REVIEW
Cancer Advances 2022;5:e22018. https://doi.org/10.53388/2022522018

1,3,4-Oxadiazole as an emerging telomerase inhibitor - a promising anticancer motif

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Competing interests
The authors declare no conflicts of interest.

Abbreviations
PCR, polymerase chain reaction; IC_{50}, half maximal inhibitory concentration.

Peer review information
Cancer Advances thanks all anonymous reviewers for their contribution to the peer review of this paper.

Citation
Kumar D, Kumar V, Kumar H, Deep A, Marwaha RK. 1,3,4-Oxadiazole as an emerging Telomerase Inhibitor- A promising anticancer motif. *Cancer Adv*. 2022;5:e22018. doi: 10.53388/2022522018.

Executive editor: Guang-Ze Ma.
Received: 28 July 2022; **Accepted:** 15 August 2022; **Available online:** 22 August 2022.

Abstract
Currently, cancer is the most rapidly growing life-threatening disease after cardiovascular in the world, posing a major threat to human life. Telomerase promotes tumorigenesis and development in most cancers and dyskerin plays a crucial role in telomere maintenance. Cancer molecules are being developed continuously as a result of continuous research. A series of novel anticancer agents have been developed using telomerase inhibitors with improved specificity and pharmacokinetics. As medicinal chemistry advances, heterocyclic-based drugs find increasing applications, including anticancer agents. These properties have led to the development of five-membered aromatic rings of oxadiazoles. In order to enhance their anticancer activity, oxadiazole scaffolds can be modified. In this review, we discuss the functions and mechanism of action of the telomerase enzyme. The paper also summarizes the interaction between 1,3,4-oxadiazole inhibitors and telomerase enzymes.

Keywords: 1,3,4-Oxadiazole derivatives; telomerase enzyme inhibitors; anticancer drugs; growth factors

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Background

The rapid growth of cancer in our era poses a major threat to human life. During the course of the disease, cancer evolves and accumulates mutations [1]. According to the American Cancer Society, approximately 2.6% of people die from cancer each year. Based on statistics reported by the American Cancer Society, around 268,600 people were detected with breast malignancy in 2019 and 41,760 died from it out of all leading cancer types [2, 3]. Mammalian cells contain a ribonucleoprotein called telomerase, which is responsible for maintaining the length and stability of the telomere in frequently dividing cells. There have been many studies that confirm dyskerin, a fragmented protein of telomerase, allows telomerase activity, allowing mature human telomerase RNA to be assembled and stabilized [4, 5]. It is believed that dyskerin plays an imperative role in telomere maintenance and considering most cancers depend on the telomerase (holoenzyme) for tumorigenesis and cancer development. Therefore, anticancer drugs could be developed targeting this protein telomerase (holoenzyme) [6] (Figure 1).

In contrast, millions of hybrid products can be prepared by combining parts from different organic products. New anticancer drugs could be developed with this new approach since some hybrid compounds will have greater biological activity than their parent compounds [7]. A variety of approaches are used to treat cancer, including chemotherapy, radiation therapy, surgery and immunotherapy. As a result of its lack of targeting, chemotherapy uses anti-neoplastic agents that kill more cancer cells but have a number of adverse side effects [8].

Organic heterocyclic rings containing N, O or S atoms became the centre of attraction in the field of synthetic chemistry for developing novel medicinal compounds for their therapeutic potentials, i.e. anticancer activity. 1,3,4-Oxadiazoles are nitrogen (two atoms) and oxygen (one atom) containing heterocyclic moiety. Due to presence of heterocyclic atoms, 1,3,4-oxadiazole shows good aqueous solubility, better thermal stability, metabolic stability and less lipophilic character than the other isomeric oxadiazoles. The ability of 1,3,4-oxadiazole to form hydrogen bonds with receptor sites has led to a significant interest in chemical, medicinal and pharmaceutical research for the development of novel drugs [9].

