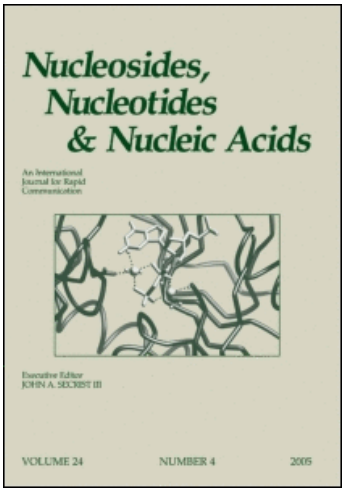

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Inducement of G-Quadruplex DNA Forming and Down-Regulation of Oncogene *C-MYC* by Bile Acid-Amino Acid Conjugate—BAA

Mingyue Tian[a]; Xiufeng Zhang[b]; Yan Li[c]; Yong Ju[c]; Junfeng Xiang[b]; Changqi Zhao[a]; Yalin Tang[b]
[a] Beijing Key Lab of Gene Engineering Drugs & Biological Technology, College of Life Sciences, Beijing Normal University, Beijing, P. R. China [b] the Chinese Academy of Sciences, Institute of Chemistry, Beijing, P. R. China [c] Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Ministry of Education, Department of Chemistry, Tsinghua University, Beijing, P. R. China

Online publication date: 19 April 2010

To cite this Article Tian, Mingyue , Zhang, Xiufeng , Li, Yan , Ju, Yong , Xiang, Junfeng , Zhao, Changqi and Tang, Yalin(2010) 'Inducement of G-Quadruplex DNA Forming and Down-Regulation of Oncogene *C-MYC* by Bile Acid-Amino Acid Conjugate—BAA', Nucleosides, Nucleotides and Nucleic Acids, 29: 3, 190 — 199
To link to this Article: DOI: 10.1080/15257771003704875
URL: http://dx.doi.org/10.1080/15257771003704875

Nucleosides, Nucleotides and Nucleic Acids, 29:190–199, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257771003704875

INDUCEMENT OF G-QUADRUPLEX DNA FORMING AND DOWN-REGULATION OF ONCOGENE *C-MYC* BY BILE ACID-AMINO ACID CONJUGATE—BAA

Mingyue Tian,[1] Xiufeng Zhang,[2] Yan Li,[3] Yong Ju,[3] Junfeng Xiang,[2] Changqi Zhao,[1] and Yalin Tang[2]

[1]*Beijing Key Lab of Gene Engineering Drugs & Biological Technology, College of Life Sciences, Beijing Normal University, Beijing, P. R. China*
[2]*Institute of Chemistry, the Chinese Academy of Sciences, Beijing, P. R. China*
[3]*Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Ministry of Education, Department of Chemistry, Tsinghua University, Beijing, P. R. China*

□ *Human c-myc gene is a central regulator of cellular proliferation and cell growth, and G-quadruplexes have been proven to be the transcriptional controller of this gene. In this study, the interaction of bile acid-amino acid conjugate (BAA) with G-quadruplexes in c-myc was investigated by circular dichroism spectroscopy, nuclear magnetic resonance (NMR) measurement, and quantitative real-time polymerase chain reaction (PCR) assay. The experimental results indicated that BAA has the ability to selectively induce the formation of parallel G-quadruplexes in c-myc, which leads to down-regulation of c-myc transcription in the human breast cancer cell MCF-7.*

Keywords c-myc gene; G-quadruplexes; bile acid-amino acid conjugate; down-regulation of transcription

INTRODUCTION

Human c-myc gene or its product is a central regulator of cellular proliferation and cell growth. The aberrant overexpression of c-myc is related to the increasing of cellular proliferation in a variety of different malignant tumors, including carcinomas of the breast, colon, and cervix, as well as small-cell lung cancer, osteosarcomas, glioblastomas, and myeloid leukemias.[1–4] The transcription of c-myc is under the complex control of multiple promoters. The nuclease hypersensitivity element III_1 ($NHEIII_1$), upstream of the P1 promoter of c-myc, controls 80% to 90% of the total transcriptional activity of this gene.[5–9] The NHE III_1, a G (guanine)-rich strand of the DNA

