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SYNTHESIS AND DNA CLEAVAGE ACTIVITY OF 4,2':4',4'':2'',4'''-QUATERTHIAZOLES

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Abstract- A series of 4,2':4',4'':2'',4'''-quaterthiazole derivatives (**2**) were readily synthesized *via* a novel five-steps reaction. The key reaction involved the condensation of 1,4-dibromobutane-2,3-dione with two equimolar amounts of 2-(Boc-protected aminoalkyl)-thiazole-4-carbothioamide. The obtained 2,2'''-bis-(3,6-diazahexyl)- and 2,2'''-bis(3,6,9-triazanonyl)-4,2':4',4'':2'',4'''-quaterthiazole (**2b** and **2c**) exhibited significant affinity for double-stranded DNA, such as calf thymus DNA, as well as marked DNA cleavage activity in the presence of Co(II) ions under physiological conditions. Furthermore, the formation of a Co(II)-complex with **2b** and **2c** was found to be necessary for the DNA cleavage activity.

INTRODUCTION

The first synthesis of quaterthiazoles, such as 2,5':2',2'':5'',2'''- and 5,4':2',2'':4'',5'''-quaterthiazoles, was described almost six decades ago by Karrer and Graf.[1] Traupel, Erlenmeyer and Mijovic then reported the synthesis of analogous quaterthiazoles as different ring assemblies, such as 2,4':2',4'':2'',4'''-, 2,2':4',4'':2'',2'''- and 5,2':4',4'':2'',5'''-quaterthiazoles.[2] A common feature of all these synthetic schemes is the Hantzsch's thiazole method, involving the reaction of a α-halocarbonyl compound with a thioamide.[1,2] Recently, Yu and coworkers demonstrated an alternative synthetic strategy of 2,4':2',4'':2'',4'''-quaterthiazole derivatives using a Stille coupling reaction.[3] Curtis and coworkers have reported the synthesis of 2,2':5',5'':2'',2'''-quaterthiazoles by the dimerization of monolithiated 2,2'-bithiazoles in the presence of $Fe(acac)_3$.[4] Erlenmeyer and coworkers originally described the synthesis of the 4,2':4',4'':2'',4'''-quaterthiazole derivatives by the condensation of 2,2'-bis(chloroacetyl)-4,4'-bithiazole with thioacetamides in

1946.[5] However, no subsequent investigations concerning the synthesis and properties of 4,2':4',4'':2'',4'''-quaterthiazoles have appeared in the literature.

In a series of studies on the synthesis and properties of bithiazole derivatives (**1**), we demonstrated that 2,2'-bis(aminoalkyl)-4,4'-bithiazoles (**1**) can form a Co(II)-complex to cleave plasmid DNA.[6] In particular, 2,2'-bis(2-aminoethyl)-4,4'-bithiazole (**1a**) was able to induce thymocyte apoptosis of rat under physiological conditions.[7] Our investigations involving bithiazoles (**1a-c**) are also applicable to those of quaterthiazoles, which possess two sets of aminoalkyl groups in the side chains of a quaterthiazole ring. Newly synthesized quaterthiazoles, such as 2,2'''-bis(aminoalkyl)-4,2':4',4'':2'',4'''-quaterthiazole (**2**), were selected because the synthetic method of **1** is readily applied to their preparation from 3-(Boc)aminopropanethioamide (**3a**), 4,7-di(Boc)-4,4-diazaheptanethioamide (**3b**), and 4,7,10-tri(Boc)-4,7,10-triazadecanethioamide (**3c**) as common starting materials. The new quaterthiazole **2** possesses two 2,4'-bithiazole rings directly connected to each other at the 4-position and two sets of polyaminoalkyl side chains as cationic centers at the 2- and 2'''-positions. The 2,4'-bithiazole rings, which comprise a part of the C-terminus of Bleomycin,[8] together with the cationic centers were predicted to interact with DNA as an anionic polymer.[9]

