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Unique behavior of 2,6-bis(bromomethyl)naphthalene as a highly active organic DNA crosslinking molecule

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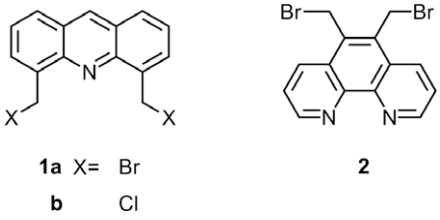
ABSTRACT

Among 14 bis-halomethylated naphthalenes and quinolines, 2,6-bis(bromomethyl)naphthalene was found to have highly active crosslinking activity on DNA. The unique behavior of high microbial mutagenicity, even though it had a low propensity to form double-strands in linearized plasmid DNA, suggested that it would offer a new seed, capable of forming intrastrand crosslinks similar to cisplatin. The electron withdrawal extent of the halogen atoms, the substitution patterns of two halomethyl groups, and the introduction of a nitrogen atom into the aromatic nucleus had remarkable effects on the activity of the molecule.

1. Introduction

From our recent studies on DNA-interacting planar organic heterocycles, two classes of tricyclic heteroaromatic halides emerged: 1,8-bis(halomethyl)acridines [**1a**: X = Br, **1b**: X = Cl][1] and 5,6-bis(bromomethyl)phenanthroline[2] (**2**) (Fig. 1). In spite of the similar crosslinking activity of these molecules on double-stranded DNA by virtue of two highly reactive benzylic positions, they exhibited quite contrasting properties in the aqueous phase. While the half-life of acridine **1a** was as short as 2 h, the phenanthroline **2** showed indefinite stability against spontaneous hydrolysis. The unexpectedly low sensitivity of **2** to water molecules was attributable to the proximity of the two halomethyl groups. The electron-withdrawing nature of the bromine atoms interferes with the mesomeric resonance of the benzylic cation with aromatic electrons.

This observation prompted us to imagine a molecule with very high crosslinking activity, whose halomethyl groups are located as remotely as possible. We turned our attention to 2,6-bis(bromomethyl)naphthalene **3a**. This compound was first synthesized in 1958;[3] however, since then there have been no reports on its effects on any biomolecules.



1a X= Br
b Cl

2

Figure 1. Organic DNA crosslinking bis-benzylic halides with planer heterocycles.

2. Results and discussion

In preliminary experiments, 10 positional isomers **4**–**12** (Fig. 2)[3] involving the target molecule **3a** were treated with linearized pBluescript® DNA for the evaluation of in vitro interstrand crosslinking activity.[4] To our disappointment, all attempts with over 100 μM **3a** resulted in no crosslinking to form double-stranded DNA.

Instead, the 1,4-disubstituted derivative **6a** exhibited remarkable crosslinking, even at as low as 10 μM as shown in Figure 3. Figure 3 also shows the crosslinking of **6a** to a certain extent at lower concentration (1 μM).

The two electron-withdrawing bromomethyl groups in **6a** are located rather closely on the same side of naphthalene, and would weaken one other's reactivity toward nucleophilic substitution. Once one of them is alkylated by a guanine base however, the resulting substituent in the *para* position becomes less of an electron-withdrawing group. The newly formed substituent facilitates the second C–N bond formation on the other bromomethyl group