A wide range of biological actions have been observed in 1,3,4-oxadiazole substituted compounds. 1,3,4-Oxadiazole ring is commercially available in several effective drugs i.e. Furamizole, antibacterial effects, Nesapidil and Tiodazocin, antihypertensive effects, Raltegravir, an antiviral drug, Setileuton as selective 5-lipooxygenase inhibitor and United States Food and Drug Administration approved Zibotentan anticancer drug (Figure 2) [10, 11].

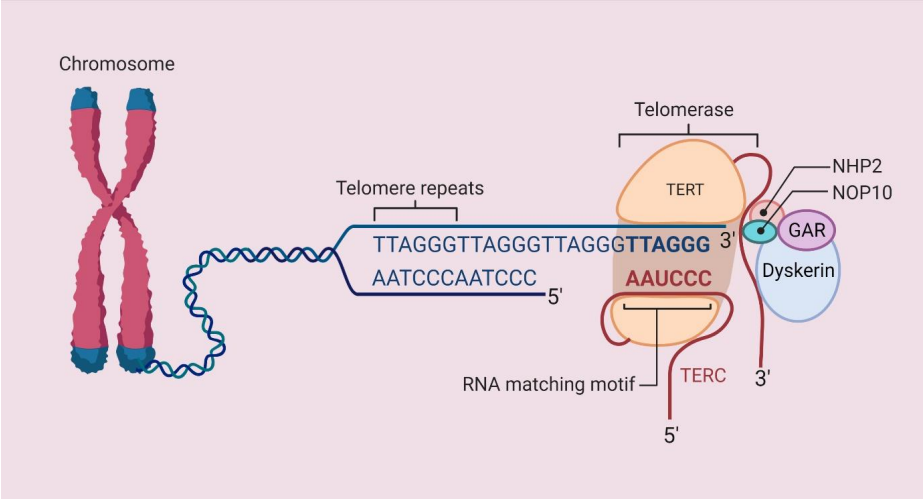

Figure 1 Telomeres and telomerase

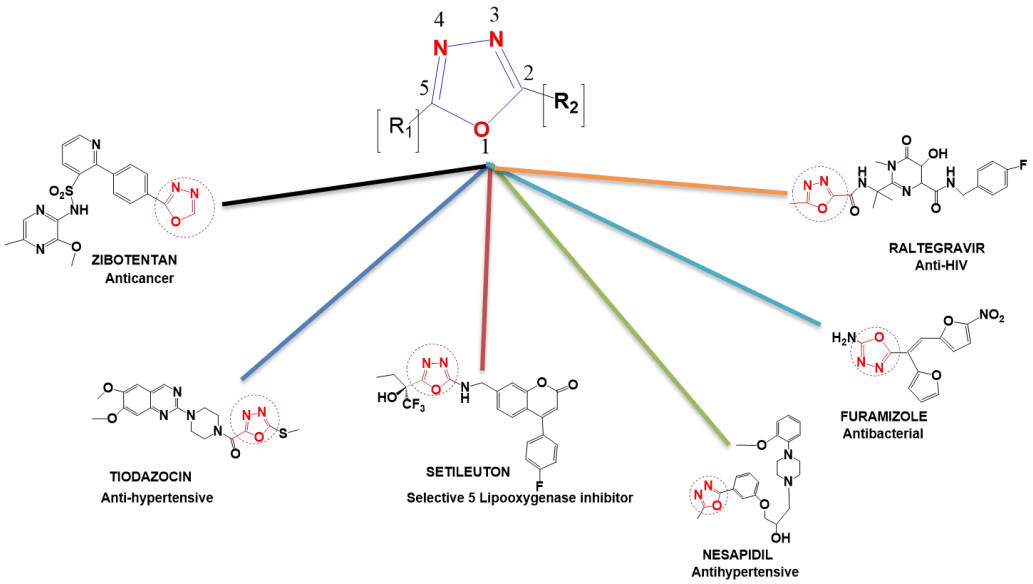

Figure 2 1,3,4-Oxadiazole containing commercially therapeutic drugs

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Telomerase enzyme

In all human chromosomes, telomerase contains a sequence of six repetitive nucleotides, TTAGGG. It plays an imperative role in maintaining genomic stability. In senescence, telomeres become short during cell division, which prevents a cell from continuing to divide, causing it to age and die [12–14] (Figure 3).

