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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1660–1664

Biological studies of photoinducible phenol quaternary ammonium derivatives

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Received 15 September 2005; revised 27 November 2005; accepted 6 December 2005
Available online 27 December 2005

Abstract—Three water-soluble DNA cross-linking phenol quaternary ammonium derivatives **3**, **4**, and **5** could inhibit the transcription in vitro by photoactivation. DNA interstrand cross-linking action might be the key factor to inhibit transcription by these compounds. Further tumor cell apoptosis was observed by flow cytometry it indicated that cross-linking agent **5** could significantly induce the late apoptosis of tumor cells.

Transcription is a critical step in protein synthesis. During this cellular process, mRNA is assembled from a DNA template.[1] As the inhibition of transcription could induce coding of RNA and proteins incompletely and then lead to cell death finally, it has been found to be potentially useful in the treatment of various cancers and searching for antiviral or anti-tumor agents such as cisplatin, actinomycin, etc.[2–5] The active functions of some such agents are involved in DNA interstrand cross-linking mechanism.[6–8] It has been speculated that DNA interstrand cross-links might affect cells by disrupting transcription.[9] Meanwhile, photocrosslinks of DNA by irradiation under mild conditions and without any additives such as metals, oxidative agents, and reducing agents will offer considerable potential application in medicine.[6] Recently, we have reported a biphenol diquaternary ammonium **5** that is a potent, water-soluble, and photoinducible DNA cross-linking agent.[10] Electrophoresis of the reaction mixtures showed that compound **5** was 100-fold more efficient than compound **4** at effecting DNA cross-linking. Cross-linked DNA was present at concentrations of compound **5** as low as 1.0 μM.[10] Under irradiation, it could form an *o*-quinone methide (*o*-QM) intermediate, which is an active electrophile in biological and chemical processes,[11–13] and then induce DNA cross-link. More recently, Freccero's group found that binol quaternary ammonium derivatives could cross-link DNA under illumination by the benzo-QM pathway.[14] In the present work, we report our new findings about their inhibitions of transcription in vitro by phenol quaternary ammonium derivatives **3**, **4**, and **5**. We have found that DNA interstrand cross-linking action might be the key factor to inhibit transcription. Furthermore, we have observed that compound **5** could induce tumor cell apoptosis, which could be potentially available modulating drugs for treating cancer in future.

To investigate the structure–activity relationship of DNA cross-linking abilities and inhibition of transcription, we chose compounds **3**, **4**, and **5** as our studied substrates. Synthesis of compounds **4** and **5** was reported in our previous paper.[10] 1,4-Dihydroxyl-benzene diquaternary ammonium **3** was a biphenol diquaternary ammonium analogue and had a similar structure as compound

Keywords: DNA cross-linking agent; Photoinducible; Interstrand; Apoptosis.
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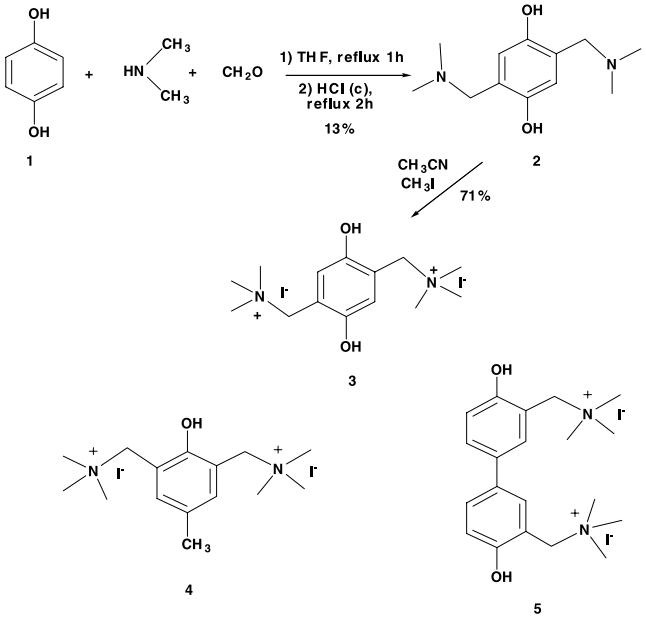
doi:10.1016/j.bmcl.2005.12.007



Scheme 1. Synthesis of **2** and **3**.

