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

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ABSTRACT

In present study, a series of new 1,3,4-oxadiazole derivatives containing 1,4-benzodioxan moiety (**6a**–**6s**) as potential telomerase inhibitors were synthesized. The bioassay tests demonstrated that compounds **6k**, **6l**, **6m**, 6n and **6s** exhibited broad-spectrum antitumor activity with IC_{50} concentration range from 7.21 μM to 25.87 μM against the four cancer cell lines, HEPG2, HELA, SW1116 and BGC823. Moreover, all the title compounds were assayed for telomerase inhibition using the TRAP-PCR-ELISA assay. The results showed compound **6k** possessed the most potent telomerase activity (IC_{50} = 1.27 ± 0.05 μM). Docking simulation was performed to position compound **6k** into the active site of telomerase (3DU6) to determine the probable binding model.

1. Introduction

Cancer chemotherapy has entered a new field of molecularly targeted therapeutics, which is highly selective and not associated with the serious toxicities of conventional cytotoxic drugs.[1] Telomerase keeps its activity in the early stages of life cycle maintaining telomere length and the chromosomal integrity of frequently dividing cells, but becomes dormant in most somatic cells during adulthood.[2] 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.[3] Therefore, telomerase has been proposed as a potential and highly selective target for the development of a novel class of anticancer agents.[4]

Oxadiazole derivatives play a significant role in various pharmaceutical applications.[5–9] As an important class of heterocyclic compound, 1,3,4-oxadiazoles show broad spectrum of bioactivities.[10–15] Among these, a few differently substituted 1,3,4-oxadiazoles have exhibited potent antitumor activities particually.[16–18] Compounds containing a 1,4-benzodioxan template also have received significant attention in chemical, medicinal and pharmaceutical research as this structural scaffold is found in a variety of drugs.[19] As shown in Figure 1, the mesylate salt of doxazosin (A), marketed as CARDURA in USA,[20] is an efficient anti-hypertensive drug. Fluparoxan (B) has been verified to have potent antidepressant properties.[21] And the 6-position substituted 1,4-benzodioxan (C) is known as a kind of nonsteroidal anti-inflammatory drug (NSAID).[22] Recently, some reports claimed that a number of other 1,4-benzodioxan template-containing compounds also have ability as potential anticancer drugs with excellent bioavailability and low cytotoxicity.[22–25]

In this paper, we described the synthesis and the structure relationships (SAR) of series of novel 1,3,4-oxadiazole derivatives possessing 1,4-benzodioxan moiety as potential antitumor agents, which was based on molecular modeling and the investigation of SAR between new inhibitors and the X-ray crystallographic structure of the telomerase. Biological evaluation was also carried out for screening potential telomerase inhibitors among the synthesized compounds.

2. Results and discussion

2.1. Chemistry

In this study, 19 oxadiazole derivatives containing 1,4-benzodioxan moiety were synthesized. The synthetic route of compounds **6a**–**6s** was shown in Scheme 1.

They were synthesized from 5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1,3,4-oxa-diazole-2-thiol (**4**) and different kinds of halogen

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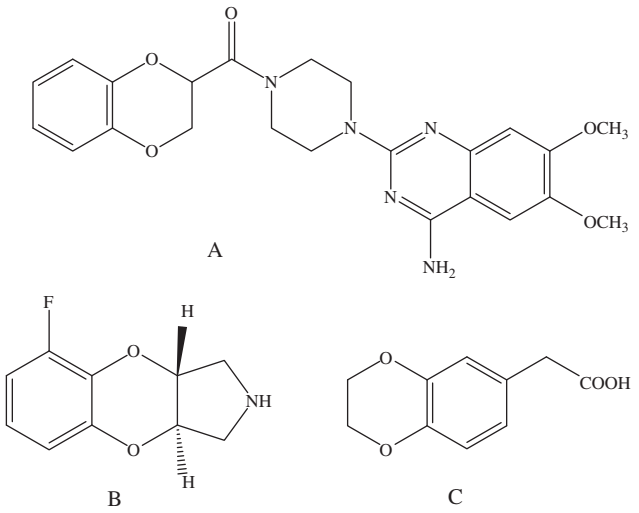


Figure 1. The structure of compounds A, B and C.

substituted bromotoluene.[26] The key intermediate **4** was prepared in three steps. Esterification of the 2,3-dihydrobenzo[*b*]-[1,4]dioxine-6-carboxylic acid (**1**) with methanol and concentrated sulfuric acid afforded the corresponding ester **2**. The aroyl hydrazide **3** was obtained by reacting **2** with 85% hydrazine monohydrate in ethanol. Treatment of the hydrazide **3** with carbon disulfide in the presence of KOH and 95% ethanol under reflux gave the key intermediate **4**. The synthesis of compounds **6a–6s** were accomplished by refluxing **4** with halogen substituted bromotoluene in the presence of NaOH in acetonitrile. All the target compounds were reported for the first time.

