Diazapyrenes: interaction with nucleic acids and biological activity

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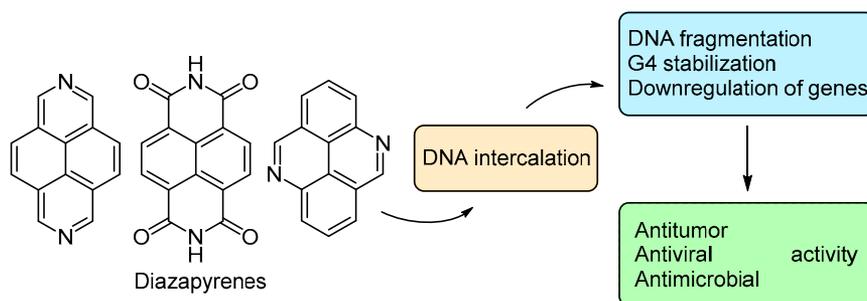
Diazapyrenes

The review summarizes data on the practical aspects of the interaction of nucleic acids with diazapyrene derivatives. The information on biological activity is given and the probable mechanisms underlying the action of diazapyrenes are analyzed. It contains 119 references.

Keywords: diazapyrene, DNA, naphthalenediimide, RNA, biological activity, intercalation, G-quadruplex.

DNA is the pharmacological target for many drugs or compounds currently undergoing clinical trials.[1] Developing new drugs and improving the effects of existing drugs is a complex task that goes beyond improving the specificity of a drug with respect to its biological target. Since in many cases only a small fraction of the administered drug compound reaches the pharmacological target, it is necessary to develop strategies for increasing the accumulation of the drug near cellular DNA. The drug delivery strategy should be aimed at increasing circulation time, enhancing tissue-specific accumulation, increasing the efficiency of cellular and nuclear uptake in addition to high specificity and affinity to DNA. The complexity of biological systems makes it difficult to simultaneously incorporate all of these factors into a single drug development algorithm. However, focusing on specificity is relatively simple because the structure of DNA is well known.[2]

Currently, there are many strategies of exploiting the known DNA structure for specific binding of drug compounds. One approach is to use small molecules that can recognize nucleic acids and bind to specific areas of DNA, major or minor grooves, or integrate between DNA bases arranged in specific nucleotide sequences.

One of the well-studied DNA targets of drugs is the G-quadruplexes (G4).[3] G4 are secondary nucleic acid structures that can form in single-stranded guanine-rich sequences under physiological conditions.[4] Four guanines are paired *via* hydrogen bonds to form G-quadruplexes. These structures are further stabilized in the presence of metal ions such as potassium ions. Studies of their stability showed that these noncanonical secondary DNA structures are capable of destabilizing the double helix, since many G4 structures are thermodynamically more stable than double-stranded DNA (dsDNA), and their denaturation rate is significantly lower.[5]

G4 sequences in eukaryotes play key regulatory functions, including transcriptional regulation of pro-moters[5b] and gene enhancers,[5b,6] translation,[5b,6,7] epigenetic regulation of chromatin[8] and DNA recombination.[5b,8]

In viruses, G4 are involved in key stages of the life cycle.[9] The presence of functionally significant G4 in HIV is known,[10] binding to which determines the antiviral effect of certain drugs.[10a,b,11] G4 were also found in the genomes of herpes virus,[12] SARS coronavirus and human papillomavirus,[13] Zika, Ebola, and hepatitis C viruses.[14] In prokaryotes, G4 sequences were described in *E. coli*,[6,15] *D. radiodurans*,[16] *Xanthomonas* and *Nostoc* sp.,[17] as well as in the genes of *E. coli*, *C. difficile*, and *Bacteroides* sp., encoding helicases Pif1 and RecQ.[18] Bacterial G4 are involved in antigenic variability of the protein pilin of the cell wall of *Neisseria gonorrhoeae*.[19]

The mechanism of action of small molecules on DNA is based on covalent (e.g., cisplatin and bis-β-chloro-ethylamine derivatives)[20] or noncovalent binding with the formation of hydrogen bonds, electrostatic, hydrophobic, and/or π–π interactions. Noncovalent binders are divided into two types: compounds that bind to minor grooves (distamycin, netropsin, and Hoechst 33258), and intercalators (doxorubicin, acridine orange, etc.).[21] Inter-calators are molecules containing a flat fragment, usually consisting of several condensed rings, which interacts with dsDNA by inserting a flat fragment between adjacent base pairs of the double helix, while hydrophobic interactions and π-stacking stabilize the formed complex.[22]

It has been shown by many studies that intercalating compounds with antibacterial and antitumor activity are inhibitors of the enzymes topoisomerase I,[23] topoisome-rase II,[24] topoisomerase IV, and DNA gyrase.[25] These enzymes are able to "relax" supercoiled DNA molecules by introducing single- or double-stranded breaks with subsequent recovery. Intercalation at the site of the interaction of DNA and topoisomerase leads to an increase in the distance between adjacent pairs of nucleotides and does not allow the enzyme to perform the final operation of suturing the cut ends,[26] and the accumulation of breaks in the DNA chain, especially double ones, in turn triggers the process of apoptosis in the cell. Since replication processes are ongoing in tumor tissues, these cells are the most vulnerable to the action of topoisomerase inhibitors.[27]

Attention to azapyrenes is associated both with theoretical aspects and with the results of practical application. Polyazapyrenes are actively used to create molecular devices,[28] redox sensors,[29] compounds with a topological bond,[30] host–guest molecules,[31] and macro-complexes with transition metal cations.[32] An increase in interest in such structures is associated, among other things, with an increase in the number of methods for the synthesis of aza- and polyazapyrenes.[33] In addition, triazapyrene derivatives, representatives of the aaptamine alkaloids family, were isolated from sea sponges of the South China Sea.[34] Aaptamines exhibit a wide range of biological activity: antifungal, antibacterial, antitumor, antiviral, including antiHIV.[35] This review presents data on the formation of complexes, including intercalation complexes, of diazapyrenes with nucleic acids and analyzes the biological activity of diazapyrenes mainly in the light of interaction with DNA.

1. FEATURES OF THE INTERACTION OF DIAZAPYRENES WITH NUCLEIC ACIDS

The interaction of 2,7-diazapyrenes and their quaternary salts with DNA was investigated by Becker et al. 2,7-Diazapyrene (**1**), *N*-methyl-2,7-diazapyrenium cation (**2**) and *N,N'*-dimethyl-2,7-diazapyrenium dication (**3**) bind to dsDNA by intercalation with an approximately perpendicular orientation to the axis of the DNA helix. The thermodynamic parameters of the binding of 2,7-diazapyrenes to DNA are significantly different. Binding of 2,7-diazapyrene (**1**) proceeds with a negative change in enthalpy ΔH and a negative change in entropy ΔS, while the binding of *N,N'*-dimethyl-2,7-diazapyrenium dication (**3**) is entropically favorable, but enthalpically disadvantageous. *N*-Methyl-2,7-diaza-pyrenium cation (**2**) occupies an intermediate position[36] (Table 1).