5. Compound **3** was prepared by methylation of compound **2**[16] in CH_3CN with the yield of 71% (Scheme 1). It was fully characterized by ^1H NMR, ^{13}C NMR, and elemental analysis.

Inhibition of transcription by compounds **3**, **4**, and **5** was tested. The experiments selected target DNA consisting of T7 promoter that was in a completely controlled in vitro transcription system by T7 RNA polymerase.[15] Prior to transcription, the target DNA was mixed to a given concentration of DNA cross-linking agents **3**, **4** and **5**, respectively, at room temperature and was irradiated for 30 min using a 50-W high-pressure mercury lamp at the distance of 20 cm. Transcription with T7 RNA polymerase was performed according to the protocol recommended by Promega (P1300). For the control experiment, RNA was synthesized in the dark or under the irradiation of target DNA alone in the absence of drug or in the presence of drug without illumination. The results are shown in Figure 1.

Concentration dependence of **5** for inhibition of transcription had also been investigated (Fig. 1B). The inhibition of transcription increased with the concentration of compound **5** relative to the target DNA. At a concentration of 5 μM, the compound **5** could almost inhibit the transcription completely (Fig. 1B, lane 4). We had compared their abilities to inhibit transcription by compounds **3**, **4**, and **5** and the results are shown in Figure 3C. At a concentration of 10 μM, compound **4** had no significant inhibition (Fig. 1C, lane 2), and compound **3** had significant ability of inhibition (Fig. 1C, lane 3). However, at this concentration, compound **5** completely inhibited the transcription process at all (Fig. 1C, lane 4). These results indicated that compound **5** had apparently the best ability to inhibit transcription among three agents.

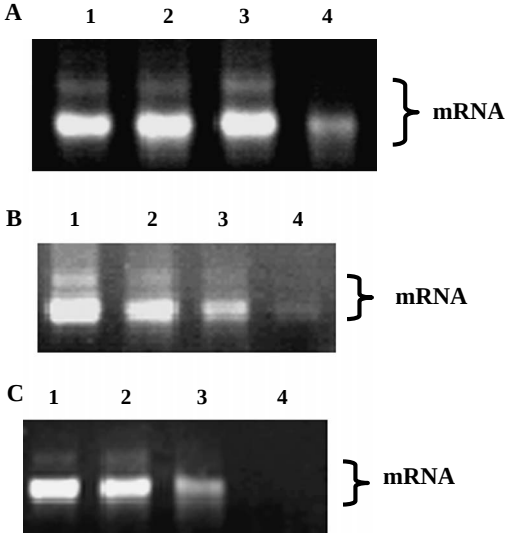
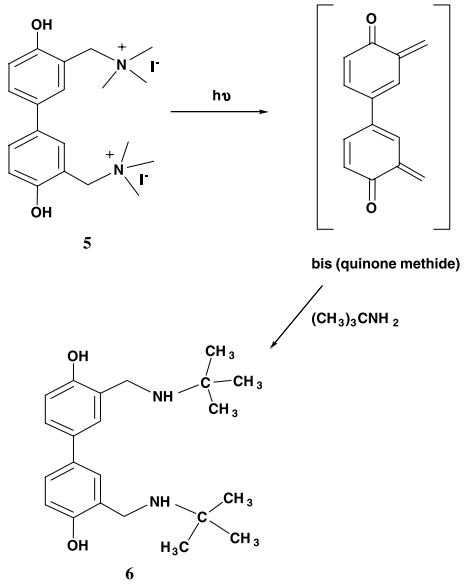


