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In silico design of telomerase inhibitors

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Telomerase is a reverse transcriptase enzyme involved in DNA synthesis at the end of linear chromosomes. Unlike in most other cells, telomerase is reactivated most cancerous cells and, therefore, has become a promising new anticancer target. Despite extensive research, direct telomerase inhibitors have yet not been introduced to the clinics because of the complexity of this enzyme. Structures of this protein from simple organisms and human homology models are currently available and have been used in structure-based drug design efforts to find potential inhibitors. Different *is silico* strategies have been applied and different chemical groups have been explored. Here, we provide an overview of recent discoveries.

Introduction

Telomeres are complexes of noncoding fragments of DNA with the six proteins collectively called shelterin [1,2]. Telomeric DNA in vertebrates comprise many repeats of six nucleotides (TTAGGG), and its length varies from 5 to 15 kbp in humans and up to 100 kbp in rodents [3]. The main function of telomeres is protection of the terminal fragments of linear chromosomes. The length of the telomere decreases on each cell division. Critical shortening of the telomeres leads to arrest of the proliferation of cells and eventually to their senescence or apoptosis. As a result, after a fixed number of population doublings (PDs), cells stop proliferating and the number of cells plateaus. The estimated maximum number of PDs to reach cellular senescence was defined as a Hayflick limit and equals ~50 PDs [4].

As a reverse transcriptase, telomerase comprises a RNA template (TER) with its binding domain (TRBD) and reverse transcriptase unit (TERT). In most somatic cells of adult organisms, telomerase becomes dormant. By contrast, in cancerous cells, telomerase is reactivated to result in the replicative immortality of cells [5,6]. Overexpression of TERT alone is not sufficient to lead to the tumorigenesis; it also requires the loss of tumor suppressor genes, such as transformation-related protein 53 (*TP53*), phosphatase and tensin homolog (*PTEN*) and retinoblastoma protein (*RB*). By contrast, various studies have shown that, in the presence of other oncogenic factors, it promotes cancer progression [1]. Thus, telomerase appears to be a major factor in maintaining the balance between normal cellular differentiation and aberrant carcinogenic proliferation, including immortality [7].

Telomerase inhibition

Given that telomerase is reactivated in most cancerous cells, it has become a promising target for anticancer chemotherapy [5,8,9]. However, although telomeres of cancerous cells are usually shorter than those of the surrounding cells, decreasing telomere lengthening by inhibiting telomerase would not have an immediate cytotoxic effect [10]. Nevertheless, there are studies showing that TERT silencing leads to a reduction in cellular proliferation and growth, with no visible effect on telomere length [11]. Furthermore, the inhibition of telomerase activity induced apoptosis in cells from ovarian cancer [12].

Methods of inhibition

Telomerase is a challenging but attractive target because inhibiting or blocking its functions related to telomere maintenance can be performed at various stages and with the use of various mechanisms [13–15]. One approach, which is now less popular, is based on blocking telomerase access to DNA by stabilizing G-quadruplexes formed by a 3′ DNA overhang, which is thought to disable

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telomere elongation [16–19]. Most compounds used in this approach are not highly selective because G-quadruplex structures can also occur in other parts of the DNA rich in G sequences [13]. Moreover, stabilizing those structures in other DNA regions can lead to adverse effects in cells other than cancer cells [13]. Such compounds also exhibit limited absorption [18]. One promising G-quadruplex stabilizer (BRACO-19) showed significant shortening of the telomeres in a uterine cancer cell line (UXF1138L) [20]. This compound was docked *in silico* to a G-quadruplex structure [21] and later patented as a potential anticancer drug. Another approach leading to inhibition of the enzymatic active site of telomerase includes the use of compounds working in antisense to the TER. Compound GRN163 (later GRN163L) was selected from chemically modified nucleotides and tested on cell lines of human melanoma (Caki-1), lung (A431), and prostate (DU145) cancer [22,23]. The compound was later patented as a potential anticancer drug. Possibly the most promising approach is using compounds to directly block the active center of the enzyme (Fig. 1) in the catalytic subunit [24]. One such compound, first proposed in 2001, is BIBR1532, which selectively inhibits telomerase activity [25]. This compound has been patented as a potential anticancer drug [26]. Thus, here we focus on direct inhibition of TERT as the most promising approach for blocking the action of telomerase.

Models for structure-based drug design

A structure-based drug design strategy can be used to design potential inhibitors of the telomerase active site. For this approach, accurate models of the human telomerase structure [27]