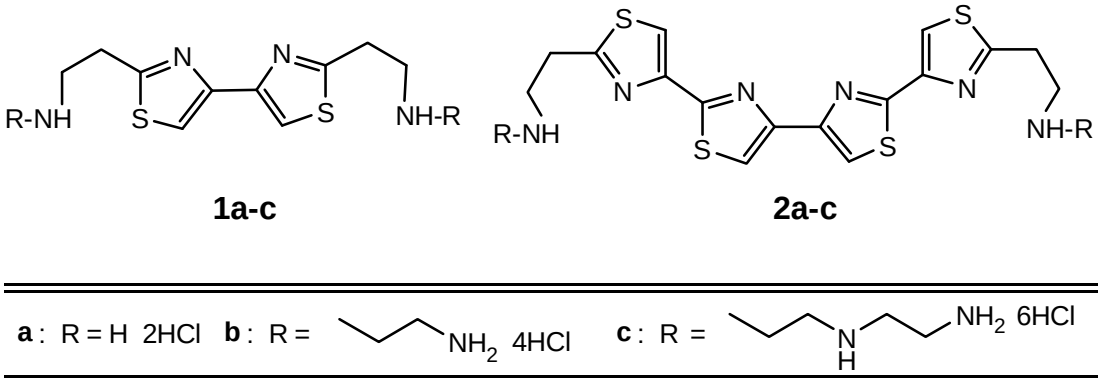


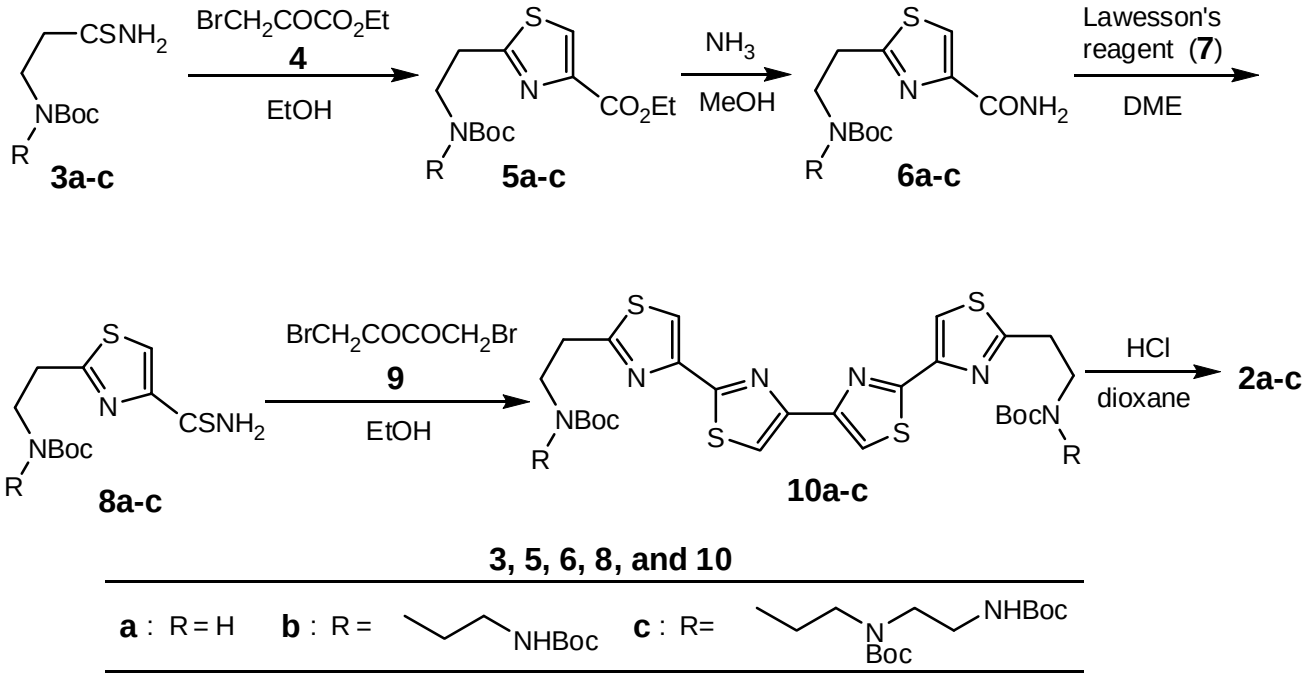
Figure 1

In this report we describe the preparation of new type of 4,2':4',4'':2'',4'''-quaterthiazole (**2**) by a novel synthetic route involving the condensation of 1,4-dibromobutane-2,3-dione with 2-(aminoalkyl)thiazole-4-carbothioamides as a key reaction step. Furthermore, we report that the quaterthiazoles (**2b** and **2c**) exhibit a strong affinity for double-stranded DNA, such as calf thymus (CT)-DNA. Significant DNA cleavage activity for supercoiled plasmid DNA (pBR322) under physiological conditions was observed in the presence of Co(II) ions. This study is the first to demonstrate DNA cleavage activity using quaterthiazole derivatives (**2**).

Synthesis of the new quaterthiazoles (2a-c)

4,2':4',4'':2'',4'''-quaterthiazoles (**2a-c**) were conveniently prepared in five-steps from thioamides (**3a-c**) as

shown in Scheme 1. That is, the condensation of a thioamide (**3a-c**)[6a] with ethyl bromopyruvate (**4**) in dry EtOH was carried out to afford the corresponding thiazole-4-carboxylic acid ethyl esters (**5a-c**). Esters **5a-c** were treated with concentrated aqueous ammonia to give thiazole-4-carboxamides (**6a-c**). Subsequently, Boc protected quaterthiazoles (**10a-c**) were obtained by the thiation of amides **6a-c** with 2,4-bis(4-methoxy-phenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent) (**7**) in dry DME to afford thiazole-4-carbothioamides (**8a-c**), followed by the condensation of **8a-c** with 1,4-dibromobutane-2,3-dione (**9**).



Scheme 1

The acidic deprotection of amino groups of **10a-c** with hydrogen chloride (HCl) in dioxane to afford quaterthiazoles (**2a-c**) was achieved in 20%, 14% and 18% total yields from **3a-c**, respectively. The obtained quaterthiazoles (**2a-c** and **10a-c**) were characterized by ^{1}H-NMR, IR, Matrix Assisted Laser Desorption Ionization (MALDI)-Time of Flight (TOF)-MS and by elemental analysis. This new synthetic route to 4,2':4',4'':2'',4'''-quaterthiazoles (**2a-c**) is superior to the Erlenmeyer's method[5)] because key intermediates, 2-(Boc-protected aminoalkyl)thiazole-4-carbothioamides (**8a-c**) were readily prepared from **3a-c**. In Erlenmeyer's method, preparation of 2,2'-bis(chloroacetyl)-4,4'-bithiazoles as a key precursor was considerably more difficult.

Affinity of the new quaterthiazoles (2a-c) for calf thymus DNA

At pH 7.0 quaterthiazoles (**2**) possess two or more positively charged nitrogen atoms on both side chains of the quaterthiazole ring and two sets of 2,4'-bithiazole ring systems, which are known to interact with DNA.[9]

Thus, affinity of **2** for CT-DNA was investigated by means of a fluorescence quenching method using a DNA-ethidium bromide (EtBr) complex. In these studies the C_{50} value, defined as the concentration of added compound required to reduce the fluorescence intensity of the DNA-EtBr complex by 50%, was used as an indicator of affinity.[10] We found that the C_{50} values of the quaterthiazoles (**2a**: 7.4 μM, **2b:** 1.6 μM, and .**2c**: 0.13 μM) are less than those of the corresponding bithiazoles (**1a**:105 μM, **1b:**16 μM, and **1c:**1.5 μM),[6a] respectively. These results show that quaterthiazoles **2** can bind to DNA more efficiently than the corresponding bithiazoles **1**. Moreover, it can be seen from the C_{50} values that **2c** displays the highest affinity for DNA. Indeed, of all the compounds tested **2c** possesses the longest side chain (i.e., 3,6,9-triazanonyl groups) at both side chains of the quaterthiazole ring. These findings suggest that the directly connected 2,4'-bithiazole rings, each substituted with long aminoalkyl groups on both side chains, play an important role in terms of defining the level of affinity for double-stranded DNA.

DNA cleavage activity of the new quaterthiazoles (2a-c)

