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Identification of human telomerase inhibitors having the core of *N*-acyl-4,5-dihydropyrazole with anticancer effects

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ABSTRACT

Eight human telomerase inhibitors (**5a**–**5h**) having the core of *N*-acyl-4,5-dihydropyrazole with anticancer effects were identified in this study. Biological results revealed that a few compounds had potent anticancer activities against three common tumor cell lines (SGC-7901, HepG2 and MGC-803). Among them, compound **5c**, with a molecular weight of only 272.2 Da, had antiproliferative activities against SGC-7901 and MGC-803 with EC_{50} values of 2.06 ± 0.17 and 2.89 ± 0.62 μM, respectively, better than 5-Fluorouracil. Compound **5c** inhibited the enzyme of telomerase with an IC_{50} value of 1.86 ± 0.51 μM, surpassing the control compound, ethidium bromide. Modeling study showed that this compound can reside in the binding pocket of the telomerase/TNA:DNA hairpin complex. When the moiety of *N*-acyl was changed to *N*-sulfonyl, the gotten compounds (**8a**–**8i**) had deteriorative activities against both these three cancer cell lines and the enzyme of telomerase.

Telomerase has been validated as an anticancer drug target because of the following facts: (1) telomerase is active in the early stages of life to maintain telomere length and therefore the chromosomal integrity of frequently dividing cells, and it becomes dormant in most somatic cells during adulthood;[1] (2) this enzyme is up-regulated in 80–90% of various cancer cells isolated from principal human tumors but it is absent in neighboring cells of healthy tissue.[2–4] Consequently, telomerase has gotten considerable attentions for developing anticancer drugs.

The catalytic subunit of this enzyme is termed as human telomerase reverse transcriptase (hTERT),[5] which is expressed at a high level in malignant cells, but at a very low level in normal cells. Accumulating evidences about hTERT indicate that hTERT might be a therapeutic target as well and its inhibitors have potential applications for cancer treatment.[6,7] In addition, hTERT may relate to other age-associated disorders.[8] Many hTERT inhibitors were identified,[2,9] and some of them, including BIBR1532 (**1**, Fig. 1),[10–12] showed promising anticancer effects.

Dihydropyrazole derivatives are potential leads for drug discovery,[13] and they have shown biological activities against cannabinoid receptor 1, monoamine oxidase, tumor necrosis, among others. Many hTERT inhibitors with the core of dihydropyrazole were reported recently.[14–17] In this study, we report the identification, biological and modeling studies of novel human telomerase inhibitors with the core of *N*-acyl-4,5-dihydropyrazole. Among them, compound **5c** had antiproliferative activities against SGC-7901 and MGC-803 cell lines with EC_{50} values of 2.06 ± 0.17 and 2.89 ± 0.62 μM, respectively, better than the positive control compound, 5-Fluorouracil (5-FU).[18,19] Compound **5c** showed inhibitory activity against telomerase with an IC_{50} value of 1.86 ± 0.51 μM, surpassing the positive control compound, ethidium bromide (**2**, Fig. 1).[20]

To carry out rational drug design, BIBR1532 (**1**) was docked into a three-dimension human telomerase model to explore the binding mode of this compound. We then designed drug-like hTERT inhibitors which are easy to synthesize and incorporate the moiety of dihydropyrazole to try to discover novel potent hTERT inhibitors. The designed compounds were subsequently docked into the model. The compounds which had similar interactions as BIBR1532 were then picked up for synthesis.

The synthesis of *N*-acyl-4,5-dihydropyrazole derivatives (**5a**–**5h**) was presented in Scheme 1. The synthesis of compound **3** started from substituted-salicylaldehyde catalyzed by C_2H_5ONa. Compounds **4a**–**4h** were obtained from hydrazine monohydrate and α,β unsaturated ketone **3**. The catalyst of DMAP was proved to be an efficient alternative for the synthesis of the target compounds **5a**–**5h**. According to Scheme 2, compounds **8a**–**8i** were synthesized. These compounds were purified by column chro-

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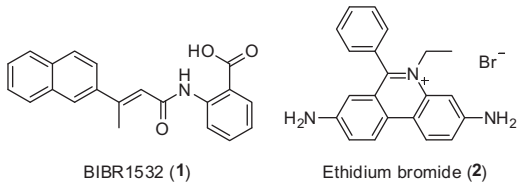


Figure 1. Chemical structures of BIBR1532 (**1**) and ethidium bromide (**2**).

matography, using acetone/petroleum ether as eluent to afford title colorless solids **8a**–**8i**.

