Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3259–3261

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Benzotrithiole 2-Oxide: A New Family of Thiol-Activated DNA-Cleaving Functionalities

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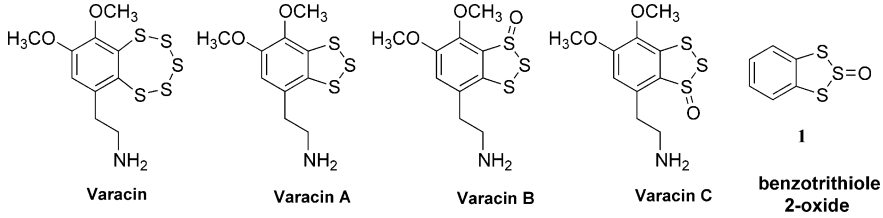
Received 13 June 2002; accepted 29 August 2002

Abstract—It was demonstrated in our studies that benzotrithiole 2-oxide was capable of causing efficient DNA cleavage in the presence of 2-mercaptoethanol or glutathione and exhibited potent cytotoxic properties against certain cancer cell lines.

Sulfur-containing organic cyclic compounds have attracted considerable interest in recent years due in part to their unique molecular structural features as well as their interesting chemical and biological properties.[1–3] These sulfur-containing antibiotics isolated from the natural sources include varacin,[1a,4] lissoclinotoxin A and D,[2a,5] lenthionine,[6] leptosins A, B, E and F,[7] sirodesmins B and C,[8] 5-methylsulfanyl varacins and 3,4-desmethylvaracin.[9] Inspired by the discoveries that many polysulfide and sulfur-containing compounds were thiol-dependent DNA-cleaving agents,[10] we have recently examined the DNA-cleaving activity and cytotoxic properties of benzotrithiole 2-oxide (**1**) and herein report the results of our studies (Scheme 1).

It was initially reported by Rasheed et al. in 1980 that benzotrithiole 2-oxide (**1**) was a biologically useful molecule that acted as herbicides, fungicides, acaricides, nematocides and insecticides.[11] This sulfur-containing compound used in our DNA-cleaving studies was synthesized according to the procedures reported in literature[12,13] with certain modifications and its DNA-cleaving ability was tested by monitoring the conversion of circular supercoiled DNA (form I) to circular relaxed (form II) DNA. Plasmid pBR322 was accordingly incubated with **1** in different buffer solutions with their pH values ranged from 5.0 to 7.5. As shown in Figure 1, benzotrithiole 2-oxide caused single stranded DNA cleavage effectively in the presence of glutathione at pH 5.0 (81%, lane 2), 5.5 (89%, lane 4) and 6.0 (76%, lane 6) while such a DNA-cleaving activity decreased slightly as the pH of the corresponding buffer solutions increased to 6.5 (55%, lane 8), 7.0 (44%, lane 10) and 7.5 (31%, lane 12). In addition, benzotrithiole 2-oxide



Scheme 1. Molecular structures of varacin, varacins A, B, C and benzotrithiole 2-oxide (**1**).

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†See second funding acknowledgement.

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00721-7

generated detectable formation of form II DNA at a concentration at 250 μM at pH 5.5 (lane 2, Fig. 2) and in a concentration-dependent fashion. It should be pointed out that the efficiency of this DNA-cleaving process decreased with increasing the concentration of benzotrithiole 2-oxide from 25 to 250 μM (lanes 4, 3 and 2). One of the possible causes of this observation could be that benzotrithiole 2-oxide was capable of acting as an inefficient oxygen radical scavenger, the effect of which manifested itself only when its concentration increased. It is certain that alternative causes of this observation are also possible. In addition, the amount of DNA cleavage by benzotrithiole 2-oxide increased with increasing the concentration of added 2-mercaptoethanol (Fig. 3) while such a DNA-cleaving activity was not observable in the absence of added thiol (Lane 1), which could be the indication that 2-mercaptoethanol functioned as the activating reagent in the DNA-cleaving process.