The DNA cleavage reactions using **2** were carried out by incubation with the plasmid pBR322 DNA[11] in the presence or absence of metal ion at 37°C for 1 h in various buffer solutions. The cleavage of plasmid DNA was followed by monitoring the conversion of supercoiled plasmid DNA (form I) into nicked circular DNA (form II) and linear DNA (form III). As shown in Figure 2, **2a-c** alone (lanes 2, 8, and 14) as well as Co(II) alone (lane 16) show no DNA cleavage activities at pH 6.0, in comparison to the DNA control (lane 1). However, **2b** or **2c** (10 μM) in the presence of Co(II) ions display significant DNA cleaving activity (Figure 2; lanes 4 and 10, respectively). No DNA cleaving activity was detected when Co(II) was substituted for other metal ions such as Mn(II), Ni(II), Cu(II) or Zn(II) (Figure 2; lanes 3 and 5-7 and lanes 9 and 11-13, respectively). By contrast, DNA cleavage activity of **2a** was not observed even at elevated concentrations (100 μM) in the presence of Co(II) ions (Figure 2; lane 15) or indeed any of the other metal ions included in this study (data not shown). The pH dependency of the DNA cleavage mediated by **2b** and **2c** was examined by performing the reaction in a range of different buffer solutions (pH values 5.0-9.0). As shown in Figure 3, **2b** caused cleavage of form I DNA to the corresponding form II DNA at pH 6.0 (91%, lane 3) and 7.0 (85%, lane 4). However, this reaction was barely detectable at pH 5.0 (lane 2), 8.0 (lane 5) and 9.0 (lane 6). By contrast, **2c** converts form I DNA to form II DNA under all pH conditions examined (lanes 7-11). Moreover, at pH 6.0 a small amount of form III DNA (7%) was observed along with a considerable amount of form II DNA (lane 8). Therefore, the optimal pH for DNA scission by **2b** or **2c** was pH 6.0. As shown in Figure 4, DNA cleavage activity of **2c** at pH 6.0 is slightly more efficient compared to that of **2b** (i.e., at 10 μM, **2c** gives form III DNA whereas **2b** does not; at 5 μM, **2c** affords much more form II DNA than **2b**).

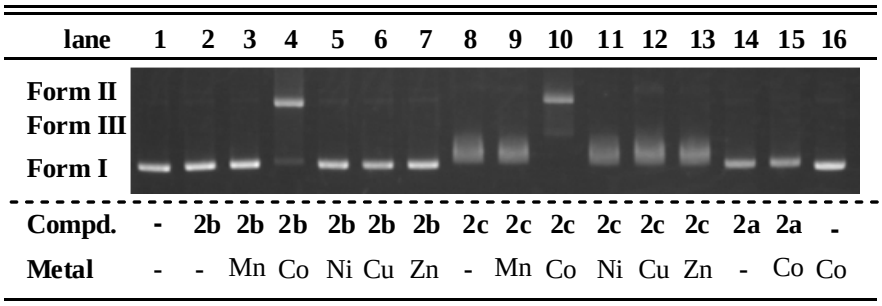


Figure 2. DNA cleavage reactions by **2a** (100 μM), **2b** (10 μM), and **2c** (10 μM) in the presence or absence of metal ion (100 μM) at pH6.0 and 37°C for 1 h.

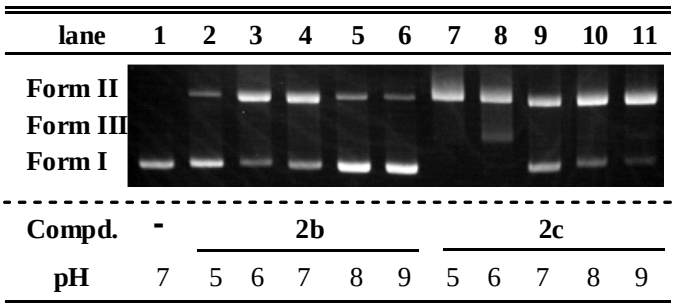


Figure 3. pH dependence of the DNA cleavage reaction by **2b** and **2c**. Concentrations of **2b**, **2c**, and Co(II) are10 μM, 10 μM, and 100 μM, respectively. All reactions were carried out at 37 °C for 1 h.

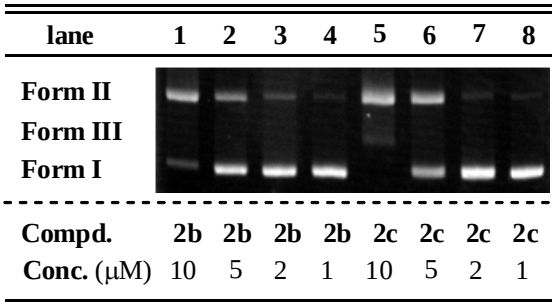


Figure 4. Comparison of DNA cleavage activities between **2b**and **2c** in the presence of Co(II) (100 μM). All reactions were carried out at pH 6.0 and 37°C for 1 h.

Complexation ability and stoichiometry of the complex of the new quaterthiazoles (2a-c) with Co(II) ions

Given that Co(II) is required for the DNA cleavage reactions of quaterthiazoles (**2b** and **2c**), we anticipated the formation of a Co(II)-complex. In order to confirm the formation of Co(II)-complexes **2b** and **2c**, we analyzed the electronic absorption spectra of the two complexes in the absence and presence of Co(II) ions at pH 7.0 (Fig. 5). In the absence of Co(II) ions, the UV spectra of **2b** and **2c** at pH 7.0 is characterized by two very strong absorption peaks at 257 nm (log ε = 4.40 and 4.41, respectively) and 300 nm (log ε = 4.33) and the visible absorption spectrum of Co(II) ions indicate very weak absorption at 512 nm (log ε = 6, data not shown). The electronic spectra of **2b** and **2c** in the presence of Co(II) ions at pH 7.0 exhibit a bathochromic shift (11 nm) along with a small hyperchromic effect for the absorption at 300 nm. In particular, in the presence of Co(II) ions a new absorption at 466 nm and a new shoulder around 380 nm are evident in the visible spectra of **2b** and **2c**, respectively**,** whereas **2b** and **2c** in the absence of Co(II) ions have no absorption in the visible region. These findings strongly suggest that the new quaterthiazoles (**2b** and **2c**) form complexes with Co(II) ions at pH 7.0.