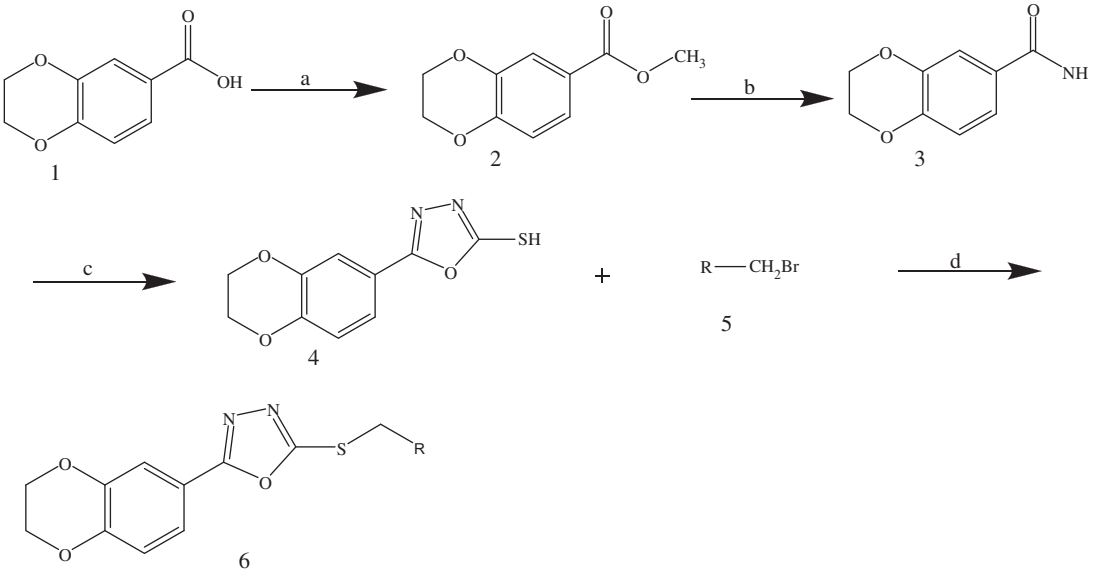
All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures. Additionally, the structure of compound **6c** was further confirmed by X-ray diffraction. Its crystal data are presented in Table 1, and Figure 2 gives a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

2.2. Biological activity

2.2.1. Antiproliferation assay

All the synthesized 1,3,4-oxadiazole derivatives **6a–6s** were evaluated for their antiproliferative activity against the HEPG2 (human liver cancer cell), HELA (human cervical cancer cell), SW1116 (human colorectal carcinoma cell) and BGC823 (human gastric cancer). The results were summarized in Table 2.

As exhibited in Table 2, the active analogs showed a distinctive potential pattern of selectivity as well as broad-spectrum antitumor activity. As for selectivity against individual cell line, a number of 1,3,4-oxadiazole derivatives possessing 1,4-benzodioxan moiety showed powerful effectiveness against HEPG2. Among these compounds, compound **6s** displayed the most potent antitumor activity (IC_{50} = 7.21 μM). Compounds **6k**, **6l**, **6m**, **6n** and **6s** with IC_{50} concentration range of 18.97–25.87 μM comparable to 5-fluorouracil (110.16 μM) were proved to be able to inhibit proliferation of HELA cells. For SW1116 cells, higher inhibitory efficiencies were observed with IC_{50} values range of 12.21–24.46 μM compared with the positive control 5-fluorouracil (95.17 μM). However the result



6a=Ph-
6b=3-Me-C_6H_4-
6c=4-NO_2-C_6H_4-
6d=2-F-C_6H_4-
6e=2-NO_2-C_6H_4-
6f=4-Me-C_6H_4-
6s=2-I-C_6H_4-
6g=4-F-C_6H_4-
6h=3-NO_2-C_6H_4-
6i=4-Cl-C_6H_4-
6j=4-I-C_6H_4-
6k=2-Me-C_6H_4-
6l=2-Br-C_6H_4-
6m=3-Br-C_6H_4-
6n=4-Br-C_6H_4-
6o=2-Cl-C_6H_4-
6p=3-Cl-C_6H_4-
6q=2,6-2F-C_6H_4-
6r=2,4-2F-C_6H_4-

Scheme 1. General synthesis of compounds (**6a–6s**). Reagents and conditions: (a) methanol, concentrated sulfuric acid; 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.

