Bioorganic & Medicinal Chemistry Letters 24 (2014) 4410–4413

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Crystalline guanine adducts of natural and synthetic trioxacarcins suggest a common biological mechanism and reveal a basis for the instability of trioxacarcin A

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ARTICLE INFO

Article history:
Received 24 June 2014
Revised 1 August 2014
Accepted 5 August 2014
Available online 14 August 2014

Keywords:
Antiproliferative agents
Nucleic acids
DNA
Natural products
Crystallography

ABSTRACT

X-ray crystallographic characterization of products derived from natural and fully synthetic trioxacarcins, molecules with potent antiproliferative effects, illuminates aspects of their reactivity and mechanism of action. Incubation of the fully synthetic trioxacarcin analog **3**, which lacks one of the carbohydrate residues present in the natural product trioxacarcin A (**1**) as well as oxygenation at C2 and C4 yet retains potent antiproliferative effects, with the self-complimentary duplex oligonucleotide d(AACCGGTT) led to production of a crystalline covalent guanine adduct (**6**). Adduct **6** is closely analogous to gutingimycin (**2**), the previously reported guanine adduct derived from incubation of natural trioxacarcin A (**1**) with duplex DNA, suggesting that **3** and **1** likely share a common basis of cytotoxicity. In addition, we isolated a novel, dark-red crystalline guanine adduct (**7**) from incubation of trioxacarcin A itself with the self-complimentary duplex oligonucleotide d(CGTATACG). Crystallographic analysis suggests that **7** is an anthraquinone derivative, which we propose arises by a sequence of guanosine alkylation within duplex DNA, depurination, base-catalyzed elimination of the trioxacarcinose A carbohydrate residue, and oxidative rearrangement to form an anthraquinone. We believe that this heretofore unrecognized chemical instability of natural trioxacarcins may explain why trioxacarcin analogs lacking C4 oxygenation exhibit superior chemical stabilities yet, as evidenced by structure **3**, retain a capacity to form lesions with duplex DNA.

The trioxacarcins are structurally complex bacterial metabolites that exhibit potent cytotoxicity in cultured human cancer cells and have demonstrated antibacterial and antimalarial activities.[1–3] Trioxacarcin A (**1**, Fig. 1) is the most potent natural trioxacarcin yet identified, with subnanomolar IC_{70} values in many human cancer cell lines.[3] The biological activity of the trioxacarcins is thought to arise from their ability to irreversibly alkylate N7 of guanosine residues of duplex DNA mediated by their spiro epoxide function. Compelling evidence supporting this proposal was obtained by X-ray crystallographic analysis of a 2:1 covalent adduct of trioxacarcin A (**1**) and a self-complementary DNA duplex oligonucleotide containing two guanosine residues.[4] Furthermore, it was shown that under physiological conditions, or more rapidly upon heating, depurination of this DNA lesion occurred to provide a 1:1 adduct of trioxacarcin A and guanine (gutingimycin, **2**), which has also been characterized crystallographically.[5] Recently, we reported a fully synthetic, component-based route to trioxacarcin A and used this platform to prepare a number of non-natural trioxacarcin analogs.[6] Many of these analogs were substantially structurally modified relative to trioxacarcin A (**1**) yet retained potent cytotoxicity. For example, the monoglycoside analog **3** is deoxygenated at the C2- and C4-positions (cf. **1**), and lacks the trioxacarcinose A glycosyl residue, but is nevertheless a potent cytotoxic agent (GI_{50} 19 ± 7 nM, H460 cells).[6,7] In this work, we provide evidence that analog **3** also produces DNA lesions by alkylation of guanosine residues within a self-complementary 8-mer duplex DNA, by crystallographic characterization of a 1:1 adduct of **3** and guanine. In addition, we have crystallographically characterized a novel guanine adduct of trioxacarcin A (**1**), which reveals a heretofore unrecognized pathway for molecular degradation of **1**, by elimination of the trioxacarcinose A residue and aromatization/oxidation to form an anthraquinone.