Figure 1. (A) Ethidium bromide-stained agarose gel (0.9%) of transcribed mRNA by compound **5**. Lane 1, from DNA template (0.05 μg); lane 2, from DNA template (0.05 μg) + hν (30 min); lane 3, from DNA template (0.05 μg) + **5** (5 μM); lane 4, from DNA template (0.05 μg) + **5** (1 μM) + hν (30 min). (B) Concentration dependence of **5** for transcribed mRNA (ethidium bromide-stained agarose gel (0.9%)). Lane 1, from DNA template (0.05 μg) + **5** (5 μM); lane 2, from DNA template (0.05 μg) + **5** (0.2 μM) + hν (30 min); lane 3, from DNA template (0.05 μg) + **5** (1 μM) + hν (30 min); lane 4, from DNA template (0.05 μg) + **5** (5 μM) + hν (30 min). (C) Comparison of compounds **3**, **4**, and **5** for transcribed mRNA (Ethidium bromide-stained agarose gel (0.9%)). Lane 1, from DNA template (0.05 μg); lane 2, from DNA template (0.05 μg) + **4** (10 μM) + hν (30 min); lane 3, from DNA template (0.05 μg) + **3** (10 μM) + hν (30 min); lane 4, from DNA template (0.05 μg) + **5** (10 μM) + hν.

The twisted form and two quaternary ammonium groups could anchor DNA strands and facilitate binding of DNA strands that cross-linking between strands then proceeded through a bis(quinone methide) intermediate.[16] However, for compounds **3** and **4**, they might involve in tandem formation of *o*-quinone methide intermediate[13] under illumination after they bound to DNA. Therefore, compound **5** might cross-link DNA with ease after photoactivation.

Our further demonstration about existence of bis(quinone methide) intermediate was performed by trapping reaction with *tert*-butyl amine under illumination (Scheme 2). Stronger nucleophilic agent *tert*-butyl amine could react with bis(quinone methide) intermediate and form secondary amine **6**. Further DNA cross-linking experimental data indicated those of, compared with compounds **3** and **4**, compound **5** had the strongest ability to cross-link DNA and compound **4** had the weakest ability (gel not shown here).

DNA cross-linked by these compounds might be one important factor to inhibit transcription. As the most efficient DNA cross-link agent, compound **5** could strongly prohibit the formation of the 'open' structure of DNA by holding two strands together. Therefore, it might possess the strongest inhibition ability of transcription among them. The transcription abilities of



Scheme 2. Trapping reaction of bis(quinone methide) intermediate.

inhibition corresponded also to their DNA cross-linking formations.[6] Apparently, interstrand DNA cross-link is the key step to block DNA strand opening and then inhibition of transcription occured.[6]

To identify DNA–DNA cross-link by compound **5** involved in interstrand formation, we mixed both linearized pBR322 (4363 bp) and pUC19 (2686 bp), and checked them by denaturing alkaline agarose gel electrophoresis reported by Cech[17] and Tepe and Williams.[18] Lambda DNA/*Hin*dIII was employed as a molecular weight marker (Fig. 2, lane 1). pBR322 DNA cross-linking band was observed in lane 4, and pUC19 DNA cross-linking band in lane 5. When mixing pBR322 and pUC19 with compound **5**, two DNA cross-linking bands were observed after illumination (Fig. 2, lane 6). The possible formations of DNA cross-links between pBR322 and pBR322, pUC19 and pUC19, and pBR322 and pUC19 were illustrated in Scheme 3. If DNA interstrand cross-link happened only, we could observe two cross-linking bands, one was 2686 + 2686 (bases), and another was 4363 + 4363 (bases) in the gel. If DNA interstrand cross-link and interhelical cross-link both occurred, we would observe three cross-linking bands, 2686 + 2686

