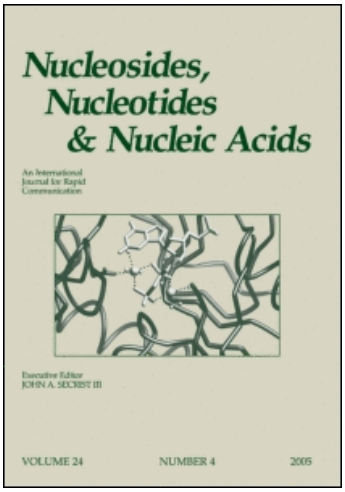


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

New Peptidyl-Anthraquinones: Synthesis and DNA Binding

Giuseppe. Zagotto[a]; Giovanni. Mitaritonna[a]; Claudia. Sissi[a]; Manlio. Palumbo[a]
[a] Department of Pharmaceutical Sciences, University of Padova, Padova, Italy

To cite this Article Zagotto, Giuseppe. , Mitaritonna, Giovanni. , Sissi, Claudia. and Palumbo, Manlio.(1998) 'New Peptidyl-Anthraquinones: Synthesis and DNA Binding', Nucleosides, Nucleotides and Nucleic Acids, 17: 9, 2135 — 2141
To link to this Article: DOI: 10.1080/07328319808004756
URL: http://dx.doi.org/10.1080/07328319808004756

NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 2135-2141 (1998)

NEW PEPTIDYL-ANTHRAQUINONES: SYNTHESIS AND DNA BINDING

Giuseppe Zagotto, Giovanni Mitaritonna, Claudia Sissi and Manlio Palumbo.

Department of Pharmaceutical Sciences, University of Padova, Via Marzolo, 5, 35131 Padova (Italy).

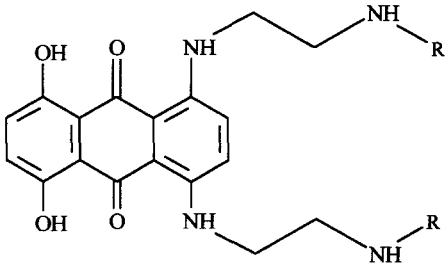
Abstract: Aminoacyl- hydroxy-anthraquinones bearing glicyl, valyl, lysyl and tryptophanyl residues in the side-chain were synthesized as new potential DNA-directed drugs. These compounds bind very tightly to double-stranded DNA by intercalating their planar portion into the nucleic acid and further stabilizing the complex through electrostatic contacts with the backbone phosphates. All protonated groups in the side-chains participate in the latter process. The free energy of DNA-binding corrected for the electrostatic contribution is similar for the lysyl and glicyl derivatives, which points to a common geometry of intercalation.

Among the most widely used anticancer drugs, DNA-interacting compounds of the anthraquinone structural type represent a major class[1,2]. In fact, naturally-derived agents, such as the anthracyclines, and synthetic compounds, such as mitoxantrone, are extensively used in clinical practice since many years[3]. Their mode of action relates to their ability of damaging the nucleic acid, thereby inducing cell death. In fact, they can produce reactive species such as free radicals or carbonium ions, which cleave or alkylate DNA, and/or they can interfere with the topoisomerase-DNA cleavable complex, causing protein-associated DNA-strand scission [4-9].

An important limitation to a fully successful treatment of neoplasia with anthracenediones is represented by their lack of selective recognition of the DNA target, which will unavoidably lead

to severe systemic toxicity[3]. Clearly, a drug exhibiting appropriate sequence specificity for the genomic sequences mutated in cancer cells would generate a substantial improvement in the therapeutic index. To address this issue, the toxic agent must be linked to a targeting element able to confer the desired DNA-sequence specificity. A number of relatively short peptidic motifs are extensively utilised in nature to interfere specifically with nucleic acids[10]. Indeed, such motifs could well act as appropriate DNA/RNA-targeting moieties when bound to cytotoxic compounds.