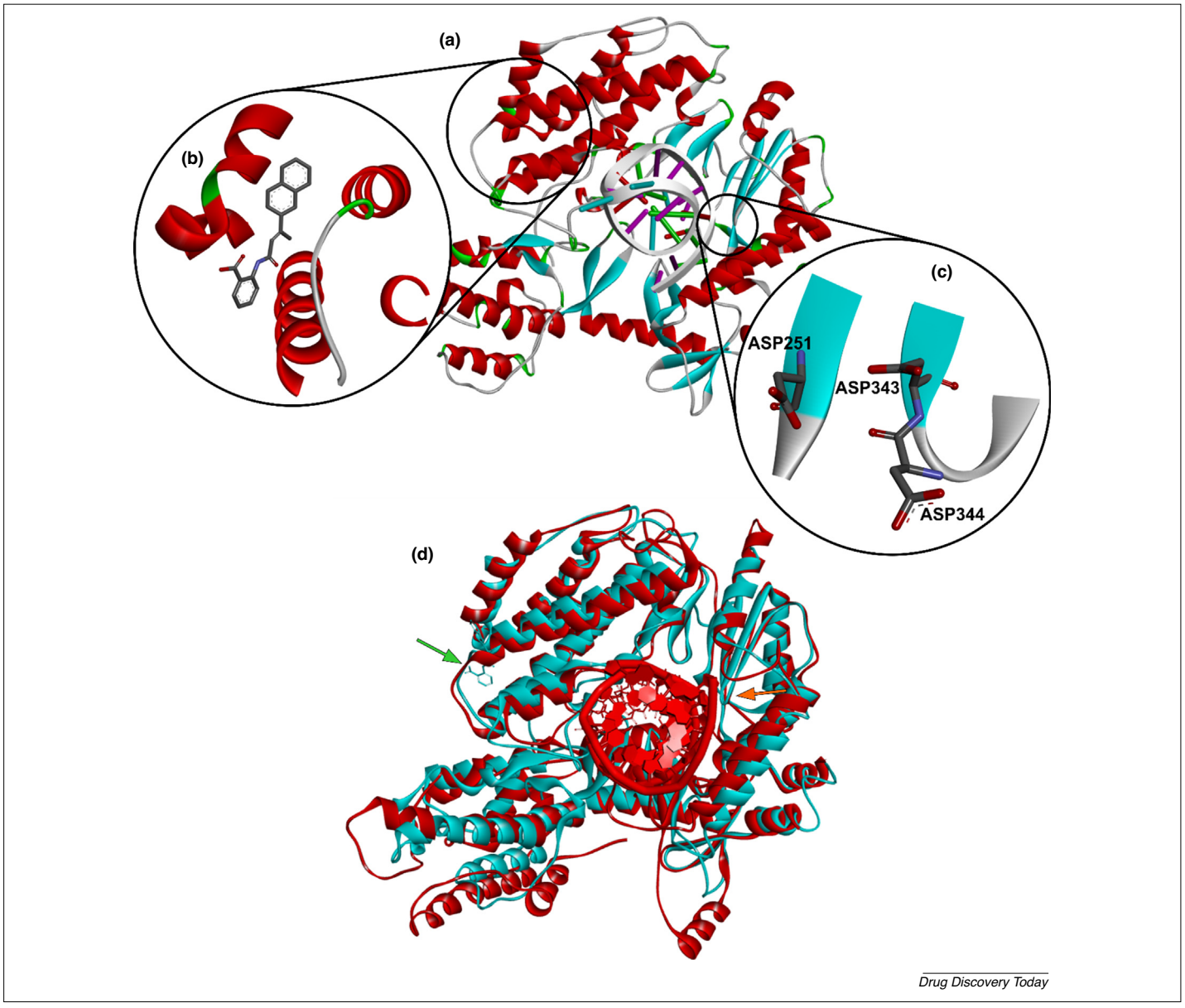


FIGURE 1

Schematic of the *Tribolium castaneum* telomerase model. **(a)** *T. castaneum* telomerase catalytic subunit TERT [Protein Data Bank (PDB) 3KYL]. **(b)** Magnified view of the FVYL pocket of the *T. castaneum* telomerase catalytic reverse transcriptase unit (TERT) bound to BIBR1532 (green arrow; PDB 5CQG). **(c)** Magnified view of the active center of *T. castaneum* telomerase with three aspartate residues highlighted that form the catalytic triad (orange arrow; PDB 3KYL). **(d)** Superimposed structure of the proposed human telomerase model (red) and *T. castaneum* telomerase structure (PDB 5CQG) (cyan).

TABLE 1

Available models of telomerase structure

PDB entry code	Source organism	Remarks	Refs
2R4G	*Tetrahymena thermophila*	RNA-binding domain with resolution 1.71 Å; X-ray	[28]
3DU5 and 3DU6	*Tribolium castaneum*	Catalytic subunit with 2.71 Å resolution; X-ray	[29]
3KYL	*T. castaneum*	Catalytic subunit in complex with RNA-DNA hairpin construct with 2.7 Å resolution; contains magnesium ion in active center; X-ray	[30]
N/A[a]	*Homo sapiens*	TERT, TEN, and TRBD with DNA substrate bound with RNA template; homology modeling	[31]
5CQG	*T. castaneum*	Catalytic subunit in complex with BIBR1532 compound bound in FVYL pocket with 2.3 Å resolution; X-ray	[32]
6D6V	*T. thermophila*	Telomerase bound to telomeric DNA obtained by cryoelectron microscopy with 4.8 Å resolution	[33]
EMD-7521	*H. sapiens*	Obtained by cryoelectron microscopy; comprises substrate-bound human telomerase with 10.2 Å resolution	[34]
6E53	*T. castaneum*	Catalytic subunit of telomerase in complex with RNA–DNA hairpin construct and telomerase inhibitor with 2.8 Å resolution; X-ray	[35]