The telomerase length of most somatic cells becomes short during DNA replication. In malignant cells, the telomerase enzyme is activated, restoring and stabilizing the length of telomere by adding sequence (TTAGGG). Telomere shortening can halt tumour progression [15–18]. Early-stage tumour progression is associated with DNA damage response associated with telomere dysfunction. Inhibits tumour progression by activating DNA damage response, inducing apoptosis and suppressing cell proliferation. Because telomeres prevent replication senescence and enable continuous division of cells, telomeres are considered one of the potential causes of cellular immortality and carcinogenesis [19] (Figure 4).

Human telomerase reverse transcriptase, an enzyme that activates telomerase enzymes, is phosphorylated by protein kinase B and other growth factors [20]. Another enzyme histone-deacetylase inhibitors can reduce protein kinase activity and limit tumour growth by suppressing human telomerase reverse transcriptase phosphorylation. Consequently, telomerase has been suggested as a preferred target for new anticancer drug development [21–23].

Mechanism of action of telomerase

As telomeres lengthen, TERT-mediated stabilization occurs and telomeres lengthening does not affect cell lifespan, thereby averting senescence and telomere crisis. Telomerase prevents DNA damage to telomeres by overlaying both sides of telomeres [24, 25]. The G end and C end of telomere (5*and 3* strands) are extended by telomerase during telomere lengthening (Figure 5).