Received 15 December 2009; accepted 22 January 2010.
Address correspondence to Changqi Zhao, Beijing Key Lab of Gene Engineering Drugs & Biological Technology, College of Life Sciences, Beijing Normal University, Beijing 100875, P. R. China. E-mail: zhaochangqi60724@yahoo.com.cn

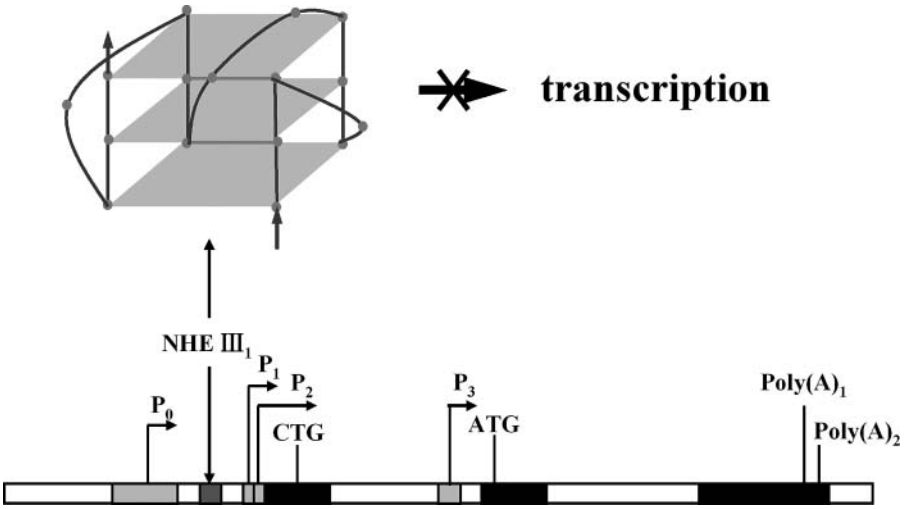

FIGURE 1 Location of the NHE III1 in the c-myc gene and proposed biological function of G-quadruplex in this region.

containing a 27 base-pair sequence, can form intramolecular G-quadruplex structures and function as a transcriptional repressor element[10] (Figure 1). Consequently controlling the transcription of c-myc via the G-quadruplex structure had emerged as an attractive target for anti-cancer therapeutic strategies.

The strategy to find new ligands that are able to selectively interfere with c-myc expression by the formation/stabilization of the specific G-quadruplexes had potential applications. A number of G-quadruplex ligands, such as TMPyP4[11] and Se2SAP[12] had been developed and shown to induce and/or stabilize the G-quadruplex structure. Otherwise, the small molecule- quarfloxin (CX-3543) designed to target the c-MYC promoter quadruplex has already entered Phase II clinical trials.

Bile acids are natural occurring compounds and play a prominent role in biological systems.[13] Bile acid could be applied in design of antimicrobial and antitumor drugs, for example, in Regen's group, a large number of bile acid-polyamine conjugates were synthesized and revealed impressive biological activities.[14] Based on the above viewpoints, we designed and synthesized a new bivalent linkages of bile acid-amino acid conjugate—BAA (Figure 2), and tested the antitumor activity of the compound. The preliminary bioactivity tests showed that the compound could inhibit the growth of human breast cancer cell MCF-7 and the IC50 values were less than 10 μM.[15] It is worth studying if the anticancer activity of BAA is related to proto-oncogene c-myc. Thus, the interaction of BAA with NHE III1 DNA was investigated.