[a] Structure modeled and, therefore, not available in PDB database.

are required (Table 1). In 2007, the high-resolution structure of the TRBD of *Tetrahymena thermophila* was published [Protein Data Bank (PDB) 2R4G] [28]. This domain is essential for the activity of the enzyme and showed no structural homologs at that time. The first model of the full-length *Tribolium castaneum* catalytic subunit of telomerase was obtained by X-ray in 2008 (PDB 3DU5 and 3DU6) [29]. This model comprises three highly conserved domains creating a ring-like structure. Motifs associated with substrate binding and catalysis located in the interior of the structure can contain seven to eight base pairs of DNA. Structural comparison of the TRBD from *T. castaneum* with that from *T. thermophila* showed some similarities [29]. A model of telomerase published in 2010 showed the full-length *T. castaneum* catalytic subunit of the enzyme in complex with a RNA–DNA hairpin construct (PDB 3KYL) [30]. A nucleotide hairpin was designed to resemble the RNA-template region and fragment of telomeric DNA. This crystal structure contains a magnesium ion in the active center. In 2011, a theoretical approach to obtain the entire 3D structure of the human TERT, TEN, and TRBD with DNA substrate bound with RNA template was reported. The sequence of the translation of human telomerase transcript variant 1 mRNA (Gen Bank: NM_198253.2) was used in homology modeling and the resulting 3D models were then assembled manually [31]. The first structure showing an inhibitor was published in 2015, revealing the *T. castaneum* full-length catalytic subunit of telomerase in complex with BIBR1532 (PDB 5CQG), which showed that the studied ligand occurs outside of the active center [32]. This structure changed our understanding of the expected mechanism of activity of this compound and possibly other active site-directed molecules. A model obtained by X-ray displayed the binding of the ligand to a highly conserved hydrophobic pocket (FVYL) motif on the outer surface of the thumb domain of telomerase. The FVYL motif is described as being near to the TRBD residues that bind the activation domain of the telomerase catalytic domain [32]. Fig. 1d shows the superimposed structures of the human and 5CQG model to provide an overview of the target hot spots. One of the most recently published models depicts active *Tetrahymena* telomerase bound to telomeric DNA obtained by cryoelectron microscopy (cryo-EM) (PDB 6D6V) [33]. The model comprises a TERT domain that interacts with the TEN domain, which is believed to physically enclose TER and regulate its activity [33]. Another model obtained by cyro-EM comprised substrate-bound human telomerase, and was the first published architectural characterization of a human telomerase (PDB EMD-7521). The structure is of low resolution and, as far as we are aware, has not yet been used in any published molecular docking studies [34]. In 2019 another X-ray structure of the *T. castaneum* catalytic subunit of telomerase in complex with telomerase inhibitor was published (PDB 6E53) [35]. This structure also contained the RNA–DNA hairpin construct and the location of the inhibitor in the active site blocked DNA synthesis (Fig. 2).

Compounds

Various compounds designed using an *in silico* approach have been tested as potential inhibitors of the catalytic subunit of telomerase (Table 2). *In silico* approaches enable researchers to consider compounds that have not been previously synthesized. Development of new molecules based on *in silico* rational structure-based drug design (selected hit molecules) should be followed by confirmation of activity for selected hits. Such identification of hits will help in the rational design of more efficient telomerase modulators, providing that comparison databases are created [36].

Benzylidene-hydrazone analogs

To predict the binding affinity and mode of action, docking into the catalytic subunit of the telomerase was performed (PDB 3DU6 [29]). There studies revealed that the benzylidene-hydrazones show various hydrophobic, electrostatic, covalent, and Van der Waal's interactions with the active center of the telomerase [37]. The most active compounds (3e and 3l) exhibited low binding energies, which were significantly lower than those of the moderately active compounds (3h and 3m) [37]. The higher binding affinities showed by compounds 3d, 3e, 3l, and 3n could have been caused by not only hydrogen bonding, but also additional π-sulfur and π–π interactions with amino-acid residues of the telomerase active site. Results of total binding energies calculated by the docking studies and the cytotoxic potencies values (IC_{50}) determined experimentally showed high correlation [37].

