

SHORT COMMUNICATION

DNA-BINDING PROPERTIES AND CYTOTOXICITY OF EXTENDED AROMATIC BISAMIDINES IN BREAST CANCER MCF-7 CELLS

Krzysztof Bielawski^{1, #}, Sławomir Wołczyński², Anna Bielawska¹

¹Department of Medicinal Chemistry and Drug Technology, Medical Academy of Białystok, Mickiewicza 2, PL 15-230 Białystok, Poland, ²Department of Gynecological Endocrinology, Medical Academy of Białystok, Skłodowskiej 24A, PL 15-276 Białystok, Poland

DNA-binding properties and cytotoxicity of extended aromatic bisamidines in breast cancer MCF-7 cells. K. BIELAWSKI, S. WOŁCZYŃSKI, A. BIELAWSKA. Pol. J. Pharmacol., 2001, 53, 143–147.

The DNA binding properties of three novel extended aromatic bisamidines (**1–3**) possessing different dicationic terminal side chains were studied. Data from the ethidium displacement assay showed that bisamidines **1–3** have significant affinity for DNA. We studied the cytotoxic activity of bisamidines **1–3** and Hoechst 33258 in the cultured breast cancer MCF-7 cells. These data show that in broad terms the cytotoxic potency of bisamidines **1–3** in the cultured breast cancer MCF-7 cells decreases with the size of the alkyl group substituent (cyclopropyl > isopropyl > cyclopentyl), in accord with their increases in DNA affinity, as shown by the binding constant values. The bisamidines **1–3** showed comparable antitumor activity to Hoechst 33258.

Key words: bisamidines, DNA binding, breast cancer MCF-7 cells, cytotoxicity

[#] *correspondence*; e-mail: kbiel@amb.ac.bialystok.pl

INTRODUCTION

The aromatic bisamidines exhibit a wide spectrum of antimicrobial, antiviral, and antitumor properties [1, 3, 7]. However, the precise genomic targets and modes of action of these ligands are not known. Most studies have focused on the abilities of bisamidines to inhibit the binding of regulatory proteins to oligonucleotide length recognition sequences that are rich in A and T base pairs. For example, these drugs have been reported to inhibit the DNA binding of certain restriction enzymes [1], the TATA box binding protein [5], the antennapedia homeodomain protein [8], the ubiquitous octamer binding protein, and the erythroid specific GATAAG protein [4]. The biological effects of bisamidines are presumably due to their inhibitory effects on transcription and replication [1, 2]. The lack of quantitative correlation between DNA binding and antimicrobial and antitumor activity for these molecules in all of the organisms studied can be attributed to the idea that DNA binding is only the first step in a multistep process.

This paper reports the DNA binding affinity and base pair specificity for three extended aromatic bisamidines **1–3** possessing different dicationic terminal side chains (Fig. 1). The DNA-binding ability of these bisamidines was evaluated by an ethidium displacement assay using calf thymus DNA, poly(dA-dT)₂ and poly(dG-dC)₂. We report here on the cytotoxic effects of bisamidines **1–3** in the cultured breast cancer MCF-7 cells, and we used these data to compare the cytotoxicity of bisamidines **1–3** with Hoechst 33258. Hoechst 33258 is a cytological DNA staining agent and is also used as a therapeutic agent for solid tumors [10]. It has been shown to bind to the minor groove of DNA at AT-rich DNA sequences nominally four or five consecutive AT base pairs in length [14].

MATERIALS and METHODS

Materials

The synthesis of compound **1** (1,4-bis{2-[5-(4-[(N-cyclopropyl)amidinophenyl]furyl)-2-carboxamido]}butane dihydrochloride), compound **2** (1,4-bis{2-[5-(4-[(N-isopropyl)amidinophenyl]furyl)-2-carboxamido]}butane dihydrochloride) and compound **3** (1,4-bis{2-[5-(4-[(N-cyclopentyl)amidinophenyl]furyl)-2-carboxamido]}butane dihydrochloride) has been detailed in the previous paper [2]. Hoechst