In their seminal studies, Pfoh et al. characterized by X-ray crystallography the 2:1 covalent intercalation complex of trioxacarcin

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http://dx.doi.org/10.1016/j.bmcl.2014.08.016
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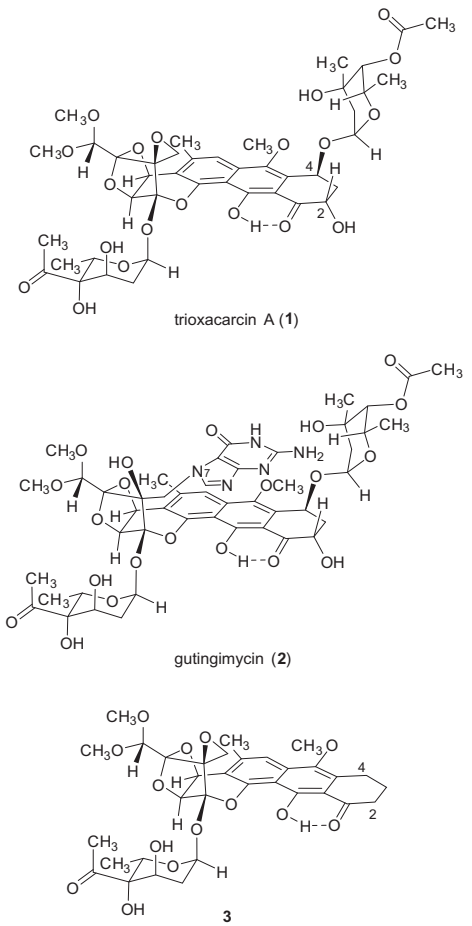


Figure 1. Structures of trioxacarcin A (**1**), gutingimycin (**2**), and the fully synthetic trioxacarcin analog **3**.

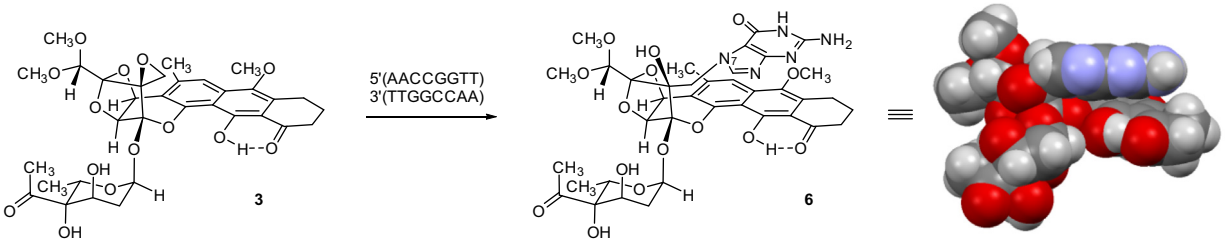
A (**1**) with the self-complementary duplex oligonucleotide d(AACCGGTT).[4] To prepare the crystalline complex, the DNA duplex was treated with trioxacarcin A (2.2 equiv) in 25% methanol–water at 4 °C for 3 days. Hanging drops of the resulting solution were equilibrated against an aqueous solution containing 1.55 M ammonium citrate and 30% dimethyl sulfoxide at 40 °C for 24 h, producing yellow crystals of the alkylated intercalation complex.

We conducted DNA-alkylation experiments with the fully synthetic trioxacarcin analog **3** by a similar protocol. Incubation of analog **3** with the self-complementary duplex oligonucleotide d(AACCGGTT) in 25% methanol–water for 3 days at 4 °C followed by equilibration with an aqueous solution containing 1.65 M triammonium citrate (pH 7.0) and 30% DMSO (2 weeks at 40 °C and 2 weeks at 23 °C) produced crystals of guanine adduct **6** suitable for X-ray diffraction. Crystallographic analysis[8] established that adduct **6** was structurally similar to gutingimycin, supporting a common mechanism of action (Scheme 1).