When complexes of 2,7-diazapyrenium dication **3** (Table 1) and bis(2,7-diazapyrenium) dication **4** (Fig. 1) with supercoiled DNA are irradiated with visible light (λ >395 nm), breaks in the polynucleotide chain are observed. Intercalation of *N,N'*-dimethyl-2,7-diaza-pyrenium dication (**3**) in this case leads to single breaks in the chain with the formation of damaged ring and linear DNA. Irradiation of bidentate complexes leads to a total cleavage of DNA with the formation of small fragments due to multiple cuts of both chains.[37]

By combining the DNA oligonucleotide and the 2,7-diazapyrenium fragment in one molecule, selective DNA cleavage was achieved.[38] As a result, it was found that the DNA chain is cleaved at a distance of 2-3 nucleotides from the point of addition of the 2,7-diazapyrenium ring, mainly in G and GG units.[39] In addition, functionalized oligonucleotides form a more

Table 1. Thermodynamic parameters of complexes of 2,7-diazapyrene (**1**) and its cations **2**, **3** with DNA
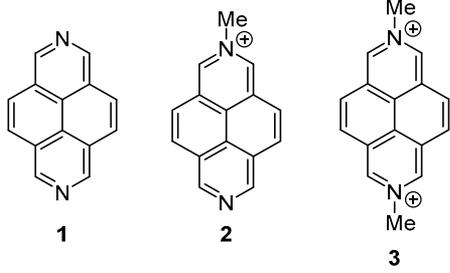
Parameter*	Compound/cation		
	1	**2**	**3**
ΔH, kcal·mol⁻¹	–9	3	5.2
ΔS, cal·mol⁻¹	–7	12	43
ΔG, kcal·mol⁻¹	–6.9	–7.4	7.1
K_S, 10⁵·M⁻¹	1.3	3.2	1.9

* ΔH – change of enthalpy upon complex formation with DNA, ΔS – change of entropy upon complex formation with DNA, ΔG – change of Gibbs free energy upon complex formation with DNA, K_S – DNA binding constant was determined by spectrophotometric titration.
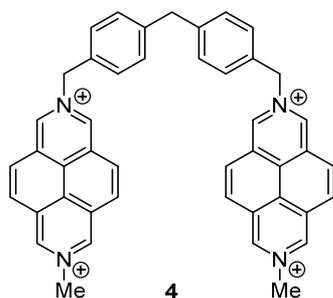
Figure 1. Bis(2,7-diazapyrenium) dication **4**.

stable DNA duplex with complementary strand compared to unmodified oligonucleotides.

Scheme 1
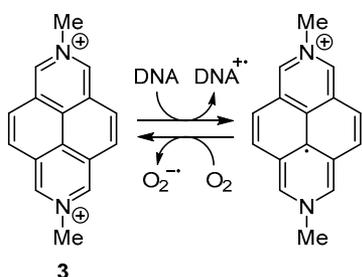
DNA cleavage occurs, most likely, through photo-oxidation of the ribose fragment, followed by hydrolysis of the phosphoester bond. The oxidation mechanism includes the excitation of the 2,7-diazapyrenium dication to the singlet state and the formation of an ion-radical pair with nucleic acid bases due to the single-electron transfer reaction (Scheme 1). Further, the radical cation of 2,7-diazapyrenium dication can be oxidized to a dication with the formation of active forms of O_2.[40]

The ease of the reduction of the quaternary salts of 2,7-diazapyrenium dication is most likely due to the stability of the corresponding radical cation and low reduction potential in comparison with structurally similar compounds[41] (Table 2). The radical cation of 2,7-diazapyrene was characterized by UV spectroscopy and EPR spectroscopy. The intense fluorescence of 2,7-diazapyrenium salts observed in dilute aqueous solutions is quenched by DNA intercalation due to the separation of electrons from neighboring nucleotides.[40a,42]

DNA photooxidation with the formation of radical ion pairs also occurs with the participation of naphthalene-1,4,5,8-tetracarboxylic acid diimides (NDIs).[43] In the case of *N,N*'-(3-hydroxypropyl)diimide of naphthalene-1,4,5,8-tetracarboxylic acid, mainly guanosine-5'-monophosphate and adenosine-5'-monophosphate undergo oxidation. Oxidation of nucleotides G and A in DNA using di-cation **5** containing dimethylamino groups occurs *via* the singlet excited state, while dianion **6** with phosphate groups (Fig. 2) oxidizes nucleotides G and A through the triplet excited state.[44] It is also noted that

Table 2. Redox potential of *N,N*'-dimethyl-2,7-diazapyrenium dication (**3**) and similar dications

Cation	Redox potential, V
3	−0.064
	−0.596
	−0.640

photooxidation of thymine dimer using NDI opens the cyclobutane ring. The destruction of the thymine dimer occurs either by direct photooxidation or by charge transfer using DNA over a distance of 22 Å between the NDI and the cyclobutane ring.[45]

4,9-Diazapyrenium salts form complexes with nucleic bases with a stoichiometry of 1:1 (log K_s 1.6–2.8). The prevalence of aromatic π-stacking over electrostatic interaction in the stabilization of such complexes was established. The large aromatic system of 4,9-diazapyrenium, compared with ethidium bromide (**7**), does not cause a significant increase in the binding constant with nucleotides (log K_s <1–2.3).[46] However, ethidium bromide (**7**) binds single-stranded polynucleotides 100–1000 times less efficiently than its structural analog **8**, the affinity of salts **7** and **8** (Fig. 3) for dsDNA and dsRNA turned out to be approximately equal (binding constants were determined by fluorimetric titration).[47] The presence of substituents in the 4,9-diazapyrenium system does not significantly affect binding to nucleotides, with the exception of two phenyl groups that reduce affinity, probably due to steric interference.[48]

Bis(4,9-diazapyrenium) cation **9** (Fig. 3) forms monointercalated complexes with dsDNA and dsRNA.[49] Additional interactions of the nonintercalated part are largely dependent on the secondary structure of the polynucleotide. Cation **9** exhibits a greater affinity for
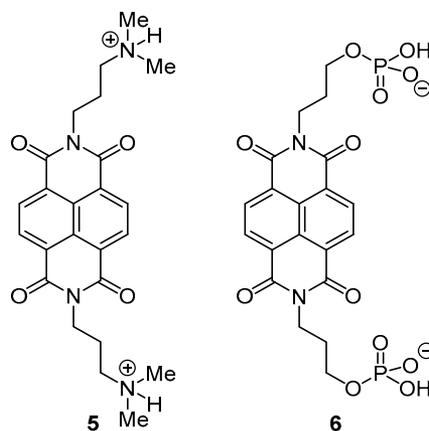
Figure 2. NDI-based dication **5** and dianion **6** used to oxidize G and A nucleotides in DNA.
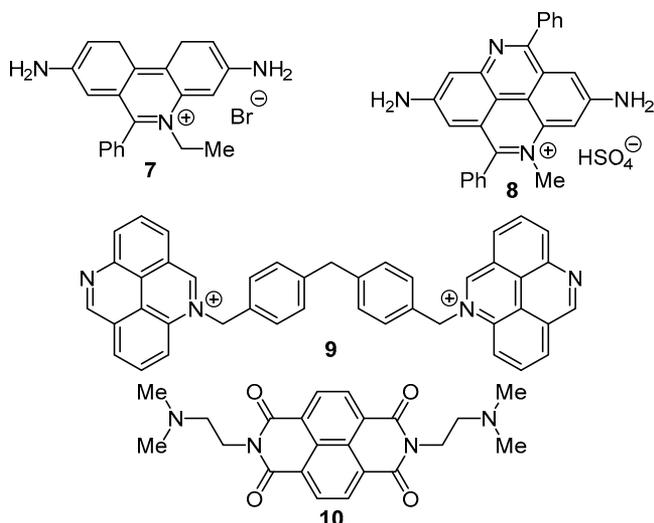
Figure 3. Ethidium bromide (**7**), its structural analog 4,9-diazapyrenium hydrosulfate **8**, bis(4,9-diazapyrenium) cation **9**, and 2,7-diazapyrene derivative **10**, which form complexes with nucleic bases.

