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New 1,4-anthracene-9,10-dione derivatives as potential anticancer agents

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Abstract

The amino-substituted anthracene-9,10-dione (9,10-anthraquinone) derivatives represent one of the most important classes of potential anticancer agents. To better understand the basic rules governing DNA sequence specificity, we have recently synthesized a new class of D- and L-aminoacyl–anthraquinone derivatives. We have tested these new compounds as cytotoxic agents, and we have correlated their activity with the configuration of the chiral aminoacyl moiety. Molecular modeling studies have been performed to compare the test drugs in terms of steric overlapping. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Anthracene-9,10-dione; DNA; Specificity; Topoisomerase; Molecular modeling

1. Introduction

The amino-functionalized anthracene-9,10-dione (9,10-anthraquinone) derivatives represent one of the most important classes of anticancer agents. For example, mitoxantrone (1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) is now licensed for clinical use in a number of countries against breast cancer and acute leukemias. As already extensively reported, the main target of these 9,10-anthraquinone derivatives in the cell appears to be DNA, with which they interact by intercalation. However, the mode of action of anthracene-9,10-dione derivatives is believed to involve the production of double-strand DNA breaks mediated by DNA topoisomerase II. DNA topoisomerases are essential enzymes that modify the topology of nucleic acids. Agents able to interfere with the DNA–enzyme recognition mechanisms have been recently synthesized [1–5] and their DNA-binding and antitumor properties evaluated. To better understand the basic rules of the DNA sequence specificity [6] we have recently investigated a new class of D- and L-aminoacyl–anthraquinone derivatives [7,8]. In this paper, as a confirmation of an investigation aimed at defining the role of aminoacyl substitution and chirality on biological activity, we have synthesized a series of tyrosine derivatives both in the L- and D-configuration.

2. Experimental

Melting points were determined by a Gallenkamp apparatus, and are uncorrected. FT-IR spectra were recorded on a Perkin–Elmer System 1760 FT-spectrometer using KBr mulls. ^{1}H NMR spectra were recorded on a Varian Gemini 200 (200 MHz) apparatus. Elemental analyses (CHN) were within 0.40% of the calculated values and were performed on a Carlo Erba 1016 Elemental Analyser. Optical purities of the amino acid derivatives were determined by a Perkin–Elmer 141 Polarimeter and compared, when possible, with known values [9,10]. UV–Vis spectra were recorded on a Perkin–Elmer Lambda 5 spectrophotometer and fluorescence measurements on a Perkin–Elmer Luminescence Spectrometer LS 50. Reagents and solvents were used as purchased without further purification. Silica gel and TLC plates were from E. Merck.

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Melting points are in °C, IR frequencies in cm^{-1}, ^1H NMR chemical shifts (δ) in ppm and coupling constants (J) in Hz. IR wave numbers are in cm^{-1} and UV frequencies in nm.

2.1. Synthesis

L- and D-tyrosine methyl esters were obtained by refluxing the amino acid in anhydrous methanol with HCl as catalyst, and reducing the alcohol with sodium borohydride in an ethanol–water solution.

2.1.1. General procedure for the synthesis of the anthraquinone derivatives

Potassium carbonate (10 g, 72 mmol) in water (150 ml) was warmed up to 80°C and oxygen was carefully removed from the solution by fluxing dry nitrogen. To the deaerated solution sodium hydrosulfite (sodium dithionite, 8.7 g, 50 mmol) and 1,4-dihydroxyanthraquinone (quinizarine, 7.5 g, 31 mmol) were added. During the reaction, further sodium hydrosulfite (5 g, 29 mmol) was added in small portions. The suspension was filtered and the solid washed and dried to give 7 g (93%) of 2,3-dihydroquinizarine.

A solution of the tyrosyl derivative, ester or alcohol, (2.6 mmol) dissolved in 100 ml of DMF was deaerated and triethylamine (2.6 mmol) was dropped in to give the free amino acid. 2,3-Dihydroquinizarine (1.3 mmol) was added to the solution. The reaction mixture was warmed up to 120°C for 5 h and the advancement of the reaction was determined by TLC (DCM). When equilibrium was reached the reaction mixture was cooled, the solvent was removed under reduced pressure and the residue partitioned in DCM and water. The organic phase was washed three times with water, dried over sodium sulfate and evaporated to dryness. The residue was purified by column chromatography (silica gel, from DCM to 95:5 DCM–ethyl acetate).