In order to determine the properties of the reactive species involved in the DNA cleaving process by benzotrithiole 2-oxide, the inhibitory effects of certain free radical scavengers and nucleophile on the DNA-cleaving process were investigated.[14] As shown in Figure 4, addition of 4-methylaniline to the corresponding mixtures did not slow down the rate of the reactions (lane 10), which could be the sign of the absence of active electrophilic species involved in the DNA-cleaving process. Conversely, the known superoxide radical ($O_2^{\cdot-}$) scavengers Tiron[15a] (lane 3) and DTT[15b,c] (lane 4) inhibited this DNA-cleaving reaction efficiently. In addition, the rate of this DNA-cleaving course was reduced by catalase[14,16] (lane 5) and potassium iodide[15b,c] (lane 6) that decrease the concentration of hydrogen peroxide (H_2O_2) in solution. Moreover, hydroxyl radical ($OH^{\cdot}$) scavengers ethanol (lane 7) and mannitol (lane 8) exhibited effective inhibitory effect on the DNA-cleaving process.[14,16] Interestingly, addition of diethylenetriamine pentaacetic acid (DETAPAC) also slowed down the rate of this DNA-cleaving process by benzotrithiole 2-oxide. DETAPAC is a known metal chelator that seizes traces of transition metal ions thus preventing the conversion of peroxide to hydroxyl radical.[10,14] Based on these observations, it can be assumed that hydrogen peroxide was generated from the reaction of benzotrithiole 2-oxide with molecular oxygen activated by the addition of thiols through the intermediate superoxide radical. The produced hydrogen peroxide

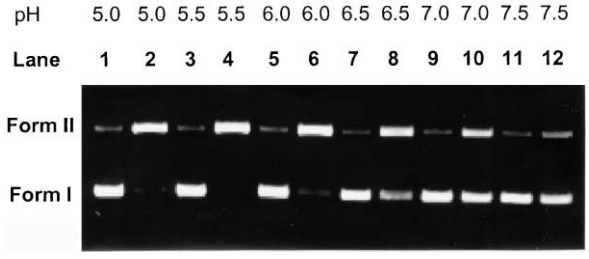

Figure 1. DNA cleavage by benzotrithiole 2-oxide in buffer solutions possessing different pH values. Reaction mixtures were incubated at 37 °C for 4 h in 50 mM sodium phosphate buffer solution containing 25 μM **1**, 10% v/v acetonitrile and 38 μM (bp) supercoiled pBR322 DNA in the presence or absence of 1 mM glutathione. Lane 1, pH 5.0; lane 2, pH 5.0 in the presence of glutathione; lane 3, pH 5.5; lane 4, pH 5.5 in the presence of glutathione; lane 5, pH 6.0; lane 6, pH 6.0 in the presence of glutathione; lane 7, pH 6.5; lane 8, pH 6.5 in the presence of glutathione; lane 9, pH 7.0; lane 10, pH 7.0 in the presence of glutathione; lane 11, pH 7.5; lane 12, pH 7.5 in the presence of glutathione.

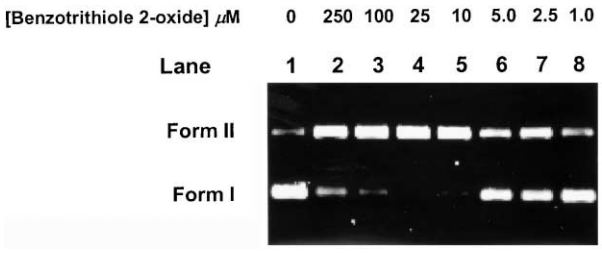

Figure 2. DNA-cleavage by various concentrations of benzotrithiole 2-oxide. Reaction mixtures were incubated at 37 °C for 4 h in 50 mM sodium phosphate buffer solution (pH 5.5) containing **1**, 10% v/v acetonitrile, 38 μM (bp) supercoiled pBR322 DNA and 1 mM glutathione. Lane 1, pBR322 alone; lane 2, 250 μM **1**; lane 3, 100 μM **1**; lane 4, 25 μM **1**; lane 5, 10 μM **1**; lane 6, 5 μM **1**; lane 7, 2.5 μM **1**; lane 8, 1 μM **1**.

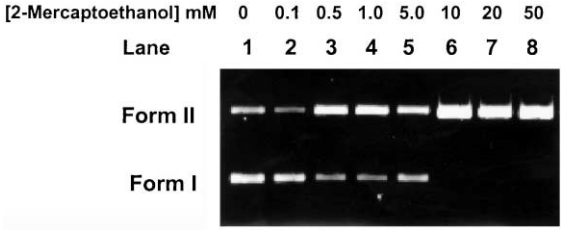

Figure 3. Thiol-dependent cleavage of supercoiled pBR322 DNA by benzotrithiole 2-oxide in various concentrations of 2-mercaptoethanol. Reaction mixtures were incubated at 37 °C for 12 h in 50 mM sodium phosphate, pH 5.5, containing 10% v/v acetonitrile, 38 μM (bp) supercoiled pBR322 DNA and 25 μM **1** in the presence of 2-mercaptoethanol. Lane 1, pBR322 alone; lane 2, 100 μM 2-mercaptoethanol; lane 3, 500 μM 2-mercaptoethanol; lane 4, 1 mM 2-mercaptoethanol; lane 5, 5 mM 2-mercaptoethanol; lane 6, 10 mM 2-mercaptoethanol; lane 7, 20 mM 2-mercaptoethanol; lane 8, 50 mM 2-mercaptoethanol.

