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2,2'-BIS(2-AMINOETHYL)-4,4'-BITHIAZOLE SYNTHESIS OF A NOVEL DNA CLEAVING AGENT ACTIVATED BY Co(II)

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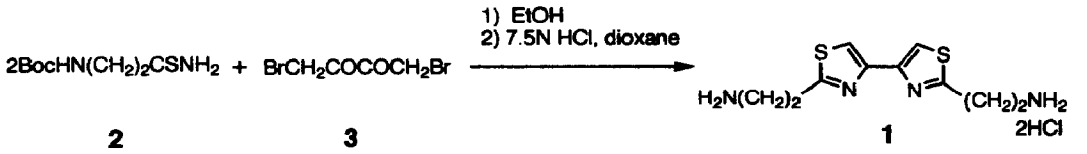
Abstracts: A novel DNA cleaving agent, 2,2'-bis(2-aminoethyl)-4,4'-bithiazole (**1**) was synthesized. Only in the presence of Co(II), a significant cleaving activity of **1** for plasmid DNA was observed at 10μM concentration. Some inhibition reactions indicated that scavengers of hydroxyl radical and singlet oxygen, superoxide dismutase, and catalase could not affect the present DNA cleavage.

In the site specific strand scission of DNA by bleomycin, 2,4'-bithiazole moiety with a positive charge center has played a key role to interact with DNA double strand.[1] As a part of distamycin analog, the thiazole moiety linked by amide bond to *N*-methylpyrrole rings could be used as a recognition site of specific DNA sequences by hydrogen bonds.[2] Therefore, of much interest is the design and synthesis of novel thiazole and bithiazole derivatives with functional group such as an amino or an amide group in order to interact with DNA strand.[3] This communication describes synthesis of a novel DNA cleaving agent, 2,2'-bis(2-aminoethyl)-4,4'-bithiazole (**1**) having simple and readily available 4,4'-bithiazole moiety and demonstrates Co(II)-activated DNA cleaving activity of **1** under the physiological condition.

As shown in Scheme 1, the title compound **1** was prepared by the condensation of two equivalents of *N*-Boc β-alaninethioamide (**2**)[4] with 1,4-dibromobutane-2,3-dione (**3**), followed by the acidic deprotection of amino groups in 62% total yield from **2**. The structure of **1** was confirmed by the spectral data and analysis.[5] ^{1}H NMR (400MHz, D_2O) spectrum of **1** shows ethylene protons signal as a multiplet around

Scheme 1



2 **3** **1**

3.54ppm and thiazole protons signal at 5 and 5' positions as a singlet at 7.87ppm. IR spectrum indicates a characteristic absorption of thiazole C-H stretching at 3120cm^{-1}.

The DNA cleaving ability of **1** in the presence or absence of a metal was investigated using supercoiled plasmid pBR322 DNA[6] by the incubation in MOPS buffer (40mM, pH7.0) at 37°C for 1.5h. Interestingly, as shown in Figure 1, whereas **1** or Co(II) alone shows no DNA cleavage (lanes 2 and 3) as compared with DNA control (lane 1), **1** could efficiently cleave DNA only in the presence of Co(II) (lane 4), that is, supercoiled DNA (form I) completely disappeared and nicked circular DNA (form II) was produced along with a small amont of linear DNA (form III). In contrast, no DNA cleavage by **1** was observed in the presence of other metals, such as Ni(II), Cu(II), and Zn(II) (lanes 5, 6, and 7, respectively). The addition of excess EDTA (1mM) to the reaction mixture of lane 4 efficiently inhibited the DNA cleavage by **1** and Co(II) (lane 8). In addition, at 100μM concentration of **1** in the presence of Co(II), the plasmid DNA was degraded to small pieces but Co(II)-activated DNA cleavage was not observed at 1μM concentration of **1** (data is not shown). It is clear that the complex formation of **1** with Co(II) is necessary to the present DNA cleavage.

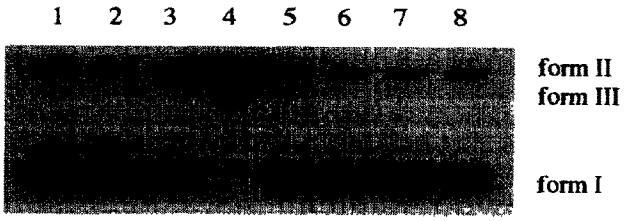
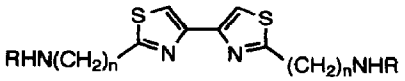


Figure 1: Cleavage of supercoiled plasmid pBR322 DNA(form I) by **1**:
Reaction solution contained 0.1μg of supercoiled plasmid pBR322 DNA in 40mM MOPS (pH 7.0) buffer. All cleavage reactions were run at 37°C for 1.5h , and the electrophoresis was carried out at 50V (1.8h) on a 1.2% agarose gel. The gel patterns were developed by soaking the gels in ethidium bromide buffer solution (1mg/1ml). The concentrations of **1** and all metals are 10μM and 100μM, respectively. lane 1, DNA control; lane 2, **1** alone; lane 3, Co(II) alone; lane 4, **1**+Co(II); lane 5, **1**+Ni(II); lane 6, **1**+Cu(II); lane 7, **1**+Zn(II); lane 8, **1**+Co(II)+ EDTA (1 mM).

