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Design and synthesis of 2-phenylpyrimidine coumarin derivatives as anticancer agents

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ABSTRACT

A series of 2-phenylpyrimidine coumarin derivatives with potential telomerase-inhibiting activity was designed and synthesized. All of the compounds were screened for antiproliferative activity against CNE2, KB, and Cal27 cell lines *in vitro*. The results showed that most of the derivatives had a favorable effect on resisting tumor cell proliferation; compound 13, 3-(4-amino-5-oxo-5H-chromeno[4,3-*d*]pyrimidin-2-yl)phenyl 4-(dimethylamino)benzenesulfonate, exhibited the best activity. Flow cytometry revealed that compound 13 can inhibit CNE2 proliferation. Telomerase inhibition and *in vitro* antitumor activity were consistent among the compounds, but compound 13 showed the best telomerase-inhibiting activity and could inhibit telomere extension. Molecular docking results indicated that compound 13 bonded with telomerase reverse transcriptase (TERT) through multiple interactions, including hydrogen bonding and hydrophobic interactions. The results of the study provide further information on 2-phenylpyrimidine coumarins, expanding the types of telomerase inhibitors as the parent structures.

Telomerase, as a ribonucleoprotein enzyme with reverse transcriptase activity, can use its own RNA as a template to synthesize telomere DNA sequences.[1–3] Any abnormalities in telomerase structure and behavior are closely related to cell senescence, tumorigenesis and development.[4,5] A large quantity of data confirms that more than 85% of human tumor cells have high levels of telomerase activity, but most somatic cells and benign tumor cells lack telomerase activity.[6,7] Therefore, most scholars believe that the proliferation of some tumor cells can be effectively inhibited through the inhibition of telomerase activity.[8,9]

Coumarin, namely, benzopyrone, is a substance with an aromatic odor. Coumarin and its derivatives constitute important organic heterocyclic molecules with diverse physiological activities. As early as the 1960s, scientists found that coumarin compounds had antitumor metastasis effects.[10] As large quantities of coumarin compounds have been separated and synthesized, more and more coumarin compounds have been found to have antitumor effects.[11–14] In recent years, Liu's group reported on multiple series of coumarin derivatives, which were found to have favorable telomerase-inhibiting effects and inhibiting effects on cell proliferation in multiple tumors.[15–17] Aromatic sulfonate antitumor drugs are widely used in the clinic, and aromatic sulfonyl groups are important pharmacophores.[18,19] Based on the above-mentioned studies and for the continuous extension of structural types of coumarin telomerase inhibitors, our research used 2-phenylpyrimidine coumarin as a parent nucleus. In addition, aromatic sulfonyl groups were introduced in the substituted phenyl *meta*-position, which was expected to increase the antitumor activity and interactions with telomerase. Then, a series of 2-phenylpyrimidine coumarin derivatives was obtained (Fig. 1), and their antitumor effects and telomerase-inhibiting activities were evaluated.

Fig. 1 shows the synthesis route. Parent compound 2 was obtained through the "one-pot" method, namely, ethyl cyanoacetate, salicylic aldehyde, 3-hydroxybenzaldehyde, and ammonium acetate were put under reflux for 2 h.[20] During the reaction process, large quantities of yellow solids precipitated, and then parent compound 2 was obtained through a simple after-treatment. Parent compound 2 can be directly applied in the next step without further purification, and it reacts with the substituted sulfonyl chloride to obtain the target product.

To test the antitumor activity of the synthesized compounds, we evaluated the activity of compounds 3–15 against human nasopharyngeal carcinoma cells (CNE2), human oral epidermoid carcinoma cells (KB), and human oral squamous cells (Cal27).

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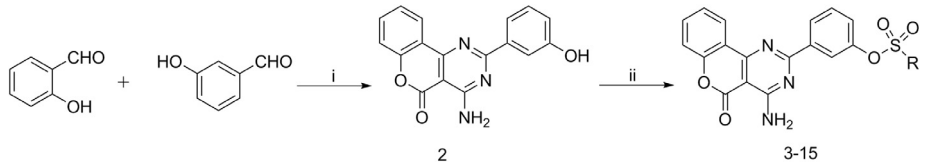
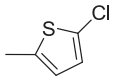
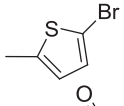
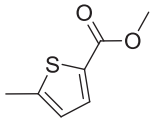
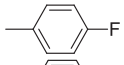
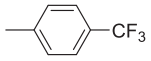
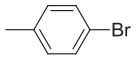
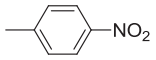
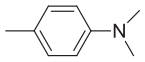
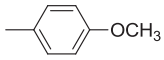
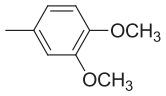


Fig. 1. Synthesis of 3′-sulfonate-substituted 2-phenyl-benzopyranopyrimidine derivatives. Reagents and conditions: (i) $CNCH_2COOC_2H_5$, NH_4OAc, C_2H_5OH, reflux, 2 h and (ii) $ClSO_2R$, DMF, rt, and 5 h (R is shown in Table 1).

