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Telomerase inhibition by new di- and trisubstituted acridine derivatives

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Aim. To study a series of new acridine derivatives containing two basic fragments able to bind to quadruplex DNA at C-4 and C-9 positions as potential telomerase inhibitors. **Methods.** TRAP assay was used to determine the activity of compounds *in vitro*. **Results.** A number of acridines inhibiting the enzyme at micromolar concentrations were found, with $IC_{50} = 2.6 \mu M$ for the most active compound. **Conclusions.** The introduction of a highly basic N,N-dimethylaminoalkyl group at the C-9 position of the acridine core results in a strong increase of biological activity of compounds, and a 5-methyl substituent further enhances it.

Keywords: telomerase inhibitors, acridines, quadruplex DNA, TRAP

Introduction

Telomerase is a unique reverse transcriptase synthesizing the telomeres using its own RNA component as a template and single-stranded telomeric DNA as a primer [1]. This enzyme is responsible for maintaining the telomere length and re-synthesizes the telomeric sequences partially lost upon each cellular division. Telomerase is active in a majority of tumor cells, which results in their immortalization. Increased telomerase activity was detected in 85–90 % of human tumors [2]. In contrast, it is inactive in normal somatic cells. So telomerase inhibitors have a significant potential as anticancer drugs [2, 3].

Guanine-rich telomeric DNA sequences can fold into specific four-stranded structures called G-quadruplexes (G4) formed by the stacks of guanine quartets linked by non-canonical systems of hydrogen bonds [4–6]. Compounds able to specifically bind and stabilize G4 structures were found to inhibit telomerase and demonstrated antitumor activity. A variety of compounds, mostly containing a large heteroaromatic core, were shown to be G4-stabilizing ligands [4, 6, 7].

A number of known telomerase inhibitors are based on acridine scaffold. Most of them contain pairs of basic substituents, e.g. at C-3 and C-6 or C-4 and C-5 positions of the ring, and quite often an aromatic fragment at C-9 [4, 6–11]. In this work we have studied a series of novel 4,9-di and 4,5,9-trisubstituted acridines as potential telomerase inhibitors. Their biological testing was performed using the modification of classic *in vitro* TRAP (Telomeric Repeat Amplification Protocol) assay [12].

Materials and Methods

Acridone carboxamides **1**, **2** were obtained as previously described [13]. Acridine derivatives with aliphatic basic 9-substituents were prepared from these compounds or their 5-methyl-substituted analogues (synthetic details will be reported in a separate paper).

3-([3-Cholamidopropyl]-dimethylammonium)-1-propanesulfonate (CHAPS), ethyleneglycol-bis-O-(β -aminoethyl)-N,N'-tetraacetic acid (EGTA) and SYBR Green I were obtained from Sigma (USA), *Taq*-DNA-polymerase and deoxynucleoside triphosphates from Fermentas (Lithuania).

Telomerase was extracted from exponentially growing MCF-7 tumor cells by lysing in a CHAPS/EGTA-based buffer [14].

TRAP analysis was performed according to TRAPeze protocol [TRAPeze® Telomerase Detection Kit. Chemicon International, Temecula, CA, USA, 2005. 39 p.] with deoxyoligonucleotide primers 5'-GTGCCCTTACCCTTACCCTTACCCTAA (CXext) [15] and 5'-AATCCGTCGAGCAGAGTT (TS) from Eurogentec (Belgium). Inhibitors were tested in a concentration range from 40 to 0.625 μM using a serial 2-fold dilution. After the telomerase extension reaction the tested compounds were removed from the mixture by phenol/chloroform extraction followed by ethanol precipitation. PCR-amplified DNA products were analyzed by gel electrophoresis. 20 μL sample was mixed with 2 μL of loading buffer (5x TBE buffer, 20 % sucrose, 0.2 % bromophenol blue) and loaded onto a standard 12 % non-denaturing polyacrylamide gel (19:1) prepared in 0.5x TBE. Electrophoresis was run for 0.5 h at 160 V and then for 0.5 h at 300 V. Gel was stained with 1x SYBR Green I for 30 min at room temperature. DNA bands were visualized on a ChemiDoc imager (Bio-Rad, USA), and images were processed

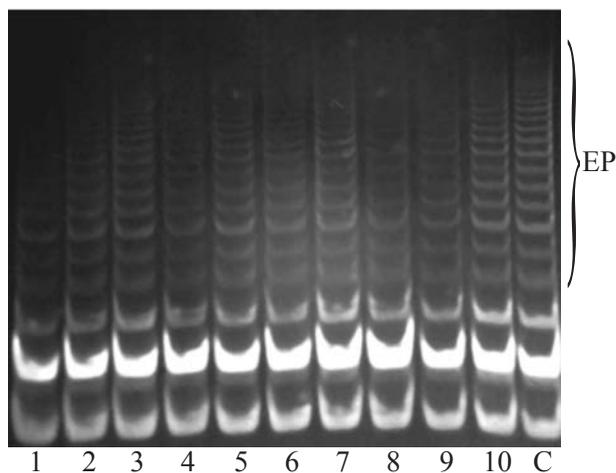


Fig. 1. TRAP analysis of the effect of acridines on telomerase activity. Lanes 1–3 – compound **5** (20, 10 and 5 μM); lanes 4–5 – compound **6** (10 and 5 μM); lanes 6–7 – compound **8** (2.5 and 1.25 μM); lanes 8–10 – compound **7** (2.5, 1.25 and 0.625 μM); C – control reaction. EP – elongation products.

with TotalLab v2.01 software (Nonlinear Dynamics, UK).