DNA than for RNA, due to significant differences in the structure of the helix.[50]

Derivatives of 2,7-diazapyrene and NDI are characterized, as a rule, by higher binding constants with DNA than 2,7-diazapyrenium salts. It was shown that the binding strength of compound **10** (Fig. 3) with DNA depends on the nucleotide composition, the complex of 2,7-diazapyrene **10** with poly(G–C)$_2$ being 20–25 times more stable than complex with poly(A–T)$_2$.[51]

The selectivity of binding of macrocyclic NDIs **11**–**17** (Fig. 4) with quadruplex DNA is higher than that of the noncyclic derivative **5a**, which is most likely caused by steric destabilization of the NDI complex with dsDNA[52] (Table 3).

The binding of macrocyclic derivatives **11** and **12** to the double helix of DNA was proved by circular dichroism spectroscopy and inhibition of topoisomerase I. Thermodynamic parameters (ΔG, ΔH, and ΔS) show that exothermic and entropy-dependent hydrophobic binding were crucial for interaction with DNA (Table 4). Due to the longer linker, compound **11** showed a higher binding selectivity, more entropy-favorable interaction and much slower dissociation from complexes with dsDNA than compound **12** (binding constants were determined by spectrophotometric titration).[53]
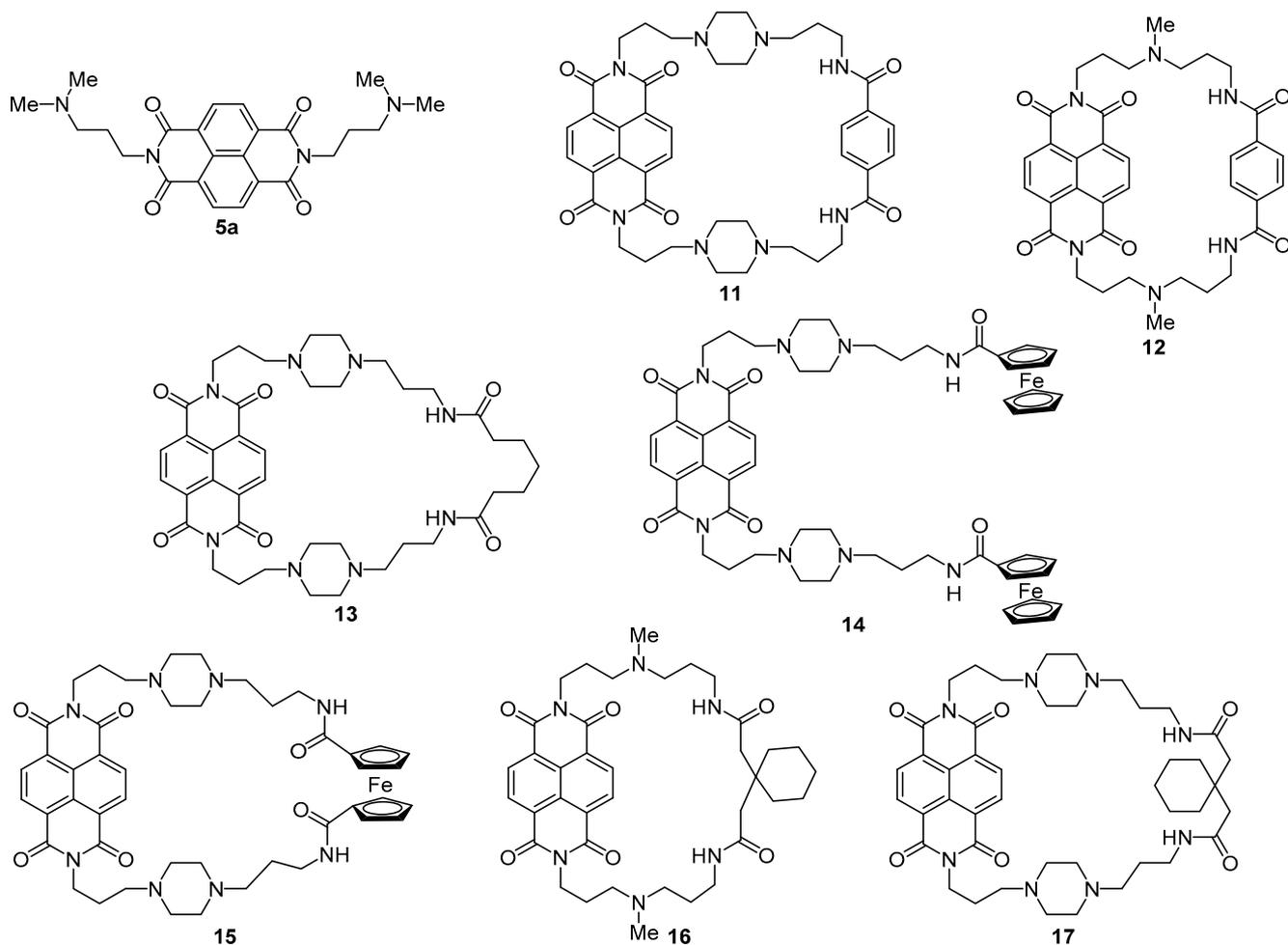
Figure 4. Derivative of NDI **5a** containing diamino groups and macrocyclic NDI derivatives **11**–**17**.

Table 3. Binding constants of NDIs **5a** and **11**–**17** with G4 and dsDNA

Compound	K_S*, 10^6 M^{-1}	
	G4	dsDNA
5a	1.4	1.0
11	3.7	2.6
12	0.5	0.12
13	6.3	0.007
14	2.0	1.0
15	3.4	0.075
16	8.6	0.33
17	1.5	0.3

* Determined by spectrophotometric titration.

Table 5. Binding constants of NDIs **18**–**21** with dsDNA

18–21

Cation	R	K_S*, 10^6 M^{-1}
18	Me	15 ± 3
19	Et	31 ± 8
20	*n*-Pr	43 ± 9
21	*n*-Bu	78 ± 23

* Determined by isothermal titration calorimetry.

Compound **12** exhibits a high affinity for binding to DNA G4 (K_S 10^6–10^7 M^{-1}) in comparison with dsDNA. The binding constants of NDI derivative **12** with G4 of various types (human telomeric DNA, quadruplexes in the promoter region of the *KRAS*, *c-MYC*, and thrombin-binding aptamer genes) are up to 270 times greater than the binding constant of NDI **12** with dsDNA (binding constants were determined by spectrophotometric titration).[53b]

The electrostatic interaction of the side chain of NDI with DNA makes a significant contribution to the stability of the intercalation complex. For the quaternary ammonium salt with cation **5**, K_S equals $6.6 \cdot 10^6$ M^{-1}. A negatively charged phosphate group destabilizes the complex (K_S <10^2 M^{-1} for the complex with cation **6** (Fig. 2)) (binding constants were determined by spectrophotometric titration).[44] Moreover, the nature of substituents of the quaternary ammonium has a great influence on the binding of cations **18**–**21** to DNA. As the size of the substituent increases, the binding constant with dsDNA accordingly increases[54] (Table 5).

The role of the imidazolyl fragment of the side chain of NDI in DNA intercalation was investigated using UV spectroscopy, competitive binding and fluorescence dye bias analysis, circular dichroism, isothermal calorimetry, and computational analysis. The studies revealed ligand **22** (Fig. 5), which forms a complex with dsDNA, characterized by a half-life of 51 min, which was determined using the dissociation kinetic analysis.