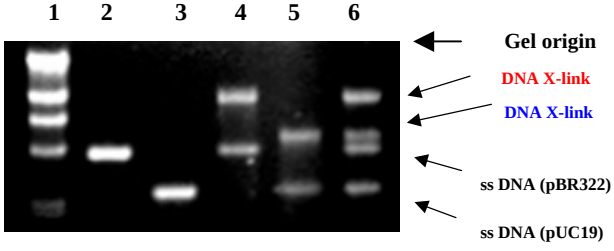
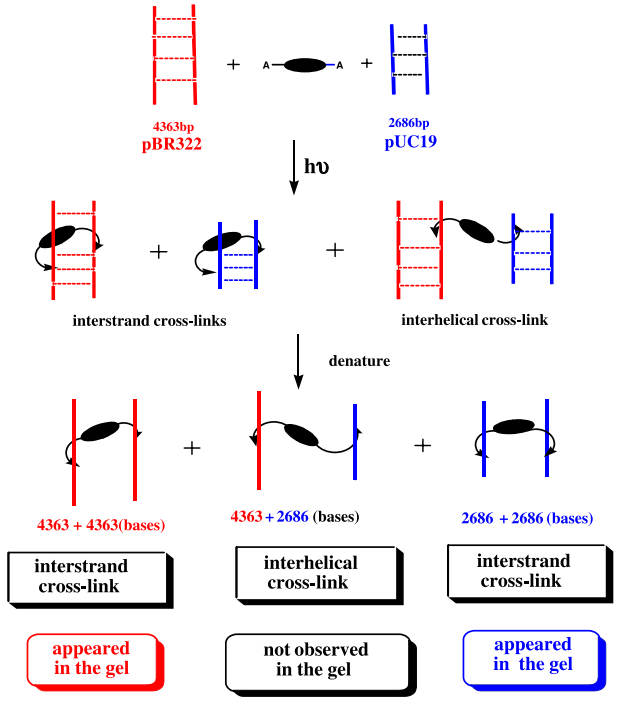


Figure 2. Denaturing alkaline agarose gel (0.9%) for DNA cross-link by compound **5**. Lane 1, Lambda DNA/*Hin*dIII (molecular weight standard, 1.5 μg); lane 2, pBR322 (control, 0.7 μg); lane 3, pUC19 (control, 0.7 μg); lane 4, pBR322 (0.7 μg) + **5** (10 μM) + h*ν* (30 min); lane 5, pUC19 (0.7 μg) + **5** (10 μM) + h*ν* (30 min); lane 6, pBR322 (0.7 μg) + pUC19 (0.7 μg) + **5** (20 μM) + h*ν* (30 min).



Scheme 3. Proposed mechanism of DNA cross-linking agents.

(bases), 4363 + 2686 (bases) and 4363 + 4363 (bases). Our experimental data clearly indicated that only two cross-linking bands, 2686 + 2686 (bases) and 4363 + 4363 (bases) (Fig. 2, lane 6), were observed. It suggested that only DNA interstrand cross-link by compound **5** happened (Scheme 3).

Interesting result was found by our investigation for tumor cell apoptosis induced by compound **5**. We mixed both compound **5** and THP-1 cell with or without illumination. Detection of apoptosis in THP-1 cells was carried out by flow cytometry using annexin V-FITC kit, which was based on the observation that soon after initiating apoptosis. Most cell types translocated phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS could be easily detected by staining with a FITC conjugate of annexin V, a protein that has a strong natural affinity for PS. Externalization of PS occurs earlier than the nuclear change associated with apoptosis.[19e,g] While propidium iodide (PI) dye could stain necrotic cells, it could not stain viable cells because of the integral cell membranes (Fig. 3).