2.1.2. 2-(4-Hydroxy-9,10-dioxo-9,10-dihydro-anthracen-1-ylamino)-3-(4-hydroxyphenyl)-propionic acid methyl ester (L, D)

NMR ($CDCl_3$): 13.6 (s, 1H), 10.6 (d, $J = 7.7$, 1H), 8.4 (m, 2H), 7.8 (m, 2H), 7.1 (m, 2H), 6.8 (m, 4H), 4.9 (s, 1H), 4.5 (m, 1H), 3.8 (s, 1H), 3.2 (m, 2H). IR: 3600, 3300, 3060, 1741, 1617, 1611, 1448, 1500, 1245, 1065. UV: 583.2, 545.8, 278.6, 249.9, 227.7, 202.5; $\varepsilon_{545.8} = 10\,692$ (ethanol). Mass $M^+ = 418$.

2.1.3. 1-Hydroxy-4-[2-hydroxy-1-(4-hydroxybenzyl)-ethylamino]anthracene-9,10-dione (L, D)

NMR ($CDCl_3$): 13.7 (s, 1H), 10.6 (d, $J = 7.4$, 1H), 8.30 (m, 2H), 7.9 (s, 1H), 7.7 (m, 2H), 7.1 (m, 4H), 6.7 (m, 2H), 3.9 (m, 1H), 3.7 (m, 2H), 2.9 (m, 3H). IR: 3404, 1614, 1585, 1514, 1289, 1244, 1168, 1027. UV: 599.7, 558.9, 251.1, 249.7, 228.4, 204.9. Mass $M^+ = 373$.

2.2. DNA binding

Measurements were carried out at 25°C in ETN buffer (1 mM EDTA, 10 nM Tris, pH 7.0). Binding was monitored spectrophotometrically or fluorimetrically in the ligand absorption or emission region.

2.3. Cell toxicity

2.3.1. Cell lines and in vitro experiments

Cytotoxic effects were evaluated on the following cell lines: H460 human non-small cell lung carcinoma (kindly provided by Dr Gazdar, National Cancer Institute and Naval Hospital, Bethesda, MD, USA; PC3 human prostate carcinoma (obtained from American Tissue Culture Society). All cell lines were maintained in RPMI-1640 containing 10% fetal calf serum.

To perform the experiments cells were seeded at a density of 3000–5000 cell/well, 100 μl/well in 96-well plates, and treated 24 h later for 24 h with the drugs. Then the medium was washed out and cells were incubated in drug-free medium for a further 48 h. Then cell survival was evaluated by sulforhodamine B (SRB) assay according to Skehan et al. [11]. Samples were fixed with 10% (w/v) trichloroacetic acid and cellular proteins were stained with 0.4% SRB dissolved in acetic acid. Protein-bound dye was solubilized with 10 mM Tris, pH 10.4 and optical density at 550 nm was measured with a microplate reader (EAR 400 AT; SLT-Labinstruments, Austria). IC_{50} was defined as the drug concentration causing a 50% reduction of absorbance at 550 nm.

2.4. Computational methods

All anthracenyl–aminoacid models were constructed using the 'Molecule Builder' module of Molecular Operating Environment (MOE 1998.10) [12]. These structures were minimized using the MMFF94 force field [13–17], until the rms value of the Truncated Newton method (TN) was < 0.001 kcal/mol/Å.

The optimized geometries of all anthracenyl–amino-acid structures were fully minimized using the RHF/AM1//RHF/3-21G(*) ab initio level of Gaussian 98 [18].

Superimposition of the geometry-optimized D- and L-TyrOMe structures was carried out using the 'Interactive Superposition' algorithm method implemented in Molecular Operating Environment (MOE 1998.10) [12].

