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# The Analogues of DNA Minor-Groove Binders as Antineoplastic Compounds

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## 1. Introduction

Chemotherapy and hormonal therapy play important role in the treatment of breast cancer, a leading cause of cancer death in women. Although there are a lot of effective medicines applied, a significant number of patients do not respond to these therapeutic agents. Drug resistance, in addition to side effects of chemotherapy and hormonal therapy, necessitates the search for new specific tumor targeting compounds. Breast cancer cell lines have been widely used not only to investigate breast cancer pathobiology, but also to screen and characterize new therapeutics. Especially MCF-7 and MDA-MB-231 breast cancer cell lines often serve as *in vitro* models in cancer research. In this chapter, some of new compounds which showed antiproliferative and cytotoxic effects against MCF-7 and/or MBA-MB-231 breast cancer cell lines are presented.

## 2. Minor groove binders (MGB)

The minor groove of double helical B-DNA is becoming a site of a great interest for developing new drugs since it is the site of non-covalent high sequence specific interactions for a large number of small molecules. Minor groove binders are one of the most widely studied class of agents characterized by a high level of sequence specificity and possessing varied biological activities. Most of them exhibit antiviral, antibacterial and antiprotozoal properties. Furthermore, some of these have shown antitumor activity.

The focus of this chapter will be on anti-cancer compounds, active against MCF-7 and/or MBA-MB-231 breast cancer cell lines, which are derived from the non-covalent minor groove binders such as netropsin, distamycin A and related compounds, the Hoechst 33258, DAPI, berenil or pentamidine (Fig.1). These compounds interact only physically with DNA and cause only reversible inhibition of DNA-dependent functions. They possess an inherent curvature that matches approximately the helical curve of the minor groove of B-DNA.

The DNA binding process in the minor groove can be described by two steps (Bailly & Chaires, 1998). In the first, electrostatic and hydrophobic interactions transfer the ligand from solution into the DNA minor groove. In the case of positively charged compounds, such as distamycin, this results in DNA counter-ion exchange. In the second step, various specific interactions are established between the bound ligand and the functional groups of the base pairs of the DNA. The interactions usually include a combination of hydrogen

bonds, hydrophobic and van der Waals contacts, and electrostatic interactions (Gallmeier & König, 2003).

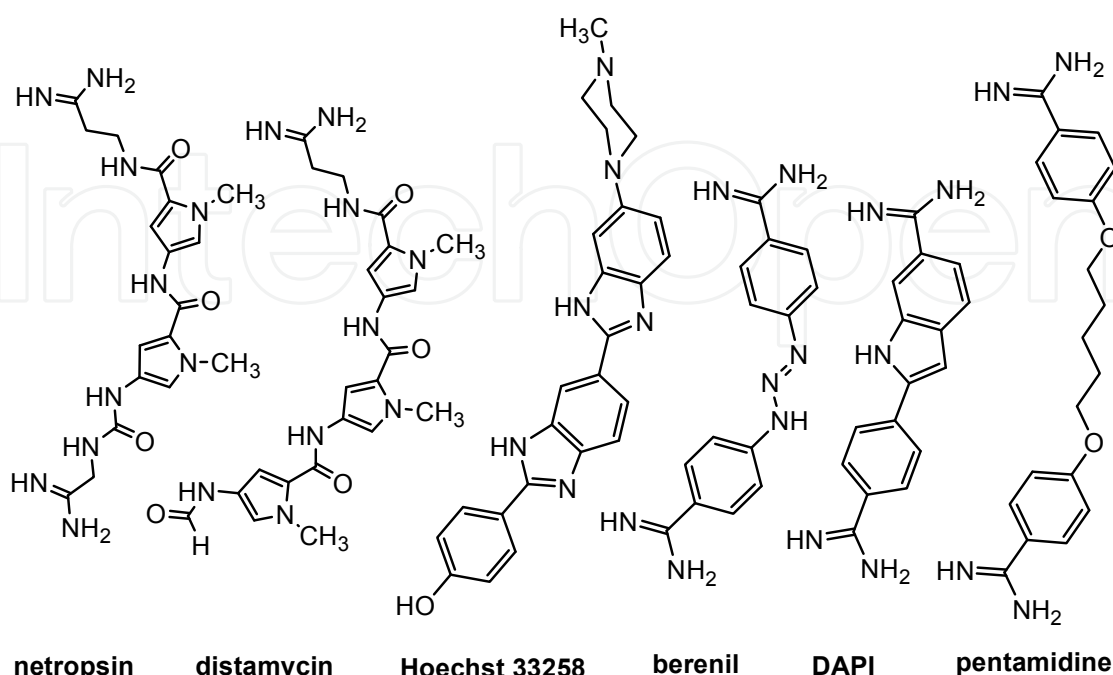


Fig. 1. Structures of minor groove binders.

Although DNA minor groove binding drugs have been extensively reviewed in the last years, defining the chemical and biological aspects of the newly synthesized compounds, only few of them have shown antitumor activity and reached clinical trials.

## 2.1 Carbocyclic analogues of distamycin and netropsin

Distamycin and netropsin have been shown to be highly DNA sequence-specific and bind preferentially to AT-rich regions of DNA. These oligoamides are highly polar compounds that nevertheless show significant cytotoxic properties.

DNA-binding model of netropsin and distamycin become the inspiration to searches of new compounds with similar interaction to DNA. The class of synthetic heteroaromatic oligopeptides, projected after the models the netropsin and distamycin, received the name lexitropsins (Kopka et al., 1985). Lexitropsins connected with molecules of different known drug, e.g. alkylating agents, are called combilexins (Sondhi et al., 1997). Until now thousands of MGB analogues have been synthesized and some reviews about recent results about analogues of netropsin, distamycin and of some lexitropsins and combilexins or related hybrid molecules with sequence reading, intercalating or alkylating activity were described and evaluated for prospective applications (Bailly & Chaires, 1998; Baraldi et al., 2004; Nelson et al. 2007; Pindur et al, 2005; Reddy et al. 2001). Here the carbocyclic lexitropsins are presented.

The derivatives containing benzene in place of N-methylpyrrole rings, with a minor modification of cationic heads, bind to AT sequences less strongly than the extensively studied MGB, however these compounds show sequence selectivity. It is worth noting that carbocyclic analogues of netropsin and distamycin are readily available, can be modified easily, and are stable under most experimental conditions.