33258 (2-[2-(4-hydroxyphenyl)-6-benzimidazolyl]-6-(1-methyl-4-piperazinyl)benzimidazole), ethidium bromide, netropsin, distamycin, calf thymus DNA, homopolymers poly(dA-dT)·poly(dA-dT), and poly(dG-dC)·poly(dG-dC) were also purchased from Sigma. Stock cultures of breast cancer MCF-7 were purchased from the American Type Culture Collection, Rockville, MD. Dulbecco's minimal essential medium (DMEM) and foetal bovine serum (FBS) used in cell culture were products of Gibco (USA). Glutamine, penicillin and streptomycin were obtained from Quality Biologicals Inc. (USA). [³H]Thymidine (6.7 Ci/mmol) was the product of NEN (USA).

Ethidium displacement assay

Fluorescence was measured on a Hitachi spectrophotometer F-2500 FL at room temperature. The DNA-ethidium complex was excited at 546 nm and the fluorescence was measured at 595 nm. To 2 ml of ethidium bromide buffer, pH 7.4, 25 μl of DNA solution ($A_{260} = 2$) was added, and the maximum fluorescence was measured. Aliquots of a 10 mM stock drug solution (1 mg of drug to be tested and the appropriate volume of distilled water to make a 10 mM solution) were then added to the DNA-ethidium solution, and the fluorescence was measured after each addition until a 50% reduction of fluorescence had occurred. If the 10 mM stock solution lowered the percent fluorescence too quickly, the solution was further diluted to 1 mM prior to titration. Theoretical curves were fitted to the fluorescence intensity data points with one or two different iterative nonlinear least-squares computer routines. The apparent binding constant was calculated from $K_{EtBr}[EtBr] = K_{app}[drug]$, where [drug] = the concentration of the drug at a 50% reduction of fluorescence and K_{EtBr} is known [11, 12]. The bisamidines **1–3** and their complexes with DNA showed neither optical absorption nor fluorescence at 595 nm and did not interfere with the fluorescence of an unbound ethidium bromide.

MCF-7 cell culture

Stock cultures of breast cancer MCF-7 cells [15] were maintained in continuously exponential growth by weekly passage in DMEM supplemented with 10% FBS, 50 μg/ml penicillin, 50 μg/ml streptomycin at 37°C in humid atmosphere containing 5% CO₂ in an incubator. Cells were cultured in Costar flasks and subconfluent cells were

detached with 0.05% trypsin, 0.02% EDTA in calcium-free phosphate-buffered saline. The study was carried out using cells from passages 3 to 7, growing as monolayer in 6-well plates (Nunc) (5×10^5 cells per well).

Cytotoxic assay

To examine the effect of the studied compounds on cells proliferation MCF-7 cells were seeded in 6-well plates and grown as described above. Cell cultures were incubated with varying concentrations of bisamidines **1–3**, Hoechst 33258 and $0.5 \mu\text{C}$ of [^3H]thymidine for 24 h at 37°C . The cells were then harvested by trypsinization and washed (with cold phosphate-buffered saline) with centrifugation for 10 min at 1500 g several times (4–5) until the dpm count in the washes were similar to the reagent control. Radioactivity was determined by liquid scintillation counting. [^3H]thymidine uptake was expressed as dpm/well.

Statistical analysis

In all experiments, the mean values for three assays \pm standard deviations (SD) were calculated. The results were submitted to statistical analysis using the Student's *t*-test. Differences were considered significant when $p < 0.05$. Mean values, SD and the number of measurements in the group (*n*) are presented in the figures.

RESULTS and DISCUSSION

The apparent DNA binding constants (K_{app}) of the bisamidines (Fig. 1) to calf thymus DNA, poly(dA-dT) $_2$ and poly(dG-dC) $_2$ were determined using the ethidium displacement assay [6, 12] and were compared to those of distamycin and netropsin (Tab. 1). These data demonstrate that all compounds can bind to the studied DNAs. As can be seen from Table 1, the binding constant (K_{app}) for bisamidines **1–3** vary from $1.8 \cdot 10^5 \text{ M}^{-1}$ for compound **1** to $1.0 \cdot 10^5 \text{ M}^{-1}$ for compound **3**. Binding of bisamidines **1–3** was weaker than that of netropsin and distamycin. The enhanced flexibility of bisamidines **1–3** would lower the probability of their occupation of the correct region of conformational space and increase the entropy loss upon binding, both of which will lead to decreasing binding affinities. It may explain the lower DNA-binding properties of bisamidines **1–3** compared to netropsin and distamycin (Tab. 1).