3. Results and discussion

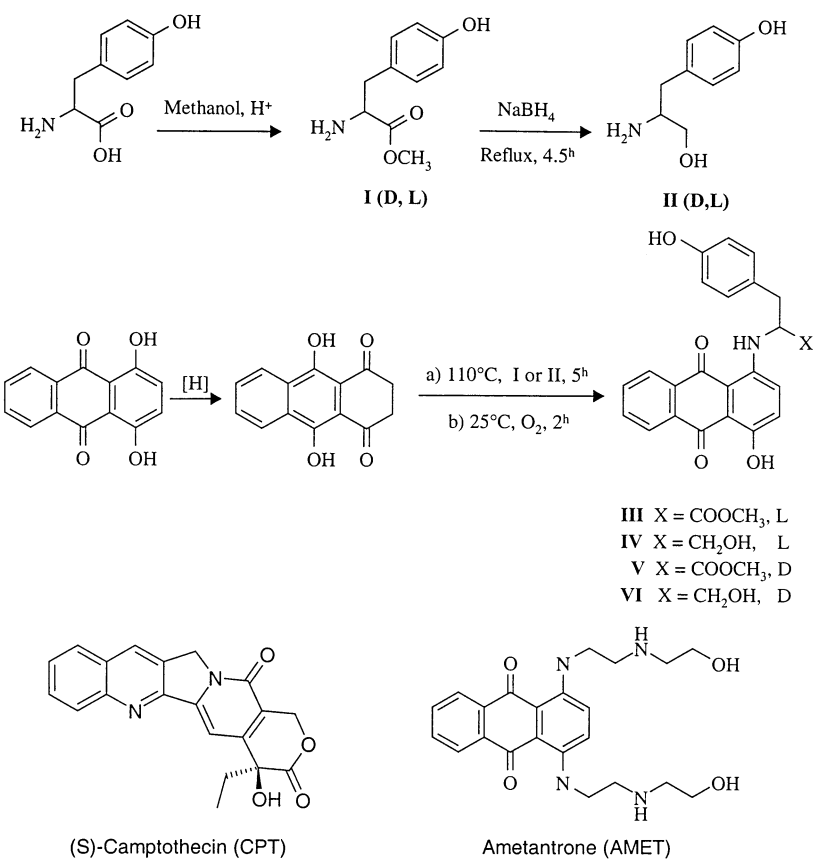
Three novel compounds have been synthesized and chemically characterized. The synthetic procedure is

shown in Scheme 1. The chemistry is quite straightforward [19] and the low solubility of the anthraquinone derivatives was the only difficulty that had to be overcome throughout the entire work. The ester reduction, as well as the reaction of the amino acid derivative with the dihydroquinizarine, was performed in slightly alkaline medium to avoid racemization.

Solubility problems arose when attempting to evaluate the DNA–anthraquinone binding constants. In fact, even at micromolar concentrations of the test compounds, a precipitate was observed, which prevented us from making a safe quantitative evaluation.

It is likely that the carboxylate arising from the ester hydrolysis, catalyzed by esterases, represents the active drug in this class [20,21]. In this connection, the ester derivatives should be regarded as prodrugs and a long exposure of the cells to the drug is necessary to achieve the desired cytotoxicity [22]. Since two of the compounds considered in the present work are not esters, the cells were exposed to them for 24 h. Furthermore, the compounds were tested against a Topoisomerase I inhibitor (Camptothecin, CPT) and a Topoisomerase II inhibitor (Ametantrone, AMET); this is because analogs of the test derivatives have been reported as being active on both enzymes [23,24]. Results on two cancer cell lines, a human prostate carcinoma (PC3) and lung carcinoma (H460), are reported in Figs. 1 and 2. In both cell lines CPT proves to be more potent. This is in agreement with the fact that cell lines from solid tumors are particularly sensitive to Topo I inhibitors. Overall, compounds III–IV are poorly active on the test systems, their IC50 largely exceeding 1 mM. Although the D-enantiomers V and VI appear to perform slightly better than their L-analogs, the differences do not appear to be significant enough to draw safe conclusions.

Molecular modeling studies have been employed to rationalize the above observations. Superposition between the geometry-optimized D- and L-TyrOMe structures is shown in Fig. 3. Eight equivalent atoms from the anthraquinone ring system were overlaid, leaving the amino acid substituent in its preferred minimum-energy conformation for each structure. The inversion of the α-amino acid chiral center does not seem to dramatically alter the three-dimensional arrangement of the two structures. In fact, the internal H-bonds between the NH of both D-/L-amino acids and the oxygen atom of the carbonyl in position 9 of the anthraquinone moiety freeze the position of the phenyl



Scheme 1.