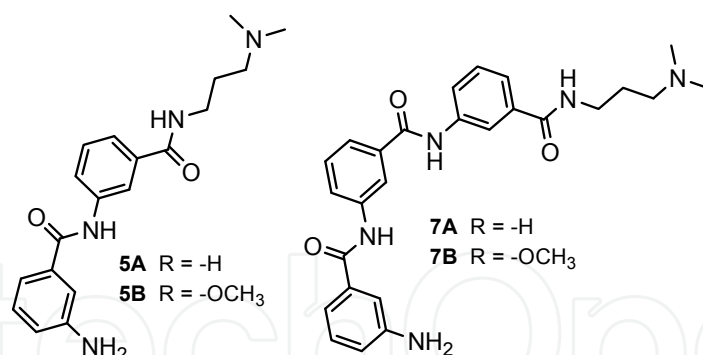


Fig. 2. Structures of carbocyclic netropsin and distamycin analogues.

The showed compounds **5A** and **5B** possess a dimethylamino group in place of the amidinium moiety normally present in netropsin. The synthesis of such C-terminus-modified analogues provides a number of advantages. First, the compounds containing a modified terminus are chemically stable, and thus the synthetic methodology is readily adaptable to preparation of further analogues. Second, they are not hygroscopic and are easy to handle. Third, the dimethylamino group is uncharged, and thus column chromatography or recrystallization can readily purify products and intermediates. Finally, with a  $pK_a$  about 9.3, this moiety would be protonated at physiological pH of 7.4 to provide favorable electrostatic attraction to the negative electrostatic charge of DNA. The compounds **7A** and **7B** have the requisite charged end groups and number of potential hydrogen-bonding loci equal to that of distamycin. To obtain information on the DNA binding modes of these types of compounds, additional derivatives, **5B** and **7B**, substituted in *ortho* position to amide moieties of each phenyl ring with a methoxy group were synthesized and designed to provide improved distinction between minor-groove and intercalation binding modes. The methoxy group protrudes from the plane of the aryl ring and would unfavourable clash with the aromatic rings of the base pairs in the intercalation cavity of DNA.

Compounds of this type have the potential for development as carriers for the groove-specific delivery of functionalized groups to DNA and as template inhibitors of transcription. Described compounds were tested for their antitumour activity in the standard cell line of the mammalian tumour MCF-7. The compounds concentration, which inhibits 50% of colony formation, is in the range 24.43 – 105.35  $\mu\text{M}$ , whereas  $IC_{50}$  for netropsin studied in the same cell line, is 5.40  $\mu\text{M}$  and for distamycin is 56.95  $\mu\text{M}$ .

During the past years, studies have indicated that antitumour activity of DNA-binding drugs is, at least in part, the result of the inhibition of enzymes that regulate DNA topology: the topoisomerases. Compounds **7A** and **7B** inhibited topoisomerases activity contrary to **5A** and **5B**. This fact indicates that topoisomerase inhibition is selective and sensitive to the number of repeating benzene carboxamide units – a minimum of three benzene carboxamide units are necessary for the inhibition of topoisomerases.

On the basis of molecular modelling it seems that the structure of benzene oligopeptides might be a useful starting framework for synthesis of selective DNA minor groove binding molecules. Molecular modelling of their interaction with  $d(\text{CGCGAATTCGCG})_2$  showed that their structure are effectively isohelical with DNA minor groove however with decreased affinity for the minor groove of AT-rich regions in comparison to netropsin and distamycin. From the energetic analysis it appeared that van der Waals and electrostatic interactions are more important than specific hydrogen bonds in stabilizing the ligand-

duplex complexes. Compounds **5A** and **5B** are effectively isohelical with the DNA minor groove. The superior DNA-binding afforded by **5A** and **5B** in comparison to **7A** and **7B** results from their more effective penetration into minor groove and smaller perturbation of molecular structure upon complex formation (Bielawski et al., 2000).

In continuation of rational drug design program aiming to develop distamycin analogues, potential minor-groove binders, and inhibitors of topoisomerases, compounds **1C** - **8C** and **1D** - **6D** (Fig. 3) were synthesized and examined. This compound skeleton was combined the structural features of distamycin and furamidine.

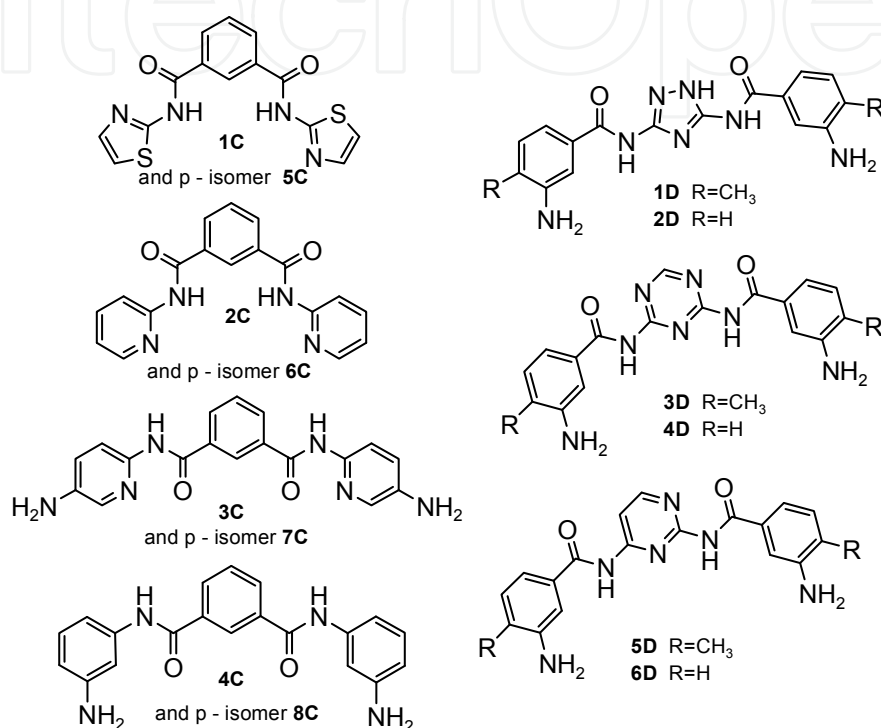


Fig. 3. Structures of distamycin analogues.

