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Novel trisubstituted acridines as human telomeric quadruplex binding ligands

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ABSTRACT

A novel series of trisubstituted acridines were synthesized with the aim of mimicking the effects of BRACO19. These compounds were synthesized by modifying the molecular structure of BRACO19 at positions 3 and 6 with heteroacyclic moieties. All of the derivatives presented in the study exhibited stabilizing effects on the human telomeric DNA quadruplex. UV–vis spectroscopy, circular dichroism, linear dichroism and viscosimetry were used in order to study the nature of the DNA binding in more detail. The results show that all of the novel derivatives were able to fold the single-stranded DNA sequences into antiparallel G-quadruplex structures, with derivative **15** exhibiting the highest stabilizing capability. Cell cycle analysis revealed that a primary trend of the "braco"-like derivatives was to arrest the cells in the S- and G_2M-phases of the cell cycle within the first 72 h, with derivative **13** and BRACO19 proving particularly effective in suppressing cell proliferation. All studies derivatives were less toxic to human fibroblast cell line in comparison with HT 29 cancer cell line.

1. Introduction

The non-canonical nucleic acid structures known as G-quadruplexes show an extremely high level of stability which is derived in part from the stacking together of G-quartets through π–π interactions to form stable quadruplex motifs [1,2]. G-rich telomeric DNA folded into quadruplex has been the focus of much recent interest as a potential anti-cancer target due to its interaction with the ribonucleoprotein telomerase, an enzyme which maintains chromosomal integrity and which is up-regulated in approximately more than 85% of various human cancer cells [3]. As the inhibition of telomerase induces cell senescence, this process has become a target for potential anticancer therapeutic intervention [4,5] both *in vitro* and *in vivo* [6,7]. The telomerase substrate must be single-stranded in order to be recognized by the telomerase RNA template prior to the catalytic step in the telomere elongation cycle. Indirect inhibition of telomerase can be achieved by the telomeric DNA substrate folding into four stranded guanine quadruplex structures, using small molecule quadruplex-binding compounds [8]. The resultant folded substrate is unable to hybridize with the telomeric RNA template and the ability of telomerase to catalyze the synthesis of further telomeric DNA repeats is inhibited. The majority of quadruplex-binding compounds contain an extended planar aromatic chromophore [9]. It has been reported that quadruplex-binding trisubstituted acridines have exhibited rapid antitumor effects [10–12] involving several parallel mechanisms, including telomere uncapping [13–15], direct or indirect telomerase inhibition with the characteristic induction of senescence and of apoptosis. Recent research has also shown that G-quadruplexes selectively target both telomerase and telomere in cancer cells [16–18].

BRACO19 has shown some promising results in studies involving tumor cell cultures and mouse xenografts. It has been reported that treatment with BRACO19 resulted in both telomerase inhibition and also in general telomere dysfunction that led to atypical mitosis and consequently to apoptosis [1].

In this paper, we present a novel series of acridine based compounds as structural analogs of BRACO19. Based on findings from

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our previous results [19–23], the side chains at positions 3 and 6 of the initial braco structure were modified with thiourea, urea and guanidine. Molecular dynamics simulations were also used prior to the empirical examination of the interaction forces of the synthesized structures.

Although the synthesis of 3,6,9-trisubstituted acridines using 2,2′,4,4′-tetranitrobenzophenone has been described previously [24], we also aimed to elaborate the synthetic pathway including Ullmann-Jourdan coupling which would allow us to utilize a broad structural diversity of commercially available reactants in our future research.

2. Materials and methods

2.1. Chemicals and instruments

All chemicals and reagents were purchased of reagent grade and used without further purification. Ethidium bromide, dimethyl sulfoxide (DMSO), Triton X 100, Tris(hydroxymethyl) aminomethane (Tris) and calf thymus DNA were obtained from Sigma–Aldrich Chemie (Germany). EDTA, RNase A and proteinase K were purchased from Serva (Germany), and all other chemicals were purchased from Lachema (Czech Republic). DNA oligomers were obtained from Metabion. Braco19 was synthetized according to previously described procedure [24].