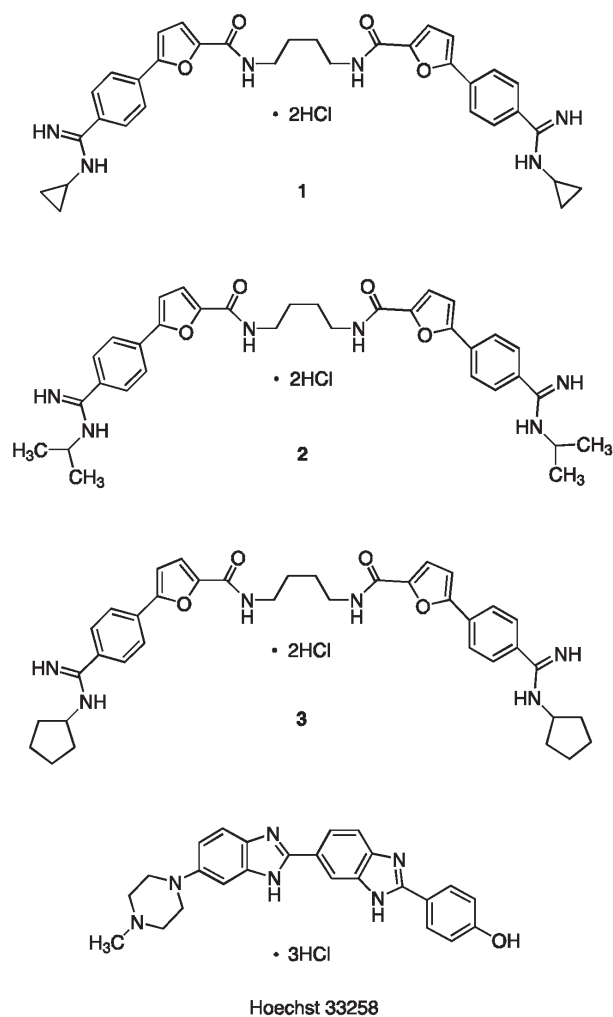


Fig. 1. The chemical structure of the aromatic bisamidines **1–3** and Hoechst 33258

Table 1. Association constants ($K_{\text{app}} \times 10^5 \text{ M}^{-1}$) of the binding of the ligands to polynucleotides

Ligand	Calf thymus DNA	poly(dA-dT) $_2$	poly(dG-dC) $_2$
Ethidium bromide	100	95	99
Netropsin	8.7	875	2.5
distamycin	7.5	340	2.0
1	1.8	7.9	0.7
2	1.4	7.5	0.6
3	1.0	7.1	0.8

The error for determination of netropsin, distamycin and the compounds **1–3** is $\pm 0.2 \times 10^5 \text{ M}^{-1}$

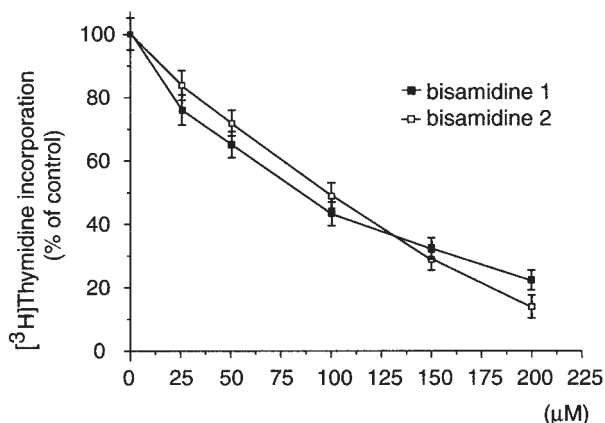


Fig. 2. Cytotoxic effects of bisamidines 1–2 on the cultured breast cancer MCF-7 cells as measured by inhibition of [³H]thymidine incorporation into DNA. Mean values ± SD of 3 independent experiments (n = 4) done in duplicates are presented