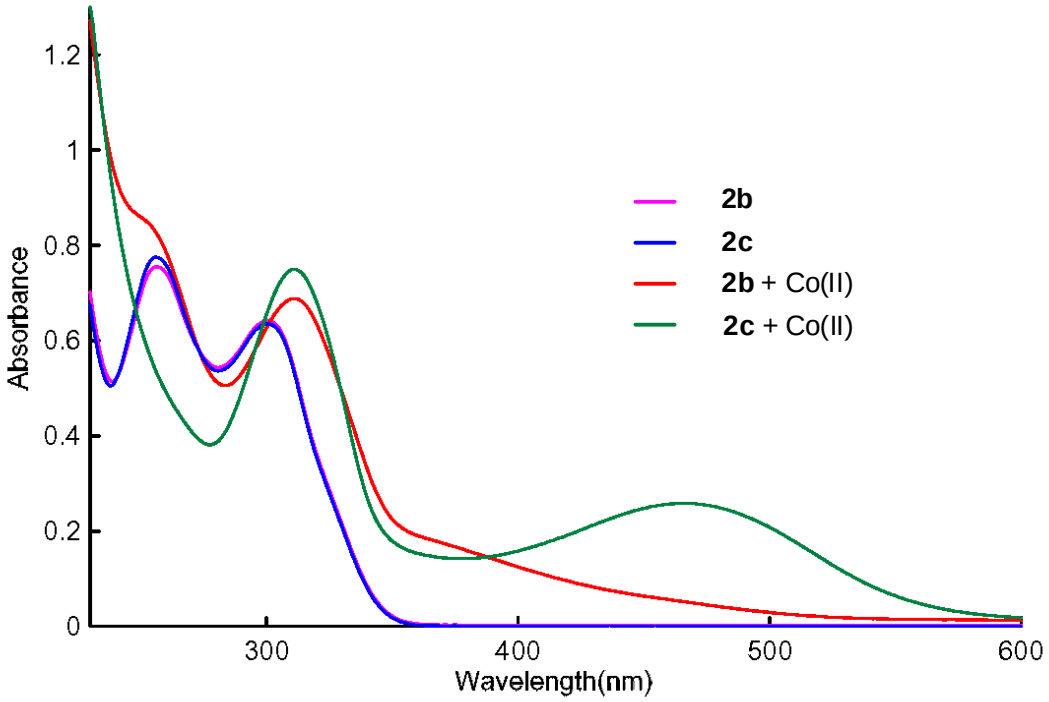


Figure 5. The electronic spectra of **2b**, **2c**, and their Co(II)-complexes at pH 7.0. The concentrations of **2b**, **2c**, and $CoCl_2$ are 0.3 μM, 0.3 μM, and 0.6 μM, respectively.

Table 1. The absorption maxima (log ε) and shoulder [380 nm (log ε)] of the Co(II)-complexes of **2b** and **2c** at various pH conditions.

pH	5.0	6.0	7.0	8.0	9.0
2b + Co(II)	255 (4.40) 302 (4.32) 380 (3.10)	308 (4.33) 380 (3.63)	311 (4.36) 380 (3.75)	311 (4.36) 380 (3.74)	310 (4.35) 380 (3.74)
2c + Co(II)	256 (4.37) 305 (4.36) 467 (3.62)	311 (4.38) 466 (3.92)	311 (4.41) 466 (3.94)	311 (4.41) 466 (3.93)	310 (4.36) 465 (3.84)

The concentrations of **2b**, **2c**, and $CoCl_2$ are 0.3 μM, 0.3 μM, and 0.6 μM, respectively.

Furthermore, the Job method of continuous variation[12] was used to determine stoichiometry of the metal complex of quaterthiazoles (**2b** and **2c**) with Co(II) ions. As shown in Figure 6 (B), the Job plot strongly suggested that **2c** forms a 1:2 complex with Co(II) ions, as was the case for **1c**.[6a] Furthermore, the Job method [Figure 6 (A)] predicted that **2b** could also form a 1:2 complex with Co(II) ions. Importantly, **2a**, which showed no DNA cleavage activity, was not predicted to form a complex with Co(II) ions.

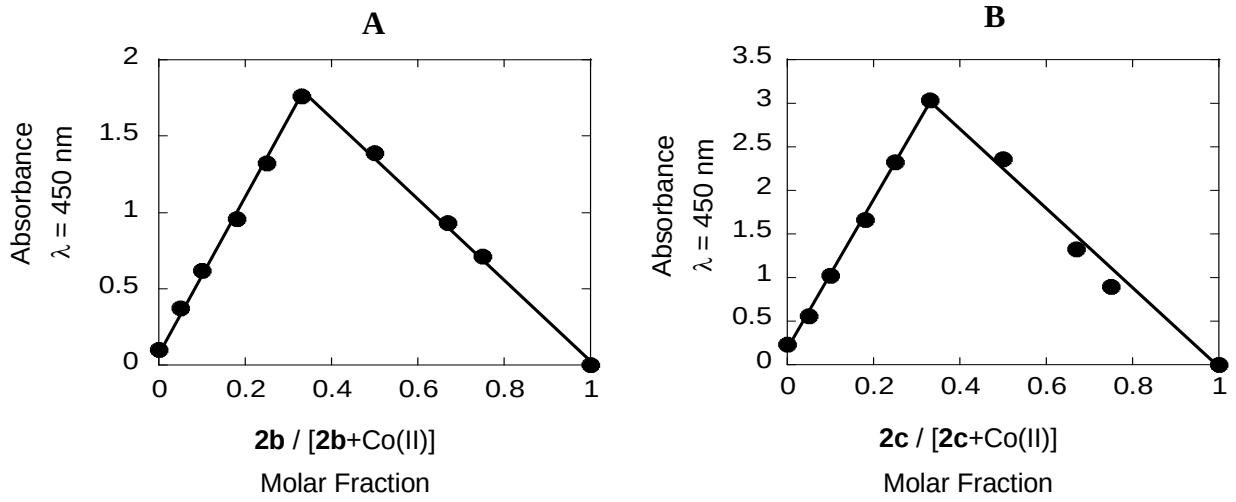


Figure 6. Job plots for **2b** and Co(II) (A) and **2c** and Co(II) (B). The sums of the concentrations of **2b** and Co(II) and **2c** and Co(II) were kept constant at 2.0 mM and 1.0 mM, respectively. The measurements were carried out at pH 7.0 in MOPS buffer (100 mM). (A) 1:2 binding ratio between **2b** and Co(II). (B) 1:2 binding ratio between **2c** and Co(II).

The pH dependency of complexation of the new quaterthiazoles (**2b** and **2c**) with Co(II) ions was also examined by analyzing the electronic spectra (Table 1). The absorption maxima and log ε values of **2b** and **2c** in the presence of Co(II) ions under neutral to moderately alkaline conditions were almost constant, indicating that the complexation abilities of the two species was unchanged. Formation of a Co(II)-complex with **2b** was markedly reduced under acidic conditions (pH 5.0), presumably due to the increased number of positively charged amino groups on both side chains. However, our results clearly show that complex formation between **2c** and Co(II) occurs more readily at pH 5.0 compared with **2b**. Interestingly, **2c** bears two uncharged amino groups on both side chains, which can coordinate Co(II) ions at pH 5.0. Therefore, it was found that the structure of the quaterthiazoles (**2b** and **2c**) significantly influence the formation of a Co(II)-complex.