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

Compound	**6c**
Empirical formula	$C_{17}H_{13}N_3O_5S$
Formula weight	371.06
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	7.199(2)
b (Å)	8.002(3)
c (Å)	14.547(5)
α (°)	96.722(3)
β (°)	94.865(3)
γ (°)	105.649(3)
V (Å)	795.5(5)
Z	10
D_{calcd} (g/cm^3)	1.567
θ range (°)	2.67–28.37
F(0 0 0)	380
Reflections collected/unique	6880/3708 [R_{int} = 0.0180]
Data/restraints/parameters	3708/0/235
Absorption coefficient (mm^{-1})	0.750
R_1; wR_2 [$I > 2\sigma(I)$]	0.0417/0.1055
R_1; wR_2 (all data)	0.0584/0.1168
GOOF	0.939

of the compounds against BGC823 cells was seemed to be less effective to compounds **6k**, **6l**, **6m**, **6n** and **6s** with IC_{50} concentration range of 18.90–22.57 μM. It was concluded that compounds **6k**, **6l**, **6m, 6n** and **6s** showed broad-spectrum antitumor activity with IC_{50} concentration range of 7.21–25.87 μM against the mentioned four cancer cell lines. However compound **6q** was proved to be less effective against them.

The structure–activity relationships in these 1,3,4-oxadiazole derivatives demonstrated that compounds with substituted benzene ring displayed powerful inhibitory activity. Meanwhile, a comparison of the substitution on benzene ring were demonstrated as follows: when the compounds were *ortho*-position-halogen substituted derivatives, the potency order was I > Br > Cl > F (e.g., **6d, 6l, 6o, 6s**) and among the *meta*-position-halogen-substituted compounds the potent inhibitory action order could be summarized as Br > Cl (e.g., **6m, 6p**); while when the substitute happened on the *para*-position of benzene ring, the potency order was Br > Cl > I > F (e.g., **6g, 6i, 6j, 6n**). However, the potent inhibitory activity order of compounds with electron-donating groups on the benzene ring (e.g., **6b**, **6f**, **6k**) was *ortho* > *meta* > *para*, which is notably different from the derivatives with electron-withdrawing groups (e.g., **6c**, **6e**, **6h**). The biological results also demonstrated the bioactivity of compounds was greatly affected by the number and position of fluorine atoms on benzene ring, just as Table 2 showed, the potency order of fluorine substituted derivatives was summarized as *para* > *ortho* > 2,4-disubstituted > 2,6-disubstituted.

2.2.2. Telomerase inhibitory assay

The telomerase inhibitory of the 1,3,4-oxadiazole derivatives for SW1116 were studied via TRAP-PCR-ELISA assay. The results were presented as mean ± SD. As shown in Table 3, most of the tested compounds displayed good telomerase inhibitory, and among them compound **6k** displayed the most potent inhibitory with IC_{50} of 1.27 ± 0.05 μM which is much lower than staurosporine. These results suggested that Compound **6k** was an excellent candidate as anticancer agents with potent telomerase inhibitory. The results of telomerase inhibitory activity of the tested compounds were correspondent to SAR of their anticancer activities. It was demonstrated that the potent anticancer activities of the synthetic compounds were probably correlated to their telomerase inhibitory activities.

2.3. Binding model of compound 6k into telomerase structure

In an effort to elucidate the mechanism by which the title compounds can induce anticancer activity and to establish an SAR based on our experimental studies, molecular docking of the potent inhibitor **6k** into ATP binding site of telomerase was performed to simulate a binding model derived from telomerase structure (3DU6 PDB). The binding model of compound **6k** and telomerase are depicted in Figure 3. In the binding model, **6k** is nicely bound to the telomerase with its oxygen atom of oxadiazole group projecting toward the amino hydrogen of LYS 372, with the hydroxyl group forming a more optimal H-bond (H–N···H: 2.131 Å, 151.768°) interaction, and the oxygen atom of benzene group of **6k** also forms hydrogen bond (H–O···H: 1.91 Å, 137.128°) with hydrogen atom of LYS406. Meanwhile, there are three π–cation interactions between compound **6k** and the telomerase, two of which are formed between the protonated amino group of LYS 406 and the 1,4-benzodioxan ring as well as the other benzene ring of **6k**, respectively. By the protonated amino group of LYS 372 projecting toward the oxadiazole group of **6k,** it shaped the third π–cation interaction. The enzyme assay data and the molecular docking results suggested that compound **6k** was a potential inhibitor of telomerase.