Computational simulation confirmed the intercalation of the naphthalene ring between the base pairs and the binding of the imidazolyl fragment to the grooves in DNA through electrostatic interaction and hydrogen bonding. As shown by experimental and calculated data, the interaction of positively charged imidazolium fragments with a negatively charged phosphate backbone of DNA contributes to a favorable change in enthalpy. In addition, intercalation caused a significant slowdown of the DNA band when analyzing changes in electrophoretic mobility.[55]

In contrast to *N,N'*-disubstituted analogs, 4- and 4,9-substituted NDIs **23** and **24** (Fig. 6) form more stable complexes with dsDNA and also show significant selectivity with respect to G4.[56] Selectivity is associated on the one hand with unfavorable steric effects in complexes with dsDNA. On the other hand, electrostatic interactions and the formation of hydrogen bonds between phosphate groups in DNA and amino groups in the side chains stabilize G4.[57]

Another water-soluble 4-substituted NDI chelates the Cu^{2+} ion, and the resulting complex **25** (Fig. 7) is able to bind to G-quadruplex DNA.[58] In addition, complex **25** is capable of reducing H$_2$O$_2$ in the presence of sodium ascorbate to form active O$_2$ forms that do not diffuse in solution and cleave DNA in G4 units. Selective cleavage of G4 DNA was also observed for the LTR-III16 and un2 sequences in DNA of respectively HIV-1 and HSV-1.[12a,58]

Table 4. Thermodynamic parameters of DNA complexes with NDIs **5a**, **11**, and **12**

Parameter	Compound		
	5a	**11**	**12**
ΔH, kcal·mol^{-1}	−6.7 ± 0.7	7.8 ± 1.0	9.2 ± 1.0
ΔS, cal·mol^{-1}	3.0 ± 2.0	53.0 ± 3.3	54.0 ± 3.0
ΔG, kcal·mol^{-1} (25°C)	−7.6	−8.0	−7.0

22

Figure 5. NDI-based ligand **22** containing an imidazolium fragment and forming a complex with dsDNA.

Figure 6. NDIs **23** and **24**, which form complexes with dsDNA.

Figure 8. NDIs **27** and **28**, highly effective O_2 photosensitizers.

Figure 7. Complex **25** of 4-substituted NDI with Cu^{2+} ion, which interacts with G-quadruplex DNA, and imide **26**, participating in nonoxidative hydrolysis of DNA and RNA.

Figure 9. Bis(hydroperoxy) derivative of NDI **29** and its structural analogs **30** and **31** used in the study of dsDNA cleavage by UV radiation and a supramolecular complex **32** of platinum and a 2,7-diazapyrene ligand that binds to dsDNA.

On the other hand, Young et al. showed that the dimethylaminoethyl fragment of compound **26** (Fig. 7) is involved in the nonoxidative hydrolysis of DNA and RNA at the sites 5'-GG-3 'and 5' GC-3'.[59]

Compounds **27** and **28** (Fig. 8) were used as highly effective O_2 photosensitizers for photooxidation of 1,5-di-hydroxynaphthalene and 3,3'-(anthracene-9,10-diyl)dipropionic acid with singlet oxygen ($^1O_2$).[60]

Bis(hydroperoxy) derivative of NDI **29** (Fig. 9) specifically cleaves dsDNA in the 5' site of the 5'-GG-3' sequence by the action of UV radiation. Breaking of the DNA chain occurs by the action of hydroxyl radicals formed during the photolysis of diimide **29**. It is interesting that upon irradiation of derivatives **30** and **31** (Fig. 9), DNA cleavage is not observed even in the presence of piperidine.[61]

The binding constant for supramolecular complex **32** of platinum and the 2,7-diazapyrene ligand (Fig. 9) with dsDNA is an order of magnitude higher than that of the free ligand (K_S $7 \cdot 10^6$ and $5 \cdot 10^5$ M^{-1}, respectively, binding constants were determined by fluorimetric titration).

Inhibition of polymerase chain reaction (PCR) by complex **32** at concentrations higher than $0.1 \cdot 10^{-6}$ M is explained by the malfunction of *Taq* polymerase upon binding of complex **32** to DNA.[62]

NDI-based ligand **33** (Fig. 10) and its complex with Zn^{2+} ions were studied with respect to the telomeric sequences TAGGG(TTAGGG)$_3$ and AGGG(TTAGGG)$_3$, which form different G4 structures depending on conditions. Both compounds have high binding constants with the hybrid type G4 formed in the presence of K^+ ions: K_S $(1.1–2.8) \cdot 10^6$ and $(6.1–9.1) \cdot 10^6$ M^{-1} for ligand **33** and its complex, respectively (binding constants were determined by spectrophotometric titration). The higher affinity for DNA of the complex formed from Zn^{2+} ions and ligand **33** is

33

Figure 10. NDI-based ligand **33**, Zn^{2+} complex of which binds to DNA.

likely due to the formation of an additional electrostatic or coordination interaction between positively charged Zn^{2+} ions and negatively charged phosphate groups of DNA.[63]

NDI-based tetradentant intercalators with peptide linkers are able to specifically bind to specific sequences in the DNA nucleotide chain. Ligand **34** (Table 6) selectively intercalates the GATAAGTACTTATC sequence, the complex with which is $4 \cdot 10^4$ times more stable than a random nucleotide sequence. Kinetic studies have shown a multistep association process with a specific sequence. The dissociation rate constant of the limiting stage of dissociation of ligand complex **34** with DNA is extremely small and corresponds to a half-life of 16 days.[64] The study of the intercalation complex by NMR spectroscopy showed that NDI fragments are located between the base pairs, and the peptide linker is alternately in minor, major, and minor grooves.[65] It was found that the rate of association of the intercalator with DNA depends on the structure of the

peptide linker, but not the nucleotide sequence. The overall C_2 symmetry of DNA-binding cation **34**, apparently, contributes to a higher rate of association, compared with asymmetric ligands **35** and **36** (Table 6). At the same time, the dissociation rate constant depends on the nucleotide sequence: the replacement of one or two nucleotides leads to a significant (up to 30 times) change in the dissociation rate.[66]

NDI derivative **37** containing a chiral epoxy group (Fig. 1) selectively alkylates a guanine fragment in DNA, and in the sequence 5'-GG-3', (*S*)-epoxide alkylates the 5'-G site, whereas the (*R*)-epoxide – only the 3'-G site.[67]

Based on naphthalene-1,4,5,8-tetracarboxylic acid *N,N'*-bis-[3-(1*H*-imidazol-1-yl)propyl]diamide complex with ruthenium **38** (Fig. 11), an ultrasensitive mRNA biosensor was developed.[68] The affinity of NDI derivatives with a ferrocene fragment for DNA duplexes and quadruplexes was used in biosensors to determine dsDNA,[69] and the affinity for DNA–RNA heteroduplex was employed for determination of mRNA[70] and telomerase activity.[71]

Takenaka et al. developed an electrochemical hybrid-dization method for the analysis of a single-nucleotide polymorphism or deletion in the human lipoprotein lipase gene. PCR products of 350 base pairs containing a wild or mutated type sequence were hybridized with a 13 or 15 base DNA probe immobilized on a gold electrode, then differential pulse voltammetry was performed before and after hybridization in the presence of ferrocenyl naphthalene diimide.[72]

Table 6. Tetradentant intercalators **34–36** based on NDI with peptide linkers

34–36

Substituent	Ligand		
	34	**35**	**36**
R^1			
R^2			
X	(β-Ala)$_3$Lys	(β-Ala)$_3$Lys	(β-Ala)$_3$Lys
Y		Gly$_3$Lys	Gly$_3$Lys

Figure 11. The NDI derivative **37**, selectively alkylating the guanine fragment in DNA, and naphthalene-1,4,5,8-tetracarboxylic acid *N,N'*-bis[3-(1*H*-imidazol-1-yl)propyl]diamide complex with ruthenium **38**, on the basis of which an ultrasensitive mRNA biosensor has been developed.