Affinity of the Co(II)-complexes of the new quaterthiazoles (2b and 2c) for CT-DNA

The extent of affinity of the Co(II)-complexes of **2b** and **2c** for CT-DNA was estimated in a range of different buffer solutions (pH 5.0-9.0) by means of fluorescence spectroscopy. Figure 7 indicated that the fluorescence intensity of EtBr bound to DNA significantly decreaset upon addition of **2b**, **2c** and their Co(II)-complexes to CT-DNA pretreated with EtBr at pH 7.0. The extent of fluorescence quenching at pH 5.0-9.0 is given in Table 2. Our results show that a Co(II)-complex of **2b** or **2b** in the absence of Co(II) ions tends to reduce quenching of fluorescence of EtBr as the pH increases. Affinity of the Co(II)-complex of **2c** for DNA remains constant over a wide pH range (5.0-9.0). By contrast, **2c** in the absence of Co(II) ions results in a decrease in quenching as the pH increases. These findings suggest that an increase of positive charge arising from Co(II) ions and/or the structure of Co(II)-complexes presumably influences the affinity of Co(II)-complexes of quaterthiazoles (**2b** and **2c**) for CT-DNA.

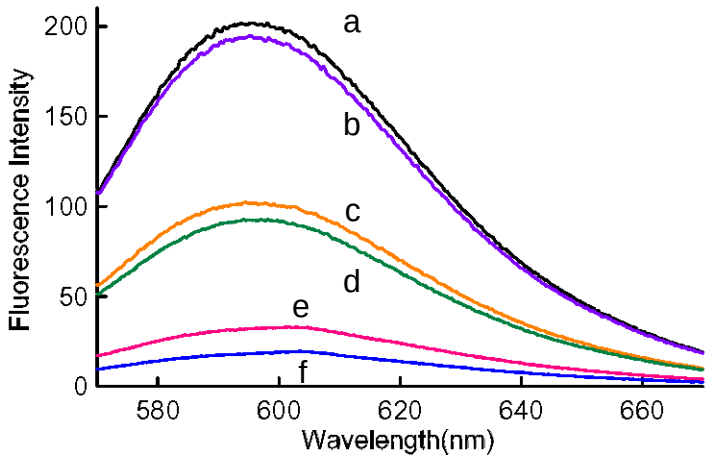


Figure 7. Fluorescence spectra of ethidium bromide in the presence of CT DNA (a), [DNA + Co(II)] (b), [DNA + **2b**+ Co(II)] (c), [DNA + **2b**] (d), [DNA + **2c**] (e), [DNA + **2c** + Co(II)] (f). The concentrations of ethidium bromide, DNA, **2b**, **2c**, and Co(II) are 250 μM, 250 μM, 1.5 μM, 1.5 μM, 3.0 μM, respectively.

Table 2. The extent of quenching (%) of fluorescence of ethidium bromide bound to DNA at various pH conditions by **2b**, **2c**, and their Co(II)-complexes.

pH	5.0	6.0	7.0	8.0	9.0
2b	84	82	49	32	30
2b + Co(II)	70	54	53	46	44
2c	83	82	84	79	54
2c + Co(II)	81	90	90	84	83

The concentrations of ethidium bromide, DNA, **2b**, **2c**, and Co(II) are 250 μM, 250 μM, 1.5 μM, 1.5 μM, 3.0 μM, respectively.

Influence of pH on DNA cleavage

The optimal pH for DNA cleavage by **2b** in the presence of Co(II) ions is pH 6.0 (see previous section). Nevertheless, **2b** is barely able to cleave DNA at pH 5.0, as shown in Figure 3. The Co(II)-complex of **2b** displays moderate affinity for DNA at pH 5.0 as indicated by the substantial quenching of fluorescence of EtBr (Table 2). However, the electronic spectral data of **2b** clearly shows little complex formation with Co(II) ions (Table 1). These observations suggest that free **2b** at pH 5.0 efficiently quenches the fluorescence of EtBr. At pH values above 8.0 the complexation ability of **2b** with Co(II) ions is high (Table 1). Nevertheless, under these conditions Co(II)-complex of **2b** displays a low affinity for DNA (Table 2) and no DNA cleavage activity was detected (Figure 3). At pH 6.0 and 7.0 the Co(II)-complex of **2b** efficiently cleaves DNA (Figure 3) and displays a high ability of complexation with Co(II) ions and a high affinity for DNA (Tables 1 and 2). Complex formation of **2c** with Co(II) ions decreased slightly at pH 5.0 compared with pH values above 6.0. However, Co(II)-complex of **2c** displayed strong DNA cleavage activity and maintained a high affinity for DNA throughout the pH range examined in this study. These findings demonstrate that the formation of a Co(II)-complex and affinity of a Co(II)-complex of the new quaterthiazoles (**2b** and **2c**) for DNA are essential for the observed DNA cleavage activity. The structure of Co(II)-complexes of new quaterthiazoles (**2b** and **2c**) are still unknown. However, the electronic spectra and stoichiometry of the Co(II)-complexes of **2b** and **2c** suggest that Co(II) is coordinated by the amino groups on both side chains and the nitrogen atoms in the quaterthiazole ring.

In conclusion, we have developed a novel synthetic route for the efficient synthesis of 4,2’:4’,4’’:2’’,4’’’-quaterthiazoles (**2**). It can be seen from the comparison of the C_{50} values that the affinities of quaterthiazoles

(**2**) for double-stranded DNA is greater than those of the corresponding bithiazoles (**1**), presumably because of presence of the two sets of 2,4'-bithiazole ring systems. We also found that the structural difference among quaterthiazoles (**2**) significantly influence the affinities for DNA. Furthermore, 2,2'''-bis(3,6-diazahexyl)- and 2,2'''-bis(3,6,9-triazanonyl)-4,2':4',4'':2'',4'''-quaterthiazoles (**2b** and **2c**) are predicted to form a 1:2 complex with Co(II) ions, resulting in considerable DNA cleavage activities at 10 μM under physiological conditions. It can be seen that the affinities of these Co(II)-complexes for DNA is dependent on pH and this plays an important role in DNA cleavage. Finally, the results of an anticancer screening study of **2c** were recently reported.[13] Although the anticancer activity of **2c** was disappointing, synthesis of related compounds is currently under way in our laboratory.

EXPERIMENTAL

All melting points were taken on a Yanagimoto micro melting point determination apparatus and are uncorrected. IR and UV spectra were recorded on a Shimadzu R8000 infrared spectrophotometer and a Jasco U550 spectrophotometer, respectively. Fluorescence spectra were recorded on a Shimadzu RF5300 spectrophotometer. ^{1}H-NMR spectra were obtained on a 400 MHz with Bruker DPX-400 spectrometers using tetramethylsilane in $CDCl_3$ and 4,4-dimethyl-4-silapentanesulufonic acid sodium salt in D_2O as internal references. MALDI-TOF MS analysis was conducted using a Kratos Kompact MALDI-IV Instrument. A saturated solution of 2,5-dihydrobenzoic acid in a 1:1 mixture of water and acetonitrile containing 1% trifluoroacetic acid was used as the matrix. The instrument was calibrated externally with a C60/70 mixture.