3. Conclusions

In this paper, a series of novel 1,3,4-oxadiazole derivatives were synthesized for comprehensive screening of potential antitumor agents. The antiproliferative activity of the compounds against four different original cancer cells (HEPG2, HELA, SW1116 and BGC823) was determined systematically. The results indicated that the compounds such as **6k**, **6l**, **6m, 6n** and **6s** exhibited excellent antitumor activity compared with the 5-fluorouracil which was widely used in treatment of cancer in clinical. We further analyzed their inhibitory effects on telomerase expressed in cancer cells which was more active in cancer cells than other normal ones and responsible for cell immortality. It was shown that compound **6k** could be more potent to disrupt telomerase activity than other compounds with the IC_{50} value of 1.27 ± 0.05 μM. Considering the results mentioned above, it could be concluded that some 1,3,4-oxadiazole derivatives were as good candidates for antitumor

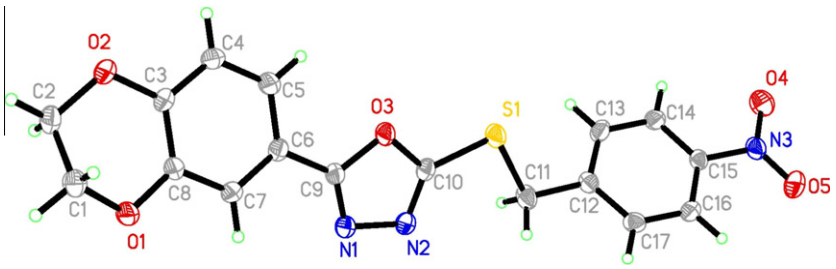


Figure 2. Molecule structure of compound **6c**.

Table 2
Antiproliferative activity of the synthesized compounds (**6a–6s**)

Compound	IC_{50} (μM)			
	HEPG2	HELA	SW1116	BGC823
6a	32.14	40.33	31.50	33.61
6b	33.37	32.79	27.20	31.23
6c	35.44	27.54	14.63	22.99
6d	23.98	41.62	21.91	28.95
6e	38.05	32.69	20.81	23.31
6f	41.90	45.43	32.52	31.61
6g	37.99	41.18	21.62	28.69
6h	38.30	35.60	30.05	23.66
6i	28.83	28.47	28.41	22.63
6j	31.19	31.09	29.23	22.67
6k	22.02	18.97	23.88	22.57
6l	19.95	22.43	14.06	21.21
6m	24.95	25.87	20.12	20.62
6n	8.54	23.59	24.46	22.25
6o	22.64	36.80	29.53	22.53
6p	33.36	31.78	25.22	26.91
6q	41.60	43.16	36.32	36.13
6r	38.70	41.69	29.61	30.33
6s	7.21	19.98	12.21	18.90
5-Fluorouracil	110.01	110.16	95.17	100.02

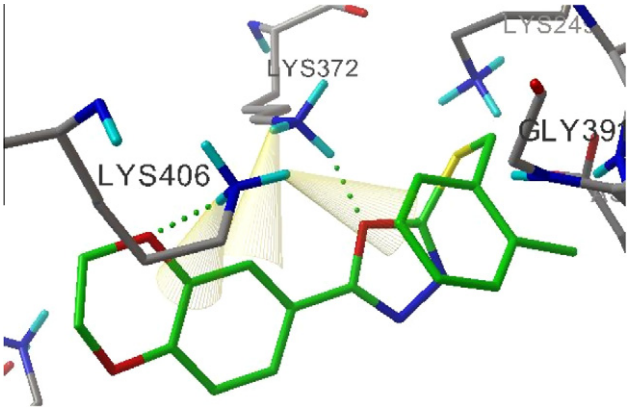


Figure 3. Molecular docking modeling of compound **6k** with telomerase: compound **6k** is nicely bound to the telomerase with its oxygen atom of oxadiazole group project toward the amino hydrogen of LYS 372, with the hydroxyl group forming a more optimal H-bond (H–N···H: 2.131 Å, 151.768°) interaction, and the oxygen atom of benzene group of **6k** also forms hydrogen bond (H–O···H: 1.91 Å, 137.128°) with hydrogen atom of LYS406. Meanwhile, three π–cation interactions are shown as yellow column.