Table 1
In vitro anticancer activity of the synthesized compounds.[a]

Compound	R	*In vitro* anticancer activity, IC_{50} (μM)[b]		
		CNE2	KB	Cal27
3	$-CH_3$	>100	>100	>100
4		4.24 ± 0.17	5.62 ± 0.35	10.12 ± 0.78
5		14.58 ± 1.98	20.86 ± 1.51	11.25 ± 0.99
6		18.64 ± 3.27	36.17 ± 1.41	18.34 ± 3.01
7		>100	38.36 ± 2.98	47.47 ± 3.26
8		3.44 ± 0.46	6.74 ± 0.24	2.58 ± 0.47
9		4.23 ± 0.18	11.59 ± 1.54	4.91 ± 0.87
10		2.34 ± 0.21	7.35 ± 0.16	4.32 ± 0.24
11		11.07 ± 1.45	15.83 ± 1.64	18.07 ± 2.21
12		16.25 ± 2.62	16.99 ± 1.60	3.54 ± 1.33
13		1.92 ± 0.13	3.72 ± 0.54	1.97 ± 0.51
14		16.57 ± 2.50	18.62 ± 1.98	9.63 ± 1.04
15		20.64 ± 2.21	29.72 ± 2.24	17.63 ± 1.85
ADM		2.12 ± 0.56	3.04 ± 0.87	1.56 ± 0.64

Negative control 0.1% DMSO, no activity.

[a] The data represented the mean of three experiments in triplicate and were expressed as means ± SD; only descriptive statistics were done in the text.

[b] The IC_{50} value was defined as the concentration at which 50% survival of cells was observed.

The results are summarized in Table 1. Following the interactions between compounds and the cells, the results revealed that the most examined compounds showed potent activity against the CNE2 and Cal27 cell lines and moderate activity against the KB cell lines. Compounds 4, 8, 10, and 13 exhibited high activity against the CNE2 cell lines, with IC_{50} values of 4.24 ± 0.17, 3.44 ± 0.46, 2.34 ± 0.21, and 1.92 ± 0.13 μM, respectively. Compounds 8, 9, 10, and 13 possessed potent activity against the Cal27 cell lines, with IC_{50} values of 2.58 ± 0.47, 4.91 ± 0.87, 4.32 ± 0.24, and 1.97 ± 0.51 μM, respectively. Compounds 4, 8, and 13 showed high activity against the KB cell lines, with IC_{50} values of 5.62 ± 0.35, 6.74 ± 0.24, and 3.72 ± 0.54 μM, respectively, which are comparable with the positive control doxorubicin (AMD).

Structure–activity relationship analysis showed that our examined compounds were divided into three series. Compound 1 has an alkyl moiety and no antiproliferative ability. Compounds 4–7 have thiophene moieties, while compounds 8–15 have aromatic-ring moieties; compounds 8–15 are better at resisting tumor cell proliferation being than compounds 4–7. In the aromatic-ring moiety series of compounds, the compounds substituted by halogen demonstrated moderate activity. Compound 13, which has *N*,*N*-dimethyl amino benzenesulfonyl moiety, had the best activity; its activity values (IC_{50}) for the CNE2, KB, and Cal27 cell lines were 1.92 ± 0.13, 3.72 ± 0.54, and 1.97 ± 0.51 μM, respectively, and the values are comparable with those of potent ADM. Therefore, for this type of structural moiety, this 2-phenylpyrimidine coumarin derivative structure warrants further optimization as a potent anticancer agent.

Next, flow cytometry was used to detect the effect of the compounds on inducing apoptosis in tumor cells. The Annexin V-FITC/PI double-staining method was used to evaluate the effect of compound 13 on inducing apoptosis of the CNE2 cells (Fig. 2). Fig. 2A shows that a blank control was not added to any of the compounds, and only 8.1% of the cells were in an apoptotic state. After compound 13 was added, and as its concentration increased, the number of apoptotic cells gradually increased; finally, the percentage of CNE2 cells in an apoptotic state had escalated to 54.9%. This result confirmed that compound 13 induced apoptosis in the CNE2 cells.

Based on the discovered coumarin skeletal structure, the structural types of telomerase inhibitors were continuously extended. Our research evaluated the telomerase-inhibiting activities of some of the examined compounds using CNE2 cell extracts with ethidium bromide as the positive control. Results are shown in Table 2. Compounds 4, 8, 13, and 15 show favorable telomerase-inhibiting activities, but compounds 3 and 12 had weak telomerase-inhibiting activity. These results are consistent with the *in vitro* antitumor effect of these compounds, and they indicate that the compounds may resist tumor cell proliferation by inhibiting telomerase activity.

Telomere length is jointly regulated by telomerase and the telomere-binding protein. Telomere length, a biological marker that measures cell senescence and apoptosis, is a good index to measure the biological characteristics of tumors.[6,21] We conducted additional research on the length change of restrictive telomere fragments to further study compound 13's mechanism in inhibiting cell proliferation and inducing cell apoptosis. Telomere restriction fragment (TRF) experimental results (Fig. 3) show that the CNE2 cells' telomere lengths after treatment with 1 μM of compound 13 for 8 days were shortened by 0.8 kb compared with the blank control group, and telomere shortening was also observed after treatment with 0.5 μM of compound 13 for 8 days. Telomere dysfunction can activate p53 to initiate cellular senescence or apoptosis to suppress tumorigenesis. In this study, senescence induction via compound 13 might be due to telomere-length short-