In this study, the interaction of the bile acid-amino acid conjugate—BAA with NHE III1 DNA was investigated using circular dichroism pectrum (CD), nuclear magnetic resonance (NMR), proliferation assay and reverse transcriptase PCR (RT-PCR). All the results showed that the BAA could

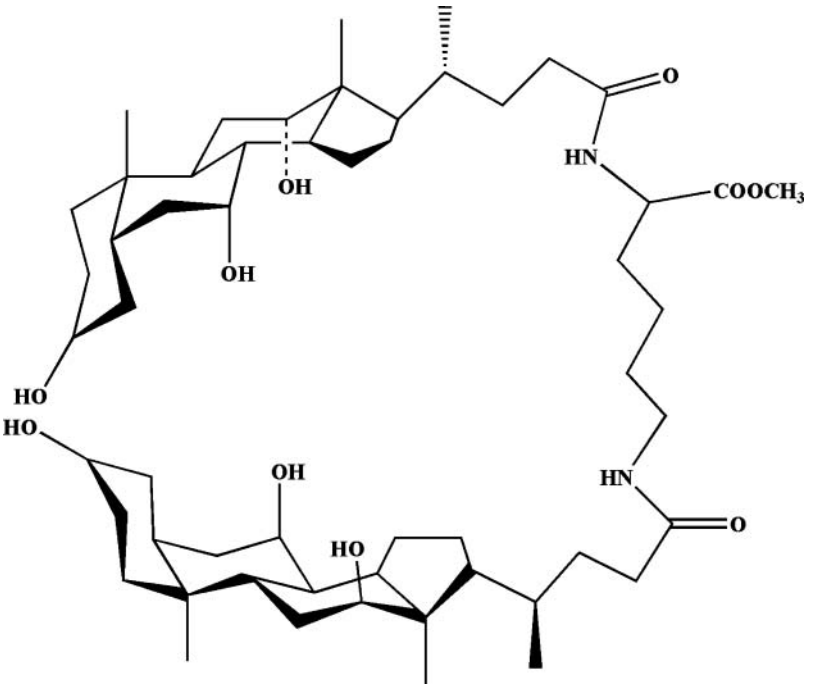

FIGURE 2 Structure of the BAA.

selectively induce the formation of intramolecular parallel G-quadruplex in c-myc DNA, and inhibit the expression of c-myc in human breast cancer cell line MCF-7.

MATERIALS AND METHODS

Materials

All oligomers/primers used in this study were purchased from Invitrogen (Beijing, China), purified by PAGE and their sequences were listed in Table 1. BAA was synthesized as previously reported.[15] The total RNA isolation kit was purchased from SBS Genetech, China. ReverTra Ace kit and SYBR Green I kit were purchased from Sikabio (China). D_2O and DEPC were purchased from Sigma Co. (USA). Tris (D11, 98%) was purchased from Cambridge Isotope Laboratories, Inc. (USA). Analytical grade inorganic salts were purchased from Sinopharm Chemical Reagent Beijing Co. (China).

CD Spectroscopy

The oligomer MYC22-18T/22T at a final concentration of 5 μM was resuspended in Tris-HCl buffer (10 mM, pH 7.4) containing the BAA to be tested. The sample solutions were equilibrated at room temperature for

TABLE 1 Sequences of oligomers (primers) used in the present study

Name of oligomer	Sequence
c-myc22	TGAGGGTGGGGAGGGTGGGGAA
Hum22	AGGGTTAGGGTTAGGGTTAGGG
c-Kit87up	AGGGAGGGCGCTGGGAGGAGGG
Bcl-2	GGGCGCGGGAGGAAGGGGGCGGG
c-mycA (Upstream primer for c-myc in RT-PCR27)	CAGCCCCGAGCCCCTGGTG
c-mycS (Downstream primer for c-myc in RT-PCR)	AGGCGCTGCGTAGTTGTGCTGATG
β-actinA (Upstream primer for β-actin as control in RT-PCR)	GTTGCTATCCAGGCTGTGC
β-actinS (Downstream primer for β-actin as control in RT-PCR)	GCATCCTGTCGGCAATGC

more than 12 hours before measurements. CD spectra were collected from 220 to 320 nm on a Jasco-815 automatic recording spectropolarimeter with a 1 cm pathlength quartz cell at 25°C. Spectra were collected with scan speed of 500 nm/min and response time of 1 second. Each spectrum was the average of four scans. A buffer blank correction was made for all spectra.