Table 3
Telomerase inhibitory activity of compounds **6a–6s**

Compound	Telomerase inhibitation IC_{50} (μM) ± SD
6a	8.21 ± 0.04
6b	3.26 ± 0.27
6c	2.71 ± 0.02
6d	2.93 ± 0.05
6e	12.15 ± 0.01
6f	5.04 ± 0.18
6g	3.67 ± 0.34
6h	4.65 ± 0.03
6i	2.91 ± 0.23
6j	3.66 ± 0.14
6k	1.27 ± 0.05
6l	2.02 ± 0.06
6m	2.45 ± 0.14
6n	1.66 ± 0.01
6o	5.89 ± 0.35
6p	2.34 ± 0.04
6q	15.78 ± 0.01
6r	2.44 ± 0.08
6s	1.83 ± 0.03
Staurosporine	8.32 ± 0.08

agents screening and research. The template 1,3,4-oxadiazole with 1,4-benzodioxan moiety was suitable to reconstruct and design for development of more potential therapeutic drugs against cancer, the greatest threat to human's health. Further research would be ongoing for synthesis and discovery more enhanced 1,3,4-oxadiazole derivatives with outstanding anticancer activity and low toxicity and side-effects.

4. Experimental protocols

4.1. Materials and measurements

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 (Tai ke 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 spectrometer at room temperature with TMS and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ). Elemental analyzes were performed on a CHN–O-Rapid instrument, and were within ±0.4% of the theoretical values.

4.2. General procedure for synthesis of the target compounds (6a–6s)

To a stirred solution of 5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1,3,4-oxadiazo-le-2-thiol **4** (1 mmol, 0.268 g) and sodium hydroxide (1 mmol) in acetonitrile (50 mL) was added dropwise acetonitrile (5 mL) containing halogen substituted compounds (1 mmol). The resulting mixture was heated under reflux for 10–24 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 saturated brine. 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–6s**.

4.2.1. 2-(Benzylthio)-5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1,3,4-oxadiazole (6a)

Light yellow crystal, yield: 60.9%, mp: 110 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.31 (m, 4H), 4.49 (s, 2H), 6.94 (d, J = 8.3 Hz, 1H), 7.29–7.35 (m, 3H), 7.45–7.49 (m, 4H). MS (ESI): 327.07 ($C_{17}H_{15}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{14}N_2O_3S$: C, 62.56; H, 4.32; N, 8.58. Found: C, 62.33; H, 4.53; N, 8.66.

4.2.2. 2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(3-methylbenzylthio)-1,3,4-oxadiazole (6b)

White crystal, yield: 66%, mp: 89 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.8 (s, 3H), 4.28–4.31 (m, 4H), 4.49 (s, 2H), 6.83–6.85 (m, 1H), 6.94 (d, J = 8.4 Hz, 1H), 7.02 (d, J = 7.8 Hz, 2H), 7.25 (s, 1H), 7.47–7.50 (m, 2H). MS (ESI): 341.09 ($C_{18}H_{17}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23. Found: C, 63.32; H, 4.73; N, 8.15.

4.2.3. 2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(4-nitrobenzylthio)-1,3,4-oxadiazol (6c)

White crystal, yield: 80.0%, mp: 196–198 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.29–4.32 (m, 4H), 4.54 (s, 2H), 6.94 (d, J = 8.9 Hz, 1H), 7.4 (s, 2H), 7.66 (d, J = 8.7 Hz, 2H), 8.19 (d, J = 8.7 Hz, 2H). MS (ESI):

372.06 ($C_{17}H_{14}N_3O_5S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}N_3O_5S$: C, 54.98; H, 3.53; N, 11.31. Found: C, 54.67; H, 3.32; N, 11.54.

4.2.4. 2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(2-fluorobenzylthio)-1,3,4-oxadiazole (6d)

Light yellow crystal, Yield: 79.5%. mp: 108 °C. ^{1}H NMR (500 MHz,$CDCl_3$) δ: 4.28–4.31 (m, 4H), 4.52 (s, 2H), 6.94 (d, J = 8.2 Hz, 1H), 7.04–7.11 (m, 2H), 7.28 (d, J = 7.8 Hz, 1H), 7.46–7.49 (m, 2H), 7.52 (q, J = 6.3 Hz, 1H). MS (ESI): 345.06 ($C_{17}H_{14}FN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}FN_2O_3S$: C, 59.38; H, 3.83; N, 8.21. Found: C, 59.20; H, 3.62; N, 8.38.