Distamycin analogues **1C** - **8C** were tested for *in vitro* cytotoxicity towards human breast cancer cells MCF-7 and MDA-MB-231. All of these compounds showed antiproliferative and cytotoxic effects against both cell lines in the range 3.47 - 12.53  $\mu\text{M}$  for MDA-MB-231 and 4.35 - 12.66  $\mu\text{M}$  for MCF-7. All of compounds demonstrated activity against DNA topoisomerases I and II at the concentration 50  $\mu\text{M}$ . Ethidium bromide assay showed that these compounds bind to plasmid pBR322 but weaker than distamycin. The most interesting seems compound **1C** with a time-dependent reduction in proliferation observed in both cell lines at concentrations: 6.38  $\mu\text{M}$  for MCF-7 and 8.79  $\mu\text{M}$  for MDA-MB-231. Compound **5C** with  $\text{IC}_{50}$  respectively 10.99  $\mu\text{M}$  for MCF-7 and 3.47  $\mu\text{M}$  for MDA-MB-231 cells is also interesting. All of investigated compounds are more potent than chlorambucil, which MCF-7  $\text{IC}_{50}$  averages 24,6  $\mu\text{M}$ . The most active analogues due to possession a free amino group can serve as potential carriers of strong acting elements, e.g. alkylating groups.

All of new oligopeptides **1D** - **6D** exhibit tumour cell cytotoxicity towards the standard cell line of the mammalian tumour MCF-7 and  $\text{IC}_{50}$  of examined compounds is in the range 183.53 - 232.50  $\mu\text{M}$ . It is similar value as other minor groove binder DAPI ( $\text{IC}_{50}$ =176  $\mu\text{M}$ ) but weaker than distamycin with  $\text{IC}_{50}$ =56.95  $\mu\text{M}$  or Hoechst 33258 ( $\text{IC}_{50}$ =55  $\mu\text{M}$ ) and presented earlier analogue of distamycin **7A** (40.73  $\mu\text{M}$ ).

Both presented groups of compounds demonstrated activity against DNA topoisomerase I and II. Ethidium bromide assay showed that these compounds bind to plasmid pBR322 but weaker than distamycin.

## 2.2 Other carbocyclic potential MGB

As a part of ongoing rational drug design programme aiming at development of carbocyclic minor groove binders six other compounds were synthesized and evaluated (Fig.4).

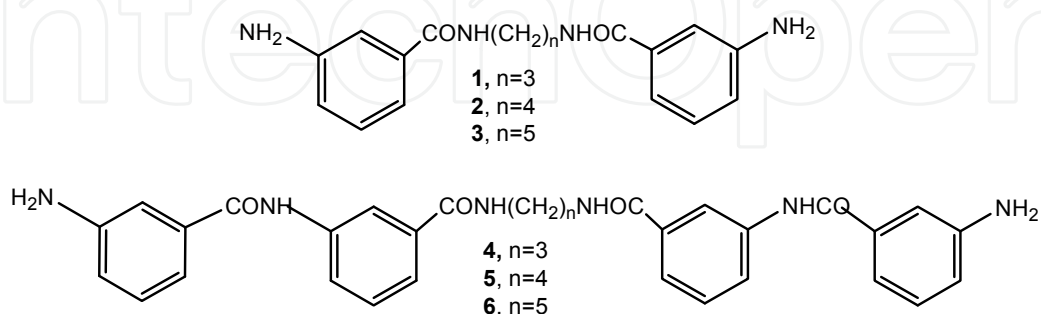


Fig. 4. Structures of carbocyclic potential MGB.

All of the tested compounds showed concentration-dependent activity. Against MDA-MB-231 cells, compounds are more cytotoxic than pentamidine with  $IC_{50} = 17.74 \mu M$  and netropsin with  $IC_{50} = 228.80 \mu M$ . The compound concentration that inhibited 50% of colony formation is in the range 8.10 to 17.52  $\mu M$ .  $IC_{50}$  against MCF-7 cell line were in the range 209.8 to 406.62  $\mu M$ , while  $IC_{50}$  of pentamidine was 14.31  $\mu M$  and netropsin 5.40  $\mu M$ . From these data we can see that the compounds 1-6 are nearly twenty times more active against MDA-MB-231 than against MCF-7 cells.

Data from relaxation assays of topoisomerase I and II demonstrated that compounds 1-6 have topoisomerase I inhibitory activity in the range from 10 to 40  $\mu M$  and topoisomerase II inhibitory activity in the range from 30 to 100  $\mu M$ .

The influence of compounds 1-6 on the amidolytic activity of urokinase, thrombin, plasmin and trypsin was also investigated. Compounds 1, 2 and 3 are ineffective as amidolytic activity inhibitors. None of investigated compounds inhibited activity of thrombin. Compounds 4-6 are inhibitors of plasmin meanwhile amidolytic activity of urokinase inhibit 5 and 6. Trypsin activity is inhibited only by compound 6.

The investigation compounds showed interesting spectrum of their activity. We can see that they bind to minor groove B-DNA and inhibit topo I and topo II activity. Some of them are also inhibitors of plasmin and urokinase. The differences in antiproliferative and cytotoxic effect against MCF-7 and MBA-MD-231 breast cancer cell lines demonstrate that mechanism of action of our compounds is not dependent only from DNA-binding mode but can be partially connected with the fact that in the case of MDA-MB-231 cells higher uPA/uPAR (urokinase plasminogen activator system) expression and high plasminogen-binding was observed than in MCF-7 cell line (Dass et al., 2008).

## 2.3 Bisamidine derivatives

The aromatic bisamidines, such as DAPI, berenil or pentamidine (Fig.1.), exhibit a wide spectrum of antimicrobial, antiviral, and antitumour properties (Baraldi et al., 2004). A number of natural and synthetic bisamidines are known to bind to B-DNA (Bailly &



Chaires, 1998). However, the precise genomic targets and modes of action 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. The lack of quantitative correlation between DNA binding and antimicrobial and antitumour 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.

To investigate DNA binding properties of bisamidines derivatives, novel extended diphenylfuran analogues **KB1-KB4** (Fig.5) possessing different dicationic terminal side chains were synthesized (Bielawski et al., 2001b). In the topoisomerase II assay, the relaxation of DNA was inhibited with all four drugs and the extent of inhibition was directly proportional to the drug concentration. Compounds **KB2-KB4** did not inhibit the topoisomerase I mediated relaxation of supercoiled DNA, compound **KB1** showed inhibiting activity at 80  $\mu\text{M}$ .