The ferrocenyl intercalator was investigated to develop an electrochemical DNA biosensor using a peptide nucleic acid (PNA) sequence as a capture probe. After hybridization with a single-stranded DNA sequence, an intercalator carrying ferrocene fragments was introduced to bind to the PNA–DNA duplex, and the electrochemical signal of ferrocene molecules was used to control DNA recognition. The developed biosensor demonstrated a linear dependence of the oxidation peak of ferrocenyl naphthalene diimide on the concentration of the target DNA in the range from 10^{-15} to 10^{-10} M with a detection limit of $11.68 \cdot 10^{-15}$ M.[73]

2. BIOLOGICAL ACTIVITY OF DIAZAPYRENES

2.1. Antiproliferative and antitumor activity of diazapyrenes

Hannon et al. reported on the *in vitro* antiproliferative effect of the metal complex **32** (Fig. 9). The antiproliferative activity of complex **32** against tumor cell lines (SISO, A-427, LCLC 103H, and 5637) was higher than that of the free ligand (half maximal inhibitory concentration (IC_{50}) $(3.1–19.2) \cdot 10^{-6}$ and $>20 \cdot 10^{-6}$ M, respectively). The metal complex **32** showed antiproliferative activity at the level of cisplatin, but with a different spectrum of activity.[62]

The antiproliferative activity of *N,N'*-dimethyl-4,9-diazapyrenium dication derivatives **39**–**41** (Fig. 12) was studied *in vitro* on four cell lines: SKBr3, HeLa, CaCo2, and SW620. The growth inhibition effect was dose-dependent and specific for different cell lines. The most pronounced antiproliferative effect at a concentration of 10^{-5} M was observed on SKBr3 cells (91.8 and 85.3% for cations **40** and **41**, respectively) and on SW620 cells (65.3% for cation **39**). The IC_{50} for the studied compounds

39 R^1 = R^2 = H
40 R^1 = Me, R^2 = H
41 R^1 = H, R^2 = Ph
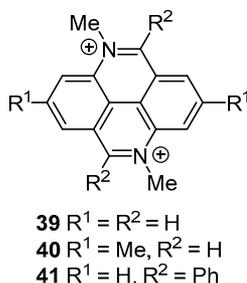
Figure 12. *N,N'*-Dimethyl-4,9-diazapyrenium cations **39**–**41**, which are part of compounds tested for antiproliferative activity.

was in the range of 0.29–52.2 μg/ml. Based on the specific fragmentation of DNA, morphological changes (reduced cell volume, round shape of cells, condensed chromatin), and inhibition of the growth of treated human tumor cells, it is assumed that the tested compounds induced apoptotic cell death.[74]

2,7-Diamino-4-methyl-5,10-diphenyl-4,9-diazapyrenium chloride inhibits the growth of tumor cells with an IC_{50} in the micromolar range for most of the tested tumor cells, with the exception of glioma cells for which the IC_{50} is in the submicromolar range.[75] In addition, 2,7-diamino-4-methyl-5,10-diphenyl-4,9-diazapyrenium hydrosulfate (**8**) (Fig. 3) exhibits fluorescence in the visible spectrum (λ 610 nm), which makes it possible to track the drug in a living cell using fluorescence microscopy. It was noted that the main part of 4,9-diazapyrenium is localized in the cell nucleus.[76]

The cations of bis(4,9-diazapyrenium) salts **9**, **42**, and **43** (Fig. 13) showed antiproliferative activity similar to monomeric derivatives. This observation, together with the DNA or RNA binding assays described above, convincingly indicates that the studied compounds owe their antiproliferative activity to the interaction with DNA and/or RNA within the cell.[49]
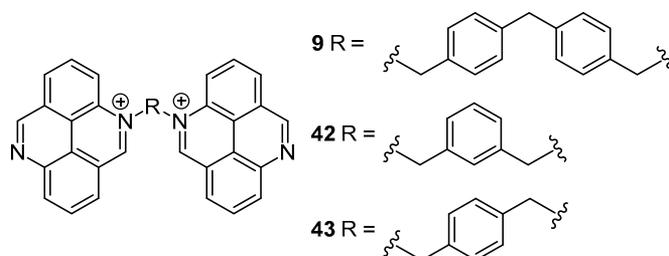
Figure 13. Cations of bis(4,9-diazapyrenium) salts **9**, **42**, and **43** with antiproliferative activity.

The mechanisms underlying the antiproliferative activity of 4,9-diazapyrenium salts are still not fully understood. It was shown that 2,7-diamino-4-methyl-5,10-diphenyl-4,9-diazapyrenium chloride significantly reduces cell clonogenicity and blocks cell proliferation in the G1 phase and also causes the induction of p53 and p21 proteins, which indicates the activation of the reaction of damaging DNA. However, growth inhibition does not require the implementation of the p53 mechanism, since pairs of isogenic p53+/– cells exhibit the same sensitivity.[75] Despite the fact that 2,7-diamino-4-methyl-5,10-diphenyl-4,9-diazapyrenium hydrosulfate (**8**) (Fig. 3) intercalates DNA and RNA, it

does not inhibit topoisomerase II. Most likely, its biological effect is due to impaired expression of the *KRAS*, *c-N-ras*, *cmos*, and *caspase-3* genes, which probably triggers the apoptosis process.[76] On the other hand, structurally close 4,9-dimethyl-5,10-diphenyl-4,9-diazapyrenium bishydrosulfate inhibits topoisomerase II, which causes the accumulation of DNA breaks and apoptosis of tumor cells.[77]

In light of the available data on the regulatory role of G4 sequences in the *KRAS* gene,[78] a possible relationship between the expression of the *KRAS* gene and the binding of 2,7-diamino-4-methyl-5,10-diphenyl-4,9-diazapyrenium cation to G4 located above the initial region of the transcription site. The driving force behind the stabilization of the 4,9-diazapyrene – DNA G4 complex may be the presence of substituents at positions 4, 5, and 10, as was shown for 4,9-disubstituted NDIs.[57] In addition, quinolinium salts with high affinity for G4 sites also have a strong antiproliferative effect on glioma cells and increase the expression of the *caspase-3* gene.[79]

A series of NDIs **5a**, **22**, **44**, and **45** that selectively bind to the hybrid form of human telomeric G4 in potassium phosphate buffer are described. The most selective ligand for this G4 is conjugate **44** based on mannose against a number of other quadruplex and duplex structures. Despite this, however, it did not show high antiproliferative activity. It was shown that ligand **45** containing the methylpiperazine fragment was more toxic for HeLa tumor cells than doxorubicin, although it was three times less toxic to lung fibroblasts of the human embryo WI-38[80] (Table 7).