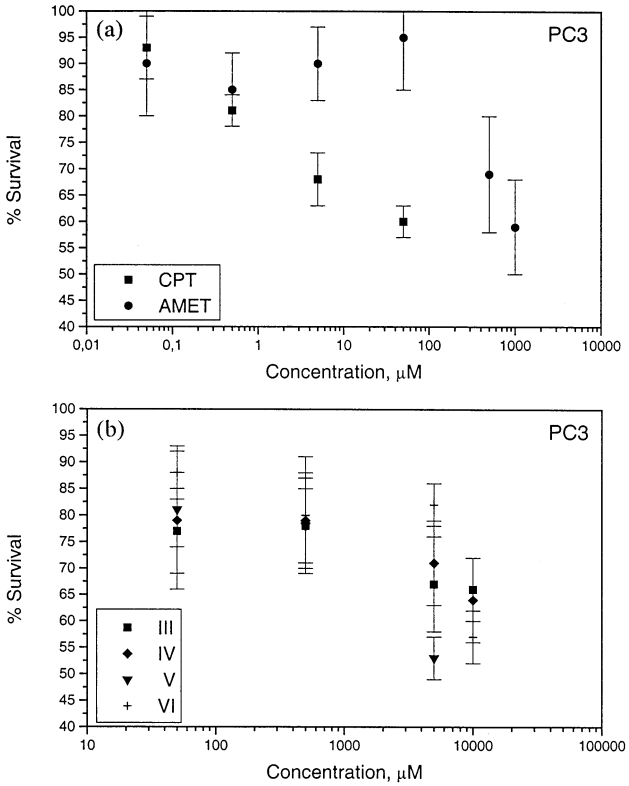


Fig. 1. Cell toxicity on a human prostate carcinoma (PC3) of the reference compounds (upper) and new synthesized compounds (lower).

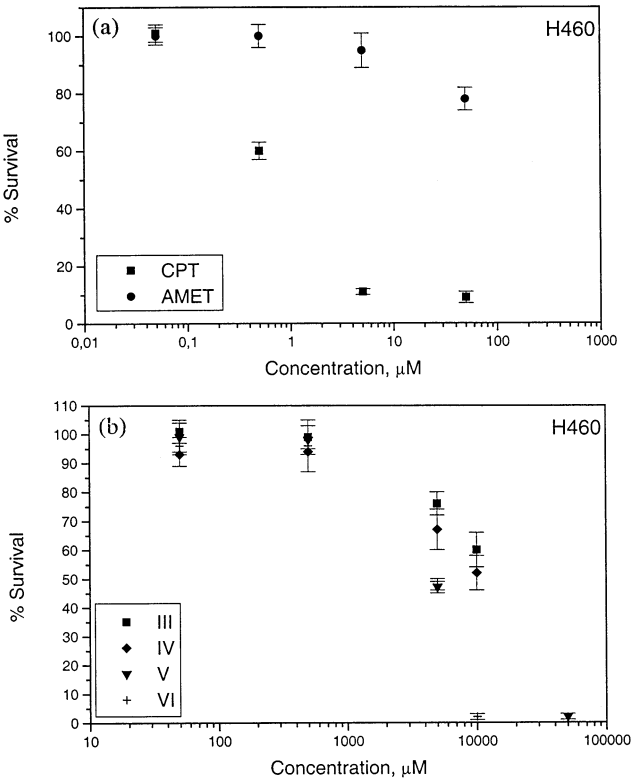


Fig. 2. Cell toxicity on a lung carcinoma (H460) of the reference compounds (upper) and new synthesized compounds (lower).

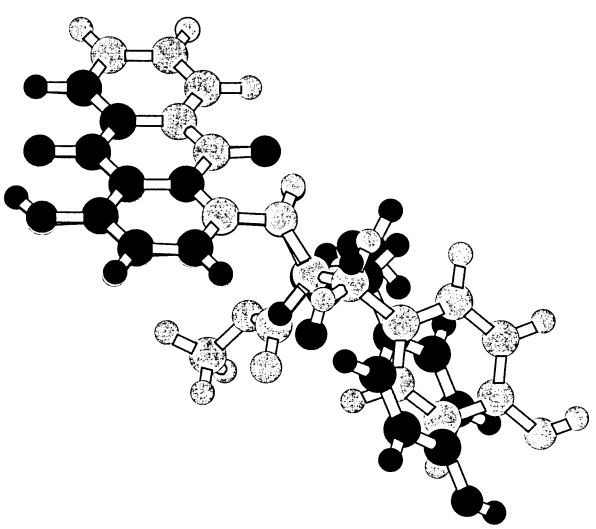


Fig. 3. Superposition between the geometry-optimized D-TyrOMe (colored in black) and L-TyrOMe (colored in gray).

ring in the same direction. Hence, they do not appear to be structurally well distinguishable. The experimental results seem to indicate that a modest chiral discrimination may occur.

In conclusion, a longer peptide chain is therefore needed to properly address the issue of chiral discrimination in DNA-binding anticancer agents.

