Recognition of Single Stranded and Double Stranded DNA/RNA Sequences in Aqueous Medium by Small Bis-Aromatic Derivatives

L.-M. Tumir and I. Piantanida*

Laboratory for Study of Interactions of Biomacromolecules, Division of Organic Chemistry and Biochemistry, "Ruđer Bošković" Institute, P. O. Box 180, HR-10002 Zagreb, Croatia

Abstract: The aim of this review is to summarize the most comprehensive results in the field of bis-aromatic compounds targeting DNA and RNA, whereby both aromatic units of small molecule bind to the polynucleotide by the aromatic stacking interactions. The most recent results about structure – DNA/RNA binding affinity, selectivity and biological implications are discussed for bis-intercalators, sterically restricted macrocyclic and threading bis-aromatics and intercalator – nucleobase conjugates.

Keywords: DNA and RNA binding, bis-intercalation, macrocyclic bis-intercalation, intercalator, nucleobase conjugate.

1. INTRODUCTION

Numerous drugs base their biological activity on interaction of low weight organic molecules with DNA and/or RNA. Small molecules of that kind are of special interest because they can more easily cross biological membranes than large molecules, and can even be delivered to cells strongly resistant to exogenous matter [1]. E.g. brain cells resist the entry of molecules with MW larger than approximately 600, thus hampering any disease treatment [1]. Actually, drugs that are currently used in cancer therapy are thought to act through the unspecific recognition of highly active DNA. This high affinity refers on replication of DNA at high frequency and therefore its relative exposition to recognition by (foreign) molecules. This feature explains the high toxicity of this class of compounds and, in addition, the slightly higher efficacy on cancer cells over healthy tissue [1]. In spite of the advances in understanding of DNA/RNA recognition, *de novo* design of sequence-selective DNA binding agents is not yet straightforward, and the design and synthesis of therapeutic compounds (e.g., antitumor drugs) remains an even more complex task. In a large measure, this difficulty in *de novo* design may be attributed to the constellation of properties that must be embodied in a single structure to provide a biologically active, therapeutically useful, sequence-selective DNA binding agent, which still has to be "small molecule" (within this review we tried to address molecules with molecular weight not significantly larger than 1000) due to aforementioned reasons [1]. Therefore design, synthesis, and biological evaluation of novel compounds that target DNA are of the utmost interest.

In contrast, few drugs are known that target RNA, and rational design of drugs that target RNA is in the beginning stages [2-5]. RNA's participate in a wide variety of biological processes: it carries information for biological functions of nucleic acids, its folded conformations can participate in complex recognition and catalytic processes similar to proteins; therefore RNA is significantly under-utilized biological target for drug design.

In general, there are three main modes of non-covalent binding of small molecules to DNA/RNA, (i) minor groove binding, (ii) intercalation and (iii) electrostatic interaction of highly positively charged molecules with nucleotide phosphate backbone [5, 6]. Although DNA intercalators have been used extensively as antitumor, antineoplastic, antimalarial, antibiotic, and antifungal agents, not all intercalators are genotoxic (defined by the ability to alter a cell's genetic material as a means of inducing a toxic effect). The presence of basic, cationic, or electrophilic functional groups is often necessary for genotoxicity [7]. Very recently, even thoroughly studied molecules as classical DNA/RNA intercalator ethidium bromide had to be re-evaluated [8-10], since it became obvious that mechanisms of non-covalent interactions between small molecule and DNA/RNA are not completely understood. A huge number of bis-aromatic derivatives were prepared with the aim of not only enhanced affinity due to the bis-intercalation into DNA/RNA, but also with the idea of introducing selectivity [11]. Many authors combined more modes of interaction in the same molecule targeting very specific goals. An extensive range of natural products bind to DNA by intercalation and they mostly form additional groove contacts contributing to their sequence preference. In fact, an important *de novo* design application of intercalators is combining them with sequence-selective groove binding molecules to enhance their affinity, selectivity, or temporal stability.

The aim of this review is to summarize the most comprehensive results in the field of bis-aromatic compounds targeting DNA and RNA, whereby both aromatic units bind to polynucleotide by aromatic stacking interactions. As this is a large area and a review is restricted to limited number of pages, we apologise in advance to any researcher whose work was not represented.

*Address correspondence to this author at the Laboratory for Study of Interactions of Biomacromolecules, Division of Organic Chemistry and Biochemistry, "Ruđer Bošković" Institute, P. O. Box 180, HR-10002 Zagreb, Croatia; Tel: +385 1 4571210; Fax: +385 1 4680 195;
E-mail: pianta@irb.hr

1389-5575/10 $55.00+.00

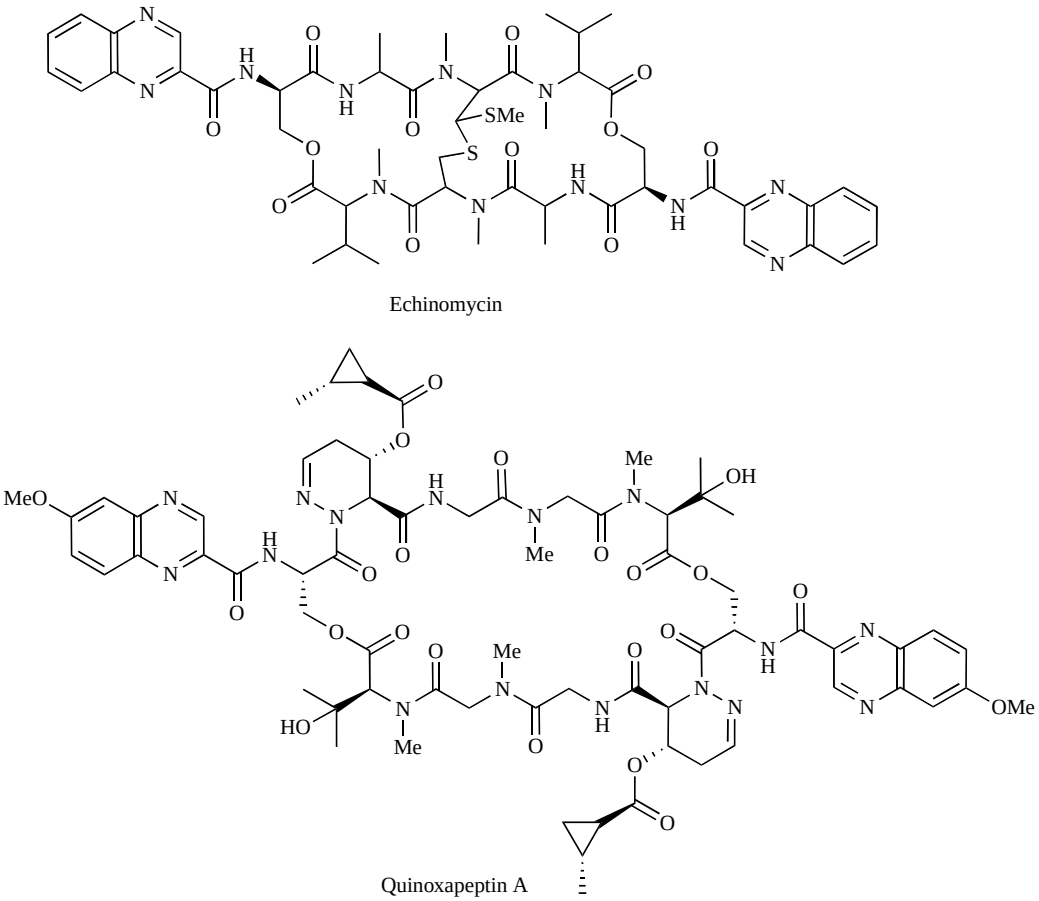

Fig. (1). Examples of the bis-intercalators isolated from the natural sources: echinomycin (top) and Quinoxapeptin A (bottom).