Ethyl 2-[2-(Boc)aminoethyl]thiazole-4-carboxylate (5a), ethyl 2-[3,6-di(Boc)-3,6-diazahexyl]thiazole-4-carboxylate (5b), and ethyl 2-[3,6,9-tri(Boc)-3,6,9-triazanonyl]thiazole-4-carboxylate (5c)

General Procedure: A solution of ethyl bromopyruvate (**4**, 10.8 g, 55 mmol) in EtOH (20 mL) was added dropwise to a stirred solution of thioamides (**4**, 50 mmol)[6a] in dry EtOH (180 mL) at rt. After the mixture was refluxed for 2 h, the mixture was concentrated under reduced pressure. A mixture of EtOAc (250 mL) and 2.0 mol/L aqueous NaOH (40 mL) was poured onto the residue and the organic layer was separated, washed twice with brine (40 mL), and then dried over anhydrous sodium sulfate (Na_2SO_4.). After the organic solvent was removed *in vacuo*, the residue was purified by a silica gel flash column with EtOAc:hexane (1:1) to give a pure product.

5a: Yield: 12.3 g (82%), colorless prisms (EtOAc), mp 140-141 ^{o}C. IR (KBr) cm^{-1}: 3393, 3104, 2976, 1720, and 1672. ^{1}H-NMR ($CDCl_3$) δ: 1.40 (3H, t, *J* =7.1 Hz, -CH_3), 1.43 (9H, s, Boc-H), 3.26 (2H, t, *J* =6.5 Hz, -CH_2-thiazole), 3.57 (2H, q, *J* =6.5 Hz, BocNHC$\underline{H_2}$-), 4.42 (2H, q, *J*=7.1Hz, -C$\underline{H_2}$$CH_3$), 4.98 (1H, br s, BocN$\underline{H}$-), 8.07 (1H, s, thiazole 5-H). MALDI-TOFMS *m/z*: 301 $(M+H)^+$, 323 $(M+Na)^+$. *Anal*. Calcd for

$C_{13}H_{20}N_2O_4S$: C, 51. 98; H, 6.71; N, 9.33. Found: C, 51.84; H, 6.42; N, 9.30.

5b: Yield: 15.7 g (71%), colorless microcrystals (Et_2O). mp 77-78 °C. IR (KBr) cm^{-1}: 3378, 3103, 2977, 1720, and 1678. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, *J* =7.1 Hz, $-CH_3$), 1.42 (18H, br s, Boc-H), 3.16-3.36 (6H, m, $-CH_2-$), 3.63 (2H, *J* =6.5 Hz, BocNHC$\underline{H_2}$-), 4.42 (2H, q, *J*=7.1Hz, $-C\underline{H_2}CH_3$), 4.80-5.10 (1H, br s, BocNH-), 8.07 (1H, s, thiazole 5-H). MALDI-TOFMS *m/z*: 444 $(M+H)^+$, 466 $(M+Na)^+$. *Anal.* Calcd for $C_{20}H_{33}N_3O_6S$: C, 54.16; H, 7.50; N, 9.47. Found: C, 54.10; H, 7.25; N, 9.46.

5c: Yield: 22.5 g (77 %), colorless microcrystals (Et_2O). mp 81-82 °C. IR (KBr) cm^{-1}: 3384, 3105, 2979, 1720, and 1687. ^{1}H-NMR ($CDCl_3$) δ: 1.40 (3H, t, *J* =7.1 Hz, $-CH_3$), 1.42 (27H, br s, Boc-H), 3.15-3.40 (10H, m, $-C\underline{H_2}-$), 3.63 (2H, br s, BocNHC$\underline{H_2}$-), 4.42 (2H, q, *J*=7.1Hz, $-C\underline{H_2}CH_3$), 4.80-5.10 (1H, br s, BocNH-), 8.07 (1H, s, thiazole 5-H). MALDI-MS *m/z*: 587 $(M+H)^+$, 609 $(M+Na)^+$. *Anal.* Calcd for $C_{27}H_{46}N_4O_8S\cdot 1/2H_2O$: C, 54.43; H, 7.95; N, 9.40. Found: C, 54.50; H, 7.77; N, 9.21.

2-[2-(Boc)aminoethyl]thiazole-4-carboxamide (6a), 2-[3,6-di(Boc)-3,6-diazahexyl]thiazole-4-carboxamide (6b), and 2-[3,6,9-tri(Boc)-3,6,9-triazanonyl] thiazole-4-carboxamide (6c)

General Procedure: Excess concentrated aqueous ammonia (0.82 mol, 50 mL) was added to a stirred solution of **5** (20 mmol) in MeOH (100 mL) at rt and then the resulting mixture was stirred for 1day at rt. After the solvent and excess ammonia was removed *in vacuo*, Et_2O (100 mL) was poured onto the gummy residue to solidify. The obtained solid was crystallized from Et_2O to give a crude product, which was used in the next step without further purification. Pure product was obtained by recrystallization from Et_2O.

6a: Yield: 2.9 g (54%), colorless microcrystals (Et_2O). mp 149-150 °C. IR (KBr) cm^{-1}: 3393, 3281, 3206, 3107, 2980, 1720, and 1672. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s, Boc-H), 3.20 (2H, t, *J*=6.5Hz, $-CH_2$-thiazole), 3.54-3.62 (2H, m, BocNHC$\underline{H_2}$-), 4.91 (1H, br s, BocNH-), 5.82 (1H, br s, $-CONH_2$), 7.18 (1H, br s, $-CONH_2$), 8.04 (1H, s, thiazole 5-H). MALDI-TOFMS *m/z*: 272 $(M+H)^+$, 294 $(M+Na)^+$. *Anal.* Calcd for $C_{11}H_{17}N_3O_3S$: C, 48.69; H, 6.32; N, 15.49. Found: C, 48.67; H, 6.16; N, 15.22.