NMR Measurement

The oligomer MYC22-18T/22T at a final concentration of 0.2 mM was resuspended in Tris-HCl buffer (10 mM, pH 7.4) containing the BAA to be tested. The sample solutions were equilibrated at room temperature for more than 12 hours before measurements. NMR experiments were performed on a Bruker AVANCE 600 spectrometer equipped with a 5 mm BBI probe capable of delivering z-field gradients and TOPSPIN software (version 2.0; Bruker, USA). All experiments were carried out at 298.2 K. The one-dimensional proton spectra were recorded by the standard pulse program p3919gp that applies 3-9-19 pulses with gradients for water suppression.

Cell Culture

The human breast cancer cell MCF-7 was obtained from the American Type Culture Collection (ATCC; USA). The cells cultures were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin in 25 cm^2 culture flasks at 37°C humidified atmosphere with 5% CO_2.

RNA Extraction

For RNA extraction, about 5×10^5 MCF-7 cells were seeded in 6-well plates, and the freshly dissolved BAA at increasing concentrations was added into the plates. After 1 day of culture, the cell pellets were lysed in TRIzol solution and the total RNA was extracted according to the manufacturer's instructions.

Quantitative Real-Time PCR

Total RNA was used as a template for reverse transcription using a Rever-Tra Ace kit. The expression levels of c-Myc mRNA level were determined by Canada-FTC2000 fluorescence quantitative PCR using SYBR Green I technology following the manufacture's protocol. The expression levels were measured using SYBR Green I kit. C-myc gene expression was quantified by the comparative cycle threshold method according to the manufacturer's instructions.

Data Analysis

One-way analysis of variation (ANOVA) was used to test for significance in differences for the expression of c-myc PCR products treated with different concentrations of BAA. The statistical test was carried out with SPSS version 13.

RESULTS AND DISCUSSION

Inducing the Forming of Parallel G-Quadruplex Structure in NHE III1 by BAA

The DNA oligomer used was TGAGGGTGGGGAGGGTGGGGAA (c-MYC22). The oligonucleotide c-MYC22 corresponded to the NHE III_1 sequence that is able to form the biologically relevant chair G-quadruplex but not the basket one, according to the literature.[16] To identify the interaction of the BAA with c-myc G-quadruplex, CD spectroscopy of c-MYC22 was performed after treatment with BAA. CD measurement has been extensively applied to study secondary structures of DNA.[17–19] It is known that parallel G-quadruplex structure gives a characteristic CD signal containing a positive band around 262 nm, while antiparallel G-quadruplex structure always shows positive and negative ones around 295 and 265 nm, respectively. As for unfolded single-strand DNA, it typically exhibits a strong positive CD signal around 256 nm. These CD spectral features help us approximately recognize different DNA motifs conveniently.

The CD spectra of c-MYC22 without any metal cations at room temperature exhibited a large positive peak at 256 nm. As shown in Figure 3, addition of BAA led to great changes of the CD spectra: The positive peak was gradually shifted from 256 nm to about 262 nm with increasing BAA concentration. The result indicated that the BAA could induce the forming of parallel G-quadruplex in the promoter region of human oncogene c-myc.

In order to provide additional evidence for the presence of guanine quartets, one- dimensional NMR was followed. Chemical shifts at 10–12 ppm for guanine imino protons are characteristic of a G-quadruplex structure.[20] The ^{1}H NMR spectra shown in Figure 4 demonstrated the presence of