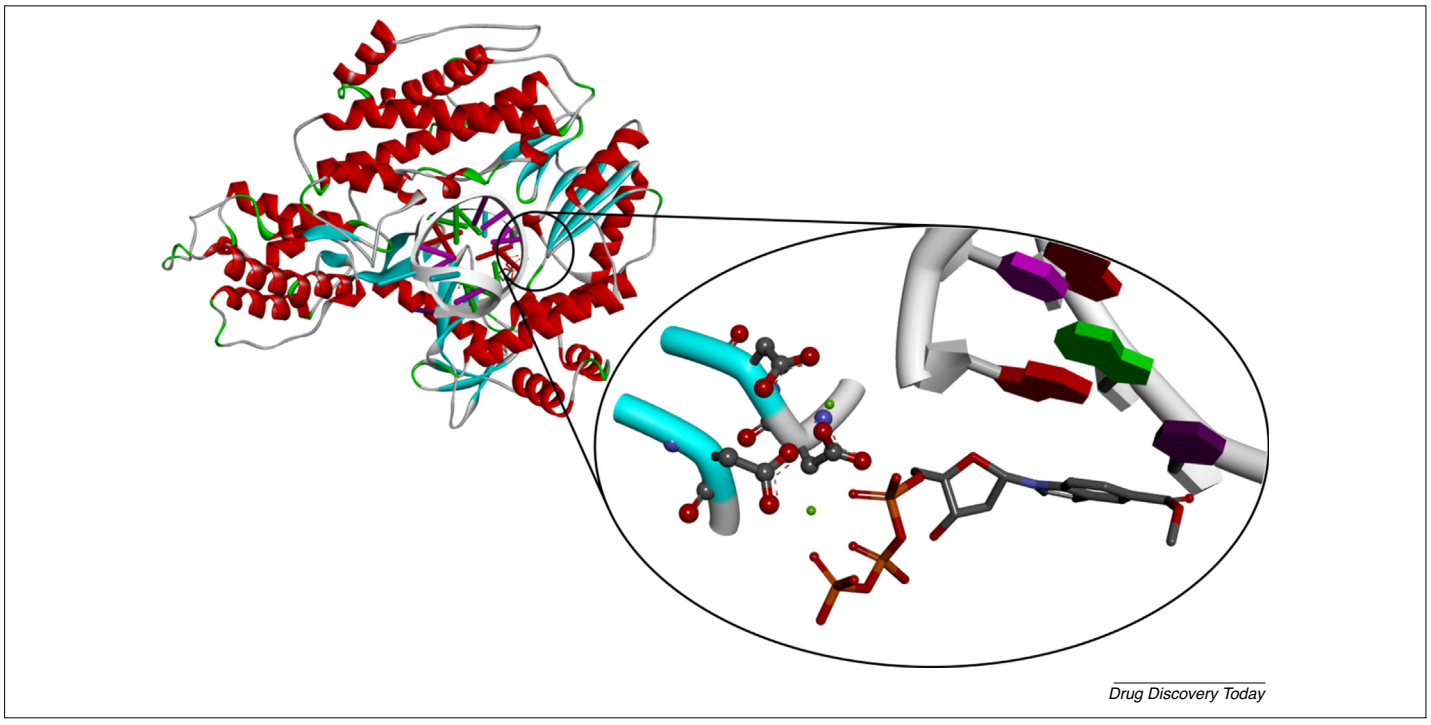


FIGURE 2

Model of the catalytic subunit of telomerase with a RNA–DNA construct in complex with a telomerase inhibitor [Protein Data Bank (PDB) 6E53]. **(a)** Structure of enzyme. **(b)** Magnified view of active center of structure, including fragment of RNA–DNA hybrid, inhibitor, fragment of protein with highlighted catalytic triad, and two magnesium ions (green).

Dihydropyrazole

Coumarin derivatives containing a 4,5-dihydropyrazole moiety were tested as potential telomerase inhibitors [38]. Bioassay tests using the human gastric cancer cell line SGC-7901 and human liver cancer cell line Hep-G2 showed that compound 3b exhibited significantly higher antitumour activity. Docking of the 3b compound to the active site of *T. castaneum* telomerase (PBD 3DU6) revealed that a coumarin containing a 4,5-dihydropyrazole moiety formed intramolecular hydrogen bonds between the ALA255 amino-acid residue and fluoride [38]. An antiproliferative assay for coumarin derivatives containing a 4,5-dihydropyrazole moiety was also performed on the SGC-7901, PC-3, and A431 human cancer cell lines [39]. The telomerase-inhibiting abilities tested by a modified TRAP assay showed that a coumarin derivative containing a 4,5-dihydropyrazole moiety (3d) and a 4,5-dihydropyrazole with bromine atom moiety (3f) exhibited potent activity. To elucidate the potential mechanism of activity of compound 3d, docking to the active site of the telomerase model (PDB 3DU6) was performed [39]. Another compound, a 2-hydroxy-phenyl-4,5-dihydropyrazole derivative (4a), was tested on the SGC-7901, Hep-G2, and PC-3 cell lines and exhibited high activity [40]. Molecular docking of 4a to a telomerase model (PDB 3DU6) showed a hydrogen bond between the oxygen and the amino hydrogen group of ARG340 and projection of a dihydropyrazole ring into a hydrophobic region (side chains of ALA220, TYR224, LYS224, THR226, and SER227), which influenced the accessibility of the hydrophobic pocket [40]. Other tested derivatives of 4,5-dihydropyrazole contained 5-phenyl-*N*-piperidine or ethanone moieties [41]. The cytotoxic activity of compounds was tested on SGC-7901, MGC-803, and Bcap-37 cell lines. Telomerase inhibition assay results showed that compound 7b exhibited the strongest telomerase inhibition, with compound 4d having the strongest activity among ethenone derivatives [41]. Visual inspection of the molecular docking of compound 4d to the telomerase model (PDB 3DU6) revealed three hydrogen bonds between nitrogen and the amino hydrogen group of LYS189, oxygen and the amino hydrogen group of ASP254, and oxygen with the amino hydrogen group of GLN308 [41].