2.1. Bis-Intercalators

As it happens quite often in the scientific research, novel ideas originate from the naturally occurring species. There are many natural products which base their biological activity on the bis-intercalation into DNA, for instance Echinomycin (Fig. **1** top), Triostins (having quinoxaline chromophores), Thiocoraline, Sandramycin, Luzopeptins, Quinoxapeptins (Fig. **1** bottom), Quinaldopeptin and many others [12, 13]. In general, two planar chromophores intercalate with no or very poor sequence selectivity between the base-pairs of duplex DNA, while the most of the recognition, selectivity and some additional affinity increase are achieved by interactions of a cyclic peptide linker (octadepsipeptide, thiodepsipeptide, decadepsipeptide, decapeptide type) within the minor groove. Many naturally occurring bis-intercalators were also in many ways modified, with a goal of increasing affinity, selectivity or biological activity. For instance, well known molecule TANDEM recently has been modified - valine residues of octadepsipeptide linker were replaced by lysines, whereby selectivity TpA was maintained, but affinity was strongly increased. Most surprisingly, novel derivative lacked antibacterial activity of starting TANDEM [14].

With a goal of improving the biological properties of already efficient naturally occurring mono-intercalators, especially their affinity and sterically controlled recognition, many dimmers were designed and synthesized. For instance, bis-derivatives in which two molecules of already well established drug daunorubicin were connected by various xylyl-linkers revealed picomolar affinity for DNA and antiproliferative effect on various cell lines at nanomolar concentrations [15]. Intriguingly, studied compounds showed mixed DNA binding (bis-intercalation and minor groove binding), which strongly depends on a pre-formed conformation of derivative sterically controlled by type of xylyl-linker (*meta*- versus *para*-xylyl-). Similar xylyl-bridged bis-daunomycin derivative was also studied by molecular modelling studies [16].

However, principally the research considering the DNA/RNA active bis-aromatic compounds was based on the fully synthetic compounds. Several extensive reviews [11, 12] and books [1, 5] on DNA/RNA bis-intercalating compounds summarized important information about structural and steric requirements for efficient bis-intercalation as well as about biological and medicinal implications. Among huge number of studied compounds several bis-intercalator families exhibited promising antitumor activities [17], the most pronounced were bis-imidazoacridone [18], bis-anthracycline [19], bis-naphthalimide derivatives [20], as well as an acridine-based derivative DACA [21].

The intercalative moiety consisting of three condensed aromatic units is mostly used as a motif for the construction of bis-intercalators. Some two decades ago large numbers of

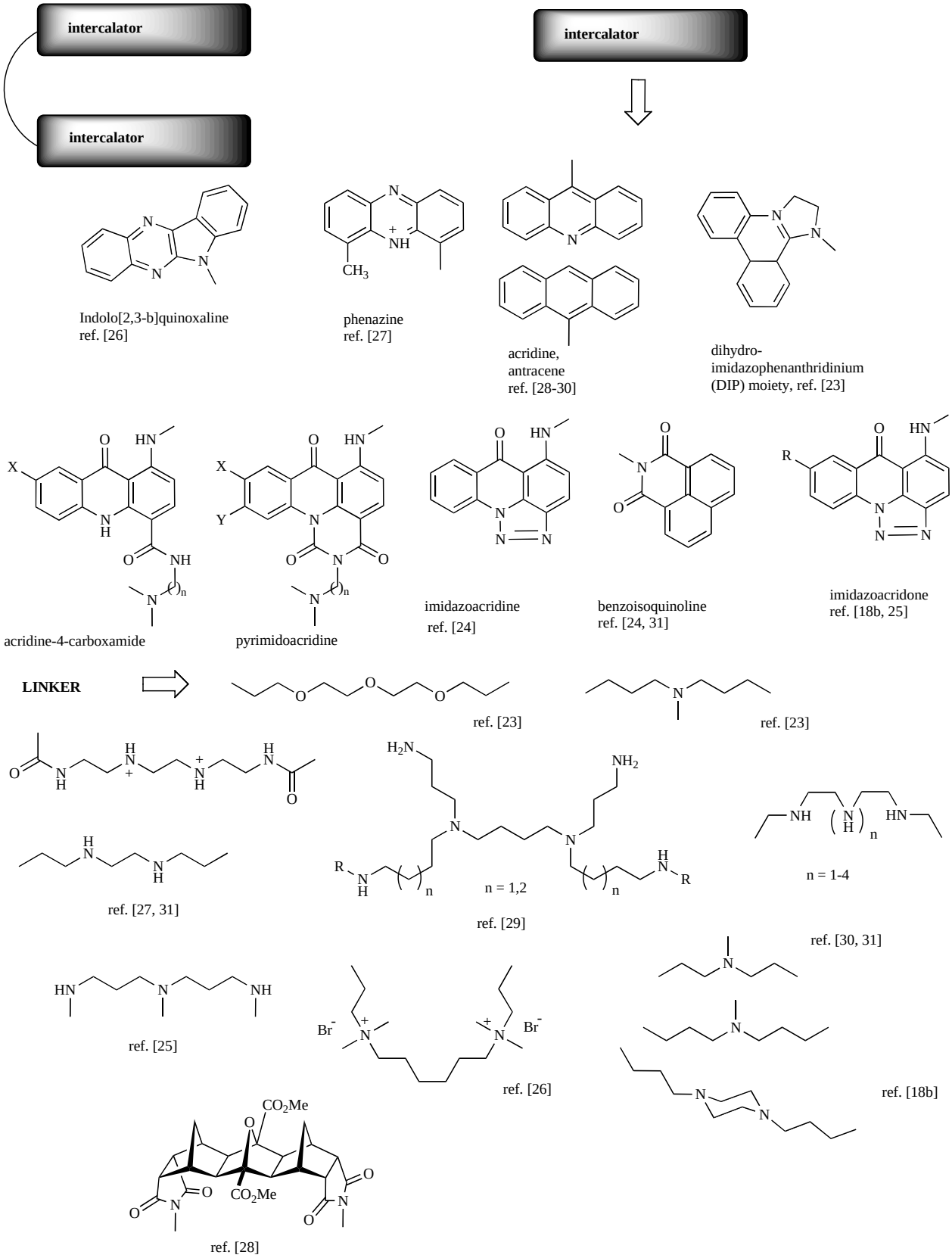

Fig. (2). Examples of aromatic moieties and DNA-active linkers exploited for a construction of recently studied heterodimeric and homodimeric bis-intercalators.

homo-dimers consisting of well known phenanthridinium and acridinium derivatives linked by various (more or less inert) spacers were studied in detail, including their interactions with DNA and screening of biological activity [11]. Obtained results led to the conclusion about the minimal length of a linker allowing bis-intercalation (the length analogous to 6-7 CH_2 groups) as well as important experience was gained about the impact of the linker rigidity and orientation of aromatic units [11].

In the last two decades the focus of the research was shifted on the heterodimers (consisting of two different intercalative moieties) and/or bis-intercalators equipped with linker, which actively contributed to the DNA/RNA binding and recognition (examples of aromatic moieties and linkers are given on Fig. **2**). Recently prepared and studied ethidium – acridine heterodimer characterized by DNA active bispyrrole linker, revealed peculiar interactions with DNA, whereby all three constituents exhibited specific type of interaction with polynucleotide [22]. However, unexpectedly, new compound didn't recognize any specific DNA sequence. At the biological level ethidium – acridine derivative accumulated in cell nuclei and stabilized topoisomerase-II-DNA covalent complexes consequently demonstrating potent cytotoxic activities. Another interestingly modified phenanthridinium analogue (dihydro-imidazophenanthridinium, DIP) also bis-intercalated into short DNA oligomers Fig. (**2**), although again without any applicable sequence selectivity [23].