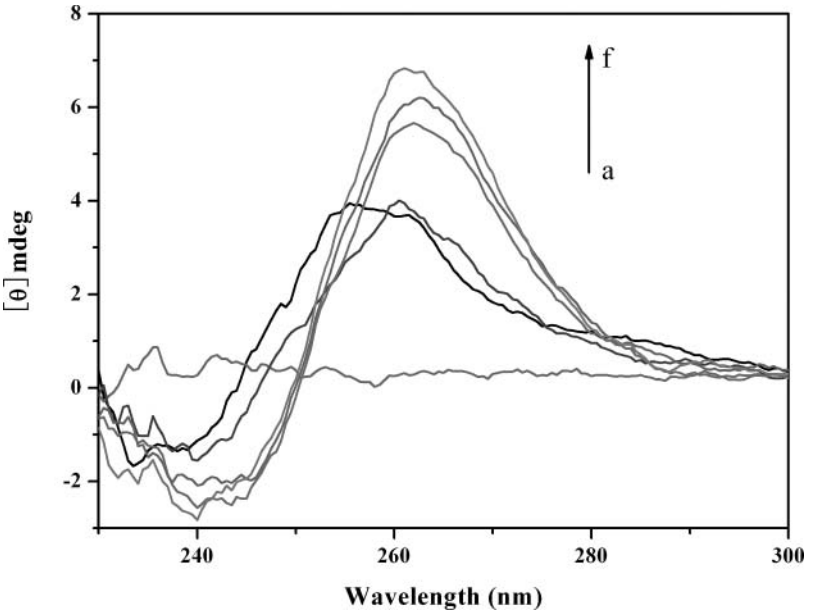

FIGURE 3 The CD spectra of c-myc22 with BAA. a) 5 μM BAA in the absence of c-myc22; b–f) The concentrations of BAA are (μM): 0, 5, 10, 20, 40, in the presence of 5 μM c-myc22.

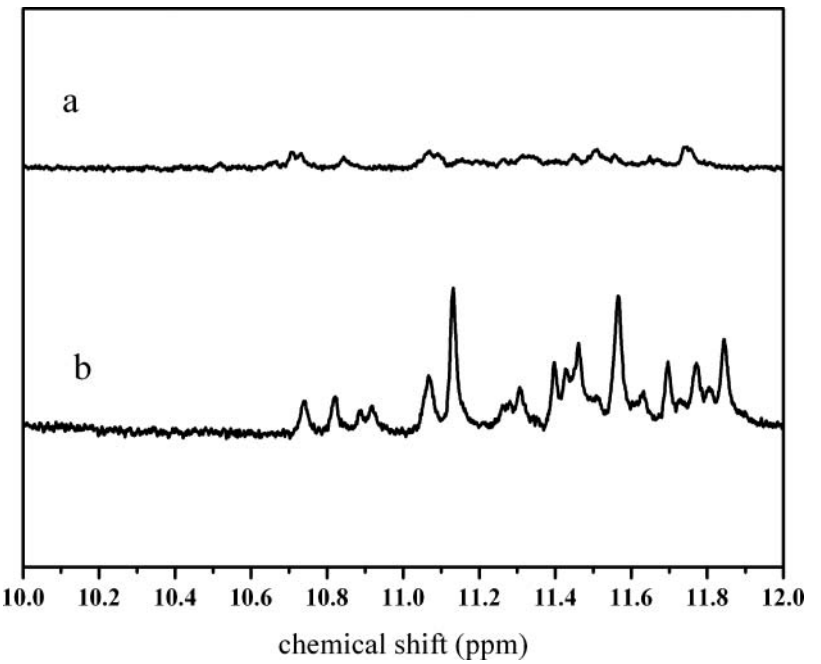

FIGURE 4 One-dimensional NMR study a solution of 0.2 mM c-myc22 with 0.2 mM BAA (b) and without BAA (a).

exchangeable protons in the chemical shift range characteristic of guanine quartet imino protons as well as exchangeable protons from the loop residues. These chemical shifts, along with the small line widths, are typical of unimolecular, folded quadruplexes.

Selectivity for C-myc G-Quadruplex DNA and Other G-Quadruplex DNA Structures by BAA

Besides human c-myc gene, telomeres, some oncogene promoter sequences such as Bcl-2,[21,22] c-Kit[23,24] and other biologically relevant regions of the genome are also rich in G (guanine) and could form intramolecular G-quadruplex structures. To identify whether the BAA could selectively induce the promoter region of human oncogene c-myc, CD spectroscopy of different G (guanine)-rich sequences were performed after treatment with BAA (Figure 5).