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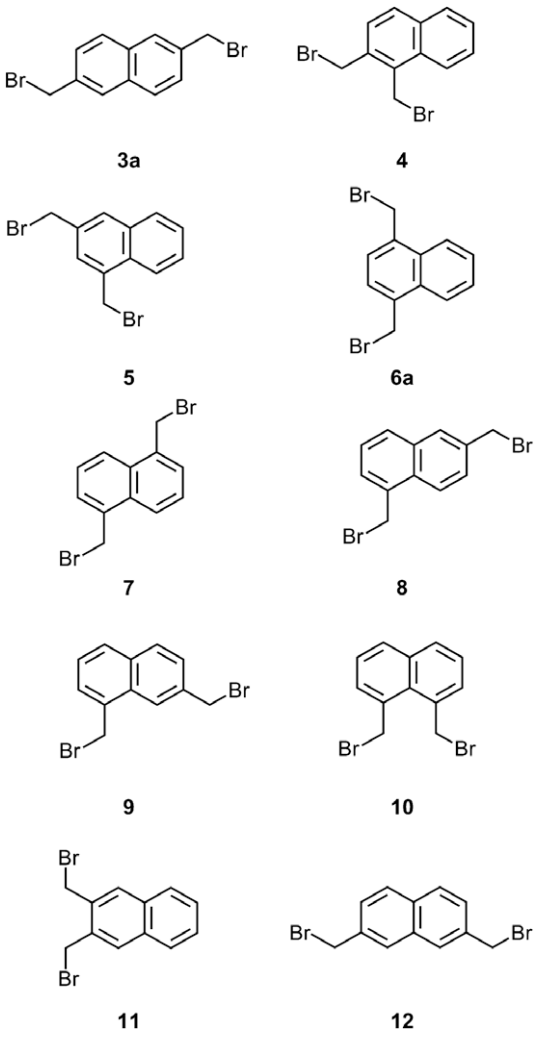

Figure 2. 2,6-Bis(bromomethyl)naphthalene **3a** and positional isomers **4**–**12**.[3]

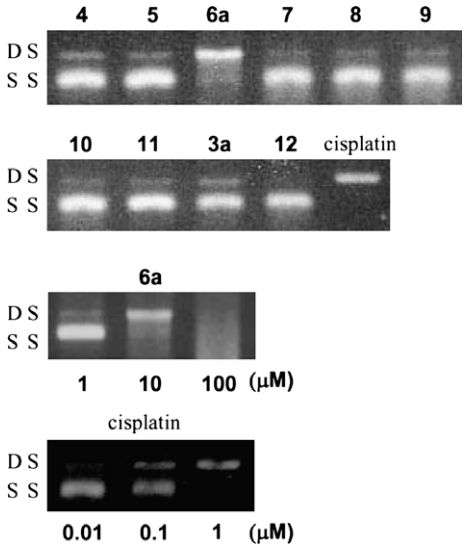

Figure 3. DNA interstrand crosslinking activity of **3a**–**12**. DS and SS indicate double-stranded and single-stranded DNA, respectively. The dose-dependencies of **6a** and cisplatin are also shown.

by stabilizing the intermediate benzylic cation, thus accomplishing crosslinking.

The situation surprisingly changed when all of the candidates were submitted to the Ames test for mutagenicity by incubation with *Salmonella typhimurium* TA92, which retains excision repair activity.[5] 2,6-Disubstituted naphthalene **3a** showed the highest mutagenicity as shown in the solid circle of Figure 4, and the activity was more than three times higher than the 1,4-disubstituted isomer **6a** (Fig. 4, solid triangle). The crosslinking formation was confirmed by the comparison between *S. typhimurium* strains, above-mentioned TA92 and TA100. The disappearance of originally sprayed bacterial cells caused by the toxicity was observed on the Ames test plates, in the latter case which lacks the excision repair activity.

This overlook of crosslinking activity for **3a** under in vitro experimental conditions raises a caveat. Such examples show that we cannot depend solely upon the DNA interstrand crosslinking assay. The low detection under in vitro conditions suggests an intrastrand crosslink formation between **3a** and single-stranded DNA chains, although the attempts for the isolation of DNA-crosslinking adducts by means of LC are under way.

Preliminary in vivo studies for the antiproliferative activity towards the human T cell leukemia cell line CCRF-HSB-2[6] showed a comparable activity of **3a** (IC_{50} 4.0 μM) with that of cisplatin (1.9 μM).

We realized that the electron-withdrawing substituent had a crucial effect on mutagenicity. As shown in Figure 4, the activities of corresponding chlorides **3b** (open circle) and **6b** (open triangle) were lower than those of bromides **3a** and **6a**, respectively (Fig. 5). The higher activities of the bromomethyl groups, especially those of **3a**, were in good accordance with their short half-life (**3a**: <10 min; **6a**: 34 min) in aqueous solution, compared with those of the chloromethyl groups (**3b**: 57 min; **6b**: 330 min).