The ability of tetracation **46** (Fig. 14) to sensitize $^1O_2$ is of interest for its application in photodynamic therapy. In addition, intense fluorescence in the red and near infrared ranges makes it possible to control penetration into cells and accumulation of tetracation **46** within the nuclei. The tetracation exhibits potential bimodal activity as a fluorescent probe and photosensitizer of reactive oxygen species when excited by light entering the so-called therapeutic window of photodynamic therapy. In the absence of light, antiproliferative effects were not observed on tumor cell lines. Irradiating the loaded NDI cells with red light reduces cell viability by up to 40% and causes a significant increase in the proportion of cells expressing γH2AX foci, which indicates DNA damage. The presence of distinct foci of DNA damage within the nucleus suggests that NDI cation **46** is capable of causing DNA damage in certain sequences.[81]

NDI polyamine derivatives inhibit topoisomerase II and *Taq* polymerase (IC$_{50}$ (2.2–8.1)·10^{-6} and (0.25–10)·10^{-6} M, respectively), and can have an antiproliferative effect on
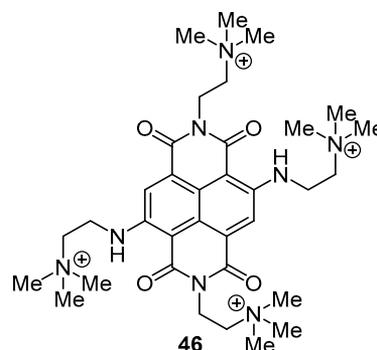
Figure 14. NDI tetracation **46** sensitizing $^1O_2$ and causing DNA damage in specific sequences.

Table 7. Stabilization of telomeric G4 F21T in potassium phosphate buffer and antiproliferative activity of NDIs **5a**, **22**, **44**, and **45** in relation to WI-38, HeLa, MCF7, MDA-MB-231 cell lines

5a, 22, 44, 45

Compound/cation	R	ΔT_{max}, °C	IC$_{50}$, 10^{-6} M			
			WI-38	HeLa	MCF7	MDA-MB-231
5a		2.3 ± 0.5	0.72	0.38	0.53	8.10
22		3.7 ± 0.3	40.22	3.63	11.77	–
44		10.1 ± 0.3	>100	>100	>100	>100
45		9.8 ± 0.6	2.28	0.41	3.09	0.81

SR, MDA-MB-468, and MOLT-4 tumor cell lines with submicromolar and nanomolar values of the concentration inhibiting the growth of 50% of cells (GI_{50}). A systematic study of the structure–activity relationship showed that an increase in activity correlates with an increase of the positive charge of the molecule.[82]

Compounds **47**–**50** are active against tumor cells in submicromolar concentrations with toxicity to normal fibroblast cells WI-38 up to 100 times lower compared to tumor cells (Table 8). Compounds **47**–**50** were found to inhibit topoisomerase IIIα and telomerases in MCF7 cells.[56,83] Inhibition of tumor cell growth is probably associated with a suppression of expression of the chaperone protein HSP90, a regulator of telomerase activity.[84] The promoter region of the *HSP90* gene contains two G4 sequences, binding to which leads to the suppression of gene expression. Tetrasubstituted NDI **50** stabilizes telomeric G4 and G4 in the promoter region of the *KIT* and *HIF-1α* oncogenes, as well as G4 in mRNA of the *BCL-2* gene.[85] Compound **48** is capable of initiating aging in tumor cells, probably as a result of telomere conformation disruption by stabilization of G4 and subsequent DNA damage, as demonstrated by the detection of γ-H2AX foci. In addition, it promotes the fusion of telomeric chromosomes, which leads to genomic instability and subsequent apoptosis.[84] Using confocal microscopy, it was found that compound **50** is able to penetrate the cytoplasm and nucleolus of a cell, which causes an increase in telomeric associations in cells that stimulate chromosomal instability.[85] With parenteral administration, NDI **48** accumulates mainly in tumors and pancreas and to a lesser extent in the lungs. Only trace amounts of NDI **48** were found in the heart, kidneys, liver, and spleen.[84]

Macrocyclic NDIs containing spermidine- and spermine-like side chains stabilize both the *KIT2* sequence and telomeric sequences (ΔT_{max} 33.1 and 26.8°C, respectively), but do not inhibit telomerase. Calculations confirmed that the most stabilizing molecule is also the best G4 binder. On this basis, an assumption was made about the electrostatic effect of the side chains as a driving force of the binding process. At the same time, the antiproliferative effect within cells turned out to be not associated with G4 stabilization (the higher ΔT_{max}, the lower the antiproliferative effect and *vice versa*) and does not have cell type selectivity, which is probably due to the physicochemical properties of the molecules.[52c]

Amino acid-functionalized NDIs were synthesized and studied as ligands for G4. One of the compounds demonstrated selective stabilization of the KIT2 sequence (ΔT_{max} 14.6°C) in combination with a slight destabilization of the KIT1 sequence *in vitro*.[86]

The crystal structures of three complexes with telomeric intramolecular human G4 showed that two of the four strongly basic *N*-methylpiperazine groups can be replaced by less basic morpholine groups without the loss of intermolecular interactions in the grooves of G4. The new compounds retain a high affinity for human telomeric quadruplex DNA, but are 10 times more effective against the pancreatic cancer cell line MIA PaCa-2 with IC_{50}

Table 8. Antiproliferative activity of NDIs **47**–**50** in relation to MCF7, A549, WI-38 cell lines

47–50
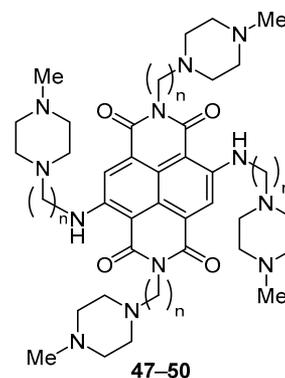
Compound	n	IC_{50}, 10^{-6} M		
		MCF7	A549	WI-38
47	2	1.01	0.47	5.59
48	3	0.17	0.11	9.04
49	4	0.10	0.07	5.50
50	5	0.20	0.26	8.43

values of ~10 nM. Compound **51** (Table 9) induces cell aging, but does not inhibit telomerase at nanomolar concentrations necessary to inhibit cell proliferation. Real-time PCR analysis of the gene array of MIA PaCa-2 cells treated with compound **51** revealed significant dose-dependent modulation of individual subgroups of genes, including strong induction of genes sensitive to DNA damage (*CDKN1A*, *DDIT3*, *GADD45A/G*, and *PPM1D*), and gene repression involved in maintaining telomeres, including *hPOT1* and *PARP1*.[87]

Tetrasubstituted NDI **51** (Table 9) has significant *in vivo* antitumor activity against the xenograft model of the MIA PaCa-2 pancreatic cancer. Intravenous administration of 15 mg/kg of compound **51** twice a week for 40 days causes a decrease in tumor growth to 80% in the group of animals with tumors. On day 40 of the experiment, in the control group and the group receiving 10 mg/kg NDI **51**, zero survival was observed, while in the group with a 15 mg/kg dose, 6 out of 8 animals survived, and 2 of them showed complete tumor regression. Further observation of them for 239 days showed no signs of tumor resumption in the absence of drug administration. The accumulation of the drug in the tumor with a primary localization in the cell nucleus was noted. The level of *BCL-2* gene expression in animal tumors under the action of compound **51** was reduced to 40%. Based on the data of molecular modeling, the authors hypothesized that ligand **51** based on NDI binds to G4 of the *BCL-2* gene in the same way as previously observed in crystalline structures with human telomeric G4. This confirms the concept that NDI **51** can bind to promoter regions containing quadruplex sequences and thus inhibit their transcription.[88]