Hum22 was the core sequence of the G-rich strand of human telomeric DNA. c-Kit87up located within −87 and −109 base pairs upstream of a

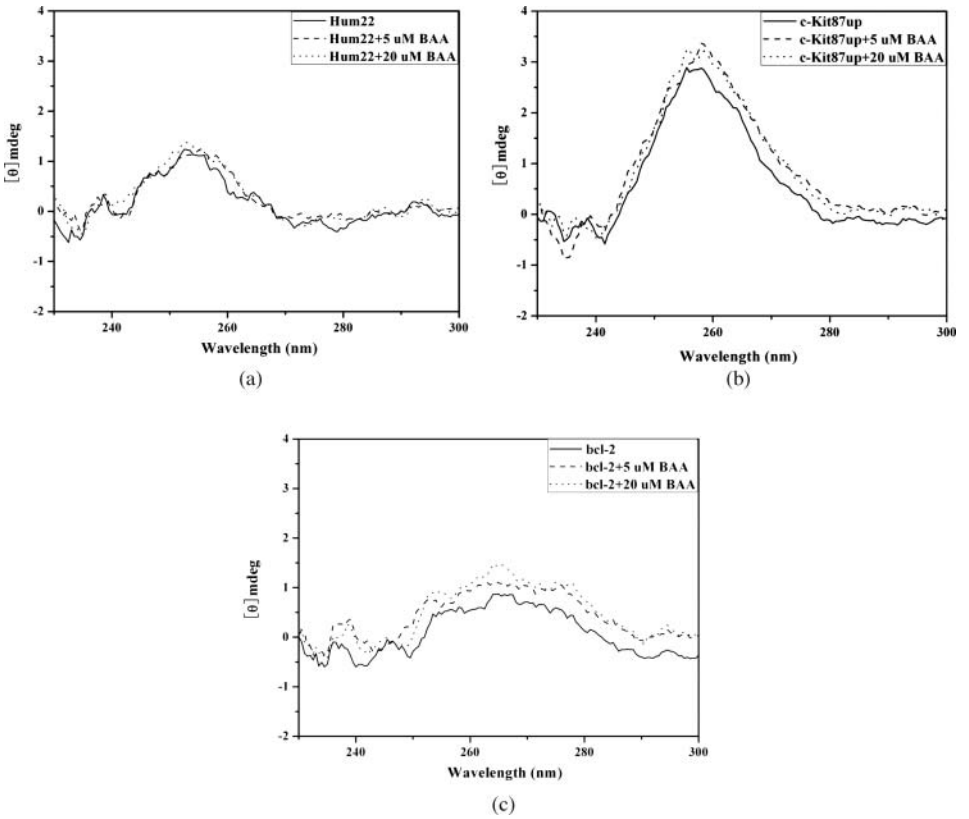

FIGURE 5 The CD spectra of different DNA sequences (5 μM), a) Hum22, b) c-Kit87up, c) Bcl-2 with BAA.

nuclease hypersensitive region of the human c-Kit promoter. Bcl-2 located in the nuclease hypersensitive region −1490 to −1451 upstream of the P1 promoter in the Bcl-2 promoter. As shown in Figure 5, the CD spectra of different DNA sequences did not show obvious change after treatment with BAA. The results indicated that the BAA could selectively induce the promoter region of human oncogene c-myc.

Inhibition of the Transcription of C-myc by the BAA in the Cancer Cell Line

To investigate whether BAA had the ability to inhibit c-myc transcription in cancer cell line, the real-time quantitative PCR was performed. The real-time quantitative PCR has been widely used to provide the quantitative estimates at the level of gene transcription.

For the transcription assay of c-myc in cancer cell lines, about 5×10^5 MCF-7 cells were seeded into six-well plates, incubated for 1 day, and then treated with BAA at 1, 10, and 50 μM for 24 hours. The total RNA was extracted and reverse transcripted to cDNA, which was then used as a template for specific SYBR Green PCR amplification of the c-myc sequence and controlled by β-actin.