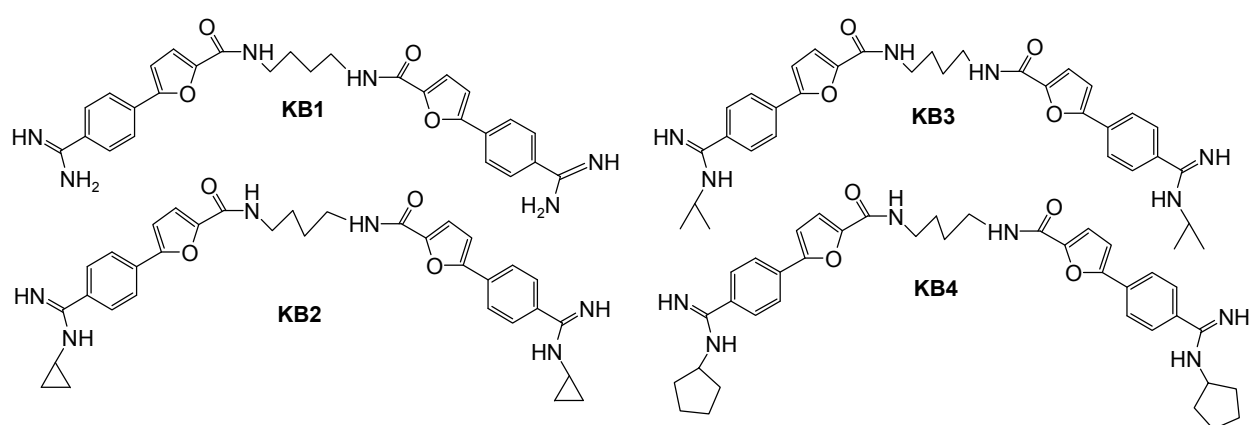


Fig. 5. Structures of novel bisamidines.

The ultrafiltration assay showed that examined compounds have significant affinity for DNA. The DNA-binding data using homopolymers poly(dA-dT) · poly(dA-dT) and poly(dG-dC) · poly(dG-dC) indicated that these compounds show moderate specificity for AT base pairs. The cytotoxicity effects of **KB1-KB4** were studied in cultured breast cancer MCF-7 cells and found to be 63  $\mu\text{M}$ , 85  $\mu\text{M}$ , 77  $\mu\text{M}$  and 97  $\mu\text{M}$ , respectively. The novel bisamidines showed comparable antitumour activity to Hoechst 33258, but were substantially more cytotoxic compared to DAPI. These data showed that in broad terms the cytotoxic potency of bisamidines **KB1-KB4** in cultured breast cancers MCF-7 cells decreases with the size of the alkyl group substituent (cyclopropyl > isopropyl > cyclopentyl), in accord with their increases in DNA affinity (Bielawski et al., 2001a). 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.

### 3. Synthetic minor groove binders as carriers for alkylating moieties

DNA alkylating agents are a major class of anticancer drugs for the treatment of various cancers including breast cancer. The first nitrogen mustard used in therapy was mechlorethamine, and the related compounds chlorambucil, melphalan, and cyclophosphamide remain in use today. A drawback common to all DNA alkylating agents is their high chemical reactivity. This can result in loss of drug by reaction with other

cellular nucleophiles, particularly proteins, and low-molecular weight thiols. This makes them vulnerable to cellular resistance mechanisms such as increased levels of glutathione. Other limitations, discussed particularly for mustards, are a lack of intrinsic DNA binding affinity of the core *N,N*-bis(2-chloroethyl)amine pharmacophore, and a requirement for bifunctional cross-linking of DNA to be fully cytotoxic. These characteristics lower their potency and the observed high ratio of genotoxic monoadducts to cross-links (up to 20:1) contributes to their known carcinogenicity. There is also evidence that the major guanine N7 adduct formed by mustards and other alkylators is readily repaired, which may also result in lower cytotoxicity (Osborne et al., 1995). For these reasons there has been much interest in the concept of specially targeting alkylating agents to DNA by attaching them to DNA affine carrier molecules, as this could in principle address these limitations. Increasing the concentration of drug in the vicinity of DNA would mean less chance of losing active drug by reaction with other cell components. Additionally, the use of DNA-affine carriers with their own defined binding geometry makes it possible to alter both the region and sequence specificity of alkylation compared with that of the alkylators

### 3.1 Distamycin related alkylating agents

Work on the targeting of nitrogen mustard alkylating agents to DNA by the use of DNA minor groove-binding ligands has shown that this strategy can greatly enhance both the *in vitro* cytotoxicity and the *in vivo* antitumour activity of the mustard moiety, when compared with untargeted mustards of similar reactivity. The main representative of this class that was clinically tested is tallimustine (Fig. 6), a benzoic acid nitrogen mustard derivative of distamycin (Cozzi, 2003).

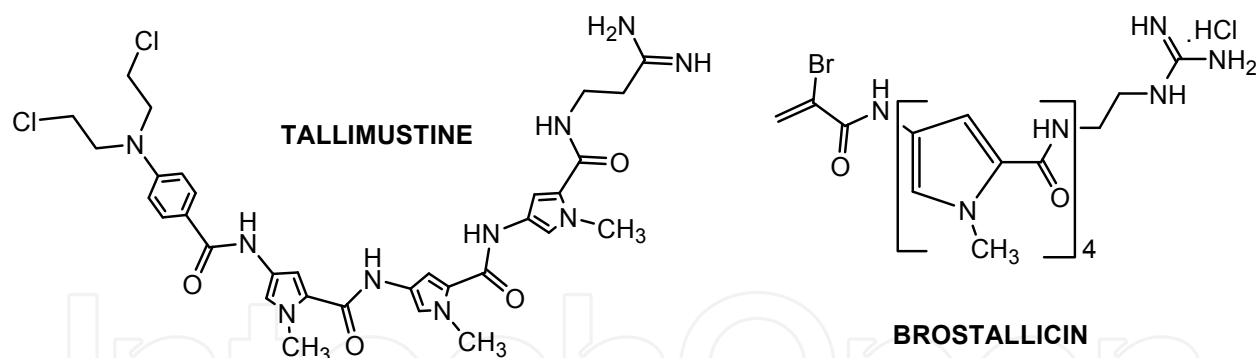


Fig. 6. Structures of tallimustine and brostallicin.