Analysis of the structural activity data of tetrasubstituted NDIs **51**–**55** containing a morpholine fragment shows that compounds with a different number of methylene units in the side chains between the morpholine and *N*-methyl-piperazine fragments tend to increase antiproliferative activity and, in some cases, exhibit excellent ability to stabilize G4 DNA (Table 9). However, no clear correlation was found between binding affinity to G4 and biological activity.[89]

A recent work presented trisubstituted NDI **56** (Fig. 15), which exerts an antiproliferative effect on human pancreatic duct adenocarcinoma cell lines.[90] Its activity was predicted by computer simulation based on the crystal structure of NDI **51** complexed with telomeric quadruplex DNA.[87] Rationally developed molecule **56** stabilized G4 *in vitro*, although not as much as the original molecule **51**, and showed significant antiproliferative activity (IC$_{50}$ $(7 \pm 2) \cdot 10^{-9}$ M) on PDAC cell lines. The authors analyzed the global genome transcript using the RNA-seq method to identify all the genes affected by the molecule. Suppression of expression was observed in a complex of genes enriched by the formation of quadruplex sequences. In addition, treatment of cells with compound **56** leads to an increase in the foci of G4-specific BG4 antibodies and double-stranded DNA damage.[90]

Tetrasubstituted NDI **57** with pyrrolidine fragments stabilizes the KIT2 sequence and telomeric sequence of human DNA with similar ΔT_{max} values (29.0 and 28.7°C,
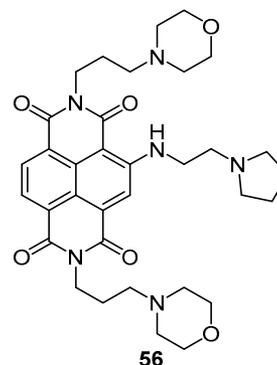
Figure 15. Trisubstituted NDI **56**, which has an antiproliferative effect on human pancreatic duct adenocarcinoma cell lines.

respectively).[56] The antiproliferative activity of compound **57** with respect to the cell lines GIST882, HGC-27, HT-29, and MCF7 was higher, than other G4 ligands, such as BRACO-19 and TMPyP4 (Fig. 16). In addition, NDI **57** reduces the transcription of the *KIT* gene by 90% and completely inhibits the expression of this gene at the dosage necessary to obtain an antiproliferative effect in cells (10^{-6} M). Given the strong inhibition of telomerase, evaluated using the TRAP-LIG assay, the authors hypothesized a double mode of action, with inhibition of telomerase and suppression of *KIT* gene expression.[91]

Table 9. Antiproliferative activity of NDIs **51**–**55** against RCC4, 786-O, MCF7, MIA PaCa-2, A549, and WI-38 cell lines
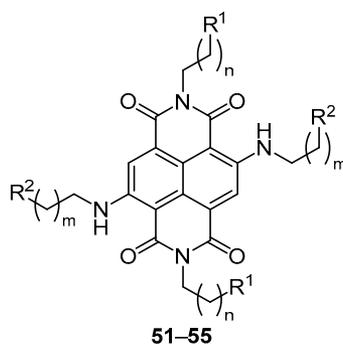
Com-pound	R^1	R^2	n	m	IC$_{50}$, 10^{-6} M					
					RCC4	786-O	MCF7	MIA PaCa-2	A549	WI-38
51	O-morpholine-N-§	Me-N-piperazine-N-§	2	2	0.56 ± 0.05	0.32 ± 0.01	0.070 ± 0.007	0.01 ± 0.01	0.019 ± 0.005	0.23 ± 0.01
52	Me-N-piperazine-N-§	O-morpholine-N-§	2	2	0.61 ± 0.07	0.44 ± 0.07	0.014 ± 0.001	0.050 ± 0.002	0.007 ± 0.001	0.300 ± 0.023
53	Me-N-piperazine-N-§	O-morpholine-N-§	1	2	0.30 ± 0.02	0.16 ± 0.02	0.021 ± 0.001	0.005 ± 0.001	0.020 ± 0.001	0.180 ± 0.002
54	O-morpholine-N-§	Me-N-piperazine-N-§	1	2	1.75 ± 0.18	0.63 ± 0.06	0.17 ± 0.01	0.04 ± 0.01	1.55 ± 0.02	0.61 ± 0.02
55	O-morpholine-N-§	Me-N-piperazine-N-§	2	1	0.28 ± 0.06	–	0.03 ± 0.01	0.01 ± 0.01	0.023 ± 0.006	2.46 ± 0.02

The mechanism of action of tetrasubstituted NDIs with pyrrolidine fragments involves binding to G4 DNA in the *BCL-2* gene and its transcript. Compound **57** strongly stabilizes the G4 series (ΔT_{max} 11.2–29°C), including DNA from the *BCL-2* promoter and G4 RNA from the 5'-untranslated region, and reduces the production of Bcl-2 protein in imatinib-resistant cells of gastrointestinal stromal tumors.[92]

Figure 17. NDI derivatives **58**, tested for antiproliferative activity against SKBR-3, CEM, and HL60 cell lines.

active (n = 2 and 3, IC$_{50}$ 0.9·10^{-6} and 1.2·10^{-6} M, respectively). The mechanism of their action is associated with the activation of caspase, the accumulation of p53 protein, and the suppression of AKT kinase.[96]

Introduction of quaternary salts of Mannich bases, *o*-quinone methide precursors into the structure of NDI made it possible to obtain selective and soft alkylating agents **59**–**61** (Fig. 18). The alkylation process with compound **59** occurs much more efficiently in the presence of DNA sequences capable of forming G4.[97] The introduction of alkylamine groups into the structure of NDI significantly increases the antiproliferative effect with respect to cell lines HT-29, A549, and SK-MEL-5 (IC$_{50}$

Figure 16. NDI **57** containing pyrrolidine moieties and G4 ligands TMPyP4 and BRACO-19.

Bifunctional compounds containing NDIs and coumarin rings showed activity against six tumor cell lines in the micromolar to submicromolar concentration range.[93]

Bisimidazole ligands based on the NDI ring inhibit the growth of A549, HeLa, and HEK 293T cell lines in the micromolar concentration range. The compound with the methylene linker between the imidazole and naphthalene diimide rings is more active than the derivative with the *p*-phenylene fragment, which is probably due to its increased stabilization.[94] In addition, these compounds inhibit telomerase and also show less antiproliferative activity with respect to normal cells in comparison with tumor cells. The biological significance of potent imidazolyl–NDI conjugates is determined by the inhibition of topoisomerase I and their cytotoxicity in HeLa cells.[95]

The antiproliferative activity of compounds **58** (Fig. 17) was studied on SKBR-3, CEM, and HL60 cell lines. It was shown that for the SKBR-3 and CEM lines, the antiproliferative effect of NDIs **58** is independent of the length of the hydrocarbon linker (IC$_{50}$ (0.2–1.7)·10^{-6} and (0.4–2.5)·10^{-6} M, respectively). However, for HL60 cells, compounds **58** with a short linker turned out to be more

Figure 18. NDI derivatives **59**–**62** – alkylating agents with antiproliferative properties.

