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.24 (d, J = 7.0 Hz, 6H), 2.97–3.00 (m, 1H), 4.54 (s, 2H), 7.47 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.5 Hz, 1H), 7.87 (d, J = 8.5 Hz, 2H), 8.00 (d, J = 8.0 Hz, 1H), 8.52 (s, 1H). MS (ESI): 346.1 ($C_{17}H_{17}ClN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{16}ClN_3OS$: C, 59.04; H, 4.66; N, 12.15. Found: C, 59.07; H, 4.61; N, 12.21.

4.2.13. 2-Chloro-5-[[(5-(*p*-aminophenyl)-1,3,4-oxadiazol-2-yl) thio]methyl]pyridine (6m)

Yellow solid, yield 58.9%, mp: 154–155 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.54 (s, 2H), 5.97 (s, 2H), 6.66 (d, J = 9.0 Hz, 2H), 7.52 (d, J = 8.5 Hz, 1H), 7.58 (d, J = 8.5 Hz, 2H), 7.96 (d, J = 8.0 Hz, 1H), 8.49 (s, 1H). MS (ESI): 319.0 ($C_{14}H_{12}ClN_4OS$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{11}ClN_4OS$: C, 52.75; H, 3.48; N, 17.58. Found: C, 52.71; H, 3.50; N, 17.55.

4.2.14.2-Chloro-5-[[(5-(*p*-dimethaminophenyl)-1,3,4-oxadiazol-2- yl)thio]methyl]pyridine (6n)

Yellow solid, yield 61.3%, mp: 151–153 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.02 (s, 6H), 4.56 (s, 2H), 6.82 (d, J = 9.0 Hz, 2H), 7.53 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 8.5 Hz, 2H), 7.98 (d, J = 8.0 Hz, 1H), 8.50 (s, 1H). MS (ESI): 347.1 ($C_{16}H_{16}ClN_4OS$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{15}ClN_4OS$: C, 55.41; H, 4.36; N, 16.15. Found: C, 55.48; H, 4.31; N, 16.18.

4.2.15. 2-Chloro-5-[[(5-(*o*-hydroxyl-p-methoxyphenyl)-1,3,4-oxadiazol-2-yl)thio]methyl]pyridine (6o)

Yellow crystal, yield 73.5%, mp: 123–124 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.80 (s, 3H), 4.57 (s, 2H), 6.61 (s, 2H), 7.53 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 9.5 Hz, 1H), 7.99 (s, 1H), 8.52 (s, 1H), 10.30 (s, 1H). MS (ESI): 350.0 ($C_{15}H_{13}ClN_3O_3S$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{12}ClN_3O_3S$: C, 51.51; H, 3.46; N, 12.01. Found: C, 51.40; H, 3.51; N, 11.97.

4.2.16. 2-Chloro-5-[[(5-(3,4-dimethoxylphenyl)-1,3,4-oxadiazol-2-yl)thio]methyl]pyridine (6p)

White solid, yield 58.1%, mp: 123–124 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.85 (s, 6H), 4.59 (s, 2H), 7.16 (d, J = 8.5 Hz, 1H), 7.40 (s, 1H), 7.53 (d, J = 9.0 Hz, 2H), 7.99 (d, J = 8.0 Hz, 1H), 8.52 (s, 1H). MS (ESI): 364.0 ($C_{16}H_{15}ClN_3O_3S$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{14}ClN_3O_3S$: C, 52.82; H, 3.88; N, 11.55. Found: C, 52.76; H, 3.91; N, 11.51.

4.2.17. 2-Chloro-5-[[(5-(3,4,5-trimethoxylphenyl)-1,3,4-oxadiazol-2-yl)thio]methyl]pyridine (6q)

Yellow crystal, yield 60.1%, mp: 119–120 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.75 (s, 3H), 3.88 (s, 6H), 4.60 (s, 2H), 7.18 (s, 2H), 7.54 (d, J = 8.5 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 8.54 (s, 1H). MS (ESI): 394.1 ($C_{17}H_{17}ClN_3O_4S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{16}ClN_3O_4S$: C, 51.84; H, 4.09; N, 10.67. Found: C, 51.75; H, 4.08; N, 10.70.