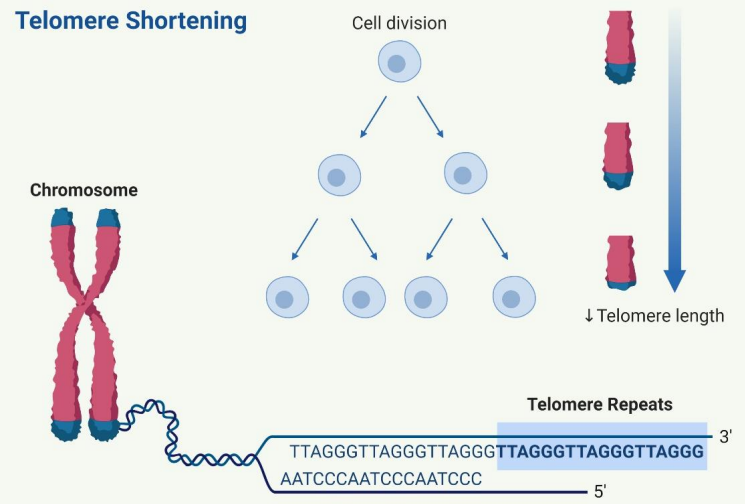

Figure 3 Telomere shortening

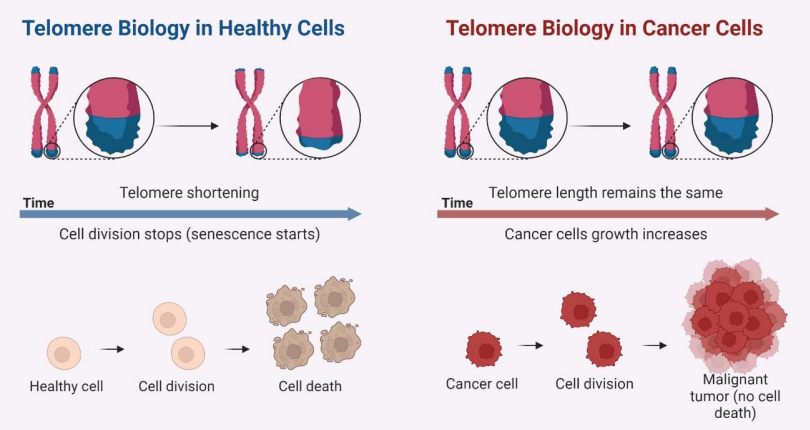

Figure 4 Study of telomere biology of Healthy vs Cancer cells

Figure 5 The G end and C end of telomere

In the telomerase complex, the G strand of newly replicated leading and lagging telomeres is extended by reverse transcription of the TERC template subunit [26–28]. A processive telomere elongation is mediated by components of the shelterin complex, such as TIN-2 and TPP-1. These components contribute to the binding of telomerase to telomeres [29, 30]. This makes inhibiting telomerase a validated treatment target for cancer. Its ultra-high sensitivity makes it one of the most widely used methods for measuring the activity of telomerase in small samples of cells or tissues. In spite of this, several polymerase chain reaction (PCR)-free methods have been developed to overcome PCR [31–34].

Significance of telomerase in cancer

A unique characteristic of telomerase is that it is absent from most somatic cells, while it is prevalent in most cancerous cells. Cancer and telomerase have complex relationships, just like aging and telomerase. There is a faster growth in understanding of telomerase's relationship with cancer than telomerase's relationship with aging. Telomerase's relationship with cancer appears to have promising clinical implications. A poor prognosis has been linked to increased telomerase activity and telomerase inhibition may suppress tumours. In the future, telomerase inhibitors should be used therapeutically in the prevention and treatment of cancer based on these fundamental findings [35–38].

Telomerase inhibitors

Zheng et al. designed new 2-chloropyridine derivatives (1) and tested their anticancer potential against SGC-7901 cell lines via telomere repeat amplification protocol [39]. 02 conjugates were found most effective against cell lines (SCG-7901-gastric cancer cell lines). Derivative (2) (2-(5-(((6-chloropyridin-3-yl)methyl)thio)-1,3,4-oxadiazol-2-yl)-5-methoxyphenol) and (3) (2-(((6-chloropyridin-3-yl) methyl)thio)-5-(naphthalen-1-yl)-1,3,4-oxadiazole) showed significant telomerase inhibitory activity i.e half maximal inhibitory concentration (IC_{50}) = 2.3 ± 0.07 μM and 2.56 ± 0.11 respectively as compared to the reference drug ethidium bromide (IC_{50} = 2.5 ± 0.23).

2-Chloropyridine derivatives linked to 1,3,4-oxadiazole ring show different anticancer activities depending on C-5 substitution. When an electron-donating group is substituted in the para position of the benzene ring, it displayed the highest potency compared to the ortho position (Figure 6).

Another pyridine clubbed 1,3,4-oxadiazole derivatives (4) was prepared by Zhang et al. [40]. The researcher evaluated their telomeres enzyme inhibitor (anticancer) potential via enzyme-linked immunoassay, telomere repeat amplification protocol-PCR-method. Among all series, conjugate (E)-N'-(3,4-dihydroxybenzylidene)-2-((5-(pyridin-4-yl)-1,3, 4-oxadiazol-2-yl) thio) acetohydrazide (5) (Figure 7) had shown maximum potency against cancer cell lines (BGC823) with IC_{50} value of 1.18 ± 0.14 μM which was much lower than the reference drug Staurosporine – (PK inhibitor) (IC_{50} = 4.18 ± 0.05 μM) and ethidium bromide (IC_{50} =2.71 ± 0.18 μM).