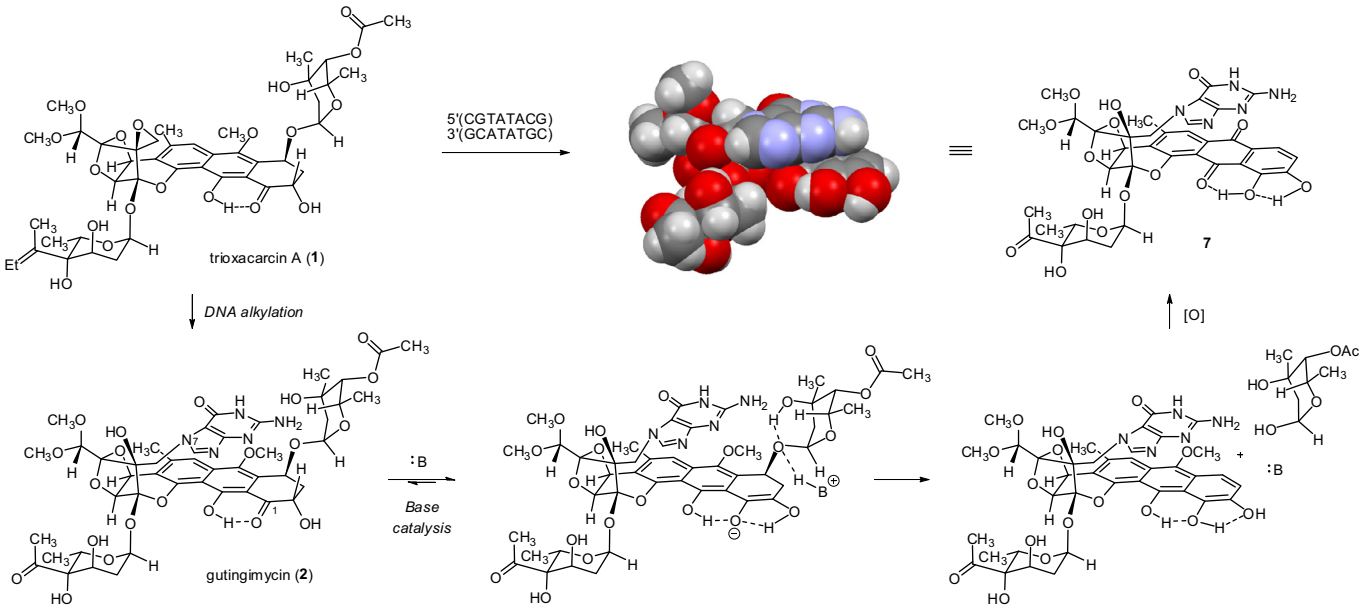
Separately, we conducted studies of the reaction of trioxacarcin A with duplex DNA oligonucleotides of different sequences. In one experiment, we incubated trioxacarcin A with the self-complementary duplex oligonucleotide d(CGTATACG) in 25% methanol–water for 3 days at 4 °C. Hanging drops of the resulting solution were allowed to equilibrate at 40 °C with an aqueous solution containing 5% 2-methyl-2,4-pentanediol, 20 mM sodium dimethylarsinate, 6 mM spermine tetrahydrochloride, 40 mM sodium chloride, and 10 mM magnesium chloride. After 2 weeks, a dark red crystalline product was obtained; X-ray crystallographic analysis[8] revealed this to be a novel guanine adduct in which the planar core of the trioxacarcin residue had undergone substantial modification to form an anthraquinone (structure **7**, Scheme 2). Although the crystal structure of adduct **7** was obtained with low resolution and may be insufficient to deduce its structure a priori, we believe it is sufficient to account for structural changes—alkylation, deglycosylation, aromatization, and oxidation—relative to the starting material trioxacarcin A (**1**), whose structure and absolute configuration are known with certainty.