4.2.5. 2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(2-nitrobenzylthio)-1,3,4-oxadiazole (6e)

White solid, yield: 70.2%, mp: 109 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.31 (m, 4H), 4.83 (s, 2H), 6.93 (d, J = 8.4 Hz, 1H), 7.44–7.49 (m, 3H), 7.58–7.60 (m, 1H), 7.85 (d, J = 10.2 Hz, 1H), 8.13 (d, J = 8.3 Hz, 1H). MS (ESI): 372.06 ($C_{17}H_{14}N_3O_5S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}N_3O_5S$: C, 54.98; H, 3.53; N, 11.31. Found: C, 54.66; H, 3.52; N, 11.49.

4.2.6. 2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(4-methylbenzylthio)-1,3,4-oxadiazole (6f)

White crystal, yield: 76%, mp: 133–135 °C. ^{1}H NMR(500 MHz, $CDCl_3$) δ: 3.4 (m, 3H), 4.29–4.32 (m, 4H), 4.47 (s, 2H), 6.94 (d, J = 8.3 Hz, 1H), 7.02 (t, J = 8.6 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.48 (d, J = 9.2 Hz, 2H). MS (ESI): 341.09 ($C_{18}H_{17}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23. Found: C, 63.30; H, 4.52; N, 8.41.

4.2.7. 2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(4-fluorobenzylthio)-1,3,4-oxadiazole (6g)

White crystal, yield: 63.4%, mp: 118 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.29–4.31 (m, 4H), 4.47 (s, 2H), 6.94 (d, J = 8.3 Hz, 1H), 7.28 (d, J = 7.8 Hz, 1H), 7.41–7.44 (m, 2H), 7.46–7.49 (m, 2H), 7.50 (m, 1H). MS (ESI): 345.06 ($C_{17}H_{14}FN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}FN_2O_3S$: C, 59.29; H, 3.81; N, 8.13. Found: C, 59.36; H, 3.57; N, 8.31.

4.2.8. 2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(3-nitrobenzylthio)-1,3,4-oxadiazole (6h)

White solid, yield: 54.7%, mp: 107–108 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.33 (m, 4H), 4.56 (s, 2H), 6.93–6.96 (m, 1H), 7.45–7.49 (m, 2H), 7.53 (d, J = 13.4 Hz, 1H), 7.86 (d, J = 12.8 Hz, 2H), 8.16 (d, J = 13.7 Hz, 1H), 8.34 (d, J = 13.6 Hz, 1H). MS (ESI): 372.06 ($C_{17}H_{14}N_3O_5S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}N_3O_5S$: C, 54.98; H, 3.53; N, 11.31. Found: C, 54.66; H, 3.41; N, 11.62.

4.2.9. 2-(4-Chlorobenzylthio)-5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1,3,4-oxadiazole (6i)

White powder, yield: 50.7%, mp: 124 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.32 (m, 4H), 4.45 (s, 2H), 6.94 (d, J = 8.4 Hz, 1H), 7.30 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 7.46–7.49 (m, 2H). MS (ESI): 361.03 ($C_{17}H_{14}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}ClN_2O_3S$: C, 56.59; H, 3.63; N, 7.76. Found: C, 56.37; H, 3.52; N, 7.98.

4.2.10. 2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(4-iodobenzylthio)-1,3,4-oxadiazole (6j)

Light yellow solid, yield: 88.3%, mp: 124 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.31 (m, 4H), 4.43 (s, 2H), 6.94 (d, J = 8.6 Hz, 1H), 6.97–7.02 (d, J = 8.1 Hz, 2H), 7.40–7.47 (m, 2H), 7.50–7.56 (m, 2H). MS (ESI): 452.97 ($C_{17}H_{14}IN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}IN_2O_3S$: C, 45.15; H, 2.90; N, 6.19. Found: C, 45.04; H, 2.57; N, 6.48.