Sun et al. designed novel quinolone conjugates (7) and screened their telomerase inhibitory potential against HepG2 (human hepatoma cells), SGC-7901 (human gastric cancer cells) and MCF-7 (human breast cancer cells) cell lines [41]. An innovative series of telomerase inhibitors sharing a quinoline core has been shown to be significantly anticancer. From the all-synthesized compounds 3-(((2-Fluorophenyl) amino) methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (8) and 3-(((4-Chlorophenyl) amino) methyl)-5-(quinolin-2-yl)-1,3,4-oxadiazole-2(3H)-thione (9) (Figure 8) shown the maximum telomerase inhibition with IC_{50} 0.8 ± 0.1 and 0.9 ± 0.0 μM. Therefore, halogenated 1,3,4-oxiadiaozle derivatives were shown to have broad-spectrum anticancer activity.

Han, Xu et al. synthesized various 2-phenyl-4H-chromone derivatives (10) clubbed with 1,3,4-oxadiazole and amide groups [42]. All derivatives were screened their anticancer activity via telomerase enzyme inhibition assay. Most synthesized derivatives of the series displayed good telomerase inhibitory activity. Among all derivatives, conjugate (11) was found most significant potency with IC_{50} < 1 μM, which was better that reference drug (staurosporine) (IC_{50} ¼ 6.41 μM). Western blotting assay which revealed, dyskerin, a fragment of telomerase, might be reduced by this compound (Figure 9).

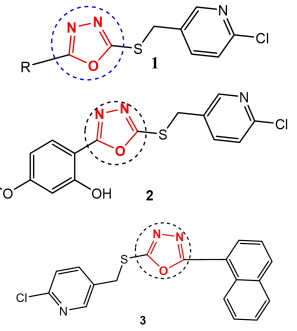

Figure 6 Telomerase inhibitors of 1,3,4-oxadiazole conjugates

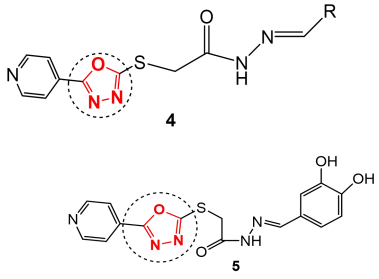

Figure 7 Telomerase inhibitors of pyridine clubbed 1,3,4-oxadiazole derivatives

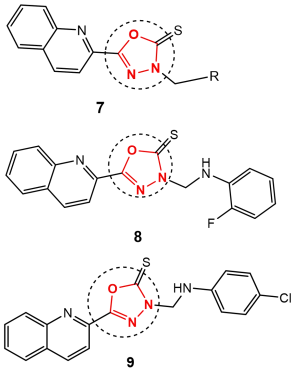

Figure 8 Novel quinolone derivatives

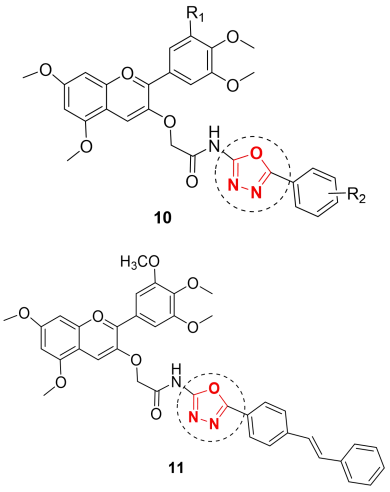

Figure 9 2-Phenyl-4H-chromone derivatives

Conclusion

This review identified 1,3,4-oxadiazole moiety as a telomerase inhibitor with anticancer properties. The review also paid attention to molecular targets and pathways involved in cancer development, mechanisms of action and structure-activity relationships. Structure-activity relationship studies have demonstrated increased activity against telomerase for 1,3,4-oxadiazoles clubbed with various other heterocyclic moieties. Introductory mechanisms showed that 1,3,4-oxadiazole compounds supressed telomerase enzyme activity by reducing dyskerin expression. The critical mechanism behind tumor suppression by 1,3,4-oxadiazole is related to the inhibition of different growth factors and kinases including telomerase enzyme. As a therapeutic agent for telomerase inhibition, 1,3,4-oxadiazoles are still under exploration in modern medicines.

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