We[6] and others[9] have observed that trioxacarcin A is unstable under basic conditions in aqueous or alcoholic solvents, made evident by rapid darkening of the initially yellow solutions to dark red. Structure **7** leads us to propose a potential chemical basis for this instability, outlined in Scheme 2. As shown, we propose that base-catalyzed enolization of the C1 ketone promotes elimination of trioxacarcinose A and aromatization (Scheme 2). Autoxidation, which we expect to be facile, would then produce anthraquinone **7**. While conceivably the dark red adduct **7** could have been formed by base-promoted elimination of the trioxacarcinose A residue prior to DNA binding and then alkylation we believe the sequence depicted in Scheme 2 is more probable. A reviewer has suggested that redox events may precede elimination of the carbohydrate residue, which is also possible.[10] Regardless of the sequencing, structure **7** suggests a link between the base instability of **1** and its heretofore unrecognized propensity to undergo elimination of the trioxacarcinose A residue followed by autoxidation. Further supporting this proposal is the observation that analog **3**, which has no capacity to undergo the elimination-autoxidation pathway, is more stable towards base and is generally much more stable than **1**. With evidence that simplified constructs such as **3** produce DNA lesions analogous to **1** yet exhibit greater chemical stability we believe that compounds of this novel chemical series present a promising avenue for further exploration in potential chemotherapeutic constructs such as antibody-drug conjugates.

In summary, we have prepared and characterized two novel adducts prepared from trioxacarcins and guanosine-containing double-stranded DNA oligonucleotides. The crystal structures of adduct **6** and anthraquinone **7** are displayed alongside that of



Scheme 1. Formation of guanine adduct **6** from the fully synthetic trioxacarcin analog **3**.



Scheme 2. Formation of anthraquinone **7** from trioxacarcin A (**1**), and a possible mechanism.