6b: Yield: 5.0 g (60%), colorless microcrystals (Et_2O). mp 100-101 °C. IR (KBr) cm^{-1}: 3429, 3377, 3090, 2982, 1715, and 1693. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (18H, br s, Boc-H), 3.20-3.72 (8H, m, $-CH_2-$), 4.85-5.15 (1H, m, BocNH-), 5.78 (1H, br s, $-CONH_2$), 7.22 (1H, br s, $-CONH_2$), 8.04 (1H, s, thiazole 5-H). MALDI- TOFMS *m/z*: 415 $(M+H)^+$, 437 $(M+Na)^+$. *Anal.* Calcd for $C_{18}H_{30}N_4O_5S$: C, 52.15; H, 7.29; N, 13.52. Found: C, 52.08; H, 7.37; N, 13.40

6c: Yield: 7.0 g (63 %), colorless microcrystals (Et_2O). mp 132-133 °C. IR (KBr) cm^{-1}: 3472, 3352, 2977, 1716, and 1682. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, br s, Boc-H), 1.46 (18H, br s, Boc-H), 3.15-3.40 (10H, m, $-C\underline{H_2}-$), 3.63 (2H, br s, BocNHC$\underline{H_2}$-), 4.70-5.20 (1H, m, BocNH-), 5.65 (1H, br s, $-CONH_2$), 7.20 (1H, br s, $-CONH_2$), 8.03 (1H, s, thiazole 5-H). MALDI-TOFMS *m/z*: 559 $(M+H)^+$, 581 $(M+Na)^+$. *Anal.* Calcd for

$C_{25}H_{43}N_5O_7S$: C, 53.84; H, 7.77; N, 12.56. Found: C, 53.98; H, 7.52; N, 12.38.

2-[2-(Boc)aminoethyl]thiazole-4-carbothioamide (8a), 2-[3,6-di(Boc)-3,6-diazahexyl]thiazole-4-carbothioamide (8b), and 2-[3,6,9-tri(Boc)-3,6,9-triazanonyl]thiazole-4-carbothioamide (8c)

General Procedure: Lawesson's reagent (**7**, 3.3 g, 8.0 mmol) was added at once to a stirred solution of amides (**6**, 15 mmol) in DME (50 mL) at rt. After the mixture was heated at 60 ^oC with stirring for 4 h, the solvent was removed *in vacuo* to give gummy residue. A mixture of EtOAc (100 mL) and 2.0 mol/L aqueous NaOH (20 mL) was poured onto the residue, the organic layer was separated, washed twice with brine (20 mL), and then dried over anhydrous Na_2SO_4. The organic solvent was removed *in vacuo* and the residue was purified by a silica gel flash column with EtOAc:hexane (1:1) to give a pure product.

8a: Yield: 2.1g (71%), colorless microcrystals (Et_2O). mp 139-140 ^oC. IR (KBr) cm^{-1}: 3350, 3248, 3151, 3117, 2974, 1688, and 1611. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s, Boc-H), 3.19 (2H, t, *J*=6.5Hz, -CH_2-thiazole), 3.55-3.62 (2H, m, BocNHC$\underline{H}_2$-), 4.86 (1H, br s, BocNH-), 7.26 (1H, br s, -$CSNH_2$), 8.34 (1H, br s, -$CSNH_2$), 8.34 (1H, s, thiazole 5-H). MALDI-TOFMS *m/z*: 288 $(M+H)^+$, 310 $(M+Na)^+$. *Anal.* Calcd for $C_{11}H_{17}N_3O_2S_2$: C, 45.97; H, 5.96; N, 14.62. Found: C, 45.94; H, 5.89; N, 14.40.

8b: Yield: 3.5 g (71%), colorless microcrystals (Et_2O). mp 116-117 ^oC. IR(KBr) cm^{-1}: 3416, 3368, 3186, ^{1}H-NMR ($CDCl_3$) δ: 1.43 (18H, s, Boc-H), 3.20-3.40 (6H, m, -CH_2-), 3.64 (2H, br s, BocNHC$\underline{H}_2$-), 4.85-5.15 (1H, m, BocNH-), 7.67 (1H, br s, -$CSNH_2$), 8.71 (1H, br s, -$CSNH_2$), 8.33 (1H, s, thiazole 5-H). MALDI-TOFMS *m/z*: 444 $(M+H)^+$, 466 $(M+Na)^+$. *Anal.* Calcd for $C_{18}H_{30}N_4O_4S_2$: C, 50.21; H, 7.02; N, 13.01. Found: C, 49.94; H, 6.79; N, 12.92.

8c: Yield: 4.4 g (77%), colorless microcrystals (Et_2O). mp 158-160 ^oC. IR (KBr) cm^{-1}: 3346, 3258, 3140, 2978, 1715, 1678, and 1603. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, br s, Boc-H), 1.46 (18H, br s, Boc-H), 3.15-3.40 (10H, m, -CH_2-), 3.63 (2H, br s, BocNHCH_2-), 4.70-5.20 (1H, m, BocNH-), 7.65 (1H, br s, -$CSNH_2$), 8.70 (1H, brs, $CSNH_2$) 8.32 (1H, s, thiazole 5-H). MALDI-TOFMS *m/z*: 587 $(M+H)^+$, 609 $(M+Na)^+$. *Anal.* Calcd for $C_{25}H_{43}N_5O_6S_2$: C, 52.33; H, 7.55; N, 12.21. Found: C, 52.16; H, 7.35; N, 12.04.

2,2'''-Bis[2-(Boc)aminoethyl]-4,2':4',4'':2'',4'''-quaterthiazole (10a), 2,2'''-Bis[3,6-di(Boc)-3,6-diazahexyl]-4,2':4',4'':2'',4'''-quaterthiazole (10b), and 2,2'''-Bis[3,6,9-tri(Boc)-3,6,9-triazanonyl]-4,2':4',4'':2'',4'''-quaterthiazole (10c)

General Procedure: 1,4-Dibromobutane-2,3-dione (**9**, 2.44 g, 10 mmol) was added in small portions to a stirred solution of thioamides (**8**, 20 mmol) in dry EtOH (50 mL) for 10 min at rt. After the resulting mixture was heated at 70 ^oC for 2 h with stirring, the reaction mixture was concentrated under reduced pressure. A mixture of EtOAc (100 mL) and 2.0 mol/L aqueous NaOH (20 mL) was poured onto the residue, organic layer was separated, washed twice with brine (20 mL), and then dried over anhydorous Na_2SO_4. The organic

solvent was removed *in vacuo* to afford a crude product, which was used in the next step without further purification. A pure product was obtained by recrystalization, followed by a silica gel flash chromatography with EtOAc:hexane (1:1).

10a: Yield: 5.0 g (81%), colorless microcrystals ($CHCl_3$), mp 219-221 °C. IR (KBr) cm^{-1}: 3360, 3138, 3105, 2978, 2923, 1686, and 1523. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (18H, s, Boc-H), 3.26 (4H, t, *J* = 6.2 Hz, -C<u>H</u>$_2$-thiazole), 3.58-3.68 (4H, m, BocNHC<u>H</u>$_2$-), 5.10 (2H, br s, BocN<u>H</u>-), 7.90 (2H, s, thiazole 5- and 5'''-H), 7.96 (2H, s, thiazole 5'- and 5''-H). MALDI-TOFMS *m/z*: 621 $(M+H)^+$, 643 $(M+Na)^+$. *Anal.* Calcd for $C_{26}H_{32}N_6O_4S_4 \cdot (1/10CHCl_3)$: C, 49.54; H, 5.11; N, 13.28 Found: C, 49.78; H, 5.06; N, 13.35.