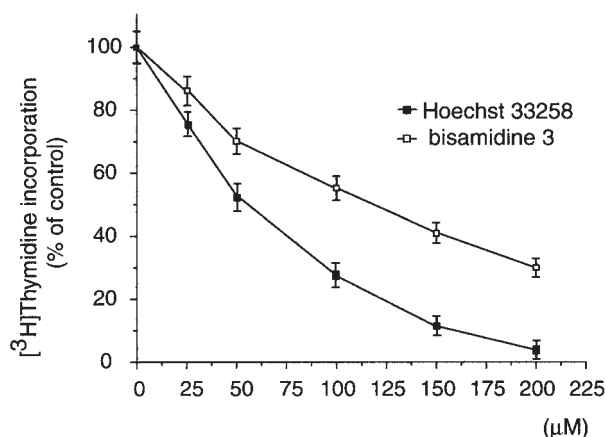


Fig. 3. Cytotoxic effects of bisamidine 3 and Hoechst 33258 on the cultured breast cancer MCF-7 cells as measured by inhibition of [³H]thymidine incorporation into DNA. Mean values ± SD of 3 independent experiments (n = 4) done in duplicates are presented

The bisamidines 1–3 bind to AT sequences more weakly than the extensively studied minor-groove binders, such as distamycin and netropsin. However, these compounds show high sequence-selectivities. The values of K_{app} for the binding of compounds 1–3 to poly(dA-dT)₂ are slightly greater than those for poly(dG-dC)₂. Since calf thymus DNA contains random sequences and therefore fewer AT sites than poly(dA-dT)₂, the selectivities of the ligands are further demonstrated by their much weaker binding to calf thymus DNA compared to poly(dA-dT)₂. The AT base pair speci-

ty shown by these bisamidines (Tab. 1) could be the result of the preferential binding of highly electro-positive bisamidines to the most electronegative region of the DNA, the AT base pairs in the minor groove [1].

The DNA binding of these bisamidines suggests that they may be effective in inhibition of regulatory protein binding in the minor groove, as has been shown for other bisamidines [1, 5, 9]. Bisamidines such as furimidazoline and furamidine have demonstrated diverse pharmacological activities [1, 3]. They show significant antiproliferative activities against various tumor cell lines, including cells resistant to cisplatin [13]. We studied the effect of bisamidines 1–3 and Hoechst 33258 on DNA synthesis in the cultured breast cancer MCF-7 cells (Fig. 2 and Fig. 3). All studied bisamidines inhibited DNA synthesis in breast cancer MCF-7 cells, in a dose-dependent manner, however with different potency. The concentrations of bisamidines 1, 2 and 3 needed to inhibit [³H]thymidine incorporation into DNA by 50% (IC_{50}) found to be 85 ± 6 M, 97 ± 5 M and 117 ± 6 M, respectively, suggesting similar cytotoxic potency of these bisamidines compared to Hoechst 33228 ($IC_{50} = 55 \pm 6$ M). These data show that in broad terms the cytotoxic potency of bisamidines 1–3 in the cultured breast cancer MCF-7 cells decreases with the size of the alkyl group substituent (cyclopropyl > isopropyl > cyclopentyl), in accord with increases in their DNA affinity, as shown by the binding constant values (Tab. 1). This suggests that DNA binding may be implicated in the cytotoxicity of these bisamidines, possibly by inhibiting interactions between cellular proteins and their DNA targets. The activity of RNA polymerases, DNA polymerases, and topoisomerases I and II can be affected by bisamidines 1–3 treatment of their DNA templates. It is also possible that the hydrophobic tetramethylene group, acting as recognition signal for hydrophobic residues in these proteins, improved the levels of such recognition. We cannot exclude the possibility that the differences in cytotoxicity are due to differences in cell uptake. Further investigations on the mechanisms of the cytotoxicity elicited by these compounds are in progress.