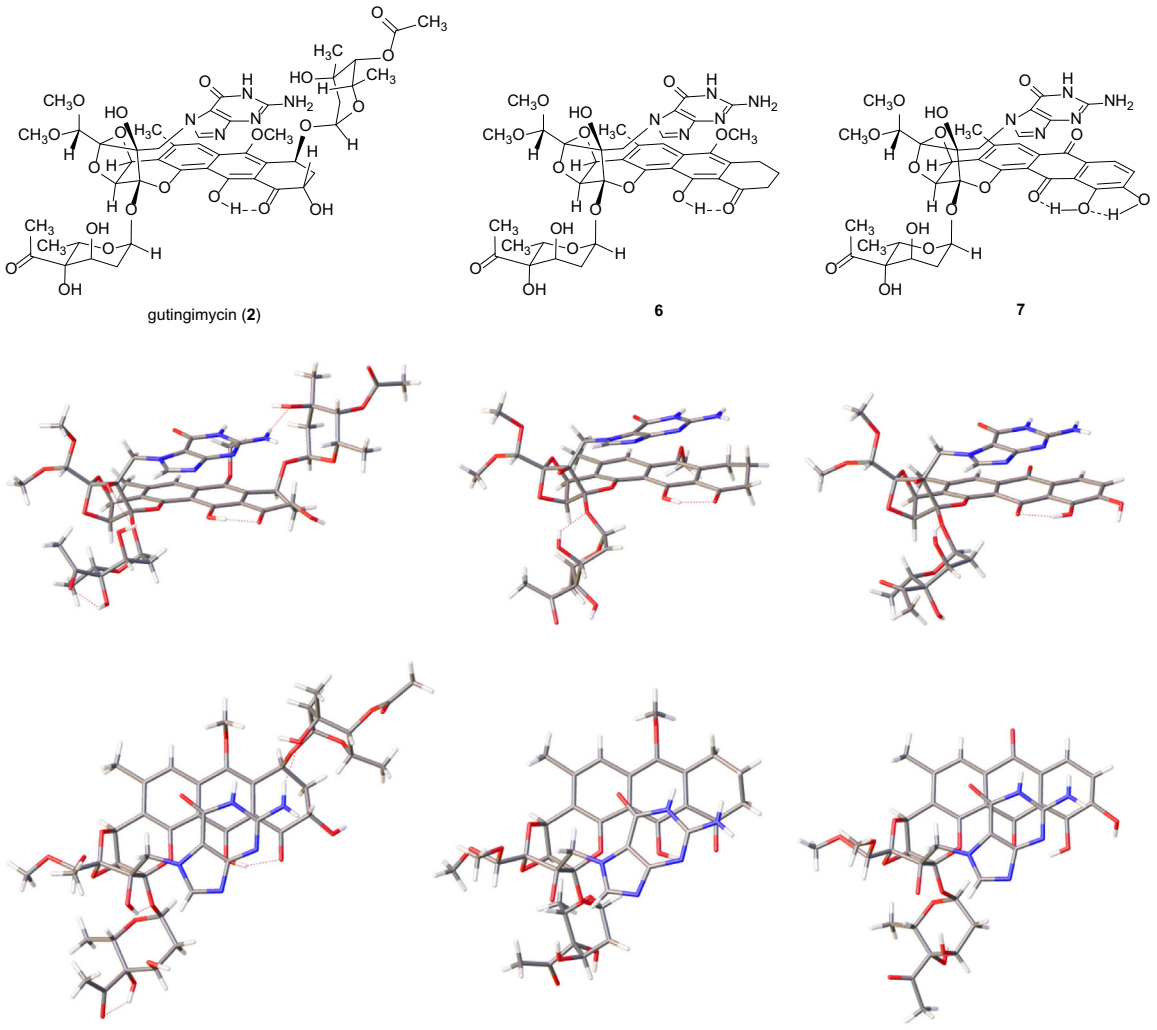


Figure 2. Comparison of the X-ray crystal structures of gutingimycin (**2**), deoxygenated adduct **6**, and anthraquinone **7**. (Adduct **7** crystallized in complex with an arsenic atom arising from the sodium dimethylarsinate additive; this is omitted from the figure for clarity.)

gutingimycin (**2**) in Figure 2 below in order to highlight their close structural similarities. Anthraquinone **7** represents a novel derivative of trioxacarcin A (**1**) and suggests a basis for the instability of **1** toward base, a liability which might limit its practical use. On the other hand, the simplified analog **3** retains a capacity to abstract guanine from duplex DNA, yet exhibits superior stability in aqueous solutions.

Acknowledgments

K.P. and B.D. thank the Deutsche Forschungsgemeinschaft DFG for funding within project DI 921/3-2. We acknowledge Theodore Betley and Shao-Liang Zheng for helpful discussions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.08.016.

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7. For trioxacarcin A (**1**), we measured a GI_{50} value of 0.85 ± 0.36 nM. See Ref. 6.
8. Crystallographic data for **6** and **7** were measured at the Swiss Light Source synchrotron at the macromolecular beamline X10SA using a wavelength of 0.6358 Å. Fine slicing data collection was performed according to the protocol established earlier (Mueller, M.; Wang, M.; Schulze-Briese, C. *Acta Crystallogr., Sect. D* **2012**, *68*, 42.). Due to the small crystal size the use of synchrotron radiation was necessary. Data integration was performed with the XDS software (Kabsch, W. *Acta Crystallogr., Sect. D* **2010**, *66*, 125.). Structure solution and refinement were carried out with SHELXS and SHELXL (Sheldrick, G. *Acta Crystallogr., Sect. A* **2008**, *64*, 112.). The X-ray crystal structure of **6** has been deposited at the Protein Data Bank (PDB ID 4HP7). The X-ray crystal structure of **7** has been deposited at the Cambridge Crystallographic Data Centre (deposition number 1004232).
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