Tallimustine (TAM) showed cytotoxicity against L1210 murine leukemia more than two orders of magnitude higher than distamycin and more than one order of magnitude higher than classical nitrogen mustard melphalan. This compound is a very sequence and regiospecific alkylator, reacting only by monoalkylation at the N3 position of the 3'-adenine in the sequence 5'-TTTTGA-3'.

Whereas the cytotoxicity of TAM is related to the ability to form interstrand cross-links in DNA with consequent inhibition of DNA replication and transcription, the mechanism of antitumour action of tallimustine, although it is not yet fully elucidated may be due to its ability to inhibit the binding of some transcription factors to their consensus sequences in DNA. The cell cycle phase perturbations caused by tallimustine and melphalan were different and can be related to the different DNA damage done by these two drug.



Tallimustine showed excellent antitumor activity in preclinical tests, but also a severe myelotoxicity (Cozzi, 2003).

A second generation DNA minor groove binder, structurally related to distamycin is brostallicin (PNU-166196), alpha-bromo-acrylamido tetra-pyrrole derivative ending with a guanidino moiety (Fig.6). This compound showed broad antitumour activity in preclinical models and dramatically reduced *in vitro* myelotoxicity in human hematopoietic progenitor cells compared with that of TAM and other MGB. Brostallicin showed a 3-fold higher activity in melphalan-resistant L1210 murine leukemia cells than in the parental line ( $IC_{50}$ -0.46 and 1.45 ng/ml, respectively) under conditions in which the cytotoxicity of conventional antitumor agents was either unaffected or reduced. This melphalan-resistant cell line has increased levels of glutathione (GSH) in comparison with the parental cells. Conversely, GSH depletion by buthionine sulfoximine in a human ovarian carcinoma cell line (A2780) significantly decreased both the cytotoxic and the proapoptotic effects of brostallicin. A 2-3-fold increase in GST-levels resulted in a 2-3-fold increase in cytotoxic activity of brostallicin. Similar results were obtained for GST-transfected human breast carcinoma cells (MCF-7).

In an *in vivo* experiment, A2780 clones were implanted into nude mice. The antitumor activity of brostallicin was higher in the GST-overexpressing tumors without increased toxicity. Regarding the mechanism of action, brostallicin interacts reversibly with the DNA minor groove TA-rich sequences but appears unreactive in classical *in vitro* DNA alkylation assays. Evidence of both covalent interaction of brostallicin with plasmidic DNA in the presence of GSH and of enhanced cytotoxicity in cancer cells characterized by high levels of GSH was obtained (Geroni et al., 2002). Brostallicin was selected for clinical development and is presently in clinical trials in Europe and the United States (Fedier et al. 2003). The phase II of studies of brostallicin in combination with cisplatin for metastatic breast cancer is currently in the stage of testing.

### 3.2 Carbocyclic lexitropsins with chlorambucil moiety

The carbocyclic lexitropsins investigated so far were not such active to be used as agent in breast cancer therapy but the application of them as potential carriers of strong acting elements was also examined. For example, derivatives with N-terminal chlorambucil group have been synthesized (Fig.7.).

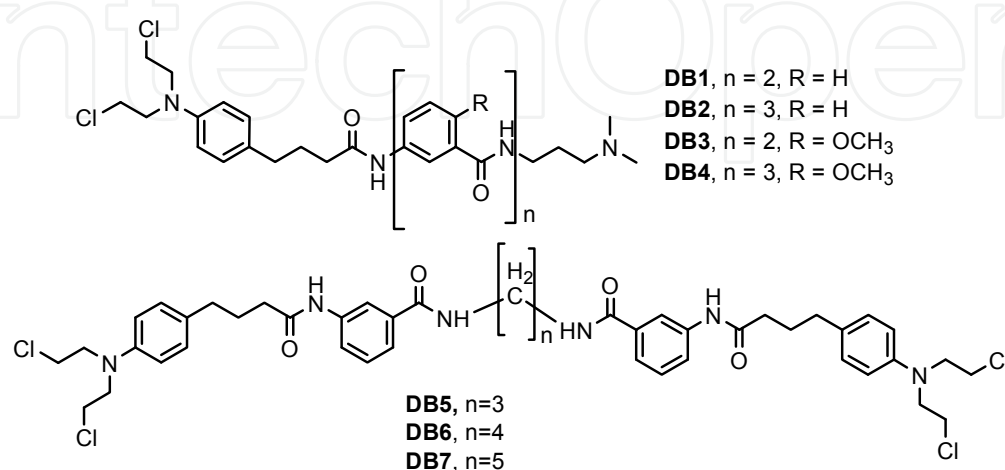


Fig. 7. Structures of carbocyclic lexitropsins with chlorambucil moiety.

After the molecular mechanics refinement calculations, energetically favoured complexes of compounds **DB1** and **DB3** with  $d(\text{CGCGAATTCGCG})_2$  were obtained (Fig.8.)

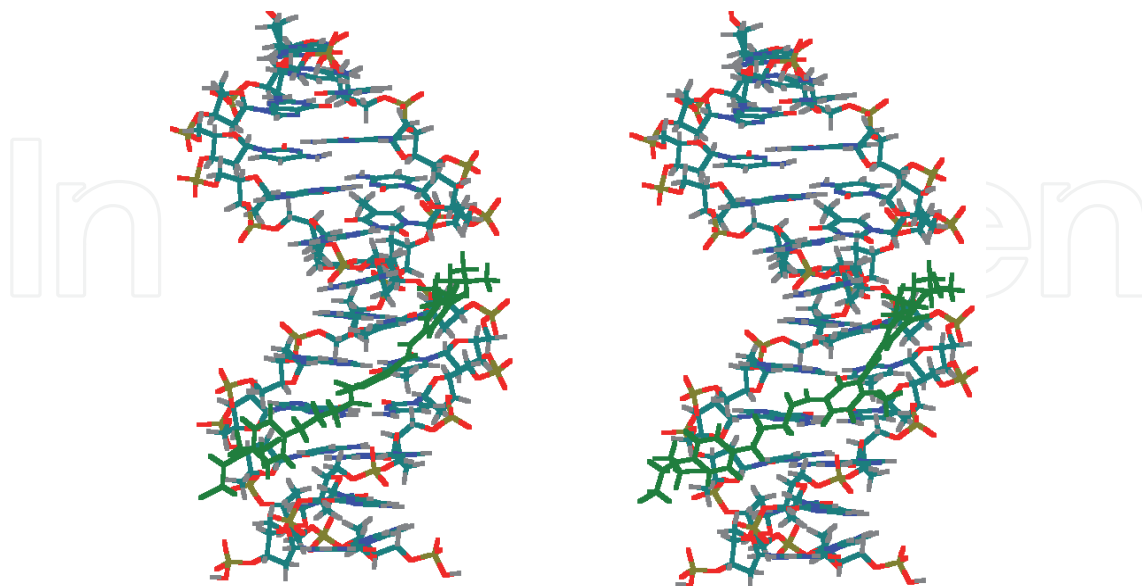


Fig. 8. Views of the low-energy complexes formed between the  $d(\text{CGCGAATTCGCG})_2$  and the carbocyclic analogues of distamycin after MD refinement. Left - **DB1**; right - **DB3**. Ligands molecules are shown in green.