However, at variance to aforementioned sequence non-selectivity, majority of other bis-intercalators showed more or less pronounced preference toward various polynucleotide sequences. For instance, some derivatives within series of bis-intercalating acridine-consisting heterodimeric bis-aromatics Fig. (**2**) [24] revealed remarkable preference to AT-rich duplexes. Bis-intercalators based on the bisimidazoacridone and indolo[2,3-b]quinoxaline linked by simple aminoalkyl linkers Fig. (**2**) [25, 26] also interacted more efficiently with the AT-rich DNA sequences. Furthermore, bis-phenazine derivative connected by carboxamide amino linker (Fig. (**2**) [27] showed basepair selective binding by strong sequence preference of poly(G-C) over poly(A-T) sequence. Moreover, compound preferred bis-intercalation at the 5'-(TpG):(CpA) site, with the aminoalkyl linker forming additional interactions within the major groove of the DNA. Bis-intercalator consisting of two aminoacridine rings linked through a rigid [3]-polynorbornane scaffold Fig. (**2**) showed binding preference for AT-rich sequences; whereby flexibility and length of linker controlled mono- or bis-intercalation of compound [28].

Series of bis-antracene, bis-acridine and bis-naphthalimide derivatives linked by a variety of different aminoalkyl linkers Fig. (**2**) [29-31] exhibited quite complex pattern of interactions with DNA and RNA. Based on this complex pattern, only general conclusion could be derived that larger aromatic moieties with long spacers between them allow bis-intercalation. However the recognition features toward some specific sequences are still unclear.

The naphthalimide moiety was quite often used as a building block for bis-intercalators due to the versatile steric reasons in respect to linker attachment, easy availability and applicable spectroscopic properties. For instance, a series of bis-naphthalimide derivatives Fig. (**3**) revealed interesting DNA binding mode by combining bis- or even poly-intercalation with threading through the DNA double helix [32-34]. Aside intercalation of naphthalimide moiety, linker structure and length as well as DNA binding ability of various linker-attached groups controlled the linker(s) orientation within major or minor DNA groove. Such combined interactions resulted in selectivity toward different DNA sequences. Even more, dimeric compound, as well as newly synthesized cyclic bis-naphthalimide derivative, exhibit positioning of linkers in both, DNA major and minor groove. Depending on a peptide sequence of the linker some compounds show preference toward specific oligonucleotide sequence, while introduction of a rigid tricyclic spiro-linker [35] yielded much more selective series of compounds, depending also on a *cis*- or *trans*- orientation of naphthalimide moiety. Moreover, a possible relationship between the ring flipping rate and the antitumor activity of a given intercalator was proposed.

A bit uncommon bis-intercalative system is formed by two porphyrins linked to peptidic nanostructures. Although large size of a system does not conform to the idea of "small molecule" it is interesting to note its high preference for G-C over AT sequences [36]. Similarly large system consisting of a bis-arginylporphyrin connected to two acridines by long flexible chains demonstrated ability of bis- and tris-intercalation accompanied by significant preference for major groove binding over minor-groove approach to DNA [37]. Also, cationic hybrid porphyrins linked to anthraquinone moiety by flexible alkyl chains of different length were binding to DNA either by bis-intercalation or by partial intercalation of anthraquinone and outside binding of porphyrin - depending on the linker length [38, 39].

As it can be seen from the structural features presented on the Fig. (**2** and **3**), the size, shape and electronic properties of aromatic (intercalating) parts of bis-intercalators still does not contribute much to the DNA-sequence selectivity. Intriguingly, aminoalkyl-linkers of various structural features are by far the most extensively used for connecting the aromatic moieties. Their applicability is generally based on the positively charged amino-groups, which either interact within minor / major groove of DNA or bind to negatively charged DNA backbone. In that way, aminoalkyl linkers do not only contribute to the overall affinity of compound, but also yield more or less pronounced sequence selectivity. Obviously, fine interplay between the structure and electronic properties of both intercalating moieties and the structural and DNA binding properties of a linker could result in substantially different polynucleotide sequence selectivity of bis-intercalator.

However, from presently available data it is still not possible to derive some more detailed information about the prediction of bis-intercalator selectivity toward some defined DNA sequence; hence presently the most of the researches are mainly focused on an elucidation of this fundamental question.

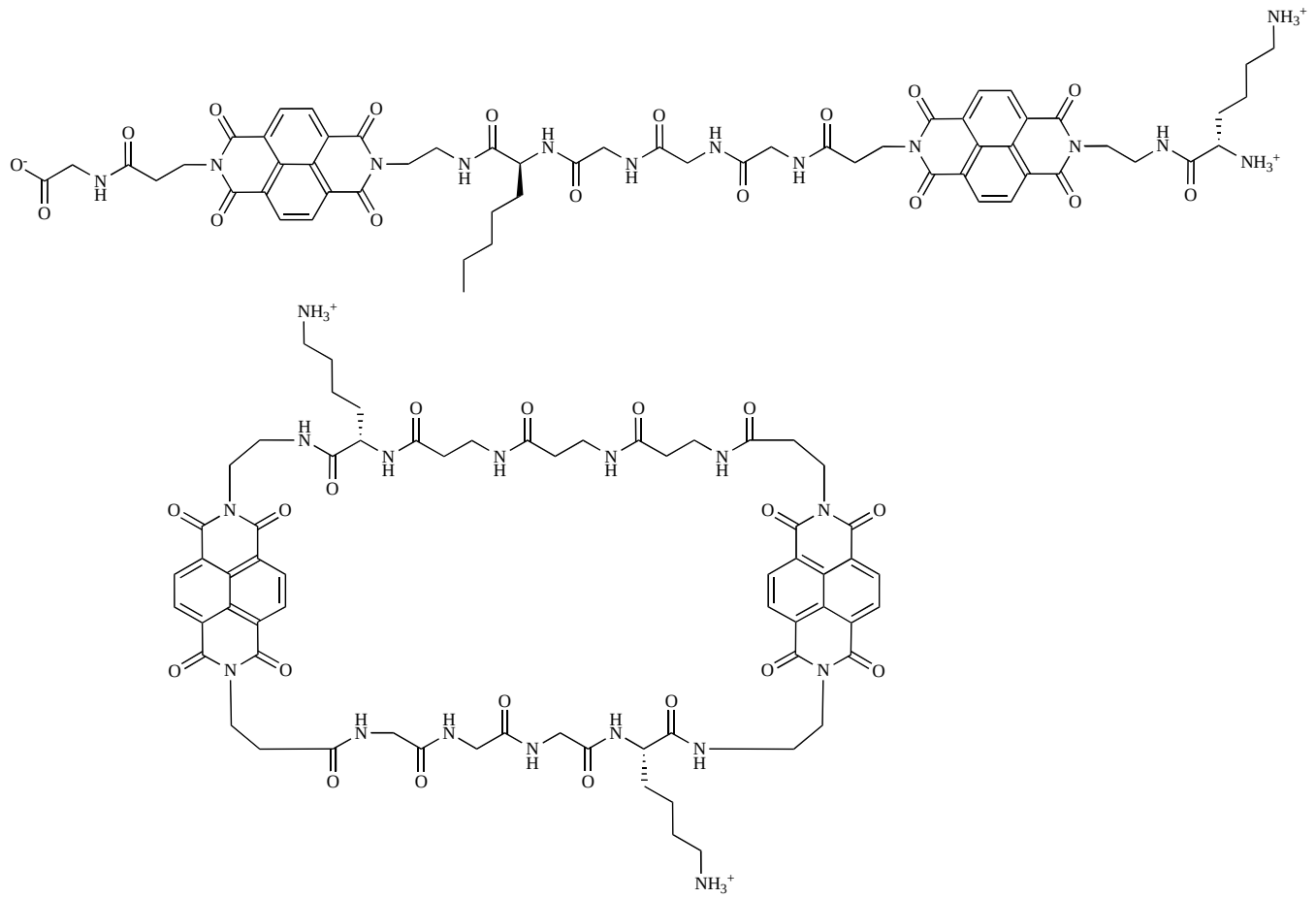

Fig. (3). The representative compounds of the series of recently studied bis-naphthalimide derivatives linked by different combinations of peptide chains.