To explore this novel avenue, we (and others) have recently examined anthraquinone compounds containing aminoacyl residues in the side-chain(s) as useful models for selective DNA-directed drugs [11-14]. In the present work we have synthesized the aminoacyl analogues of the leading drug mitoxantrone reported below:



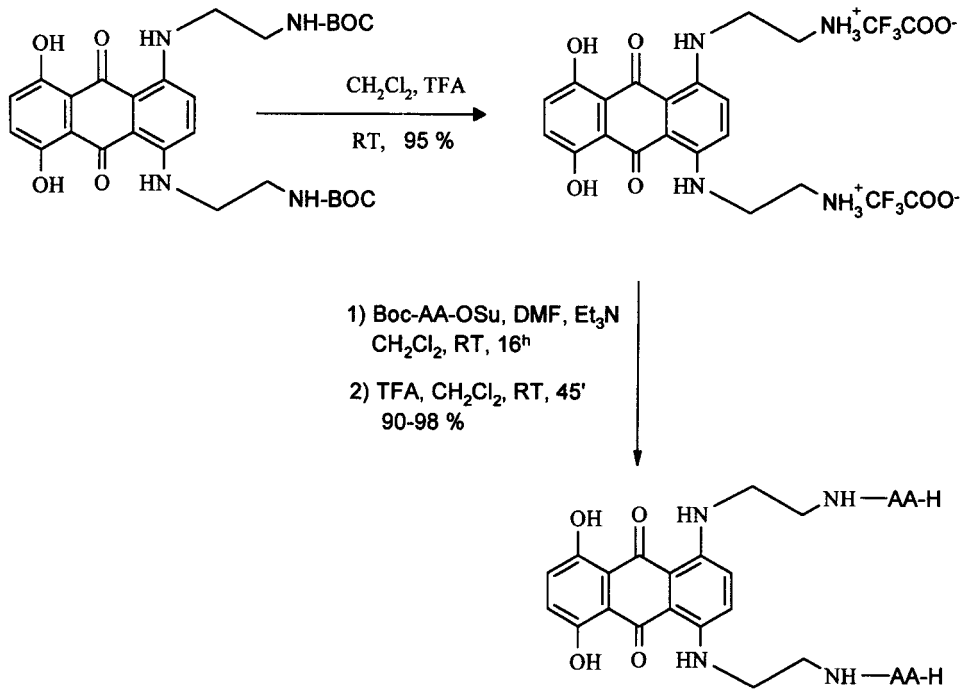
R = glicyl
valyl
lysyl
triptophanyl

R = CH_2CH_2OH in mitoxantrone

Synthesis.

The starting 1,4-bis[(2-aminoethyl)amino]-5,8-dihydroxyanthracene-9,10-dione, bis(trifluoro-acetate) was obtained from the commercially available 4,8-diamino-1,5-dihydroxyanthracene-9,10-dione-2,6-disulfonic acid (Acid Blue 45) as described previously [12,15]. The main problem was connected to the very low solubility of the products in the synthetic and purification steps. The deprotected side chains of the starting anthracene-9,10-dione were coupled to the (*tert*-butoxycarbonyl)-protected aminoacid (Boc-AA-OH) using the conventional coupling reagents dicyclohexylcarbodiimide and N-hydroxysuccinimide. The condensation product was purified by column chromatography, since it proved very difficult to purify the final compound due to solubility reasons. The Boc group was then cleaved with trifluoroacetic acid and the product stored as the trifluoroacetate salt. The obtained 1,4-bis[(2-glyclaminoethyl)amino]-5,8-dihydroxyanthracene-9,10-dione and the corresponding lysyl, triptophanyl and valyl derivatives were charaterized by ^{1}H- and ^{13}C-NMR, and elemental analyses.

The synthetic scheme is the following:



DNA binding properties.

Spectrophotometric titration experiments in the presence of double-stranded DNA were performed at neutral pH and variable ionic strength. Glycyl (compound **G**) and lysyl (compound **K**) derivatives were studied in detail, due to their better solubility profile.

A representative example is shown in Figure 1.