Novel aryl-2H-pyrazole derivatives bearing 1,4-benzodioxan or 1,3-benzodioxole moieties were tested as potential telomerase inhibitors [42]. Molecular docking of one of the derivatives (16A) showed the most potent telomerase inhibition properties. The telomerase inhibition tests showed that compound 16A exhibited inhibitory activity, with an IC_{50} value of 0.9 μM, whereas antiproliferative tests showed that this compound exhibited high activity against human gastric cancer cell line SGC-7901 and human melanoma cell line B16-F10, with IC_{50} values of 18.07 and 5.34 μM, respectively. Docking was performed using a model of the TERT protein catalytic subunit (PDB 3DU6). Visual inspection of the docking pose of 16A into the active site revealed two optimal intermolecular hydrogen bonds, and two benzene rings of the compound formed π-cation interactions, influencing the accessibility of the hydrophobic pocket that flanks the active binding site [42]. However, it has not been possible to repeat this research with the same results (E. Wieczerzak, M. Dzierzynska, M. Heldt, N. Maciejewska, M. Olszewski, M. Serocki, U. Kalathiya, M. Baginski, unpublished data, 2019).

Newly synthesized 4,5-dihydropyrazole derivatives containing a heterocyclic oxygen moiety were also tested. Results of a modified TRAP assay showed that 18 of the tested derivatives displayed

good telomerase inhibitory activity, with 10a being the compound with the highest activity [43]. All of the derivatives were docked to telomerase model 3DU6 to explore interaction models of the potential inhibitors [43].

Another compound, dihydropyrazole-chromen (13k), was tested on cancer cell line MGC-803, where it exhibited an IC_{50} value 1.41 μM and showed no toxic effects on human healthy gastric mucosa cells (GES-1). Molecular modeling was used to model the binding poses of the designed compound in a 3D human telomerase model [44].

Eight new potential human telomerase inhibitors with a core of *N*-acyl-4,5-dihydropyrazole (5a–5h) were also obtained [45]. The compounds were evaluated for anticancer activity on human gastric cell lines (MGC-803 and SGC-7901) and a human liver cancer cell line (Hep-G2). Results showed that compound 5c exhibited the most potent antiproliferative activity, followed by

TABLE 2

Structure of different scaffolds of potential inhibitors

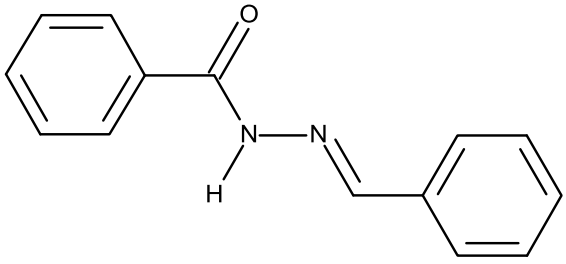
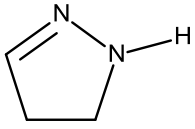
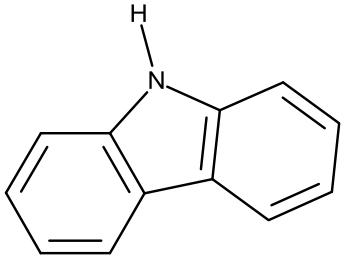
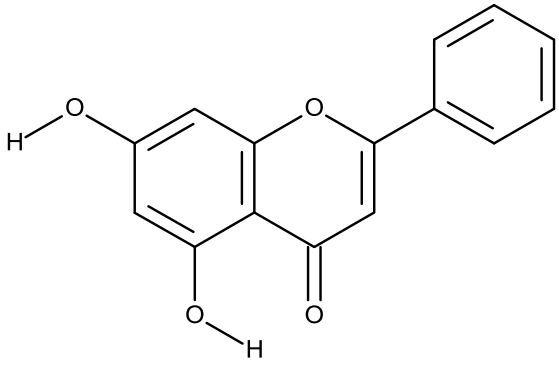
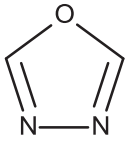
Name of the scaffold	Scaffold structure	Refs
Benzylidene-hydrazone analogues		[37]
Dihydropyrazole derivatives		[38–45]
Dibenzopyrrole derivatives (carbazole)		[46]
Flavone pyridine derivatives		[47]
Oxadiazole derivatives		[48–51]

TABLE 2 (*Continued*)