2.2. Sterically Restricted Bis-Aromatic Compounds

One of the approaches to control the selectivity of the bis-aromatic compounds toward targeted DNA or RNA sequences was design and synthesis of rigid systems characterised by sterically more or less well defined binding cavities or pre-defined orientation of intercalative moieties.

For instance, macrocyclic compounds reveal several interesting features originating from sterical rigidity of binding cavity between aromatic subunits, although additional interactions of linkers also play an important role in some cases. Mostly systems shown on Fig. (**4**) were designed as cyclobis-intercalands (aimed for binding and recognition of nucleotides and similar small molecules), however some of them revealed intriguing selectivity toward very specific single stranded regions in DNA and/or RNA.

A group of cyclobis-intercalators characterized by aliphatic amine bridges constraining distance of 7 Å between the two aromatic moieties Fig. (**4**) [40] revealed a number of intriguing interactions with polynucleotides. Namely, some of them bind to duplexes containing mismatched thymine bases with high selectivity over the fully matched ones, even revealing (naphthalene derivative) preference to pyrimidines – pyrimidine mismatches compared to all other possible base mismatches [41, 42]. Bis-acridine revealed also preference for binding to single stranded (ss) DNA in comparison with double stranded DNA [40]. Moreover, bis-acridine cleaved a ^{32}P-labeled duplex oligonucleotide containing one abasic site [43, 44] and revealed selective photo-cleavage of single-stranded nucleic acids or single-stranded regions in complex nucleic acids like hairpins [45]. A structural explanation for the observed discrimination was that insertion into fully matched DNA is disfavoured because of steric hindrance due to the macrocyclic scaffold. In addition, the short distance between the two intercalator units does not allow bis-intercalation between regular base pairs in line with the nearest-neighbour exclusion principle. The incorporation of the extended aromatic moiety like dibenzophenanthroline (quinacridine), enlarged the size of the binding cavity enough to show significant specificity for quadruplex over duplex interactions [46]. Even larger aromatic moiety like porphyrin Fig. (**4**) didn't yield any significant change of the selectivity in respect to aforementioned compounds, however due to the photoactivity of porphyrin it showed selective photocleavage of the single-stranded polynucleotides like e.g. cleavage of tRNAasp preferentially at single stranded domains [45].

Recent results have pointed out that selectivity of bis-phenanthridinium derivatives toward various DNA/RNA sequences also could be controlled by the steric effects to achieve the preference toward single stranded polynucleo-

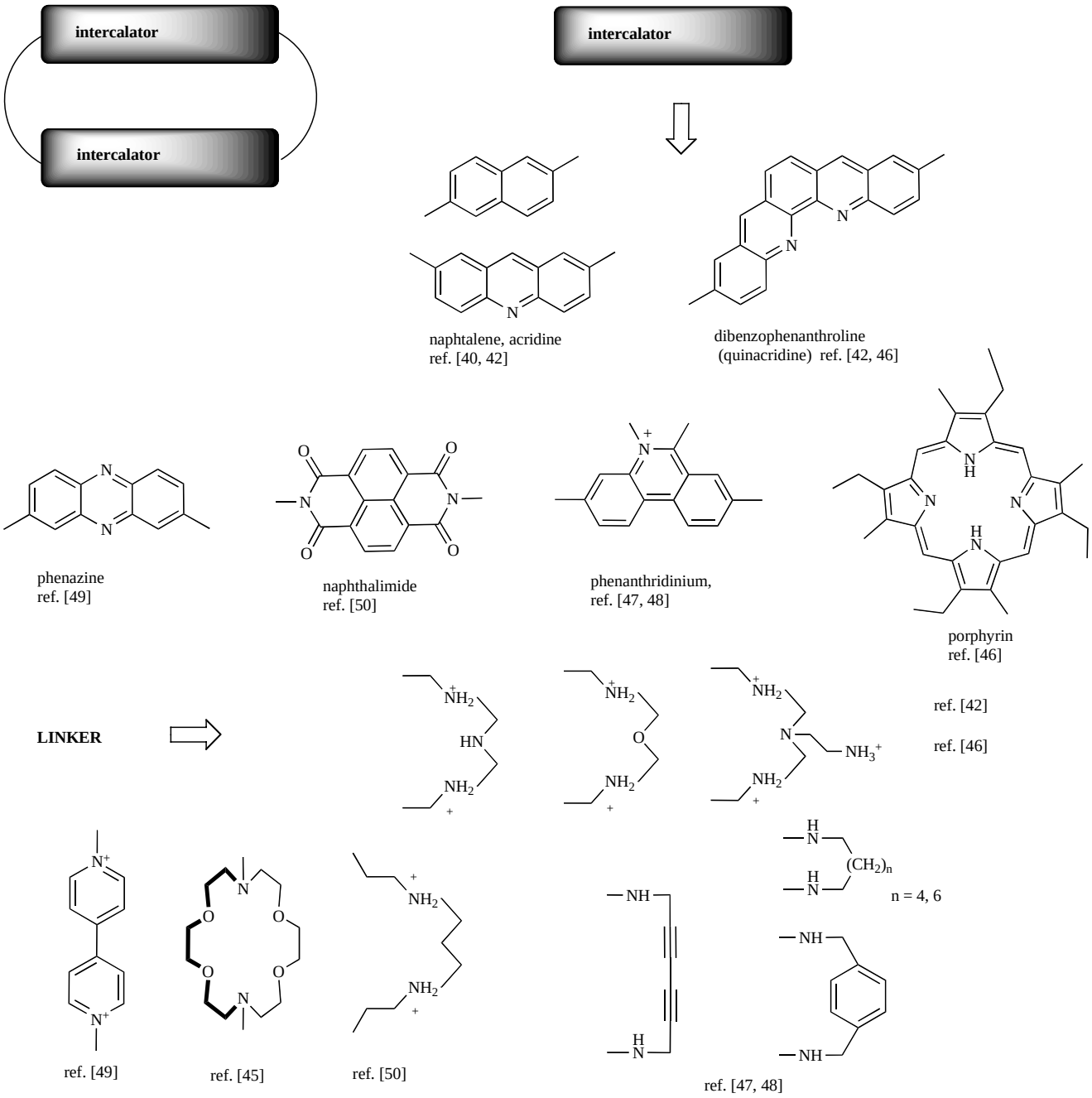

Fig. (4). Examples of macrocyclic derivatives constructed of aromatic moieties and DNA-active linkers.

tides in respect to double stranded ones [47, 48]. An intriguing macrocycle constructed of two phenazine subunits connected by viologen linkers incorporated several functions aside a well defined cavity suitable for steric recognition of specific DNA sequences: photoactive intercalating phenazine subunits, as well as viologen subunits as efficient electron mediators [49]. Consequently compound generated upon visible light irradiation DNA single strand breaks at very low concentrations.

For an naphthalimide-based cyclobis-intercalator authors proposed specific threading bis-intercalation mode to double stranded DNA, whereby the DNA base pairing is temporarily disrupted to allow insertion of a cyclic molecule and formation of a unique structure resembling to a catenane [50].

2.3. Intercalators Equipped by Aromatic Substituents which Contribute to DNA/RNA Binding and Recognition

Large number of compounds designed to be bis-intercalators actually interacted with DNA and RNA by mono-intercalation accompanied by additional interactions of non-intercalated aromatic subunit. Sometimes such interactions resulted in quite interesting DNA/RNA selectivity, observed as spectroscopic change which is dependent on a secondary structure of a polynucleotide. For example, by taking advantage of protonation of phenanthridine at pH<6

the affinity of bis-phenanthridine derivatives toward DNA/RNA was easily controlled by electrostatic (pH modulated) binding with backbone and/or nucleobases [51]. Furthermore, linker length within the urea-substituted bis-phenanthridine derivatives regulated their binding mode toward double stranded polynucleotides, consequently switching the selectivity of compounds toward ds-RNA or toward ds-DNA, as well as yielding the selective fluorescence response toward addition of G-C basepair and A-U(T) basepair containing polynucleotides [52]. However, these mono-intercalating "bis-intercalators" only proved that design of compounds targeting DNA/RNA is still highly challenging task, and due to the really large number of the corresponding examples, which have very few features in common, this family will not be discussed here.