The presence of isosbestic points allowed to treat the binding equilibrium as a two-component system, so that the amount of free and DNA-bound drug could be directly calculated from the titration curves.

Scatchard plots were derived, from which the intrinsic binding constants (K_i) and the number of base pairs occupied by a single ligand molecule (n) were extrapolated[16]. No cooperativity effects were observed. The results are summarized in Table 1.

To achieve reasonable dissociation of the complex as to perform reliable measurements, the K_i values were evaluated only at ionic strength higher than 0.5 M. Even at such unusually high salt concentrations, the binding constants range between 10^4 and 10^5 M^{-1}, which points to a very efficient interaction of the aminoacyl-anthraquinones with the nucleic acid, as it is the case for mitoxantrone[17].

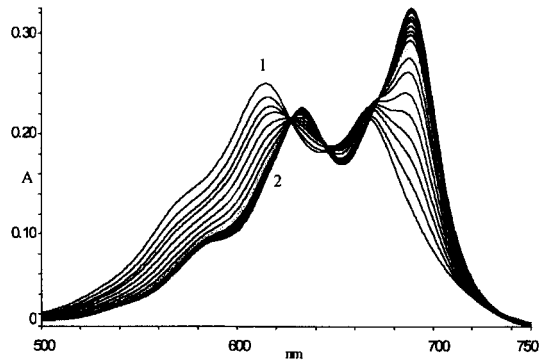


Figure 1. Spectrophotometric titration of glycyl-derivative (**G**) with dsDNA at 0.75 M ionic strength, pH 7.0 and 25°C. 1: free drug; 2: DNA-bound drug. Drug concentration = 20 μM.

Table 1. DNA-binding parameters of anthraquinones **G** and **K** at pH 7.0, 25°C and different ionic strength (IS).

Compound	I S (M)	log K_i [a]	n [b]
G	0.5	4.68 ± 0.03	2.7 ± 0.1
	0.75	4.49 ± 0.03	3.0 ± 0.1
	1.00	3.95 ± 0.02	3.1 ± 0.2
K	0.5	5.11 ± 0.05	2.4 ± 0.1
	0.75	4.41 ± 0.02	2.6 ± 0.1
	1.00	3.97 ± 0.01	2.8± 0.1

[a] intrinsic binding constant
[b] size of the binding site (base pairs)

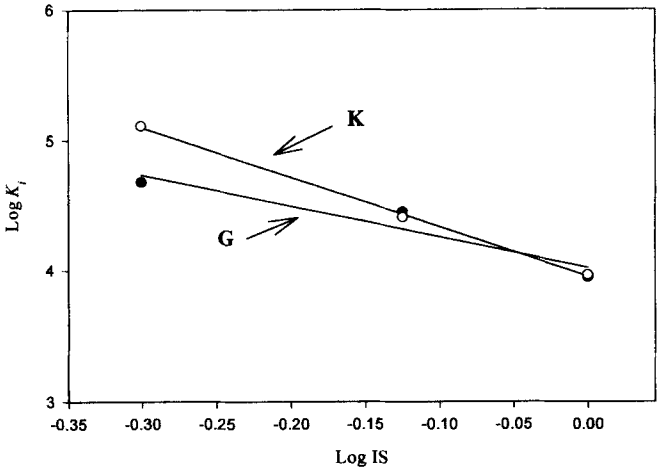


Figure 2. Ionic strength (IS) dependence of the intrinsic DNA-binding constant (K_i) for compounds **G** and **K**.

It is worth of note that *n* ranges between 2.4 and 3.1 bp, very close to the values normally found for anthracyclines and anthraquinones [17]. Considering the structural similarity of the planar portion of the above derivatives and ours and the comparable spectroscopic behaviour, it can be inferred that a normal intercalation mechanism is operating for the present aminoacyl derivatives.

The observed changes in K_i upon changing ionic strength point to the participation of electrostatic interactions in complex formation. This is conceivable considering that the protonated amino groups of the anthraquinone side-chains will be in close vicinity to the DNA phosphates when the intercalation complex forms. The Manning's condensation theory applied to nucleic acids [18,19] was used to evaluate the number (*m*) of ion pairs formed per complex unit between the DNA backbone and the drug from. The plot of log K_i vs. log (ionic strength) is presented in Fig. 2.