Inhibition activity was determined by comparing the amount of DNA produced in the test assay with that in a control reaction with no inhibitor added. For quantitative analysis, optical densities of the sets of DNA product bands on corresponding lanes of the gel were integrated. IC_{50} values (compound concentrations required for achieving 50 % inhibition of enzymatic activity) were obtained from the concentration-activity plots. At least three independent experiments were performed for each compound.

Results and Discussion

We have recently found that acridone derivatives containing pyridyl groups at 4-carboxamide fragment inhibit telomerase at 100 μM concentration. Molecular modeling suggested the introduction of additional phosphate-binding group in these compounds to improve their G4 binding and thus inhibition activity [16]. A series of new acridine derivatives with two aliphatic and aromatic basic fragments were synthesized. The structures of potential inhibitors (Table) were designed taking into account that strongly basic group like N,N-dimethylaminoalkyl introduced at C-9 position is easily protonated in water and thus could interact with G4 phosphate anions enhancing the ligand binding. In addition to phosphate binding, the basic pyridyl function at 4-carboxamide was expected to form hydrophobic bonds with G4 DNA bases further increasing the ligand affinity.

The level of telomerase inhibition was determined in TRAP assay. In this 2-step method [12], telomerase enzymatic reaction is followed by PCR amplification of its DNA products and analysis of the resulting reaction mixture by gel electrophoresis. Tumor cell lysate is used as the source of telomerase. We have employed TRAPeze protocol from Chemicon [TRAPeze® Telomerase Detection Kit. Chemicon International, Temecula, CA, USA, 2005. 39 p.], a well-established modification of the original method [12]. The inhibitors were removed from the reaction mixture before the PCR step to exclude their possi-

Table. *In vitro* telomerase inhibition activity of acridone and acridine derivatives in TRAP assay.

Compound	Structure	IC ₅₀ , μM	Compound	Structure	IC ₅₀ , μM
1		l.a.*	5		11.5±0.08
2		l.a.	6		6.5±0.02
3		20.0±0.11	7		2.6±0.01
4		23.1±0.13	8		4.8±0.03

* low activity (IC₅₀ > 40 μM)

ble effect on *Taq*-DNA-polymerase. Alternative reverse PCR primer CXext developed to minimize analysis artifacts [15] was used instead of the standard CX.

The compounds were tested in a concentration range 40–0.625 μM (Fig. 1). The activity plots built by calculating the yield of enzymatic reaction products as a function of drug concentration allowed determining the IC₅₀ values of inhibitors (Table).

The test results confirmed the idea of structure design to be correct. 9-Acridone derivatives **1–2** with *o*- and *m*-pyridyl fragments were relatively inactive as telomerase inhibitors. However, the activity of all 9-substituted analogues bearing strongly basic functions sharply increased. The inhibition data indicated a higher activity of the compounds with N,N-dimethylaminopropyl residue (**5**, **6**) as compared to their analogues containing a shorter Me₂NCH₂CH₂ group (**3**, **4**). As expected, electron-donating 5-methyl substituent significantly enhanced the biological activity

(compare the IC₅₀ values of compounds **7** and **5**, **8** and **6**), perhaps due to increasing the electronic density on acridine core which resulted in its more efficient π-π stacking with G4.

Conclusions

4,9- and 4,5,9-substituted acridines containing two basic DNA-binding moieties efficiently inhibit telomerase *in vitro* at low micromolar concentrations. Trisubstituted derivative **7** with IC₅₀ = 2.6 μM is the most active inhibitor. The key structural feature determining the activity of compounds is a presence of highly basic N,N-dimethylaminoalkyl group at C-9 position. Further optimization would allow the development of more potent acridine-based telomerase inhibitors.

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Інгібування теломерази новими ди- та тризаміщеними похідними акридину

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Мета. Дослідити серію нових похідних акридину, що містять у положеннях С-4 і С-9 два основні фрагменти, здатні зв'язуватися з квадруплексною ДНК, як потенційних інгібіторів теломерази. **Методи.** Для визначення активності сполук *in vitro* використано метод TRAP. **Результати.** Виявлено ряд акридинів, що інгібують фермент у низьких мікромолярних концентраціях, для найактивнішого з яких $IC_{50} = 2.6$ мкМ. **Висновки.** При введенні високоосновної N,N-диметиламіноалкільної групи в положення С-9 акридинового ядра біологічна активність сполук різко зростає, а 5-метильний замісник додатково збільшує її.

Ключові слова: інгібітори теломерази, акридини, квадруплексна ДНК, TRAP

Ингибирование теломеразы новыми ди- и тризамещенными производными акридина

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Цель. Изучить серию новых производных акридина, содержащих в положениях С-4 и С-9 два основных фрагмента, способных связываться с квадруплексной ДНК, как потенциальных ингибиторов теломеразы. **Методы.** Для определения активности веществ *in vitro* использован метод TRAP. **Результаты.** Обнаружен ряд акридинов, ингибирующих фермент в низких микромолярных концентрациях, для самого активного из которых $IC_{50} = 2.6$ мкМ. **Выводы.** При введении высокоосновной N,N-диметиламиноадкильной группы в положении С-9 акридинового ядра биологическая активность соединений резко возрастает, а 5-метильный заместитель дополнительно увеличивает ее.

Ключевые слова: ингибиторы теломеразы, акридины, квадруплексная ДНК, TRAP

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