Compounds **DB1** and **DB3** form centrosymmetric 4 bp complexes with the ligands displaced towards the 5' end of the 5'-AATT binding site. This displacement facilitates increased Waals contacts with the walls of the minor groove. In addition to the decreasing affinity for the 5'-AATT-3' match site, there are weaker contacts with the O2 atom of C21 indicating that the binding-site size requirement for **DB1** and **DB3** extends over slightly more than the four central AT base pairs. The energy wells for these ligands within this AT tract are narrow and the data indicate that specific interactions with flanking sequences strongly inhibit ligand translation along the minor groove. The benzene rings **DB1** and **DB3** are positioned roughly in the plane of the bases and the amide groups are located between base pairs. No regular pattern of bifurcated hydrogen bonds then exists. From the analysis of these complexes it appears that van der Waals and electrostatic interactions are more important in stabilizing the complexes than specific hydrogen bonds formation. This is consistent with the observed reduced affinity to AT pairs and increased affinity towards GC sequences of the carbocyclic lexitropsins with chlorambucil moieties in comparison with distamycin and netropsin. The protonated terminal dimethylamine nitrogen of the (dimethylamino)propyl tail is adjacent to a negatively charged phosphodiester linkage. The hydrophobic methoxy groups of **DB3** are situated outside the minor groove; therefore, the binding energies for **DB1** and **DB3** are almost the same. Compounds **DB1** and **DB3** produce an increase in groove width of ca. 1.5 Å compared with the netropsin-DNA complex (Kopka et al., 1985). Because of flexibility of the aliphatic tether of chlorambucil moiety, there is probably a limited distribution of alkylation sites derived from an individual binding complex rather than a unique alkylation site for each individual bound compound. An accurate definition by molecular modelling of the optimal binding site for the compounds studied alone has been hampered by the fact that the DNA fragment used in the model contains a limited number of binding sites.

The **DB1-DB4** compounds concentration, which inhibits 50% of breast cancer MCF-7 colony formation, is in the range 85 - 104  $\mu\text{M}$ . In the case of **DB5-DB7** compounds, this concentration is in the range 66 to 124  $\mu\text{M}$ . All of them induced cancer cell death by apoptosis and necrosis.

### 3.3 Amidine analogues of chlorambucil

Also a number of novel cyclic amidine analogs of chlorambucil (Fig.9) were synthesized and examined for cytotoxicity in breast cancer cell cultures.

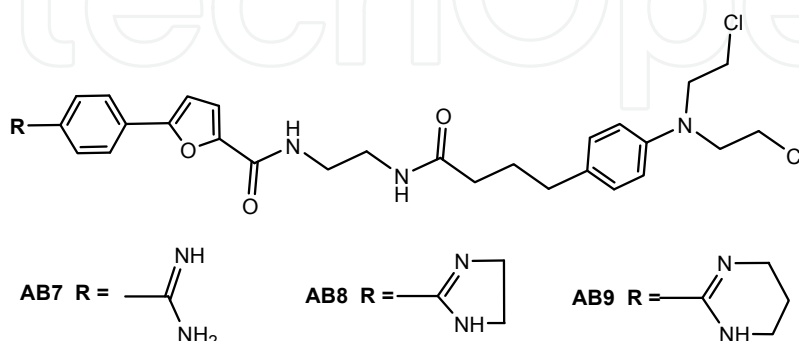


Fig. 9. Structures of amidine analogues of chlorambucil **AB7-AB9**.

In terms of reduction in cell viability, the compounds rank in both MCF-7 and MDA-MB-231 cells in the order **AB9** > **AB7** > **AB8** > chlorambucil. The values of  $\text{IC}_{50}$  were relatively higher for **AB 9** and **AB 7** which possess a cationic 4,5-dihydro-1Himidazol and amidine function, respectively. Among the derivatives, compound **AB 8** in both MDA-MB-231 and MCF-7 proved to be only slightly more potent than chlorambucil, with  $\text{IC}_{50}$  values of 70 and 76  $\mu\text{M}$ , respectively, compared to 92 and 97  $\mu\text{M}$  for chlorambucil. In contrast, compound **AB9**, which contains the 4,5-dihydro-1Himidazol moiety is clearly much more active and showed a high level of cytotoxic potency,  $\text{IC}_{50}$  22 and 18  $\mu\text{M}$  in MCF-7 and MDA-MB-231, respectively. Compound **AB9**, the most active of the series, is approximately five times more potent than chlorambucil.

The degree to which these compounds inhibited cell growth breast cancer cells was directly correlated to DNA-binding affinity.

The ability of compounds **AB7-9** to inhibit topoisomerases I and II activity was quantified by measuring the action on supercoiled pBR322 DNA substrate as a function of increasing concentration of the ligands by the use of agarose gel electrophoresis. Chlorambucil as a control was, as expected, ineffective in this assay. The investigation indicate that cyclic amidine analogs of chlorambucil are a potent catalytic inhibitor of topoisomerase II but not topoisomerase I. Compound **AB9** was the most potent topoisomerase II inhibitors, with 50% inhibitory concentration ( $\text{IC}_{50}$ ) 5  $\mu\text{M}$ . (Bielawska et al., 2004).