All the target compounds were evaluated for their antiproliferative activities against three cancer cell lines: human gastric cancer cell lines MGC-803 and SGC-7901 and human liver cancer cell line Hep-G2. Compound 5-FU, a drug which is used in the treatment of cancer in clinic, was used as the positive control. The cells were allowed to proliferate in presence of tested compounds for 48 h, and the results were reported with EC_{50} values and are shown in Table 1. It is obvious from Table 1, compound **5c** showed the most potent antiproliferative activities against SGC-7901 and MGC-803 with EC_{50} values of 2.06 ± 0.17 and 2.89 ± 0.62 μM, respectively, better than the positive control compound 5-FU. Regarding the cell line of Hep-G2, compound **5h** had the best antiproliferative activity with an EC_{50} value of 4.21 ± 0.41 μM. This compound also had antiproliferative activity against Hep-G2 with an EC_{50} values of 4.80 ± 0.81 μM, which, however, is inferior to 5-FU. Compound **5d** and **5h** showed anticancer activities against SGC-7901 with EC_{50} values of 4.18 ± 0.69 and 7.00 ± 0.77 μM, respectively, comparable to that of positive control compound 5-FU. From the data presented in Table 1, it is obvious that derivatives of *N*-acyl-4,5-dihydropyrazole (**5b**, **5c**, **5d** and **5h**) exhibited better activities against SGC-7901, Hep-G2 and MGC-803 cell lines than derivatives of *N*-benzoyl-4,5-dihydropyrazole compounds (**5e** and **5f**).

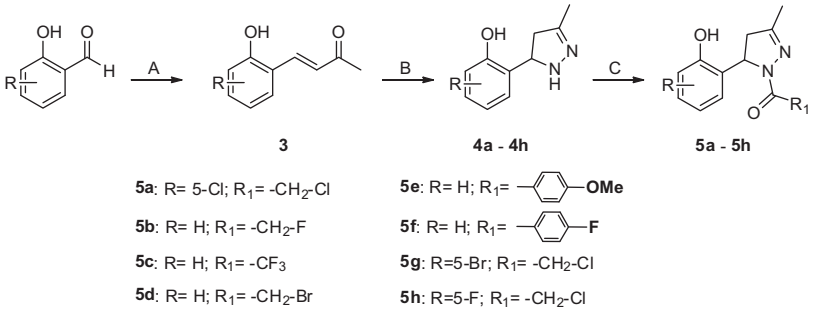
N-Sulfonyl-4,5-dihydropyrazole derivatives **8a**–**8i** were synthesized and evaluated for their antiproliferative activities against these three cancer cell lines. On the whole, the activities were not as good as the *N*-acyl compounds (**5a**–**5h**), and several of them did not show inhibitory activities even at 60 μM. The best one is **8h**, which had antiproliferative activities against SGC-7901, Hep-G2 and MGC-803 with EC_{50} values of 16.32 ± 0.99, 22.09 ± 0.99 and 30.00 ± 2.29 μM, respectively.

Table 1
Antiproliferative activities of the synthesized compounds **5a**–**5h** and **8a**–**8i** against MGC-803, SGC-7901 and Hep-G2 cell lines[a]

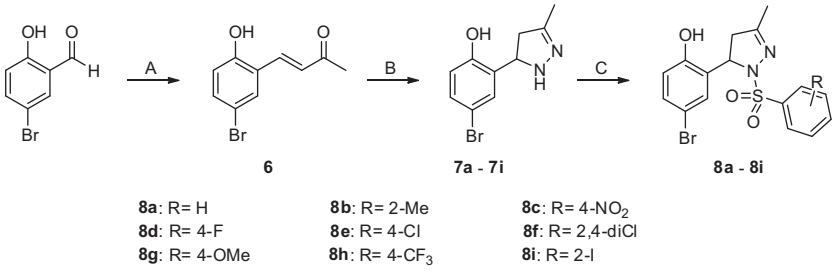
Compound	Antiproliferative activities (EC_{50}, μM)		
	SGC-7901	Hep-G2	MGC-803
5a	14.01 ± 0.58	20.29 ± 1.22	12.25 ± 0.37
5b	8.60 ± 1.11	9.41 ± 0.97	4.26 ± 0.78
5c	2.06 ± 0.17	4.80 ± 0.81	2.89 ± 0.62
5d	4.18 ± 0.69	14.40 ± 1.22	3.65 ± 0.55
5e	45.70 ± 1.98	40.33 ± 1.87	38.45 ± 1.65
5f	50.28 ± 2.20	39.20 ± 2.41	35.39 ± 1.82
5g	9.21 ± 1.02	12.29 ± 1.08	10.45 ± 1.20
5h	7.00 ± 0.77	4.21 ± 0.41	6.87 ± 1.00
8a	55.6 ± 2.44	>60	>60
8b	48.07 ± 1.93	39.27 ± 1.68	>60
8c	27.33 ± 1.28	>60	22.21 ± 0.99
8d	>60	50.11 ± 1.75	15.45 ± 1.33
8e	>60	>60	>60
8f	>60	45.20 ± 2.09	37.88 ± 2.00
8g	21.09 ± 1.50	38.20 ± 1.68	20.34 ± 1.41
8h	16.32 ± 0.99	22.09 ± 0.99	30.00 ± 2.29
8i	>60	>60	52.51 ± 2.85
5-FU	7.03 ± 0.29	2.21 ± 0.20	3.35 ± 0.18

[a] Values are means of three experiments and are expressed as means ± SD.