Its slope is related to *m*, while the intercept at 1 M salt is close to the thermodynamic binding constant, corrected for the electrostatic contribution (K^0)[19]. The results are consistent with *m* values of 2 for **G** and of 4 for **K**. Thus, all charged groups of the aminoacyl residues (2 in **G** and 4 in **K**) participate in complex formation and strengthen the intercalation complex by additional electrostatic interactions. Finally the K^0 value is close to 10^4 for both compounds. It follows that the intercalation process occurs energetically in a very close fashion for the test compounds and contributes to the total binding energy for about 22 kJ/mol.

Preliminary results obtained with the D-enantiomer of the lysyl-anthraquinone **K** indicate that this configuration of the aminoacid increases the drug affinity for the target DNA more than the L

configuration. This may be considered rather interesting in view of developing biologically effective peptidyl anthraquinones resistant to enzymatic digestion.

REFERENCES

1. *Anthracycline and Anthracenedione-Based Anticancer Agents* (1988) Lown, J.W., Ed., Elsevier, Amsterdam.

2. Zee Cheng, R. K.-Y., Cheng, C.C. (1983) *Drugs Future* **8**, 229-248.

3. Faulds, D., Balfour, J. A., Chrisp, P., Langtry, H. D. (1991) *Drugs* **41**, 400-449.

4. Kolodziejczyk, P., Reszka, K., Lown, J. W. (1988) *Free Radical Biol. Med.* **5**, 13-25.

5. Fisher, G. R, Brown, J. R., Patterson, L. H.(1989) *Free Radical Res. Commun.* **7**, 221-226.

6. Fisher, G. R.,Brown, J. R., Patterson, L. H. (1990) *Free Radical Res. Commun.* **11**, 117-125.

7. Mewes, K., Blanz, J, Ehninger, G., Gebhardt, R., Zeller, K. (1993) *Cancer Res.* **53**, 5135-5142.

8. Liu, L. F. (1989) *Annu. Rev. Biochem.* **58**, 351-375.

9. Capranico, G., Binaschi, M., Borgnetto, M. E., Zunino, F. and Palumbo, M. (1997) *Trends Pharm.Sci.* **18**, 323-329.

10. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J.D (1994) in *Molecular Biology of the Cell,* 3rd Ed, Garland Publishing Inc., New York, pp.404-416.

11. Morier-Teissier, E., Bernier, J. L., Lohez, M., Catteau, J. P., Hénichart, J. P. (1990) *Anti-Cancer Drug Des.* **5**, 291-305.

12. Morier-Teissier, E., Boitte, N., Helbecque, N., Bernier, J.-L., Pommery, N., Duvalet, J.-L., Fournier, C., Hecquet, B., Catteau, J.-P., Hénichart, J.-P. (1993) *J. Med. Chem.* **36**, 2084-2090.

13. Meikle, I., Cummings, J., Macpherson, J.S., Smyth, J.F. (1995) *Anti-Cancer Drug Des.* **10**, 515-527.

14. Gatto, B., Zagotto, G., Sissi, C., Cera, C., Uriarte, E., Palù, G., Capranico, G., Palumbo, M. (1996) *J. Med. Chem.* **39**, 3114-3122.

15. Houben-Weyl - Methoden der Organischen Chemie, *Quinone Teil III*, 170.

16. Mc Ghee, J. D., Von Hippel, P. H. (1974) *J. Mol. Biol.* **86**, 469-489.

17. Lown, J.W., Morgan, A. R., Yen, S.-F., Wang, Y.-H.,Wilson, W. D. (1985) *J. Med. Chem.* **24**, 4028-4035.

18. Manning, G.S. (1978) *Q.Rev.Biophys.* **11**,179-246.

19. Record, M.T. Jr, Anderson, C.F., Lohman ,T.M. (1978) *Q.Rev.Biophys.* **11**, 103-178.