The substitution of the naphthalene nucleus with quinoline, possessing a nitrogen atom with higher electronegativity (Fig. 6), dramatically lowered the mutagenicity of the original compounds. As the corresponding 2,6-disubsituted quinoline **13** and 5,8-substi-

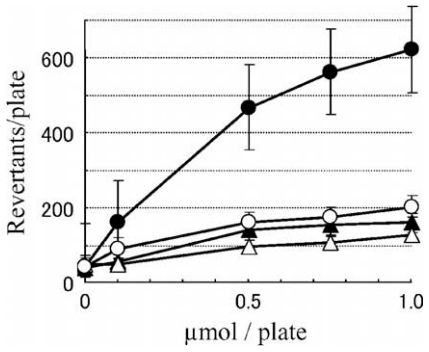

Figure 4. Mutagenicity of substituted naphthalenes, solid circle: **3a**; open circle: **3b**; solid triangle: **6a**; open triangle: **6b**.

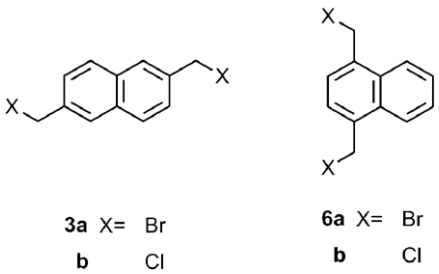

Figure 5. Substitution of bromine with chlorine atom at both benzylic positions in **3a** and **6a**.[7]

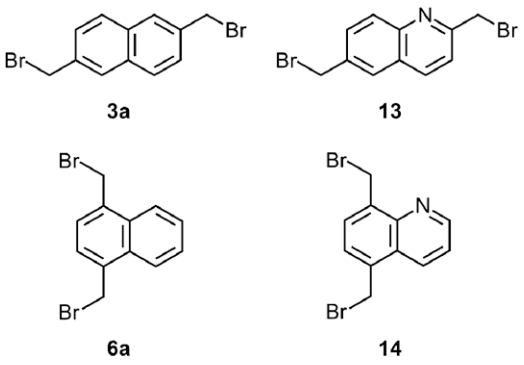

Figure 6. Substitution of naphthalene in **3a** and **6a** to quinoline to **13**[8] and **14**.[9]

tuted form **14** were quite stable (half-life: **13**: 770 min; **14**: 630 min), they no longer exhibited significant activity (Fig. 7). Although hydrogen bond formation between the lone pair electron on the nitrogen and certain protic hydrogens in DNA had been suggested, it did not work advantageously in the present quinoline derivatives **13** and **14**.

3. Conclusion

We found highly active bis-bromomethylated aromatic molecule **3a** with crosslinking activity on DNA. The unique behavior, judged from the contrasting results between the expression of microbial mutagenicity and double-strand formation on linearized plasmid DNA, suggests intrastrand crosslinking bond formation, which is well known as the major crosslinking mechanism of cisplatin. The two remotely located bromomethyl groups, which easily suffer from nucleophilic attack, are the origin of the high activity. The antiproliferative activity of a wide range of substituted naphthalenes and quinolines towards leukemia cells as mentioned above is currently being investigated, and the results will be reported in due course.

4. Experimental

4.1. Materials and methods

Linearized pBluescript® DNA was the product from STRATAGENE. Nutrient broth and Bacto agar were the products from DIFCO Laboratories. DMSO was freshly distilled from CaH_2 before use (bp_{14} 76 °C). The minimal media for mutation assays were prepared according to the reported procedure.[10] The samples **3a**–**13** were provide by a halogenations of the benzylic position with *N*-bromosuccimide[3] or 1,3-dichloro-5,5-dimethylhydantoin.[11] The quinoline skeletons in **14** was prepared under improved conditions for Skraup synthesis.[12] The cell line used in antiproliferative activity study, CCRF-HSB-2 was provided by RIKEN Cell Bank (Tsukuba, Japan).