References

[1] C. Bailly, J.B. Chaires, Sequence-specific DNA minor groove binders. Design and synthesis of netropsin and distamycin analogues, Bioconj. Chem. 9 (1998) 513–538.
[2] G. Capranico, M. Binaschi, DNA sequence selectivity of topoisomerases and topoisomerase poisons, Biochim. Biophys. Acta Gene Struct. Expr. 1400 (1998) 185–194.
[3] I.Y. Choi, I.K. Chung, M.T. Muller, Eukaryotic topoisomerase II cleavage is independent of duplex DNA conformation, Biochim. Biophys. Acta 1264 (1995) 209–214.
[4] D.B. Jacoby, P.C. Wensink, DNA binding specificities of YPF1, a Drosophila homolog to the DNA binding subunit of human DNA-dependent protein kinase, Ku, J. Biol. Chem. 271 (1996) 16827–16832.
[5] M. Palumbo, Introduction to DNA sequence-specific agents, in: M. Palumbo (Ed.), Advances in DNA Sequence Specific Agents, vol. 3, JAI Press, Greenwich, CT, 1998, pp. 1–6.
[6] D.M. Lilley, DNA–protein structural interactions, in: D.M. Lilley (Ed.), Frontiers in Molecular Biology, Oxford University Press, New York, 1995.
[7] B. Gatto, G. Zagotto, C. Sissi, C. Cera, E. Uriarte, G. Palu, G. Capranico, M. Palumbo, Peptidyl anthraquinones as potential antineoplastic drugs: synthesis, DNA binding, redox cycling, and biological activity, J. Med. Chem. 39 (1996) 3114–3122.
[8] G. Zagotto, G. Mitaritonna, C. Sissi, M. Palumbo, New peptidyl–anthraquinones: synthesis and DNA binding, Nucleosides Nucleotides 17 (1998) 2135–2141.
[9] P. Rinaldi, M. Wilk, Use of liquid crystal induced circular dichroism for absolute configurational assignments of beta-amino alcohols, J. Org. Chem. 48 (1983) 2141–2146.

[10] Y. Asano, A. Yamada, Y. Kato, K. Yamaguchi, Y. Hibino, K. Hirai, K. Kondo, Enantioselective synthesis of (*S*)-amino acids by phenylalanine dehydrogenase from *Bacillus sphaericus*: use of natural and recombinant enzymes, J. Org. Chem. 55 (1990) 5567–5571.
[11] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening, J. Natl. Cancer Inst. 82 (1990) 1107–1112.
[12] Molecular Operating Environment (MOE 1998.10), Chemical Computing Group Inc., 1255 University St, Suite 1600, Montreal, Quebec, Canada, H3B 3X3, 1998.
[13] T.A. Halgren, Merck molecular force field 1. Basis, form, scope, parameterization, and performance of MMFF94, J. Comput. Chem. 17 (1996) 490–519.
[14] T.A. Halgren, Merck molecular force field 2. MMFF94 van der Waals and electrostatic parameters for intermolecular interactions, J. Comput. Chem. 17 (1996) 520–552.
[15] T.A. Halgren, Merck molecular force field 3. Molecular geometries and vibrational frequencies for MMFF94, J. Comput. Chem. 17 (1996) 553–586.
[16] T.A. Halgren, R.B. Nachbar, Merck molecular force field 4. Conformational energies and geometries for MMFF94, J. Comput. Chem. 17 (1996) 587–615.
[17] T.A. Halgren, Merck molecular force field 5. Extension of MMFF94 using experimental data, additional computational data, and empirical rules, J. Comput. Chem. 17 (1996) 616–641.
[18] M.J. Frisch, Gaussian Inc., Pittsburg PA, 1988.
[19] O. Bayer, Chinone, in: J. Houben, T. Weyl (Eds.), Methoden der Organischen Chemie, vol. VII/3C, Georg Thieme Verlag, Stuttgart, 1979.
[20] J. Cummings, I. Meikle, J.S. Macpherson, J.F. Smyth, Characterization of the major metabolite of the novel topoisomerase I inhibitor NU/ICRF 505, Anti Cancer Drug Des. 11 (1996) 367–382.
[21] J. Cummings, I. Meikle, J.S Macpherson, J.F. Smyth, Determination of the novel topoisomerase I inhibitor NU/ICRF 505 and its major metabolite in plasma, tissue and tumour by high-performance liquid chromatography, J. Chromatogr. B 685 (1996) 159–164.
[22] I. Meikle, J. Cummings, J.S. Macpherson, J.F. Smyth, Induction of apoptosis in human cancer cell lines by the novel anthracenyl amino acid topoisomerase I inhibitor NU/ICRF 505, Br. J. Cancer 74 (1996) 374–379.
[23] I. Meikle, J. Cummings, J.S. Macpherson, J.F. Smyth, Development of anthracenyl–amino acid conjugates as topoisomerase I and II inhibitors that circumvent drug resistance, Biochem. Pharmacol. 52 (1996) 979–990.
[24] I. Meikle, J. Cummings, J.S. Macpherson, J.F. Smyth, Identification of anthracenyl–dipeptide conjugates as novel topoisomerase I and II inhibitors and their evaluation as potential anticancer drugs, Anti Cancer Drug Des. 10 (1995) 515–527.