To confirm if the compounds discussed herein performed anticancer activities via telomerase inhibition, five compounds (**5b**, **5c**, **5e**, **5f** and **8h**) were selected and assayed for their enzyme inhibition against the target of telomerase by a modified TRAP assay[21–23] using an extraction from MGC-803 cells. Modified TRAP is a powerful technique to determine small molecules inhibiting telomere



Scheme 1. Synthesis of compounds **5a**–**5h**. Reagent and conditions: (A) CH_3COCH_3, C_2H_5ONa, 20–30 °C, 10 h; (B) NH_2–NH_2·H_2O, C_2H_5OH, reflux, 3 h. (C) R′COOH, DMAP, 60 °C, 4 h.



Scheme 2. Synthesis of compounds **8a**–**8i**. Reagent and conditions: (A) CH_3COCH_3, C_2H_5ONa, 20–30 °C, 12 h; (B) NH_2–NH_2·H_2O, C_2H_5OH, reflux, 1 h; (C) substituted-benzenesulfonyl chloride, $CHCl_3$, 10 °C, DMAP, 3 h.

Table 2
The enzyme inhibitory activities of five selected compounds against telomerase[a]

Compound	Enzyme inhibitory activities (IC_{50}, μM)[a,b]
5b	4.00 ± 0.48
5c	1.86 ± 0.51
5e	24.95 ± 1.28
5f	29.81 ± 1.59
8h	>60
Ethidium bromide	2.18 ± 0.36

[a] Values are means of three experiments and are expressed as means ± SD.
[b] Telomerase supercoiling activity.

elongation qualitatively and quantitatively.[24] The results are summarized in Table 2. Not surprisingly, compound **5c** had the most potent activity against telomerase with an IC_{50} value of 1.86 ± 0.51 μM, better than the positive control compound, ethidium bromide (IC_{50} = 2.18 ± 0.36 μM). Compound **8h**, a *N*-sulfonyl-4,5-dihydropyrazole derivative, did not show inhibitory activity even at 60 μM.

It is worth noting that the chemical structure of compound **5c** is simple, with a molecular weight less than 300 Da, and can be considered as a fragment, hence, techniques of fragment-based drug discovery[25] may apply for to get novel and more potent telomerase inhibitors.

The single-crystal X-ray diffraction data for compound **5e** was determined on a Bruker SMART APEX CCD diffractometer using MoKα radiation (λ = 0.71073 Å) by the ω scan mode. The structure (Fig. 2) was solved by direct methods using the SHELXS program. Crystallographic data (excluding structure factors) for the structure of **5e** were deposited into the Cambridge Crystallographic Data Center with a Registered No. of CCDC-814974.

In this study, a three-dimension human telomerase model[26] and an advanced docking method—Induced Fit Docking of the Schrödinger Suite were employed to explore the binding mode of BIBR1532. The docked complex was then done a 10 ns MD simulation using the program of Desmond. Our designed compounds were then docked into the active site of the modeled hTERT–BIBR1532 complex to see if these designed compounds have similar interactions with the hTERT as BIBR1532.

One docking pose of compound **5c** may can account for the SAR of the compounds (see Fig. 3A): the phenolic hydroxyl group forms a hydrogen bond with the DNA; another hydrogen bond can be observed between one nitrogen atom of the moiety of 4,5-dihydropyrazole and Lys 710. The benzene ring may have weak hydrophobic interaction with the residue of Pro 929; the trifluoromethyl group of **5c** also has hydrophobic interactions with Pro 627, Lys 902 and Val 904. When the $-CF_3$ group was replaced by a bulkier group, such as a substituted phenyl group (**5e** and **5f**), the enzyme inhibitory activities dropped because these bulkier groups may conflict with the protein.