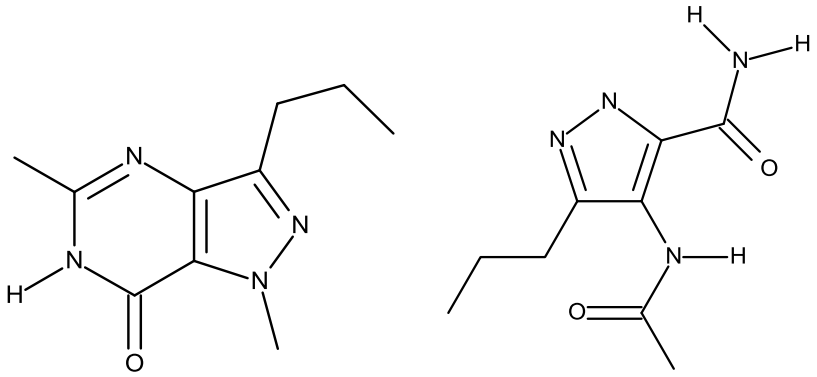
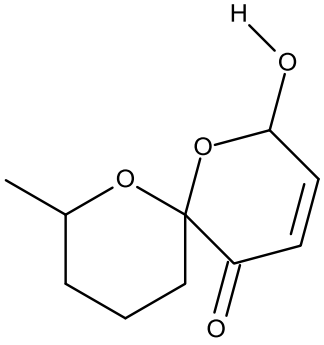
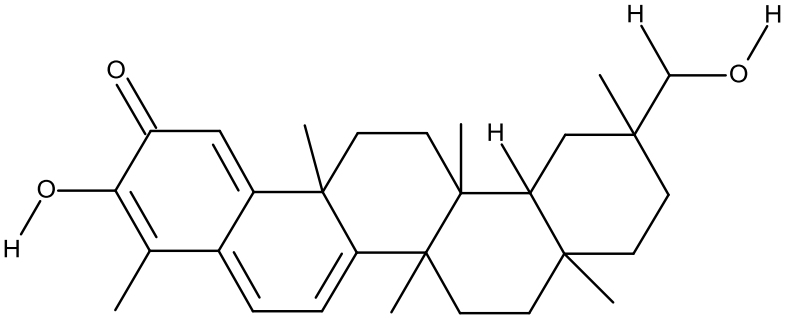
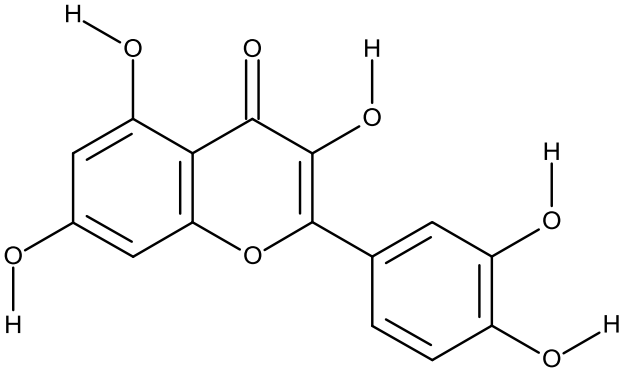
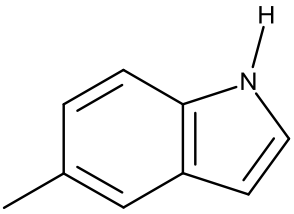
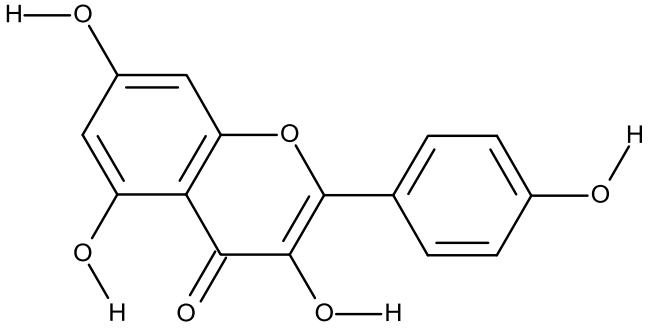
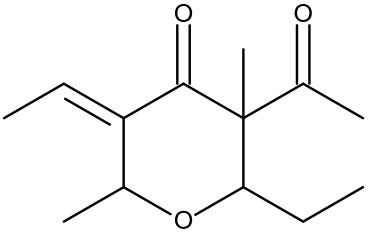
Name of the scaffold	Scaffold structure	Refs
Pyrazole derivatives		[52]
Spiroketals		[53]
Celastrol derivatives		[54]
Myricetin derivatives		[55]

TABLE 2 (*Continued*)

Name of the scaffold	Scaffold structure	Refs
Indolyl-2′-deoxynucleotide analogs		[35]
Flavonoid derivatives		[56]
Chrolactomycin derivatives		[57]

5d and 5h. Docking of the compounds to the active site of the modeled hTERT-BIBR1532 complex (5CQG) was made to determine whether these derivatives had similar interactions with the enzyme as seen with BIBR1532. Results shown that, despite the difference in the structures, the most potent compound, 5c, exhibited similar hydrophobic interactions with the enzyme as BIBR1532 [45].

Dibenzopyrrole

Another scaffold used for potential telomerase inhibitors are dibenzopyrroles. Twenty-one new derivatives were designed and tested as a potential telomerase inhibitors *in silico* with the use of the 3DU6 model. The scaffold of the compounds was modified by extending its ends groups with different functional groups [46]. The synthesis and evaluation of these compounds awaits publication (M. Szewczyk, M. Heldt, N. Maciejewska, M. Olszewski, S. Makowiec, M. Baginski, unpublished data, 2020).