Compound **AB7** and chlorambucil were compared for their effects on collagen and DNA synthesis in breast cancer MDA-MB-231 cells.  $\text{IC}_{50}$  values for chlorambucil and its amidine analogue for collagen synthesis were found to be about 44 and 19  $\mu\text{M}$ , respectively. Increased ability of **AB7** to suppress the protein synthesis, compared to chlorambucil, was found to be related to an inhibition of prolidase activity and expression. The phenomena were probably a result of disruption of  $\beta_1$ -integrin and the insulin-like growth factor-I (IGF-I) receptor mediated signaling caused by this compound. Expression of  $\beta_1$ -integrin receptor, as well as focal adhesion kinase pp125<sup>FAK</sup> (FAK), growth-factor receptor-bound protein 2

(GRB2), son of sevenless protein 1 (Sos1) and phosphorylated mitogen activated protein kinases (MAPK), extracellular-signal-regulated kinase 1 (ERK1) and kinase 2 (ERK2) but not Src and Shc proteins was significantly decreased in cells incubated for 24 h with 10  $\mu$ M **AB7**, compared to controls. Chlorambucil in the same conditions did not evoke any changes in expression of all these signaling proteins, as shown by Western immunoblot analysis. In addition, **AB7** revealed a higher antiproliferative activity than chlorambucil, accompanied by a stronger down-regulation of IGF-I receptor expression. The results were confirmed by [ $^3$ H]thymidine incorporation assay. Incubation of the cells with 10  $\mu$ M **AB7** for 12 and 24 h contributed to a decrease in DNA synthesis by about 33 and 46% of the control values, respectively, while in case of chlorambucil by about 23 and 29%, respectively. These data suggest that the amidine analogue of chlorambucil (**AB7**) disturbs MDA-MB 231 cell metabolism more potently than does the parent drug, chlorambucil. The mechanism of this phenomenon may be due to its stronger suppression of  $\beta_1$ -integrin and IGF-I receptor signaling. (Sienkiewicz et al., 2005).

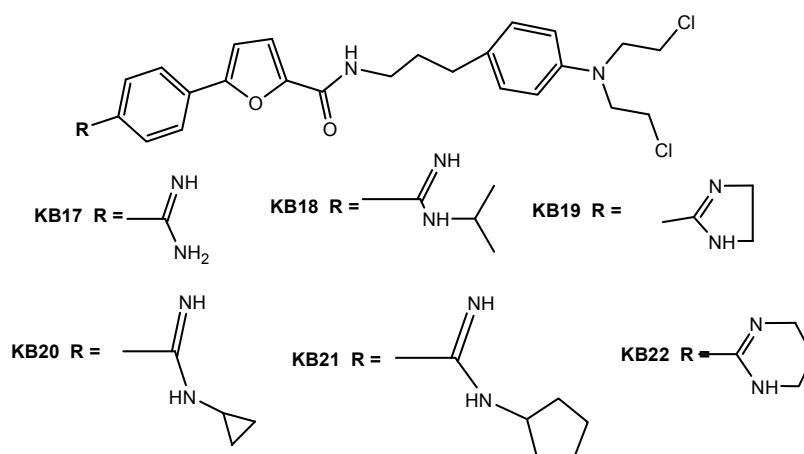


Fig. 10. Structures of amidine analogues of chlorambucil **KB17-KB22**.

As continuation of chlorambucil analogues of amidines investigations, novel nitrogen mustard agents **KB17-KB22** (Fig.10) involving 4-(N,N-bis(2-chloroethyl)-aminophenyl)propylamine linked to a 5-(4-N-alkylamidinophenyl)-2-furancarboxylic acid moiety by the formation of an amide bond have been synthesized, characterized, and evaluated for their *in vitro* cytotoxic activity against MDA-MB-231 and MCF-7 human breast cancer cells. Evaluation of the cytotoxicity of **KB17-KB22** employing a MTT assay and inhibition of [ $^3$ H]thymidine incorporation into DNA demonstrated that these compounds exhibit remarkable cytotoxic effects in comparison with 4-[bis(2-chloroethyl)amino]benzenebutanoic acid. Compounds **KB17** and **KB19**, which possess a cationic amidine and 4,5-dihydro-1H-imidazol function moiety are approximately ten times more potent than 4-[bis(2-chloroethyl)amino]benzenebutanoic acid. The new compounds were evaluated as DNA topoisomerase II inhibitors. The cytotoxicity of the compounds **KB17-KB22** correlates with their DNA binding affinities and their relative potency as topoisomerase II inhibitors (Bielawski et al., 2009).

### 3.4 Amidine analogues of melphalan

The amidine analogues of melphalan **KB6-KB10** (Fig.11) differing by the nature of terminal basic side were synthesized and examined (Bielawska et al., 2007). Evaluation of the

cytotoxicity of these compounds was employing a MTT assay in both MDA-MB-231 and MCF-7 human breast cancer cells. Although growth inhibition was concentration-dependent in either cell line, it was more pronounced at shorter times, in MCF-7 than MDA-MB-231. In terms of reduction in cell viability, the compounds rank in both MCF-7 than MDA-MB-231 cells in the order **KB7** > **KB6** > **KB8** > **KB9** > **KB10** > melphalan.

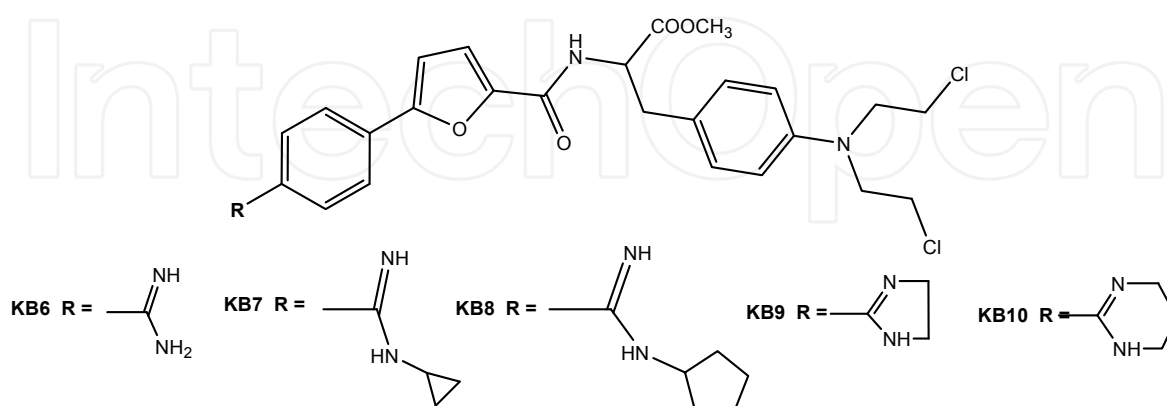


Fig. 11. Structures of amidine analogues of melphalan.