4.2.11. 2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(2-methylbenzylthio)-1,3,4-oxadiazole (6k)

White powder, yield: 60.36%, mp: 117–118 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.60 (s, 3H), 4.29–4.32 (m, 4H), 4.47 (s, 2H), 6.94 (d, J = 8.3 Hz, 1H), 7.12 (t, J = 8.6 Hz, 2H), 7.33–7.35 (m, 1H), 7.48 (d, J = 8.2 Hz, 2H), 7.70–7.73 (d, J = 3.7 Hz, 1H). MS (ESI): 341.09 ($C_{18}H_{17}N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23. Found: C, 63.40; H, 4.55; N, 8.35.

4.2.12. 2-(2-Bromobenzylthio)-5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1,3,4-oxadiazole (6l)

White powder, yield: 63.4%, mp: 128–129 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.31 (m, 4H), 4.52 (s, 2H), 6.94 (d, J = 8.2 Hz, 1H), 7.08–7.12 (m, 1H), 7.24 (d, J = 7.8 Hz, 1H), 7.46–7.49 (m, 2H), 7.52–7.56 (m, 2H). MS (ESI): 404.98 ($C_{17}H_{14}B_rN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}B_rN_2O_3S$: C, 50.38; H, 3.23; N, 6.91. Found: C, 50.20; H, 3.41; N, 6.68.

4.2.13. 2-(3-Bromobenzylthio)-5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1,3,4-oxadiazole (6m)

White powder, yield: 62.1%, mp: 112–113 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.31 (m, 4H), 4.52 (s, 2H), 6.94 (d, J = 8.2 Hz, 1H), 7.40–7.47 (m, 1H), 7.51–7.53 (m, 3H), 7.54–7.57 (m, 2H). MS (ESI): 404.98 ($C_{17}H_{14}B_rN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}B_rN_2O_3S$: C, 50.38; H, 3.23; N, 6.91. Found: C, 50.24; H, 3.57; N, 6.72.

4.2.14. 2-(4-Bromobenzylthio)-5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1,3,4-oxadiazole (6n)

Yellow crystal, yield: 65.9%, mp: 143 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.31 (m, 4H), 4.53 (s, 2H), 6.95 (d, J = 8.3 Hz, 1H), 7.20–7.25 (m, 1H), 7.35–7.43 (m, 2H), 7.53–7.54 (m, 1H), 7.68–7.71 (d, J = 9.1 Hz; 1H), 7.75 (s, 1H). MS (ESI): 404.98 ($C_{17}H_{14}B_rN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}B_rN_2O_3S$: C, 50.38; H, 3.23; N, 6.91. Found: C, 50.24; H, 3.47; N, 6.74.

4.2.15. 2-(2-Chlorobenzylthio)-5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1,3,4-oxadiazole (6o)

White powder, yield: 60.1%, mp: 108 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.32 (m, 4H), 4.45 (s, 2H), 6.94 (d, J = 8.4 Hz, 1H), 7.08–7.12 (d, J = 8.3 Hz, 2H), 7.32 (m, 1H), 7.46–7.49 (m, 2H). MS (ESI): 361.03 ($C_{17}H_{14}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}ClN_2O_3S$: C, 56.59; H, 3.63; N, 7.76. Found: C, 56.69; H, 3.42; N, 7.58.

4.2.16. 2-(3-Chlorobenzylthio)-5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1,3,4-oxadiazole (6p)

Light yellow crystals, yield: 55.9%, mp: 122–123 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.32 (m, 4H), 4.50 (s, 2H), 6.93 (d, J = 8.4 Hz, 1H), 7.23–7.25 (m, 2H), 7.32 (m, 1H), 7.46–7.49 (m, 2H). MS (ESI): 361.03 ($C_{17}H_{14}ClN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}ClN_2O_3S$: C, 56.59; H, 3.63; N, 7.76. Found: C, 56.38; H, 3.92; N, 7.68.

4.2.17. 2-(2,6-Difluorobenzylthio)-5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1,3,4-oxadiazole (6q)

Yellow crystal, yield: 54.8%, mp: 121 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.31 (m, 4H), 4.52 (s, 2H), 6.95 (d, J = 8.3 Hz, 1H), 7.30–7.25 (m, 2H), 7.35–7.43 (m, 1H), 7.53–7.54 (m, 1H), 7.68–7.71 (d, J = 9.2 Hz, 1H). MS (ESI): 363.05 ($C_{17}H_{13}F_2N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{12}F_2N_2O_3S$: C, 56.35; H, 3.34; N, 7.73. Found: C, 56.23; H, 3.12; N, 7.93.