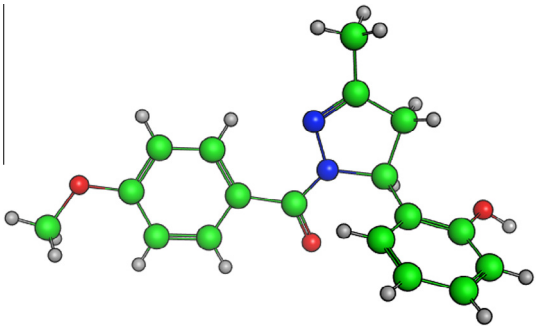


Figure 2. The 3D chemical structure of compound **5e** gotten by single-crystal X-ray diffraction (Registered No.: CCDC-814974).

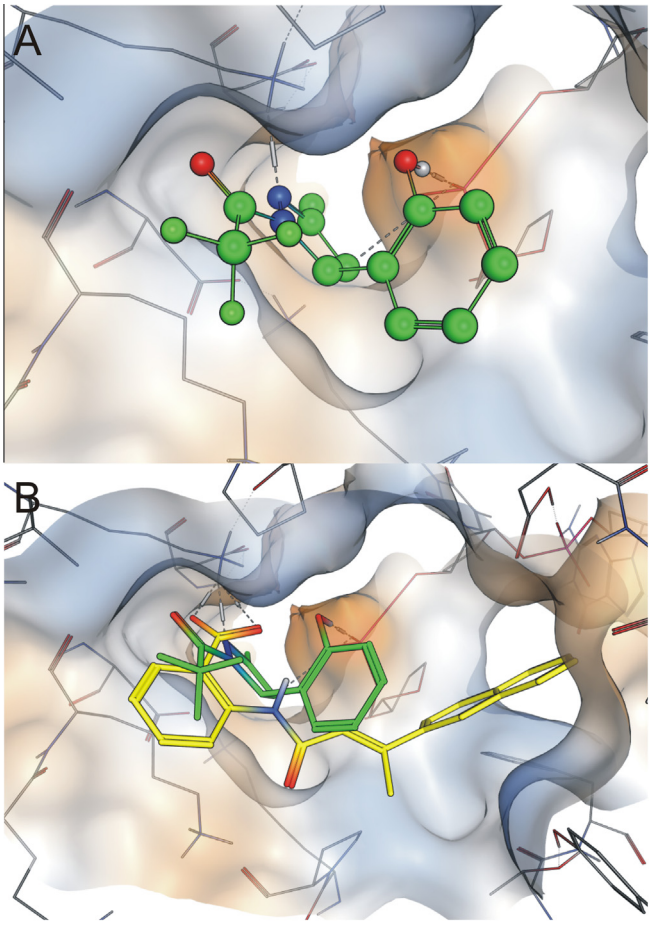


Figure 3. (A) Putative binding pose of compound **5c** (green) to a human telomerase model; (B) the superimposition of **5c** (green) to BIBR1532 (yellow) in the three-dimension human telomerase model. The surface of the binding pocket is presented and colored by lipophilicity: hydrophilic and lipophilic surfaces are shown in orange and blue, respectively.

BIBR1532 is much bulkier than compound **5c**, but these two compounds share similar hydrophobic interactions with the enzyme of telomerase in the model (Fig. 3B). Moreover, BIBR1532 can reach Lys 710 and use its carboxylic group to interact with it. This hydrogen bond also can be observed for compound **5c**. Whereas compound **5c** uses its phenolic hydroxyl group to form another hydrogen bond with the DNA. Because BIBR1532 can form more interactions with telomerase/TNA:DNA hairpin than **5c**, it is no wonder that BIBR1532 (IC_{50} value of 0.093 μM) had more potent inhibitory activity than **5c** against the target.

All in all, in this study, several human telomerase inhibitors having the core of *N*-acyl-4,5-dihydropyrazole with anticancer effects were identified. Biological results demonstrated that a few compounds had potent anticancer activities against three common tumor cell lines (SGC-7901, HepG2 and MGC-803). Among them, compound **5c** had antiproliferative activities against SGC-7901 and MGC-803 with EC_{50} values of 2.06 ± 0.17 and 2.89 ± 0.62 μM, respectively, better than the positive control compound, 5-FU. Compound **5c** could inhibit the enzyme of telomerase with an IC_{50} value of 1.86 ± 0.51 μM, better than the control compound, ethidium bromide. Modeling study showed that this compound can reside in the binding pocket of the telomerase/TNA:DNA hairpin complex. When the moiety of *N*-acyl was changed to *N*-sulfonyl, the gotten compounds (**8a**–**8i**) had deteriorative activities against both the three cancer cell lines and the enzyme of telomerase, indicating the importance of the moiety of *N*-acyl. Since the chemical structure of compound **5c** is simple, and its molecular

weight is low (272.2 Da), we may develop more potent human telomerase inhibitors by employing a diversity evolution strategy.[27]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.025.

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