Nevertheless, there are groups of bis-aromatic derivatives by purpose designed to intercalate with one aromatic unit, while other aromatic moiety had to accomplish recognition of targeted DNA/RNA sequence by both aromatic stacking interactions and hydrogen bonding. One of the most extensively studied groups are intercalator – nucleobase conjugates Fig. (**5**), whereby the intercalator should "anchor" the compound to the ds-DNA or ds-RNA by the high affinity of aromatic stacking interactions, while nucleobase should yield the recognition of the nearby abasic site by insertion and formation of hydrogen bonds with the complementary nucleobase [53].

The series of analogues presented on the Fig. (**6**) [53, 54] achieved the aforementioned goal of highly selective binding to abasic sites. Moreover, derivative with bis-guannidinium linker revealed clear synergistic effect in combination with well known anticancer drug bis-chloroethylnitrosourea (BCNU) in both, the *in vitro* experiments on the murine leukaemia L1210 and human adenocarcinoma A549 cell lines and *in vivo* experiments on the murine leukemia P388.

A number of various phenanthridinium – nucleobase conjugates characterized by inert aliphatic linkers interacted selectively with complementary polynucleotide sequences, whereby the polynucleotide hydrophobic environment most likely allowed formation of specific hydrogen bonds between nucleobase attached to intercalator and nucleobases of polynucleotide [55, 56]. Obtained results suggest that high stability and selectivity toward specific single stranded DNA/RNA sequences (like abasic sites, bulges, hairpins) could be achieved by covalently attaching the nucleobase to the bis-intercalator skeleton by linkers, which would allow insertion of nucleobase between two intercalative subunits. In such hydrophobic cleft possible recognition spot for complementary ss- sequence could be formed, whereby targeted basepair would be additionally stabilized by aromatic stacking interactions. The very recent first step toward study of bis-intercalator – nucleobase conjugates already revealed a capacity of such design for the recognition of complementary nucleotide combined with high affinity; [57] a promis-

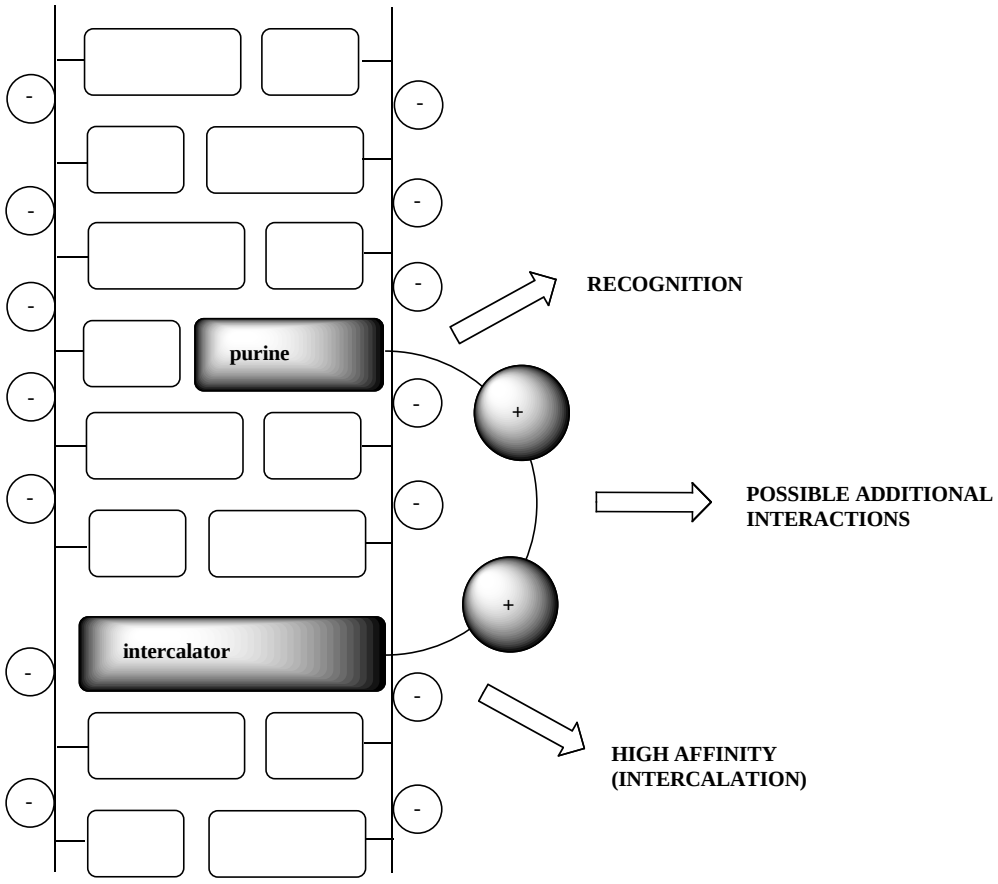

Fig. (5). Schematic presentation of the recognition at the targeted abasic site by an intercalator – nucleobase conjugate.

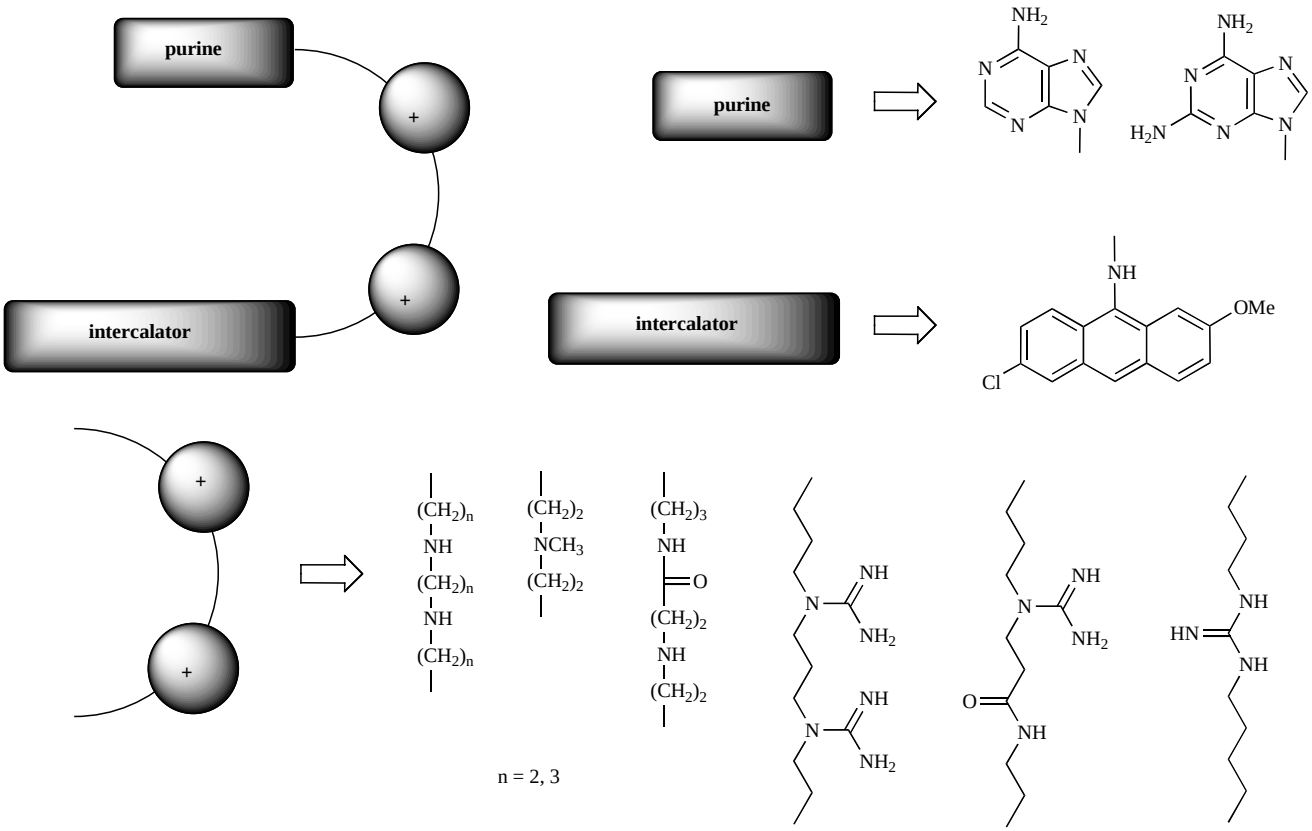

Fig. (6). Example of successful abasic site recognition by intercalator – nucleobase conjugates [53, 54].

ing result for the recognition of single stranded DNA or RNA sequences.