The decreasing of c-myc PCR products was significant when treated with BAA, and the decreasing was in a dose-dependent manner (Figure 6). As shown in Figure 6, 1 μM of BAA could inhibit the expression of c-myc obviously, and the expression of c-myc was decreased by 21% ($P < 0.05$). When

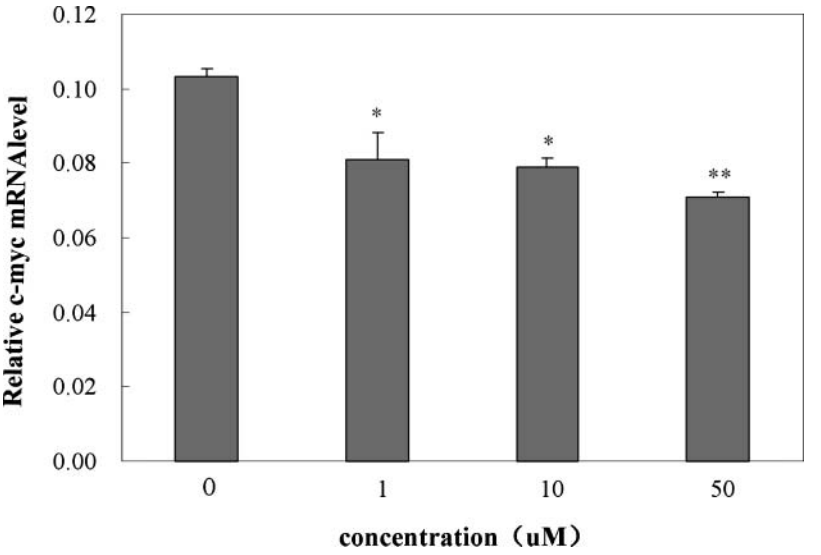

FIGURE 6 Effect of BAA on the expression of c-Myc in MCF-7 cells. Relative expression levels of c-myc mRNA in MCF-7 cells were measured by real-time PCR. The expression of β-actin gene was used to normalize the values of c-Myc (columns). *P < 0.05, **P < 0.01 (one-way ANOVA).

the concentration of BAA was 50 μM, the expression of c-myc was inhibited significantly, and decreased by 32% ($P < 0.01$). The results indicated that BAA had the ability to inhibit c-myc transcription in cancer cell line, which were consistent with the results in the above experiments.

CONCLUSION

In summary, bile acid-amino acid conjugate—BAA have been investigated on its interaction with c-myc DNA G-quadruplexes. It was found that BAA could selectivity induce the forming of parallel G-quadruplex structure in c-myc, and was able to inhibit c-myc gene transcription in the human breast cancer cell MCF-7. This study suggested that the anticancer activity of BAA might relate to the interaction of BAA with c-myc DNA G-quadruplex.