10b: Yield: 6.1 g (67%), colorless microcrystals (*tert*-butyl methyl ether), mp 215-217 °C. IR(KBr) cm^{-1}: 3346, 3119, 2976, 1710, 1686, and 1524. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (36H, s, Boc-H), 3.20-3.40 (12H, m, -CH_2-), 3.68 (4H, br s, BocNHCH_2-), 4.85-5.15 (2H, m, BocNH-), 7.88 (2H, s, thiazole 5- and 5'''-H), 7.95 (2H, s, thiazole 5'- and 5''-H). MALDI-TOFMS *m/z*: 907 $(M+H)^+$, 929 $(M+Na)^+$. *Anal.* Calcd for $C_{40}H_{58}N_8O_8S_4$: C, 52.96; H, 6.44; N, 12.35. Found: C, 53.25; H, 6.39; N, 12.06.

10c: Yield: 8.6 g (72%), colorless microcrystals (EtOAc), mp 183-185 °C. IR (KBr) cm^{-1}: 3340, 3356, 2974, 2931, 1686, 1638, and 1526. ^{1}H-NMR ($CDCl_3$) δ: 1.38-1.52 (54H, m, Boc-H), 3.20-3.40 (20H, m, -CH_2-), 3.67 (4H, br s, BocNHCH_2-), 4.85-5.15 (2H, m, BocNH-), 7.88 (2H, s, thiazole 5- and 5'''-H), 7.95 (2H, s, thiazole 5'- and 5''-H). MALDI-TOFMS *m/z*: 1193 $(M+H)^+$, 1216 $(M+Na)^+$. *Anal*: Calcd for $C_{54}H_{84}N_{10}O_{10}S_4$: C,54.34; H,7.09; N, 11.74. Found: C, 54.07; H, 6.97; N, 11.44.

2,2'''-Bis(2-aminoethyl)-4,2':4',4'':2'',4'''-quaterthiazole dihydrochloride (2a), 2,2'''-Bis(3,6-diazahexyl)-4,2':4',4'':2'',4'''-quaterthiazole tetrahydrochloride (2b), and 2,2'''-Bis(3,6,9-triazanonyl)-4,2':4',4'':2'',4'''-quaterthiazole hexahydrochloride (2c)

General Procedure: To a stirred solution of Boc protected quaterthiazoles (**10**, 5 mmol) in dry dioxane (20 mL), HCl in dioxane (4.0 mol/L, 10 mL) was added at rt. After addition the precipitate immediately appeared and the reaction mixture was stirred for 10 h at rt. The precipitate was collected by suction filtration to give a crude product, which was purified by recrystallization.

2a: Yield: 2.3 g (92%), colorless microcrystals (H_2O:EtOH=1:2), mp > 300°C. IR (KBr) cm^{-1}: 3500-2600, 1450, 1164, and 829. ^{1}H-NMR (D_2O) δ: 3.44-3.57 (8H, m, -CH_2-), 7.88 (2H, s, thiazole 5'- and 5''-H), 8.05 (2H, s, thiazole 5- and 5'''-H). MALDI-TOFMS *m/z*: 421 $(M+H)^+$, 443 $(M+Na)^+$. *Anal.* Calcd for $C_{16}H_{16}N_6S_4 \cdot 2HCl \cdot H_2O$: C, 37.57; H, 3.94; N, 16.43. Found: C, 37.59; H, 3.66; N, 16.45.

2b: Yield: 3.1 g (95%), colorless microcrystals (H_2O:EtOH=1:2), mp >300 °C. IR (KBr) cm^{-1}: 3500-2500, 1498, 1448, 1117, and 887. ^{1}H-NMR (D_2O) δ: 3.46-3.76 (16H, m, -CH_2-), 8.07 (2H, s, thiazole 5'- and 5''-H), 8.23 (2H, s, thiazole 5- and 5'''-H). MALDI-TOFMS *m/z*: 507 $(M+H)^+$, 529 $(M+Na)^+$. *Anal.* Calcd for

$C_{20}H_{26}N_8S_4 \cdot 4HCl \cdot 0.5H_2O$: C, 36.31; H, 4.72; N, 16.94. Found: C, 36.27; H, 4.51; N, 16.88.

2c: Yield: 3.7 g (91%), colorless microcrystals (H_2O:EtOH=1:1), mp > 300 ^oC. IR (KBr) cm^{-1}: 3500-2400, 1520, 1476, 1192, and 820. ^1H-NMR (D_2O) δ: 3.22-3.68 (24H, m, $-CH_2-$), 8.16 (2H, s, thiazole 5'- and 5''-H), 8.33 (2H, s, thiazole 5- and 5'''-H). MALDI-TOFMS *m/z*: 593 $(M+H)^+$, 615 $(M+Na)^+$. *Anal.* Calcd for $C_{24}H_{36}N_{10}S_4 \cdot 6HCl$: C, 35.52; H, 5.22; N, 17.26. Found: C, 35.63; H, 4.96; N, 16.98.

The Fluorescence Spectroscopic Experiments to Measure C_{50} Values of the New Quaterthiazoles (2a -c)

EtBr displacement assays were carried out as described in reference 10. Measurements were done in quartz cuvettes (1 cm pathlength) on a Shimadu R5300 spectrofluorimeter. Experiments were performed by adding increasing amounts of the quaterthiazoles to preformed EtBr/CT-DNA complex. The concentration of EtBr and CT-DNA (purchased from Sigma-Aldrich Japan Co. Ltd.) was 2.56 μM and 2.59 μM, respectively.

DNA Cleaving Reactions of the New Quaterthiazoles (2a -c)

Plasmid pBR322 DNA purchased from Toyobo Co. Ltd. as supercoiled DNA[10] was used. Each reaction solution contained 0.1 μg of supercoiled plasmid pBR322 DNA in 10 mM buffer solutions (pH 5.0-9.0). All cleavage reactions were run at 37 ^oC for 1 h and electrophoresis was carried out at 100 V (40 min) in 1.2% agarose gels in 40 mM TAE (pH 8.1) buffer. The gel patterns were developed by soaking the gels in EtBr buffer solution (1 μg/1 mL TAE buffer) and photographed with a CCD camera under UV irradiation.

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