^{1}H (400 MHz, 600 MHz) and ^{13}C (100 MHz, 150 MHz) NMR spectra were measured on a Varian Mercury Plus or a Varian VNMRS NMR spectrometers at room temperature in CD_3OD or DMSO-d_6 using TMS as an internal standard (0 ppm for both nuclei). Melting points were determined with a Koffler hot-stage apparatus and are uncorrected. Elemental analyses were performed on a Perkin-Elmer analyzer CHN 2400. Reactions were monitored by thin-layer chromatography (TLC) using Silufol plates with detection at 254 nm. Preparative column chromatography was conducted using Kiesegel Merck 60 column, type 9385 (grain size 250 nm) or aluminum oxide Merck 90 neutral (grain size 200 nm).

2.2. Synthesis of trisubstituted acridine derivatives

2.2.1. Synthesis of 3-nitroacetanilide (**2**)

3-Nitroaniline (**1**) (1 g, 7.24 mmol) was heated to 100 °C in 5 mL of acetanhydride. The reaction was monitored with TLC, using toluene–acetone mixture (5:2 v/v) as eluent. After the reaction had finished, the reaction mixture was poured into water and neutralized by ammonium hydroxide. The precipitate was filtered off and used without purification in the next reaction.

Yield: 70%, white solid, mp: 156–158 °C. Anal. Calcd for $C_8H_8N_2O_3$ (180.16): 53.33% C, 4.48% H, 15.55% N. Found: 53.45% C, 4.25% H, 15.63% N. ^{1}H NMR (400 MHz, DMSO-d_6): 10.43 (s, 1H, NH), 8.63 (t, 1H, H-2, J = 1.60), 7.90 (d, 1H, H-6, J = 2.00), 7.88 (d, 1H, H-4, J = 2.00), 7.59 (t, 1H, H-5, J = 8.00), 2.11 (s, 3H, CH_3—CO). ^{13}C NMR (100 MHz, DMSO-d_6): 169.01 (C═O), 147.89 (C1), 140.33 (C3), 130.01 (C5), 124.80 (C6), 117.44 (C4), 112.93 (C2), 23.08 (CH_3—CO).

2.2.2. Synthesis of 3-aminoacetanilide (**3**)

Acetanilide **2** (1 g, 5.66 mmol) was dissolved in 100 mL of methanol and a catalytic amount of 10% palladium on activated charcoal (ca. 0.015 g) was added. The reaction mixture was cooled by an ice bath and natrium borohydride (1 g, 26.43 mmol) was added portionly. The resulting mixture was then stirred vigorously for 1 h. The end of the reaction was monitored with TLC, using toluene–acetone mixture (5:2 v/v) as eluent. The product was chromatographed over silica gel eluated by toluene–acetone mixture (5:2 v/v).

Yield: 75%, white solid, mp: 86–88 °C. Anal. Calcd for $C_8H_{10}N_2O$ (150.18): 63.98% C, 6.71% H, 18.65% N. Found: 63.76% C, 6.50% H, 18.53% N. ^{1}H NMR (400 MHz, DMSO-d_6): 9.58 (s, 1H, NH), 6.92 (t, 1H, H-2, J = 2.00), 6.88 (t, 1H, H-4, J = 8.00), 6.64 (dd, 1H, H-6, J_1 = 0.80, J_2 = 8.00), 6.23 (dd, 1H, H-5, J_1 = 1.60, J_2 = 8.00), 5.00 (bs, 2H, NH_2), 1.99 (s, 3H, CH_3—CO). ^{13}C NMR (100 MHz, DMSO-d_6): 167.85 (C═O), 148.85 (C1), 139.85 (C3), 128.75 (C4), 109.04 (C5), 106.93 (C6), 104.67 (C2), 23.98 (CH_3—CO).