3. CONCLUSIONS AND BIOLOGICAL IMPLICATIONS

There are many biological implications of DNA/RNA active small molecules, whereby the antiviral, antitumor, antibacterial, antiparasitic activity are to most prominent [1, 5, 7, 17]. The small molecules that bis-intercalate into DNA commonly exhibit significantly increased affinity in comparison with the monomer, which again is often accompanied by the enhancement of biological activity and eventually improved medical action of novel dimmeric molecule. However, inspired by naturally occurring bis-intercalators, within last two decades a number of novel bis-aromatic derivatives were prepared characterized by incorporation of "active" linker – the general idea of achieving DNA/RNA sequence selectivity is often accompanied by intriguing biological activity and selectivity, whereby more often than not the recognition and selectivity are observed but not predicted. The most prominent examples of improved activity of bis-intercalators are given here for imidazoacridone, anthracycline, naphthalimide and acridine (DACA) dimmers [18-21].

For several special cases the compounds designed for the targeted goal really have shown expected results. For instance, intercalator – nucleobase conjugates [53, 54] and some of cyclobis-intercalators [42, 43] selectively bind to the DNA abasic site lesion, which is mutagenic or lethal if not repaired. In combination with antitumor drugs or therapies (alkylating agents, γ-rays irradiation) that are based on the massive production of alkylated nucleic bases, such specific abasic site binders would actually act as DNA repair inhibitors and might potentiate the cytotoxic effect of antitumor therapy. Interestingly, the intercalator – nucleobase conjugates which showed the strongest synergistic effect with antitumor drug, revealed the lowest *in vitro* toxicity on studied cell lines [54].

There are, however, several peculiar biological effects of cyclobis-intercalators, e.g. dibenzophenanthroline (quinacridine) derivative is a potent inhibitor of telomerase with activity in the submicromolar range (IC_{50} = 0.13 μM,), most likely due to the selective binding of the macrocycle to the telomeric regions of DNA - the driving force could be the preference of a compound toward telomere G-strand in a form of quadruplex [46].

In addition, intercalators as condensed aromatic systems usually emit strong fluorescence and small molecules emitting specific or at least highly selective signals upon binding to certain DNA/RNA sequences are of high importance for many technologies used in molecular biology and medicine. For instance, fluorescent techniques have been significantly developed during the last two decades and now represent about 60% in the detection of targets and processes [58]. There is great interest in imaging of RNA in human diseases such as various neurological and psychiatric disorders, heart and cancer diseases [59]. However, finding a RNA-selective probe for living cell imaging has proved to be difficult [60],

and only a few RNA visualization agents are currently available. Therefore, most of here presented compounds could also be useful as molecular tools and markers for research in biochemistry and molecular biology.

To conclude, presently available results reveal significant progress in the efficient design of bis-aromatics selectively targeting specific DNA/RNA sequences, although this is still not highly accurate process. Weak points are still prediction of selectivity in the biological systems and efficient design of novel molecule that has targeted spectroscopic properties upon DNA/RNA binding. Nevertheless, all aforementioned stressed the significance of the further research in the corresponding scientific field.

ACKNOWLEDGEMENTS

We thank the Ministry of Science, Education and Sport of the Republic of Croatia for financial support (Grant no. 098-0982914-2918).

REFERENCES

[1] Silverman, R.B. *The Organic Chemistry of Drug Design and Drug Action*, Elsevier Academic Press: Burlington, MA, **2004**.

[2] a) Hermann, T.; Westhof, E. RNA as a drug target: chemical, modelling, and evolutionary tools. *Curr. Opin. Biotech.*, **1998**, *9*, 66-73. b) Chow, C.; Bogdan, F.M. A structural basis for RNA-ligand interactions. *Chem. Rev*., **1997**, *97*, 1489-513.; c) Afshar, M.; Prescott, C.D.; Varani, G. Structure-based and combinatorial search for new RNA-binding drugs**.** *Curr. Opin. Biotechnol*., **1999**, *10*, 59-63.

[3] Wilson, W.D.; Li, K. Targeting RNA with small molecules**.** *Curr. Med. Chem*., **2000**, *7*, 73-98.

[4] Gallego, J.; Varani, G. Targeting RNA with small-molecule drugs: therapeutic promise and chemical challenges. *Acc. Chem. Res*., **2001**, *34*, 836-843.

[5] Demeunynck, M.; Bailly C.; Wilson, W.D. *DNA and RNA binders*, Wiley-VCH: Weinheim, **2002**.

[6] Ihmels, H.; Otto, D. Intercalation of organic dye molecules into double-stranded DNA – general principles and recent developments. *Top Curr. Chem.,* **2005**, *258*, 161-204.

[7] a) Snyder, R.D. Assessment of atypical DNA intercalating agents in biological and *in silico* systems. *Mutat. Res.,* **2007**, *623*, 72-82. b) Snyder, R.D.; McNulty, J.; Zairov, G.; Ewing, D.E.; Hendry, L.B. The influence of *N*-dialkyl and other cationic substituents on DNA intercalation and genotoxicity. *Mutat. Res.,* **2005**, *578*, 88-99.

[8] Luedtke, N. W.; Liu, Q.; Tor, Y. On the electronic structure of ethidium. *Chem. Eur. J.*, **2005**, *11*, 495-508.

[9] Kubar, T.; Hanus, M.; Ryjacek, F.; Hobza, P. Binding of cationic and neutral phenanthridine intercalators to a DNA oligomer is controlled by dispersion energy: quantum chemical calculations and molecular mechanics simulations. *Chem. Eur. J*., **2006**, *12*, 280-290.

[10] Langner, K.M.; Kedzierski, P.; Sokalski, W.A.; Leszczynski, J. Physical nature of ethidium and proflavine interactions with nucleic acid bases in the intercalation plane. *J. Phys. Chem. B,* **2006,** *110*, 9720-9727.

[11] Wakelin, L.P.G. Polyfunctional DNA intercalating agents. *Med. Res. Rev*., **1986**, *6*, 275-340.

[12] Dawson, S.; Malkinson, J.P.; Paumier, D.; Searcey, M. Bisintercalator natural products with potential therapeutic applications: isolation, structure determination, synthetic and biological studies. *Nat. Prod. Rep*., **2007**, *24*, 109-126.

[13] Lorenz, K.B.; Diederichsen, U. Solution-phase synthesis of nucleobase-substituted analogues of triostin A. *J. Org. Chem*., **2004**, *69*, 3917-3927.

[14] Hampshire, A.J.; Rusling, D.A.; Bryan, S.; Paumier, D.; Dawson, S.J.; Malkinson, J.P., Searcey, M.; Fox, K.R. DNA binding by analogues of the bifunctional intercalator TANDEM. *Biochemistry*, **2008**, *47*, 7900-7906.

[15] Portugal, J.; Cashman, D.J.; Trent, J.O.; Ferrer-Miralles, N.; Przewloka, T.; Fokt, I.; Priebe, W.; Chaires, J.B. A new bisintercalating anthracycline with picomolar DNA binding affinity. *J. Med. Chem*., **2005**, *48*, 8209-8219.