4.2.18. 2-Chloro-5-[[(5-(pyridin-2-yl)-1,3,4-oxadiazol-2-yl)thio] methyl]pyridine (6r)

White solid, yield 62.3%, mp: 135–136 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.63 (s, 2H), 7.53 (d, J = 8.5 Hz, 1H), 7.62–7.65 (m, 1H), 8.00–8.07 (m, 2H), 8.13 (d, J = 8.0 Hz, 1H), 8.54 (s, 1H), 8.77–8.78 (m, 1H). MS (ESI): 305.0 ($C_{13}H_{10}ClN_4OS$, $[M+H]^+$). Anal. Calcd for $C_{13}H_9ClN_4OS$: C, 51.23; H, 2.98; N, 18.38. Found: C, 51.29; H, 2.95; N, 18.41.

4.2.19. 2-Chloro-5-[[(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)thio] methyl]pyridine (6s)

Light yellow crystal, yield 59.3%, mp: 97–98 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.58 (s, 2H), 6.80 (s, 1H), 7.33 (s, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 8.5 Hz, 1H), 8.06 (s, 1H), 8.51(s, 1H). MS (ESI): 294.0 ($C_{12}H_9ClN_3O_2S$, $[M+H]^+$). Anal. Calcd for $C_{12}H_8ClN_3O_2S$: C, 49.07; H, 2.75; N, 14.31. Found: C, 49.16; H, 2.80; N, 14.25.

4.2.20. 2-Chloro-5-[[(5-(thiophen-2-yl)-1,3,4-oxadiazol-2-yl) thio]methyl]pyridine (6t)

White solid, yield 58.3%, mp: 93–94 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.58 (s, 2H), 7.27–7.30 (m, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.78–7.79 (m, 1H), 7.94–7.95 (m, 1H), 7.97–8.00 (m, 1H), 8.51(s, 1H). MS (ESI): 310.0 ($C_{12}H_9ClN_3OS_2$, $[M+H]^+$). Anal. Calcd for $C_{12}H_8ClN_3OS_2$: C, 46.52; H, 2.60; N, 13.56. Found: C, 46.48; H, 2.53; N, 13.62.

4.2.21. 2-Chloro-5-[[(5-(naphth-1-yl)-1,3,4-oxadiazol-2-yl) thio]methyl]pyridine (6u)

Light yellow crystal, yield 61.4%, mp: 110–111 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.66 (s, 2H), 7.55 (d, J = 8.5 Hz, 1H), 7.66–7.76 (m, 3H), 8.03 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 7.5 Hz, 1H), 8.15 (d, J = 7.5 Hz, 1H), 8.22 (d, J = 8.5 Hz, 1H), 8.57 (s, 1H), 8.99 (d, J = 9.0 Hz, 1H). MS (ESI): 354.0 ($C_{18}H_{13}ClN_3OS$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{12}ClN_3OS$: C, 61.10; H, 3.42; N, 11.88. Found: C, 61.02; H, 3.37; N, 11.92.

4.3. Crystal structure determination

X-ray single-crystal diffraction data for compound **6d** were collected on a Bruker SMARTAPEX CCD diffractometer at 293(2) K using Mo Kα radiation (λ = 0.71073 Å) by the ω scan mode. The program SAINT was used for the integration of the diffraction profiles. All the structures were solved by direct methods using the SHELXS program of the SHELXTL package and refined by full-matrix least-squares methods with SHELXL-97.[20] All non-hydrogen atoms of compound **6d** were refined with anisotropic thermal parameters. All hydrogen atoms were placed in geometrically idealized positions and constrained to ride on their parent atoms. The crystal data, data collection, and refinement parameters for the compound are listed in Table 1.

4.4. Anti-proliferation assay

The antiproliferative activity of the prepared compounds **6o–6u** against SGC-7901 gastric cancer cells was evaluated as described in the literature[21] 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 24 h before the cytotoxicity assessment. Tested samples at pre-set concentrations were added to six wells with 5-fluorouracil being employed as a positive reference. After 48 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 on 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. The results were summarized in Table 2.

4.5. Telomerase inhibitory assay

Compounds **6o** and **6u** were assayed for telomerase inhibition by a modified TRAP assay, using a SGC-7901 cell extract. The telomerase assay methods used are the same as previously described.[22] The results were reported in Table 3.

4.6. Molecular docking modeling

The GLIDE protocol (Schrodinger Inc.)[23] was used to dock compound **6o** into ATP binding site of telomerase crystal structure (3DU6.pdb, downloaded from the Protein Data Bank). No constraints were implemented during the docking calculation.

Acknowledgment

This work was supported by Jiangsu National Science Foundation (No. BK2009239).

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