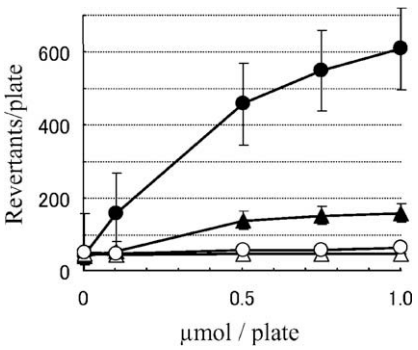

Figure 7. Mutagenicity of substituted naphthalenes and quinolines, solid circle: **3a**; open circle: **13**; solid triangle: **6a**; open triangle: **14**.

4.2. Analytical methods

Gel electrophoreses were performed on ADVANCE Mupid-2plus. HPLC data were recorded with HITACHI L-4000 UV detector.

4.3. Crosslinking assay

DNA interstrand crosslinking activity was assayed as follows. Linearized pBluescript® DNA (460 ng) was treated with the samples dissolved in DMSO at 37 °C for 6 h. Then, DNA was precipitated with EtOH (95%) and cooled for 24 h before being centrifuged for 20 min. The supernatant was removed, and the pellet was washed with EtOH (75%), and further spun for 20 min. The pellet was lyophilized to dryness, and the resulted DNA was then dissolved in separation buffer [EDTA (1 mM) in aqueous DMSO (30% v/v) solution]. The mixture was heated at 95 °C for 5 min and immediately placed in an ice bath. The DNA sample was separated by 1% agarose gel electrophoresis, and the DNA bands were visualized by staining with ethidium bromide. The results were shown in Figure 3. In the case of **3a**–**12**, plasmid was treated at 10 μM of each sample. As the reference, cisplatin was applied under 1 μM.

4.4. Ames test

S. typhimurium TA92 was grown in the medium [5 mL, nutrient broth (6 g) and of NaCl (5 g) in H_2O (1000 mL)] for 15.5 h at 37 °C. The samples for assay were diluted in DMSO. In a tube containing sodium phosphate buffer (0.1 M, pH 7.4, 0.5 mL), the bacterial culture as above (0.1 mL), and the sample solution (50 μL) were mixed, then the mixture was incubated at 37 °C for 20 min. This was mixed with separately prepared soft agar solution (0.6% agar in 0.6% NaCl, 2 mL) at 45 °C, and the total mixture was overlaid on a minimal agar plate. Each agar plate (90 mm diameter) contained the minimal medium (30 mL) with Bacto agar (1.5%). After incubation at 37 °C for 2 days, the number of colonies on the plate, which indicates the number of revertants caused by crosslinking was counted manually. The mean of the numbers from six plates for each experiment was calculated, and presented in Figures 4 and 7.

4.5. Cell proliferation assay

CCRF-HSB-2 cells were cultured in RPMI1640 supplemented with 10% fetal calf serum at 37 °C in an atmosphere containing 5% CO_2. Cells were collected when they were nearly confluent and diluted to a concentration of 3×10^5 cell/mL with culture medium. Aliquots (3 mL) of cell suspension were dispensed into each well of a 6-well plate. After incubation for 24 h at 37 °C in an atmosphere containing 5% CO_2, the cells were treated with the test samples as solution in DMSO. Each well received 3 μL of the sample solutions. After incubation for 24 h at 37 °C in an atmosphere containing 5% CO_2, Trypan blue-excluding cells were counted.

4.6. Stability in aqueous solution

The stability of the samples was examined by measuring the decomposition rate in aqueous solution. Each sample was dissolved in a mixture of sodium phosphate buffer (0.01 M, pH 7.4) and MeCN (4:1, final concentration: 10 μM). The resulting mixture (2 μL) was analyzed on a LiChrosorb RP-18 column (5 μm particle

size, 250 mm × 4.6 mm) by the elution with MeCN–H_2O or MeOH–H_2O.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.04.009.

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