[16] Trieb, M.; Rauch, C.; Wibowo, F.R.; Wellenzohn, B.; Liedl, K.R. Cooperative effects on the formation of intercalation sites. *Nucleic Acids Res*., **2004**, *32*, 4696-4703.

[17] Bischoff, G.; Hoffmann, S. DNA-binding of drugs used in medicinal therapies. *Curr. Med. Chem.*, **2002**, *9*, 321-348.

[18] a) Cholody, W.M.; Hernandez, L.; Hassner, L.; Scudiero, D.A.; Djurickovic, D.B.; Michejda, C.J. Bisimidazoacridones and related compounds: New antineoplastic agents with high selectivity against colon tumors. *J. Med. Chem*., **1995**, *38*, 3043-3052. b) Tarasov, S.G.; Casas-Finet, J.R.; Cholody, W.M.; Kosakowska-Cholody, T.; Gryczynski, Z.K.; Michejda, C.J. Bisimidazoacridones: 2. Steady-state and time-resolved fluorescence studies of their diverse interactions with DNA. *Photochem. Photobiol.*, **2003**, *78*, 313-322.

[19] a) Portugal, J.; Martin, B.; Vaquero, A.; Ferrer, N.; Villamarin, S.; Priebe, W. Analysis of the effects of daunorubicin and WP631 on transcription. *Curr. Med. Chem*., **2001**, *8*, 1-8. b) Inge, T.H.; Harris, N.L.; Wu, J.Q.; Azizkhan, R.G.; Priebe, W. WP744 is a novel anthracycline with enhanced activity against neuroblastoma. *J. Surg. Res*., **2004**, *121*, 187-196.

[20] a) Villalona-Calero, M.A.; Eder, J.P.; Toppmeyer, D.L.; Allen, L.F.; Fram, R.; Velagapudi, R.; Myers, M.; Amato, A.; Kagen-Hallet, K.; Razvillas, B.; Kufe, D.W.; Von Hoff, D.D.; Rowinsky, E.K. Phase I and pharmacokinetic study of LU79553, a DNA intercalating bisnaphthalimide, in patients with solid malignancies. *J. Clin. Oncol*., **2001**, *19*, 857-869. b) Bousquet, P.F.; Brana, M.F.; Conlon, D.; Fitzgerald, K.M.; Perron, D.; Cocchiaro, C.; Miller, R.; Moran, M.; George, J.; Qian, X.-D.; Keilhauer, G.; Romerdahl, C. A. Preclinical evaluation of LU 79553: A novel bis-naphthalimide with potent antitumor activity. *Cancer Res*., **1995**, *55*, 1176-1180.

[21] Bridewell, D.J.; Finlay, G.J.; Baguley, B.C. Topoisomerase I/II selectivity among derivatives of *N*-[2-(dimethylamino)ethyl] acridine-4-carboxamide (DACA). *Anti-Cancer Drug Des*., **2001**, *16*, 317-324.

[22] Carrasco, C.; Helissey, P.; Haroun, M.; Baldeyrou, B.; Lansiaux, A.; Colson, P.; Houssier, C.; Giorgi-Renault, S.; Bailly, C. Design of a composite ethidium-netropsin-anilinoacridine molecule for DNA recognition. *ChemBioChem*, **2003**, *4*, 50-61.

[23] Guthrie, K.M.; Parenty, A.D.C.; Smith, L.V.; Cronin, L.; Cooper A. Microcalorimetry of interaction of dihydro-imidazo-phenanthridinium (DIP)-based compounds with duplex DNA. *Biophys. Chem*., **2007**, *126*, 117-123.

[24] Antonini, I.; Santoni, G.; Lucciarini, R.; Amantini, C.; Sparapani, S.; Magnano, A. Synthesis and biological evaluation of new asymmetrical bisintercalators as potential antitumor drugs. *J. Med. Chem*., **2006**, *49*, 7198-7207.

[25] Hernandez, L.; Cholody, W.M.; Hudson, E.A.; Resau, J.H.; Pauly, G.; Michejda, C.J. Mechanism of action of bisimidazoacridones, new drugs with potent, selective activity against colon cancer. *Cancer Res.*, **1995**, *55*, 2338-2345.

[26] Wilhelmsson, L.M.; Kingi, N.; Bergman, J. Interactions of antiviral indolo[2,3-b]quinoxaline derivatives with DNA. *J. Med. Chem.,* **2008**, *51*, 7744-7750.

[27] Dai, J.; Punchihewa, C.; Mistry, P.; Ooi, A.T.; Yang D. Novel DNA bis-intercalation by MLN944, a potent clinical bisphenazine anticancer drug. *J. Biol. Chem*., **2004**, *279*, 46096-46103.

[28] Van Vliet, L.D.; Ellis, T.; Foley, P.J.; Liu, L.G.; Pfeffer, F.M.; Russell, R.A.; Warrener, R.N.; Hollfelder, F.; Waring, M.J. Molecular recognition of DNA by rigid [n]-polynorbomane-derived bifunctional intercalators: Synthesis and evaluation of their binding properties. *J. Med. Chem.*, **2007,** *50*, 2326-2340.

[29] Wang, L.; Price, H.L.; Juusola, J.; Kline, M.; Phanstiel, O. Influence of polyamine architecture on the transport and topoisomerase II inhibitory properties of polyamine DNA-interealator conjugates. *J. Med. Chem*., **2001**, *44*, 3682-3691.

[30] Lomadze, N.; Schneider, H.-J.; Albelda, M.T.; Garcia-Espana, E.; Verdejo, B. Dramatic selectivity differences in the association of DNA and RNA models with new ethylene- and propylene diamine derivatives and their copper complexes. *Org. Biomol. Chem*., **2006**, *4*, 1755-1759.

[31] Gonzalez-Bulnes, L.; Gallego, J. Indirect effects modulating the interaction between DNA and a cytotoxic bisnaphthalimide reveal a two-step binding process. *J. Am. Chem. Soc*., **2009**, *131*, 7781-7791.

[32] Lee, J.; Guelev, V.; Sorey, S.; Hoffman, D.W.; Iverson, B.L. NMR structural analysis of a modular threading tetraintercalator bound to DNA. *J. Am. Chem. Soc.*, **2004**, 126, 14036-42.

[33] Mazzitelli, C.L.; Yongjun, C.; Reczek, J.J.; Iverson, B.L.; Brodbelt, J.S. Screening of threading bis-intercalators binding to duplex DNA by electrospray ionization tandem mass spectrometry. *J. Am. Soc. Mass. Spectrom.* **2007**, *18*, 311-321.

[34] Chu, Y.J.; Hoffman, D.W.; Iverson, B.L. A pseudocatenane structure formed between DNA and a cyclic bisintercalator. *J. Am. Chem. Soc.*, **2009,** 131, 3499-508.

[35] Chu, Y.; Sorey, S.; Hoffman, D.W.; Iverson, B.L. Structural characterization of a rigidified threading bisintercalator. *J. Am. Chem. Soc.*, **2007**, 129, 1304-1311.

[36] Biron, E.; Voyer, N. Towards sequence selective DNA binding: design, synthesis and DNA binding studies of novel bis-porphyrin peptidic nanostructures. *Org. Biomol. Chem*., **2008**, *6*, 2507-2515.

[37] Far, S.; Kossanyi, A.; Verchere-Beaur, C.; Gresh, N.; Taillandier, E.; Perree-Fauvet, M. Bis- and tris-DNA intercalating porphyrins designed to target the major groove: Synthesis of acridylbis-arginyl-porphyrins, molecular modelling of their DNA complexes, and experimental tests. *Eur. J. Org. Chem.*, **2004**, 1781-1797.

[38] Zhao, P.; Xu, L.C.; Huang, J.W.; Zheng, K.C.; Liu, J.; Yu, H.C.; Ji, L.N. DNA binding and photocleavage properties of a novel cationic porphyrin-anthraquinone hybrid. *Biophys. Chem.*, **2008**, *134*, 72-83.