In order to investigate the relationship between the structures of 4,4'-bithiazole derivatives and the cleaving activity, some bithiazole analogs, 2,2'-diamino-, 2,2'-bis(aminomethyl)-, and 2,2'-bis(3-amino-propyl)-4,4'-bithiazoles (**4**, **5**, and **6**), 2,2'-bis[2-(acetamido)ethyl]-4,4'-bithiazole (**7**) and 2-(2-aminoethyl)-4-methylthiazole (**8**) structurally related to **1** were prepared by the same procedure as shown in Scheme 1.[7] Interestingly, except for **1** (Run 6), no significant DNA cleaving activity was observed by the incubations of plasmid DNA with bithiazoles (**4**-**7**) at 100μM concentration in the presence of Co(II), as listed in Table 1. The bithiazole **4**, having no ethylene chain between amino group and 2 position of thiazole ring, showed no DNA cleavage (Run 4). Furthermore, **5** and **6**, possessing the shorter or longer alkylene chain compared with

ethylene chain of **1**, could slightly cleave DNA (Run 5) or could not cleave DNA (Run 7), respectively. In the case of bithiazole **7**, having protected amino groups, no strand scission was observed(Run 8). The application of thiazole **8** even at 200μM concentration also showed no DNA cleaving activity (Run 9). These facts strongly indicate that the specific structure of 4,4'-bithiazole possessing two free 2-aminoethyl groups attached at 2 and 2' positions of each bithiazole ring and the presence of Co(II) are essential for the DNA cleavage.



4 : n = 0 R = H
5 : n = 1 R = H
6 n = 3 R = H
7 : n = 2 R = Ac

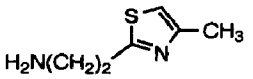


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Table 1: Cleavages of Supercoiled DNA by **1** and Related Compounds(**4-8**) in The Presence of Co(II)

Run No.	1	2	3	4	5	6	7	8	9
Compd. No.	-	**1**	-	**4**	**5**	**1**	**6**	**7**	**8**
Conc.(μM)		100		100	100	10	100	100	200
Co(II)	-	-	+	+	+	+	+	+	+
form I (%)	90	85	87	86	76	-	86	90	90
form III (%)	-	-	-	-	-	17	-	-	-
form II (%)	10	15	13	14	24	83	14	10	10

The concentration of Co(II) is 100μM. Run 1 is DNA control.

Table 2: Inhibitions of DNA Cleavage by **1** in The Presence of Co(II)

Run No.	1	2	3	4	5	6	7	8	9
1 / Co(II)	-	+	+	+	+	+	+	+	+
Inhibitors	-	-	EtOH	2-Propanol	DMSO	D-Mannitol	DABCO	SOD	Catalase
Conc.			0.2M	0.2M	0.2M	0.1M	5mM	20μg/ml	20μg/ml
form I (%)	90	-	-	-	-	-	-	-	-
form III (%)	-	11	11	11	6	11	7	10	15
form II (%)	10	89	89	89	94	89	93	90	90

The concentrations of **1** and Co(II) are 50μM and 100μM, respectively. Run 1 is DNA control. Run 2 is DNA cleavage control.

Since this DNA cleavages by **1** requires the presence of Co(II), the cleavage reaction may be considered to proceed by the oxidative degradation of DNA induced by active oxygen species. However, the addition of hydroxyl radical scavengers such as ethanol, 2-propanol, dimethyl sulfoxide, and *D*-mannitol in the incubation of **1** and DNA with Co(II) never inhibited the cleavage (Run 3-6), as listed in Table 2. A scavenger of singlet oxygen such as 1,4-diazabicyclo[2.2.2]octane (DABCO) induced no inhibition of the cleavage (Run 7). Furthermore, in the presence of large excess of superoxide dismutase (SOD) or catalase no DNA cleavages were observed (Run 8 and 9). These results indicate that this DNA cleavage by **1** in the presence of Co(II) is not caused by oxidative degradation. On the other hand, it was considered that active oxygen species could not be induced by redox reaction involving the reduction of Co(II) to Co(I) species because this DNA cleavage reaction did not require any reducing agents. Further investigations about the mechanism of the present DNA cleavage by **1**, including hydrolytic mechanism, are under progress in my laboratory.

REFERENCES AND NOTES

1. Kasai, H.; Naganawa, H.; Takita, T.; Umezawa, H.; *J. Antibiot.*, **1978**, *31*, 1316.
2. Rao, K. E.; Bathini, Y.; Lown, J. W., *J. Org. Chem.*, **1990**, 55, 728-737.
3. In the preparation of this communication, the photoactivated DNA cleavage by chlorinated 2,4'-bithiazole derivatives at 20nM concentration was reported. See: Quada, Jr., J. C.; Levy, M. J.; Hecht, S. M.; *J. Am. Chem. Soc.*, **1993**, *115*, 12171-12172.
4. As a precursor of **1**, compound **2** was prepared from β-alanine by four steps, that is, esterification, Boc protection of amino group, amidation of esters, and the thiation of amido carbonyl group by Lawesson's reagent in 45% total yield from β-alanine.
5. Analytical Data of **1**. Found: C, 36.51;H, 4.90; N, 16.98. Calcd. for $C_{10}H_{14}N_4S_2 \cdot 2HCl$: C,36.70; H,4.93; N, 17.12.
6. Commercially available pBR322 plasmid DNA contains supercoiled DNA (form I) together with a small amount of nicked circular DNA (form II) as an impurity.
7. Preparations of bithiazoles (**4-7**) and thiazole (**8**) were carried out as follows:
   4: See; Erlenmeyer, H.; Menzi, K.; *Helv. Chim. Acta*, **1948**, *31*, 2065. **5** or **6**: The reactions of **3** with *N*-Boc glycinethioamide or 3-(*N*-Boc-amino)propionthioamide, respectively, followed by deprotection gave **5** or **6** in 54% or 66% yields. **7**: Acetylation of **1** with acetic anhydride yielded **7** in 95% yield. **8**: The reaction of **2** with bromoacetone, followed by deprotection gave **8** in 70% yield. All of obtained compounds (**4-8**) were completely analyzed by spectral data.

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