4.2.18. 2-(2,4-Difluorobenzylthio)-5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-1,3,4-oxadiazole (6r)

Light yellow crystal, yield: 58.7%, mp: 157 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.31 (m, 4H), 4.52 (s, 2H), 6.95 (d, J = 8.3 Hz, 1H), 7.20–7.25 (m, 2H), 7.41–7.43 (m, 1H), 7.53–7.54 (m, 2H). MS (ESI): 363.05 ($C_{17}H_{13}F_2N_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{12}F_2N_2O_3S$: C, 56.35; H, 3.34; N, 7.73. Found: C, 56.22; H, 3.21; N, 7.90.

4.2.19. 2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(2-iodobenzylthio)-1,3,4-oxadiazole (6s)

White powder, yield: 65.0%, mp: 104 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.28–4.31 (m, 4H), 4.42 (s, 2H), 6.93 (d, J = 8.3 Hz, 1H), 7.12–7.15 (m, 2H), 7.40 (d, J = 8.1 Hz, 1H), 7.47–7.52 (m, 2H), 7.64 (m, 1H). MS (ESI): 452.97 ($C_{17}H_{14}IN_2O_3S$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{13}IN_2O_3S$: C, 45.15; H, 2.90; N, 6.19. Found: C, 45.43; H, 2.72; N, 6.32.

4.3. Crystal structure determination

Crystal structure determination of compound **6c** was carried out on a Nonius CAD4 diffractometer equipped with graphite-monochromated MoKα (λ = 0.71073 Å) radiation (Fig. 2). The structure was solved by direct methods and refined on F^2 by full-matrix least-squares methods using SHELX-97.[27] All the non-hydrogen atoms were refined anisotropically. All the hydrogen atoms were placed in calculated positions and were assigned fixed isotropic thermal parameters at 1.2 times the equivalent isotropic U of the atoms to which they are attached and allowed to ride on their respective parent atoms. The contributions of these hydrogen atoms were included in the structure-factors calculations. The crystal data, data collection, and refinement parameter for compound **6c** are listed in Table 1.

4.4. Anti-proliferation assay

The antiproliferative activity of the title compounds **6a–6s** against the four cell lines HEPG2, HELA, SW1116 and BGC823, were evaluated using a standard MTT-based colorimetric assay. Ten thousand corresponding cells per well were seeded into 96-well plates (Corning, New York, USA) and incubated at 37 °C, 5% CO_2 for 24 h. And then 100 μL a series of concentration of drug-containing medium were dispensed into wells to maintain the final concentration as 60, 20, 6.67, 2.22, 0.74, 0.25 and 0.082 μg/mL. Each concentration was in triplicate, and 5-fluorouracil (5-FU) (Sigma–Aldrich, St. Louis, USA) was used as the positive control. After 48 h incubation, cell survival was determined by the addition of 25 μL MTT (Sigma–Aldrich, St. Louis, USA) work solution (5 mg/mL MTT dissolved in PBS). After post-incubation at 37 °C for 4 h, the medium was discarded following by adding 100 μL DMSO (Sigma–Aldrich, St. Louis, USA). The plates were then votexed for 10 min for complete dissolution. The optical absorbance was measured at 570 nm. The data represented the mean of three independent experiments in triplicate and were expressed as mean ± SD. The IC_{50} value was defined as the concentration at which 50% of the cells could survive.

4.5. Telomerase inhibitory assay

Compounds **6a–6s** were tested in a search for small molecule inhibitors of telomerase activity by using the TRAP-PCR-ELISA assay. In detail, the SW116 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 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 to 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 24 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 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 microtiter plate subsequently. The immobilized DNA fragment were detected with a peroxidase-conjugated anti-DIG antibody and visualized following addition of the stop regent. The microtitre plate was assessed on TECAN Infinite M200 microplate reader (Männedorf, Switzerland) at a wavelength of 490 nm, and the final value were presented as mean ± SD.

4.6. Experimental protocol of docking study

Molecular docking of compound **6k** into the three-dimensional tolemerase complex structure was carried out using the AutoDock software package (version 4.0) as implemented through the graphical user interface Auto-Dock Tool Kit (ADT 1.4.6).[28]

First, AutoGrid component of the program precalculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitors within the macromolecules. 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 structures of telomerase complex were generated through homology modeling using MODELLER 9v7 program.[29] The template (PDB code: 3DU6) was retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). All bound waters and ligands were eliminated and the polar hydrogens and the Kollman-united charges were added to the molecule, respectively.

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