[39] Zhao, P.; Xu L.C.; Huang, J.W.; Fu, B.; Yu, H.C.; Zhang, W.H.; Chen, J.; Yao, J.H.; Ji, L.N. DNA-binding and photocleavage properties of cationic porphyrin-anthraquinone hybrids with different lengths of links, *Bioorg. Chem.*, **2008**, *36*, 278-287.

[40] Teulade-Fichou, M.-P.; Fauquet, M.; Baudoin, O.; Vigneron, J.P.; Lehn, J.-M. DNA double helix destabilizing properties of cyclobis-intercaland compounds and competition with a single strand binding protein. *Bioorg. Med. Chem*., **2000**, *8*, 215-222.

[41] David, A.; Bleimling, N.; Beuck, C.; Lehn, J.-M.; Weinhold, E.; Teulade-Fichou, M.-P. DNA mismatch-specific base flipping by a bisacridine macrocycle. *ChemBioChem*, **2003**, *4*, 1326-1331.

[42] Bahr, M.; Gabelica, V.; Granzhan, A.; Teulade-Fichou, M.-P.; Weinhold, E. Selective recognition of pyrimidine-pyrimidine DNA mismatches by distance-constrained macrocyclic bis-intercalators. *Nucl. Acids Res*., **2008**, *36*, 5000-5012.

[43] Berthet, N.; Michon, J.; Lhomme, J.; Teulade-Fichou, M.P.; Vigneron, J.P.; Lehn, J.-M. Recognition of abasic sites in DNA by a cyclobisacridine molecule. *Chem-Eur. J.*, **1999**, *5*, 3625-3630.

[44] Jourdan, M.; Garcia, J.; Lhomme, J.; Teulade-Fichou, M.P.; Vigneron, J.P.; Lehn, J.-M. Threading bis-intercalation of a macrocyclic bisacridine at abasic sites in DNA: Nuclear magnetic resonance and molecular modeling study. *Biochemistry*, **1999**, *38*, 14205-14213.

[45] Blacker, A.J.; Teulade-Fichou, M.P.; Vigneron, J.P.; Fauquet, M.; Lehn, J.-M. Selective photocleavage of single-stranded nucleic acids by cyclobisintercaland molecules. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 601-606.

[46] Teulade-Fichou, M.P.; Hounsou, C.; Guittat, L.; Mergny, J.L.; Alberti, P.; Carrasco, C.; Bailly, C.; Lehn, J.-M.; Wilson, W.D. Molecular recognition of quadruplex DNA by quinacridine derivatives. *Nucleos. Nucleot. Nucl.*, **2003**, *22*, 1483-1485.

[47] Piantanida, I.; Palm, B.S.; Čudić, P.; Žinić, M.; Schneider, H.-J. Phenanthridinium cyclobisintercalands. Fluorescence sensing of AMP and selective binding to single stranded nucleic acids. *Tetrahedron Lett*., **2001**, *42*, 6779-6783.

[48] Piantanida, I.; Palm, B.S.; Čudić, P.; Žinić, M.; Schneider H.-J. Interactions of acyclic and cyclic bis-phenanthridinium derivatives with ss- and ds- polynucleotides. *Tetrahedron*, **2004**, *60*, 6225-6231.

[49] Lorente, A.; Fernandez-Saiz, M.; Herraiz, F.; Lehn, J.-M.; Vigneron, J.P. Photocleavage of DNA by tetracationic intercalands containing phenazine and viologen subunits. *Tetrahedron Lett.*, **1999**, *40*, 5901-5904.

[50] Takagi, M.; Yokoyama, H.; Takenaka, S. Poly-intercalators carrying threading intercalator moieties as novel DNA targeting ligands. *J. Incl. Phen. Mol. Rec. Chem*., **1998**, *32*, 375-383.

[51] Malojčić, G.; Piantanida, I.; Marinić, M.; Žinić, M.; Marjanović, M.; Kralj, M.; Pavelić, K.; Schneider, H.-J. A novel bis-phenanthridine triamine with pH controlled binding to nucleotides and nucleic acids. *Org. Biomol. Chem*., **2005**, *3*, 4373-4381.

[52] Radić Stojković, M.; Piantanida, I. Tuning urea-phenanthridinium conjugates for DNA/RNA and basepair recognition. *Tetrahedron*, **2008**, *64*, 7807-7814.

[53] Lhomme, J.; Constant, J.-F.; Demeunynck, M. Abasic DNA structure, reactivity, and recognition. *Biopolymers*, **1999**, *52*, 65-83.

[54] Alarcon, K.; Demuynck, M.; Lhomme, J.; Carrez, D.; Croisy, A. Diaminopurine-acridine heterodimers for specific recognition of abasic site containing DNA. Influence on the biological activity of the position of the linker on the purine ring. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 1855-18.

[55] Juranović, I.; Meić, Z.; Piantanida, I.; Tumir, L.-M.; Žinić, M. Interactions of phenanthridinium-nucleobase conjugates with polynucleotides in aqueous media. Recognition of poly U. *Chem. Commun*., **2002**, 1432-1433.

[56] Tumir, L.-M.; Piantanida, I.; Juranović, I.; Meić, Z.; Tomić, S.; Žinić, M. Recognition of homo-polynucleotides containing adenine by phenanthridinium bis-uracil conjugate in aqueous media. *Chem. Commun*., **2005**, 2561-2563.

[57] Tumir, L.-M.; Grabar, M.; Tomić S.; Piantanida I. The interactions of bis-phenanthridinium – nucleobase conjugates with nucleotides: adenine conjugate recognizes UMP in aqueous medium. *Tetrahedron*, **2010**, *66*, 2501-2513.

[58] Trinquet, E.; Mathis, G. Fluorescence technologies for the investigation of chemical libraries. *Mol. Biosyst*., **2006**, *2*, 380-387.

[59] a) MacLaren, D.C.; Toyokuni, T.; Cherry, S.R.; Barrio, J.R.; Phelps, M.E.; Herschman, H.R.; Gambhir, S.S. PET imaging of transgene expression. *Biol. Psychiatr*., **2000**, *48*, 337-348. b) Bartlett, D.W.; Su, H.; Hildebrandt, I.J.; Weber, W.A.; Davis, M.E. Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality *in vivo* imaging. *Proc. Natl. Acad. Sci. U.S.A.*, **2007**, *104*, 15549-54. c) Tian, X.; Aruva, M.R.; Zhang, K.; Shanthly, N.; Cardi, C.A.; Thakur, M.L.; Wickstrom, E. PET imaging of *CCND1* mRNA in human MCF7 estrogen receptor–positive breast cancer xenografts with oncogene-specific [^{64}Cu]chelator-peptide nucleic acid-IGF1 analog radiohybridization probes. *J. Nucl. Med.*, **2007**, *48*, 1699-707. d) Cherry, S.R., The 2006 Henry N. Wagner lecture: Of mice and men (and positrons)–advances in PET imaging technology. *J. Nucl. Med*., **2006**, *47*, 1735-45. e) de Vries, E.F.J.; Vroegh, J.; Dijkstra, G.; Moshage, H.; Elsinga, P.H.; Jansen, P.L.M.; Vaalburg, W. Synthesis and evaluation of a fluorine-18 labeled antisense oligonucleotide as a potential PET tracer for iNOS mRNA expression. *Nucl. Med. Biol*., **2004**, *31*, 605-612. f) Perera, R.J.; Ray, A. MicroRNAs in the search for understanding human diseases. *BioDrugs*, **2007**, *21*, 97-104.

[60] Li, Q.; Chang, Y.-T. A protocol for preparing, characterizing and using three RNA-specific, live cell imaging probes: E36, E144 and F22. *Nat. Protoc*., **2006**, *1*, 2922-2932.

Received: December 10, 2009 Revised: March 13, 2010 Accepted: March 20, 2010