Flavone pyridines

An antiproliferative assay for flavone pyridine compounds was performed with the SGC07901 cell line and showed that 2-chloropyridine derivatives containing flavone moieties (6a–6f) showed significantly higher activity, with the two most potent compounds being 6e and 6f [47]. To determine the inhibitory activity of compounds against telomerase, a modified TRAP was performed, with results similar to those of the antiproliferative assays. Docking of the derivatives to the telomerase model (PDB 3DU6) of the most potent compound (6e) indicated residues in the hydrophobic region (LYS198, ASN369, ILE252, and ARG253), which are recognized as key factors in controlling telomerase selectivity [47].

Oxadiazole

A series of newly synthesized derivatives of 2-chloropyridine with a 1,3,4-oxadiazole moiety were tested as telomerase inhibitors [48]. The most potent compound as revealed by a TRAP assay (6o) exhibited telomerase activity with an IC_{50} of 2.3 μM. Binding of the 6o compound to the 3DU6 model showed the importance of three hydrogen bonds between the protein and its ligand [48].

Another novel series of 1,3,4-oxadiazole derivatives with a 1,4-benzodioxan moiety was tested on four cancer cell lines (HepG2, HeLa, SW1116, and BGC823) [49]. From the results, compounds with a substituted benzene ring displayed high inhibitory activity. Molecular docking of the most potent compound (6k) to the active center of the 3DU6 model of telomerase was performed. Hydrogen bonds were observed between the oxygen atom of the oxadiazole group of 6k and the amino hydrogen of LYS372, as well as between the oxygen atom of the benzene group of LYS372 and the hydrogen atom of LYS406 [49].

Another group of compounds tested as potential telomerase inhibitors comprised 33 heterocyclic azole derivatives containing

pyrazine. The antiproliferative activity of the compounds was tested on the HepG2, SW1116, HeLa, and BGC823 cell lines. Telomerase inhibition assay results showed moderate correlation with the antiproliferative assay, also depicting compound 8h as the most active [50]. The most potent derivative from each group (5c, 8h, and 11f) was docked to a telomerase model (PDB 3DU6) [50].

In the same project, derivatives of 1,3,4-oxadiazole with pyridine and acylhydrazone moieties were tested [50]. Structure–activity relationship studies showed that different substituents on the benzene ring affected the anticancer activity. Modified TRAP assays showed a correlation between telomerase inhibition and the antiproliferative activities of the compounds, with the highest telomerase inhibition activity being found with compound 6 s. The most potent compound was then docked into the active center of the telomerase structure (PDB 3DU6). The results suggest that introduction of pyridine, acylhydrazone, and 3,4-dihydroxyphenyl groups reinforce the interaction of ligand with the receptor [50].

Newly synthesized quinoline derivatives with oxadiazole moieties were tested on three cancer cell lines (HepG2, SGC-7902, and MCF-7) [51]. The position of the substitution on the benzene ring was significant for the activity of the compounds. A telomerase inhibitory assay was performed that showed a correlation with cell proliferation assay results, revealing compounds 4d and 4i as the most potent. Those compounds were later docked to the 3DU6 model [51].

Pyrazole

Another series of novel derivatives of pyrazole-5-carbocamide and pyrazole-pyrimidine were designed, synthesized, and tested as potential telomerase inhibitors [52]. The SGC-7901, MGC-803, and Bcap-37 cancer cell lines were chosen for antiproliferative assays. Compounds with a 5-propyl-1H-pyrazole-3-carboxamide group (8a–8e) generally exhibited better activity results than compounds with a pyrazolo[4,3-d]pyrimidin-7(6H)-one group (9a–9e). To recognize the mechanism by which most potent compound (8e) exhibited its activity, molecular docking to the 3DU6 telomerase model was performed, and showed six bonding interactions between the derivative and the enzyme [52].

Spiroketals

Antiproliferative assays of a spiroketal stereoisomeric mixture (12a–12d) was carried out using breast carcinoma (MCF-7), melanoma (M14), pulmonary carcinoma (H125), colon carcinoma (HT-29), promyelocytic leukemia (HL-60) and neuroblastoma (SH-SY5Y) cell lines and showed that the stereoisomeric mixture and also the pure enantiomers 12a and 12b, induced significant inhibition of cell proliferation in all the lines tested [53]. The assay showed that the spiroketal mixture was a potent telomerase inhibitor. Molecular docking of the stereoisomers to a telomerase model (PDB 5CQG) showed that the 12a and 12b enantiomers exhibited the strongest interaction with the enzyme [53].

Celastrol

Rational drug design of the scaffold of celastrol was performed to obtain derivatives with increased solubility or reduced toxicity by either esterification or amidification. The anticancer activity of the compounds was tested in SGC-7901, SMMC-7721 (human hepatoma cells), MGC-803, and Hep-G2 cell lines [54]. Results showed that celastrol without a substituent group showed good antiproliferative activity, whereas esterification reduced this activity. Derivatives containing a β-hydroxyl-ethylamide substituent (3c and 3d) exhibited potent anticancer activity compared with the moderate anticancer activity of the derivatives 3h and 3i, whereas the remaining compounds showed poor activity. Results of telomerase activity assay correlated well with the antiproliferative assay results. Compound 3c was docked to an active site of the 5CQG telomerase model and interactions between the ligand and the receptor indicated the importance of hydrogen bonds in those interactions [54].