2.2.3. Synthesis of 4-(acetylamino)-2-chlorotoluene (**5**)

A solution of acetyl chloride (0.83 g, 10.59 mmol) in 10 mL of dry methylene chloride was added in drops over 20 min into the solution of 4-amino-2-chlorotoluene (**4**) (1 g, 7.06 mmol) and triethylamine (1.43 g, 14.12 mmol) in 10 mL of dry methylene chloride. Once the full amount of acetyl chloride had been added, the reaction mixture was stirred until the reaction was completed according to TLC, using toluene–acetone mixture (5:2 v/v) as eluent. In order to eliminate an excess of acetyl chloride, 5 mL of methanol was then added to the reaction mixture. After 15 min, the solvent was evaporated off at reduced pressure, and the crude product was dissolved in 10 mL of methanol and slowly poured into distilled water. The thus-obtained precipitate **5** was filtered off and dried overnight.

Yield: 95%, white solid, mp: 105–106 °C. Anal. Calcd for $C_9H_{10}ClNO$ (183.64): 58.86% C, 5.49% H, 7.63% N. Found: 58.98% C, 5.35% H, 7.77% N. ^{1}H NMR (400 MHz, DMSO-d_6): 10.01 (s, 1H, NH), 7.78 (d, 1H, H-2, J = 2.00), 7.33 (dd, 1H, H-6, J_1 = 2.00, J_2 = 8.40), 7.23 (d, 1H, H-5, J = 8.4), 2.25 (s, 3H, CH_3), 2.03 (s, 3H, CH_3—CO). ^{13}C NMR (100 MHz, DMSO-d_6): 168.32 (C═O), 138.36 (C1), 132.83 (C3), 130.99 (C5), 129.41 (C4), 118.81 (C2), 117.45 (C6), 23.87 (CH_3—CO), 18.80 (CH_3).

2.2.4. Synthesis of 4-(acetylamino)-2-chlorobenzoic acid (**6**)

Acetamide **5** (1 g, 5.45 mmol) was added to a solution of potassium permanganate (2.58 g, 16.34 mmol) in 100 mL of distilled water. The reaction mixture was stirred vigorously at reflux temperature for 2 h. The reaction was monitored with TLC, using toluene–acetone mixture (3:2 v/v) as eluent. After the reaction was completed, the resultant mixture was filtered in order to remove manganese dioxide and the filtrate was acidified with diluted hydrochloric acid (1:3 v/v) thereby producing the acid **6** from its potassium salt. The precipitated product **6** was then suction-filtered though a Büchner funnel and dried overnight.

Yield: 65%, white solid, mp: 208–210 °C. Anal. Calcd for $C_9H_8ClNO_3$ (213.62): 50.60% C, 3.77% H, 6.56% N. Found: 50.70% C, 3.58% H, 6.62% N. ^{1}H NMR (400 MHz, DMSO-d_6): 10.35 (s, 1H, NH), 7.88 (d, 1H, H-3, J = 2.00), 7.83 (d, 1H, H-6, J = 8.40), 7.53 (dd, 1H, H-5, J_1 = 2.00, J_2 = 8.40), 2.09 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): 169.04 (C═O), 165.84 (COOH), 142.78 (C4), 132.93 (C2), 132.27 (C3), 124.29 (C1), 119.87 (C6), 116.68 (C5), 24.06 (CH_3).

2.2.5. Synthesis of 4-(acetylamino)-2-(3-(acetylamino)phenylamino)benzoic acid (**7**)

Dry potassium carbonate (0.32 g, 2.34 mmol), activated copper (0.029 g, 0.46 mmol) and copper(I) oxide (0.06 g, 0.43 mmol) were added to a mixture of benzoic acid **6** (1 g, 4.68 mmol) and 3-aminoacetanilide (**3**) (0.77 g, 5.15 mmol) in 8 mL of ethoxyethanol. After 4 h of vigorous stirring at reflux temperature, the reaction mixture was poured into 30 mL of distilled water and the resultant mixture was filtered. The filtrate was than acidified with hydrochloric acid (1:3 v/v) and the precipitated crude product was suction-filtered though a Büchner funnel and dried overnight.

Yield: 50%, white solid, mp: 254–255 °C. Anal. Calcd for $C_{17}H_{17}N_3O_4$ (327.34): 62.38% C, 5.23% H, 12.84% N. Found: 62.50% C, 5.15% H, 12.74% N. ^{1}H NMR (400 MHz, DMSO-d_6):