Apoptosis is a programmed process of cell death accompanied by morphological and biochemical features. Targeting apoptotic cell death pathways will provide the wide-ranging opportunities for the discovery and development of novel drugs.[19] Most traditional cancer chemotherapy drugs show their cytotoxic effects by inducing apoptosis.[20] It was shown that compound **5** could induce human tumor cell line, THP-1 cells, apoptosis. THP-1 cells with no drug and no irradiation treatment was considered as control cells (Fig. 3A, 4.9% late apoptosis rate). Compound **5** with illumination induced strongest cell apoptosis (Fig. 3D, 62.03% late apoptosis rate). Only

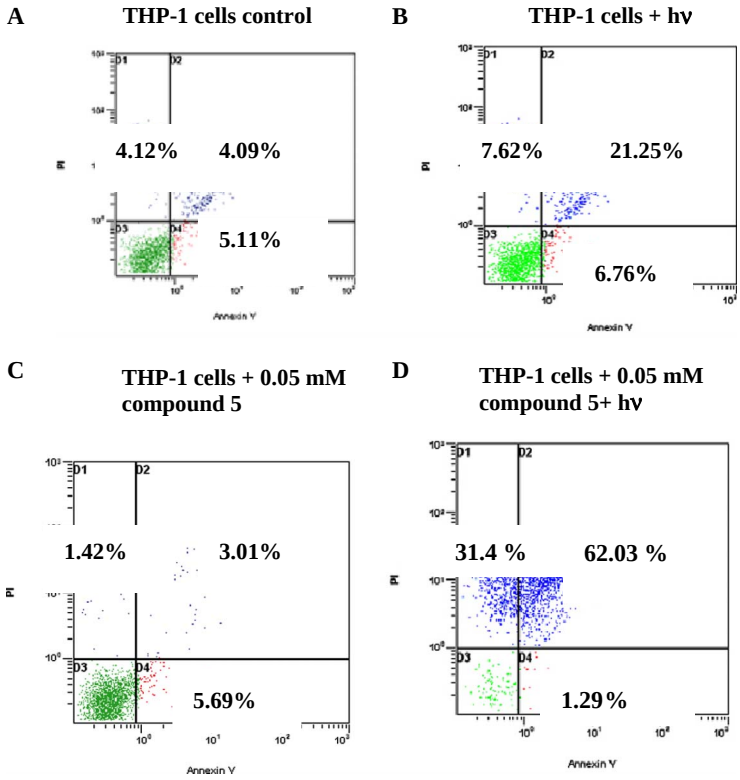


Figure 3. Tumor cell line THP-1 apoptosis induced by compound **5**. The cells were labeled with annexin V-fluorescein isothiocyanate and propidium iodide (PI). The distribution pattern of live and apoptotic cells was determined by FACS analysis. Viable cells are those with low annexin or no annexin and PI staining (lower left panel, D3). Early stage apoptotic cells are represented by high annexin and low PI staining (lower right panel, D4). Later stage apoptotic cells are represented high annexin and high PI staining (upper right panel, D2). And necrosis is represented by cells with high PI and low annexin staining (upper left panel, D1). (A) only THP-1 cells; (B) THP-1 cells illuminated for 30 min; (C) THP-1 cells treated with 0.05 mM compound **5**; (D) THP-1 cells treated with 0.05 mM compound **5** and illuminated for 30 min.

compound **5** treated cells without illumination or only illumination treated cells represented weak apoptosis (Fig. 3B and C, 21.15% and 3.01% late apoptosis rates, respectively).[21] The effect of apoptosis induced by light might be caused by the plasma membrane slightly disturbed under photoirradiation.[19c,19e]

Effective DNA cross-linking agents might be promising techniques to design new types of anti-tumor agents. Our studied indicated that phenol quaternary ammonium compounds **3**, **4**, and **5** could inhibit the transcription under illumination. Apparently, compound **5** had a good shape orientation to adopt helical conformation and could easy localize on DNA. Therefore, compound **5** had better DNA binding mode and induced DNA interstrand cross-link after illumination. This action would prevent the formation of the 'open' structure by holding two strands together and then inhibit the transcription and replication process. Furthermore, compound **5** could induce cell to undergo apoptosis. It will encourage us to investigate the apoptosis-related drug discovery in future.

Acknowledgments

X.Z. thanks the NSFC (National Science Fund of China) (No. 20272046) and National Science Fund for Distinguished Young Scholars (No. 20425206) for financial support.

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