REFERENCES

1. Pelengaris, S.; Rudolph, B.; Littlewood, T. Action of myc In Vivo—proliferation and apoptosis. *Curr. Opin. Gen. Dev.* **2000**, 10, 100–105.
2. Spencer, C.A.; Groudine, M. Control of c-myc regulation in normal and neoplastic cells. *Adv. Cancer Res.* **1991**, 56, 1–48.
3. Marcu, K.B.; Bossone, S.A.; Patel, A.J. MYC function and regulation. *Ann. Rev. Biochem.* **1992**, 61, 809–860.
4. Facchini, L.M.; Penn, L.Z. The molecular role of MYC in growth and transformation: recent discoveries lead to new insights. *FASEB J.* **1998**, 12, 633–651.
5. Sakatsume, O.; Tsutsui, H.; Wang, Y.; Gao, H.; Tang, H.; Yamauchi, T.; Murata, T.; et al. Binding of THZif-1, a MAZ-like zinc finger protein to the nuclease-hypersensitive element in the promoter region of the c-MYC protooncogene. *J. Biol. Chem.* **1996**, 271, 31322–31333.
6. Postel, E.H.; Mango, S.E.; Flint, S.J. A nuclease-hypersensitive element of the human c-myc promoter interacts with a transcription initiation factor. *Mol. Cell. Biol.* **1989**, 9, 5123–5133.
7. Siebenlist, U.; Hennighausen, L.; Battey, J.; Leder, P. Chromatin structure and protein binding in the putative regulatory region of the c-myc gene in Burkitt lymphoma. *Cell* **1984**, 37, 381–391.
8. Boles, T.C.; Hogan, M.E. DNA structure equilibria in the human c-myc gene. *Biochemistry* **1987**, 26, 367–376.
9. Tomonaga, T.; Levens, D. Activating transcription from single stranded DNA. *Proc. Natl. Acad. Sci. USA* **1996**, 93, 5830–5835.
10. Siddiqui-Jain, A.; Grand, C.L.; Bearss, D.J.; Hurley, L.H. Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. *Proc. Natl. Acad. Sci. USA* **2002**, 99, 11593–11598.
11. Grand, C.L.; Han, H.; Munoz, R.M.; Weitman, S.; VonHoff, D.D.; Hurley, L.H.; Bearss, D.J. The cationic porphyrin TMPyP4 down-regulates c-MYC and human telomerase reverse transcriptase expression and inhibits tumor growth in vivo. *Mol. Cancer Ther.* **2002**, 1, 565–573.
12. Seenisamy, J.; Bashyam, S.; Gokhale, V.; Vankayalapati, H.; Sun, D.; Siddiqui-Jain, A.; Streiner, N.; et al. Design and synthesis of an expanded porphyrin that has selectivity for the c-MYC G-quadruplex structure. *J. Am. Chem. Soc.* **2005**, 127, 2944–2959.
13. Sjövall, J. Fifty years with bile acids and steroids in health and disease. *Lipids* **2004**, 39, 703–722.
14. Zhang, J.; Jing, B.; Regen, S.L. Kinetic evidence for the existence and mechanism formation of a barrel stave structure from pore-forming dendrimers. *J. Am. Chem. Soc.* **2003**, 125, 13984–13987.
15. Li, Y.; Zhang, Z.; Ju, Y.; Zhao, C.Q. Design, synthesis and antitumor activity of dimeric bile acid-amino acid conjugates. *Lett. Org. Chem.* **2007**, 4, 414–418.
16. Siddiqui-Jain, A.; Grand, C.L.; Bearss, D.J.; Hurley, L.H. Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. *Proc. Natl. Acad. Sci. USA* **2002**, 99, 11593–11598.

17. Li, J.; Correia, J.J.; Wang, L.; Trent, J.O.; Chaires, J.B. *Nucleic Acids Res.* **2005**, 33, 4649.
18. Rujan, I.N.; Meleney, J.C.; Bolton, P.H. Vertebrate telomere repeat DNAs favor external loop propeller quadruplex structures in the presence of high concentrations of potassium. *Nucleic Acids Res.* **2005**, 2, 2022–2031.
19. Ambrus, A.; Chen, D.; Dai, J.; Bialis, T.; Jones, R.A.; Yang, D. Human telomeric sequence forms a hybrid-type intramolecular G-quadruplex structure with mixed parallel/antiparallel strands in potassium solution. *Nucleic Acids Res.* **2006**, 34, 2723–2735.
20. Ivan, S.; Richard, H.S. Lead is unusually effective in sequence-specific folding of DNA. *J. Mol. Biol.* **2000**, 296, 1–5.
21. Dexheimer, T.S.; Sun, D.; Hurley, L.H. Deconvoluting the structural and drug-recognition complexity of the G-quadruplex-forming region upstream of the bcl-2 P1 promoter. *J. Am. Chem. Soc.* **2006**, 128, 5404–5415.
22. Dai, J.; Chen, D.; Jones, R.A.; Hurley, L.H.; Yang, D. NMR solution structure of the major G-quadruplex structure formed in the human BCL2 promoter region. *Nucleic Acids Res.* **2006**, 34, 5133–5144.
23. Rankin, S.; Reszka, A.P.; Huppert, J.; Zloh, M.; Parkinson, G.N.; Todd, A.K.; Ladame, S.; et al. Putative DNA quadruplex formation within the human c-kit oncogene. *J. Am. Chem. Soc.* **2005**, 127, 10584–10589.
24. Fernando, H.; Reszka, A.P.; Huppert, J.; Ladame, S.; Rankin, S.; Venkitaraman, A.R.; Neidle, S.; et al. A conserved quadruplex motif located in a transcription activation site of the human c-kit oncogene. *Biochemistry* **2006**, 45, 7854–7860.