REFERENCES

1. Bailly Ch., Dassonneville L., Carrasco C., Lucas D., Kumar A., Boykin D.W., Wilson W.D.: Relationships between topoisomerase II inhibition, sequence-speci-

- ficity and DNA binding mode of dicationic diphenylfuran derivatives. *Anti-Cancer Drug Des.*, 1999, 14, 47–60.
2. Bielawski K., Galicka A., Bielawska A., Średzińska K.: Inhibitory effect of pentamidine analogues on protein biosynthesis in vitro. *Acta Biochim. Polon.*, 2000, 47, 113–120.
 3. Boykin D.W., Kumar A., Spychala J., Zhou M., Lombardy R.J., Wilson W.D., Dykstra Ch.C., Jones S.K., Hall J.E., Tidwell R.R., Laughton Ch., Nunn Ch.M., Neidle S.: Dicationic diarylfurans as anti-Pneumocystis carinii agents. *J. Med. Chem.*, 1995, 38, 912–916.
 4. Brogginini M., Ponti M., Ottolenghi S., D’Incalci M., Mongelli N., Mantovani R.: Distamycins inhibit the binding of OTF-1 and NFE-1 transactors to their conserved DNA elements. *Nucl. Acid. Res.*, 1989, 17, 1051–1059.
 5. Chiang S.A.Y., Welch J., Rauscher F.J., Beerman T.A.: Effects of minor groove binding drugs on the interaction of TATA box binding protein and TFIIA with DNA. *Biochemistry*, 1994, 33, 7033.
 6. Debart F., Periguad C., Gosselin D., Mrani D., Rayner B., Le Ber P., Auclair C., Balzarini J., De Clercq E., Paoletti C., Imbach J.L.: Synthesis, DNA binding, and biological evaluation of synthetic precursors and novel analogues of netropsin. *J. Med. Chem.*, 1989, 32, 1074–1083.
 7. Denny W.A.: New direction in the design of anticancer drugs. *Drug Des. Delivery*, 1988, 3, 99–124.
 8. Dorn A., Affolter M., Muller M., Gehring W.J., Leupin W.: Distamycin-induced inhibition of homeodomain-DNA complexes. *EMBO J.*, 1992, 11, 279–286.
 9. Gambari R., Nastruzzi C.: DNA-binding activity and biological effects of aromatic polyamides. *Biochem. Pharmacol.*, 1994, 47, 599–610.
 10. Kraut E.T., Fleming M., Segal J., Neidhart N., Behrens B.C., MacDonald J.: Phase II study of pibenzimol in pancreatic cancer – a southwest oncology group study. *Invest. New Drug.*, 1991, 9, 95–96.
 11. Lee M., Rhodes A.L., Wyatt M.D., D’Incalci M., Farrow S., Hartley J.A.: In vitro cytotoxicity of CG sequence directed alkylating agents related to distamycin. *J. Med. Chem.*, 1993, 36, 863–870.
 12. Morgan A.R., Lee J.S., Pulleyblank D.F., Murray N.L., Evans D.H.: Ethidium fluorescent assays. Part 1. Physicochemical studies. *Nucl. Acid. Res.*, 1979, 7, 547–569.
 13. Neidle S., Kelland L.R., Trent J.O., Simpson I.J., Boykin D.W., Kumar A., Wilson W.D.: Cytotoxicity of bis(phenylamidinium)furan alkyl derivatives in human tumour cell lines: relation to DNA minor groove binding. *Bioorg. Med. Chem. Lett.*, 1997, 11, 1403–1408.
 14. Searle M.S., Embrey K.J.: Sequence-specific interaction of Hoechst 33258 with the minor groove of an adenine-tract DNA duplex studied in solution by ¹H NMR spectroscopy. *Nucl. Acid. Res.*, 1990, 18, 3753–3762.
 15. Soule H.D., Vazquez J., Long A., Albert S., Brennan M.: A human cell line from a pleural effusion derived from a breast carcinoma. *J. Natl. Cancer Inst.*, 1973, 51, 1409–1416.

Received: December 11, 2000; in revised form: January 30, 2001.