The values of  $IC_{50}$  were relatively higher for **KB7** which possess a cationic N-cyclopropylamidine function. Among the derivatives, compound **KB10** in both MDA-MB-231 and MCF-7 proved to be only slight more potent than melphalan, with  $IC_{50}$  values of 117 and 100  $\mu$ M, respectively, and compared to 130 and 125  $\mu$ M for melphalan. In contrast, compound **KB7** is clearly much more active and showed a high level of cytotoxic potency,  $IC_{50}$  55 and 77  $\mu$ M in MCF-7 and MDA-MB-231, respectively. Compound **KB7**, the most active of the series, is approximately 2 times more potent than melphalan.

An attempt has also been made correlate the observed biological activity with topoisomerases inhibitory properties and DNA-binding properties of selected compounds. The cytotoxicity of the amidine analogues of melphalan towards cultured human breast cancer cells correlate with topoisomerase II inhibitory properties but not with DNA-binding properties.

A molecular mechanics and molecular dynamics approach was used to examine the structure of complex formed between the d(CGCGAATTCGCG)<sub>2</sub> duplex and compound **KB7**. It is predicted that this compound should have a decreased affinity for the minor groove of AT-rich regions in comparison to netropsin and furamidine. From the energetic analysis it appears that van der Waals and electrostatic interactions are more important than specific hydrogen bonds in stabilizing the ligand duplex, similarly like described earlier chlorambucil derivatives of carbocyclic lexitropsins.

These experimental studies suggest that amidine analogues of melphalan **KB6-KB10** may have other consequences for the metabolism of breast cancer cells. There were found that compound **KB7** is a more potent inhibitor of collagen biosynthesis than a parent drug, melphalan (Bielawski et al., 2006).

Melphalan for 24 h did not affect the expression of proteins involved in the signaling cascade activated by  $\beta$ -integrin receptor. In contrast, compound **KB7** inhibited expression of Shc and MAP-kinases in both estrogen receptor-positive and estrogen receptor-negative breast cancer cells. Decreased expression of FAK-kinase was found only in MDA-MB-231 cells. Another important benefit evoked by the compound **KB7** seems to be inhibition of



phospho-ERK's activation (Bielawski et al., 2006). Upregulation of those kinases was found in various breast cancers (Santen et al., 2002). Blocking these kinases was found to have proapoptotic and antiproliferative effects on MDA-MB-231, that indicates a new target in the treatment of breast malignancies (Fukazawa et al., 2002). Induction of apoptosis by **KB7** in both MDA-MB-231 and MCF-7 breast cancer cells was stronger than by the parent drug and run by activating caspase-3 (Sosnowska et al., 2009).

These results and other recent studies indicate that the amidine analogues of melphalan represent multifunctional inhibitors of breast cancer cells growth and metabolism.

### 3.5 Alkylating analogues of Hoechst 33258

A series of carbamate derivatives of Hoechst 33258 was prepared as potential anticancer agents (Fig. 12).

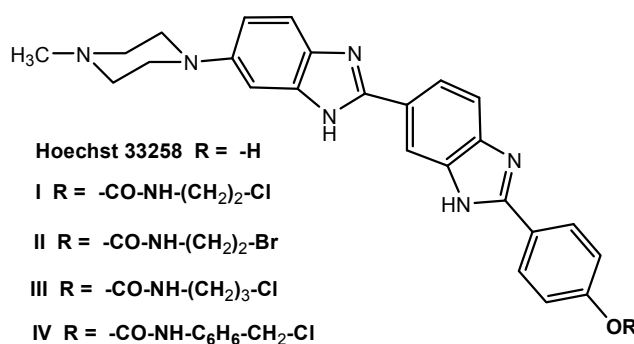


Fig. 12. Structures of alkylating analogues of Hoechst 33258.

These new compounds (**I–IV**) were readily prepared in good yields by addition of chloroethyl, bromoethyl, chloropropyl or 4-(chloromethyl)phenyl isocyanates to Hoechst 33258. Their cytotoxic activity was evaluated on human breast cancer MCF-7. Compounds **I–IV** were more cytotoxic than Hoechst 33258. In particular derivative **IV**, the most active of the series, is up to 3 times more potent than Hoechst 33258. The DNA-binding ability of these compounds was evaluated by an ultrafiltration method using calf thymus DNA. These data show that in broad terms the cytotoxic potency of **I–IV** in cultured breast cancer MCF-7 cells increases, in accord with their increases in DNA affinity, as shown by the binding constant values (Bielawski et al., 2002).

## 4. Conclusion

An understanding of the mechanism, by which minor groove binding agents interact with DNA has led to the design of agents that can reversibly bind with high selectivity to extended DNA target sequences. Until now thousands of MGB analogues have been synthesized – here has been presented only small part of all investigations.

The described results in the field of distamycin and netropsin, as well as other minor groove binders, and modifications of their structures give the expectation of obtaining a compound with required activity; which will be able to be applied as medical agent in anticancer therapy. Targeting alkylating moieties to DNA by attachment of DNA minor groove binding carrier, such as distamycin, netropsin, or Hoechst 33258 reduces the loss of active drug, due to reaction with other cell components and makes it possible to direct the alkylation both sequence specifically and regiospecifically. These compounds are able to



compete with natural substrates, such as specific transcription factors, and alter gene expression.

An overall conclusion from this review is the increasing molecular-level knowledge about how the simpler minor groove binding agents bind to DNA. This in turn has fed into the design of agents that can reversibly bind with high selectivity to longer sequences of virtually any composition, which likely occur very seldom in the genome. Such compounds are highly effective tools, which are being explored in more and more complex biological systems.

Although the biomedical sciences have recently been in intensive progress, it is difficult to find selective targets for cancer chemotherapy. Still many of drugs used today for treating cancer patients, also patients with breast cancer, are in fact practically nonselective and exhibit severe toxicity to normal tissues. Hence, each new synthesized compound gives the chance to obtain a better result than previously.

## 5. Acknowledgment

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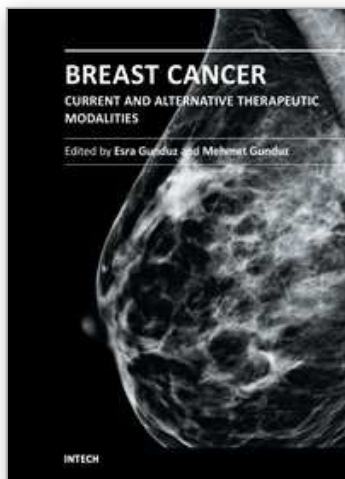
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