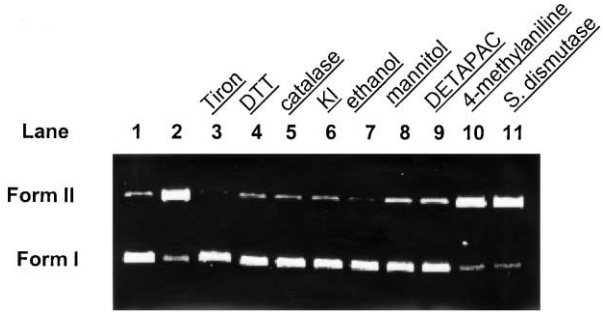

Figure 4. DNA-cleavage by benzotrithiole 2-oxide in the presence of radical scavengers or nucleophiles. Reaction mixtures were incubated at 37 °C for 7 h in 50 mM sodium phosphate buffer solution (pH 5.5) containing 25 μM **1**, 10% v/v acetonitrile, 38 μM (bp) supercoiled pBR322 DNA and 2.5 mM 2-mercaptoethanol. Lane 1, absent of benzotrithiole 2-oxide; lane 2, standard reaction in the absence of added radical scavengers or nucleophile; lane 3, 200 mM Tiron; lane 4, 80 mM DTT; lane 5, 53.2 μg/mL catalase; lane 6, 100 mM KI; lane 7, 1 M ethanol; lane 8, 100 mM mannitol; lane 9, 2.5 mM DETAPAC; lane 10, 10 mM 4-methylaniline; lane 11, 80 μg/mL superoxide dismutase.

Table 1. Cytotoxic property of benzotrithiole 2-oxide against selected cancer cell lines

Cell type	Cell line	IC_{50} (nM) benzotrithiole 2-oxide	IC_{50} (nM) doxorubicin
Colon cancer	HT-29	78.9	61.8
Prostate cancer	PC-3	62.9	49.8
Breast cancer	MDA231	627.0	1.95
Bladder cancer	UMUC3	365.6	9.2
Lung cancer	PACA2	981.9	8.7
Renal cell	A549	151.2	2.7
Human kidney carcinoma	A4982LM	849.9	52.8
Human breast carcinoma	MCF-7	209.2	1.2

was further decomposed into hydroxyl radicals that ultimately caused the DNA cleavage by abstracting hydrogen atoms from the deoxyribose backbone of DNA, a mechanism similar to the mode of action of antibiotic leinamycin.[14,17] It should be noted that addition of superoxide-decomposing superoxide dismutase (SOD) to our reaction mixture did not slow down the rate of the DNA-cleavage reaction (Lane 11, Fig. 4). This observation does not rule out the possibility that superoxide radical was involved in our DNA-cleaving process. This happened most likely because the hydrogen peroxide produced by SOD from superoxide could itself lead to DNA cleavage through a Fenton reaction[10] in which a thiol serves as the reducing reagent, a phenomenon observed previously in the DNA-cleaving reaction by leinamacin.[14,18]

The cytotoxic properties of benzotrithiole 2-oxide was evaluated on eight different human cancer cell lines, which are expressed as the concentration of this compound that inhibits 50% of cell proliferation (IC_{50}). As a positive control, the clinically used anticancer drug doxorubicin was also tested parallelly for its cytotoxic activity of the same cell lines. As shown in Table 1, the IC_{50} values of benzotrithiole 2-oxide against these eight cancer cell lines are all on the nanomolar scales.

In summary, our studies demonstrated that benzotrithiole 2-oxide was capable of causing DNA cleavage effectively in the presence of 2-mercaptoethanol or glutathione and exhibited potent cytotoxic properties against the some cancer cell lines. Further studies on the detailed mechanisms of this DNA-cleaving process by benzotrithiole 2-oxide are in progress.

Acknowledgements

The authors are grateful for the financial support of this work from The Hong Kong Polytechnic University Area of Strategic Development Fund. The Open Laboratory of Chirotechnology was funded by The University Grants Committee of Hong Kong (Areas of Excellence Scheme, AOE P/10-01).

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