Myricetin

Another group of compounds investigated are myricetin derivatives, flavonoid compounds present in various fruits and considered to have anticancer activity. Antiproliferative assays were performed on Bcap-37, MDA-MB-231, SGC-7901, and MGC-803 cancer cell lines [55]. Results indicated that most derivatives of 3-(3-(substituted)propoxy)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)-4*H*-chromen-4-one (3c, 3d, 3h, 3i, and 3j) exerted relatively high inhibitory activity, whereas, of the derivatives of 2-(5,7-trimethoxy-4-oxo-2-(3,4,5-trimethoxy-phenyl)-4H-chromen-3-yloxy)-N′-(2-substituted) acetylhydrazine, moderate activity was exhibited by compounds 6a, 6b, and 6f, whereas only one exhibited high activity (6d). Telomerase inhibitory assay results showed compounds 3h, 6a and 6d to have the most potent activity. Docking of the derivative 6d to a telomerase model (5CQG) showed interactions including a hydrogen bond between the carbonyl group of the ligand and the residue of LYS710 [55].

Indolyl-2′-deoxynucleotide analogs

Hernandez-Sanchez and colleagues tested a set of nucleotide analogs as a potential inhibitors of telomerase activity. Compounds were designed to mimic the core structure of deoxyadenosine triphosphate (dATP), screened by docking to *T. castaneum* telomerase (PDB 3KYL), and tested using *in vitro* telomerase activity assays. The compound with inhibitory activity [5-MeCITP (5-methylcarboxyl-indolyl-2′-deoxyriboside 5′-triphosphate] was co-crystallized with *T. castaneum* telomerase, showing noncanonical binding that disturbed the TER positioning, resulting in the publication of model 6E53 [35].

Flavonoid derivatives

Flavonoid-derived compounds were synthetized and screened for telomerase inhibitory activity against HeLa human cervical cancer cells, and for antiproliferative activity against human lung cancer cells (A549), human hepatoma cells (HepG2), human gastric cancer cells (MGC-803) and human gastric cancer cells (SGC-7901). Results suggested that compound 5c exhibited the most inhibitory activity with $IC_{50}<50$ μM. Compound 5c, with another compound showing no activity (6c), and BIBR1532 were docked to the *T. castaneum* telomerase model (PDB 5CQG). Results indicated that 5c and BIBR1532 showed similar stability and mode of interaction [56].

Chrolactomycin derivatives

A recently published study described an attempt to design covalent inhibitors of telomerase based on chrolactomycin derivatives

using the *T. castaneum* telomerase model (PDB 3DU5). Results showed that the lead compound (NU-1) exhibited potent irreversible enzyme inhibition and insignificant effects on telomerase-negative control cell lines [57].

Concluding remarks and future directions

The most crucial issue in the *in silico* drug design of telomerase inhibitors is the credibility of the model used. Although the active site of the human and *T. castaneum* catalytic subunit is conserved structurally (Fig. 1), it remains unclear whether studies using the nonhuman structure can be directly related to the human enzyme. Another important issue is the inclusion in *in silico* studies of RNA–DNA as well as magnesium ions. The recent X-ray structure of *T. castaneum* telomerase containing a RNA–DNA construct, magnesium ions, and inhibitor (PDB 6E53) supports the idea that the model for *in silico* studies should also take into account other components, rather than only the protein entity. By contrast, the published structure with the inhibitor BIBR1532 (PDB 5CQG) queries whether there is more than one mode of TERT inhibition. Should the conserved hydrophobic pocket (FVYL) motif on the outer surface of the thumb domain of telomerase also be explored within *in silico* studies? The studies discussed herein mark the beginning of the *in silico* search for telomerase inhibitors. Many *in silico* studies have been performed *post factum* rather than as a starting point. Moreover, in many cases, a model of telomerase without magnesium ions and RNA-DNA system was used. Also, only simple tests, usually TRAP, were performed as proof that the study compound was a potential telomerase inhibitor. Given their structure, the compounds studied might have other anticancer mechanisms of action and, therefore, typical MTT cytotoxicity tests do not need to correlate with antitelomerase activity. Despite the many structural scaffolds that have been explored and published as results of *in silico* studies within the past 4–5 years, few patents have followed, and neither have some of these studies been followed up by other groups. In addition, none of the *in silico* designed molecules have reached clinical trials. The only inhibitor of the telomerase catalytic subunit to be tested in the clinics is imetelstat [58] (https://clinicaltrials.gov), but it was not designed using *in silico* studies. However, we consider that a recent breakthrough using the *in silico* approach resulted in the inhibitor 5-MeCITP [35]. This was the first time that the molecular mechanism of telomerase inhibition in the active site was outlined and the role of the three ASP residues of telomerase interacting with magnesium ions and the RNA–DNA system was proposed. This new model and view of the inhibition mechanism could become a starting point for more intensive and effective *in silico* studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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