$(0.4–9)\cdot10^{-6}$ M).[98] Alkylation of telomeric G4 by compounds **62** containing an amide fragment (Fig. 18) occurs 100–1000 times more efficiently than by single and double-stranded oligonucleotides. Their antiproliferative activity against telomerase-positive carcinoma cells was slightly higher than with respect to telomerase-negative cell lines (half-maximum effective concentration (EC_{50}) (1.4–27.5)$\cdot10^{-6}$ and (18–40)$\cdot10^{-6}$ M, respectively).[99] The antiproliferative effect of compound **61** is associated with inhibition of the expression of the *RET* proto-oncogene. Biophysical and reporter gene analysis showed that impaired *RET* gene expression was due to G4 stabilization in the gene promoter caused by alkylation in the G4 region. In addition, NDI derivative **61** inhibits *RET* gene expression and has an *in vivo* antitumor effect without obvious signs of general toxicity.[100]

NDIs **64**–**66** with isothiocyanate groups (Fig. 19), functional analogs of the anticancer agent sulforaphane (**63**), have shown greater antitumor activity than sulforaphane (**63**). Compounds **64**–**66** exhibited antiproliferative activity against the Jurkat cell line of human T-lymphoblastic leukemia in the submicromolar concentration range. It was noted that NDI **66** inhibits the cell cycle in phase G1.[101]

A number of asymmetric derivatives of NDI were synthesized and evaluated for anticancer properties using various experimental methods. The anticancer effects of these compounds are significantly affected by their substituents. Among the analyzed NDI derivatives, compound **67** (Fig. 19) has a stronger inhibitory effect on SMMC-7721 and Hep G2 hepatoma cells than on non-tumor QSG-7701 cells, which indicates the selective action of NDI **67** against hepatoma cells. Compound **67** induces
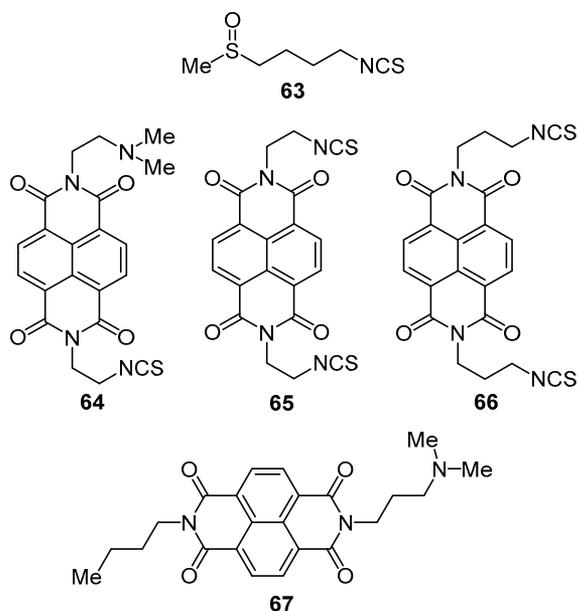
Figure 19. The anticancer agent sulforaphane (**63**), its functional analogs NDIs **64**–**66** with isothiocyanate groups, and NDI derivative **67** with selective action against SMMC-7721 and Hep G2 hepatoma cells.

autophagy and, in a concentration-dependent manner, inhibits the migration of hepatoma cells as a result of the generation of reactive oxygen species.[102]

A series of hybrid compounds was developed by Milelli et al. to bind histone deacetylases with G-quadruplex DNA by combining the structural elements of scriptaid (**68**) and NDI in one molecule. The most active of them, compound **69** (Fig. 20), binds various DNA structures, inhibits histone deacetylases both *in vivo* and *in vitro* and can cause a decrease in cell proliferation. Moreover, derivative of NDI **69** exhibits the property of reprogramming the cell phenotype – preventing the epithelial-mesenchymal transition in tumor cells, leading to cells of a less aggressive and migratory phenotype.[103]
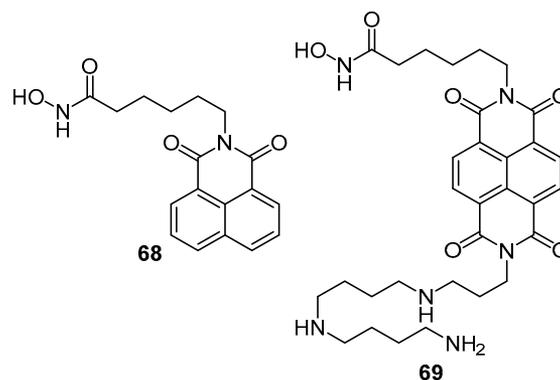
Figure 20. Scriptaid (**68**) and its NDI derivative **69** inhibiting histone deacetylase.

NDI–carbohydrate conjugates **70**–**75** showed antiproliferative activity in the micromolar concentration range (Table 10). The carbohydrate moiety appears to improve the intracellular uptake of the active compound. There is a correlation between the intensity of absorption of glycosylated NDI and their high toxicity with respect to tumor cell lines. Carbohydrate NDIs appear to be mainly translocated to tumor cells *via* glucose transporters, where GLUT4 plays a major role. Interestingly, the addition of a monosaccharide *via* a short ethylene linker to the NDI ring (compound **74**) leads to a 2–3-fold increase in cellular absorption and 5.7–8-fold increase in antiproliferative activity, as compared to compound **71**, where the carbohydrate is attached directly *via* a glycosidic bond.[104]

2.2. Antibacterial, antiviral, and antiprotozoal activity of diazapyrenes

NDI derivatives **76**–**79** with an extended aromatic ring (Fig. 21) bind and stabilize G4 to a high degree, and some of these compounds showed a certain selectivity for viral G4 compared to human telomeric G4. This feature leads to activity against HIV-1 in the nanomolar concentration range for two strains of the virus. This is one of the first examples of G4 ligands that show increased selectivity for viral G4 and exhibit significant antiviral activity.[11b]

Compound **76** (Fig. 21) was tested for antiviral activity against the HSV-1 herpes virus. It showed greater antiviral activity compared to acyclovir. Treatment of cells infected

with HSV-1 with NDI **76** at low concentrations (IC_{50} $(18.3 \pm 1.4) \cdot 10^{-9}$ M) causes significant inhibition of the virus without pronounced signs of cytotoxicity.[105]

Bioinformational analysis of the *M. tuberculosis* genome revealed more than 10,000 fragments with the potential for the formation of G4 structures. The synthetic ligand for the detected G4 based on NDI **76** has an antibacterial effect on *M. tuberculosis* in the submicromolar concentration range (the minimum inhibitory concentration for 80% of the studied strain (MIC_{80}) $1.25 \cdot 10^{-6}$ M).[106]

A theranostic compound containing two vancomycin fragments was synthesized based on the 1,8-diazapyrene ring. The drug exhibits high and selective antibacterial activity against Gram-positive bacteria, including strains resistant to vancomycin, with minimal effect on Gram-negative bacteria and mammalian cells. In addition, this preparation can be used for selective two-photon fluorescence imaging of Gram-positive bacteria.[107]
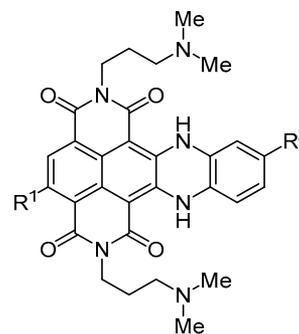
76 R^1 = H, R^2 = H
77 R^1 = Br, R^2 = H
78 R^1 = H, R^2 = NO_2
79 R^1 = Br, R^2 = NO_2

Figure 21. Condensed NDI derivatives **76–79** with antiviral activity.

Table 10. Antiproliferative activity of NDIs **70–75** against HT-29, MCF-7, HeLa, and MRC-5 cell lines

70–75

Compound	R	IC$_{50}$, 10^{-6} M			
		HT-29	MCF-7	HeLa	MRC-5
70		2.92 ± 0.48	1.19 ± 0.31	1.56 ± 0.70	1.15 ± 0.29
71		2.26 ± 1.03	1.06 ± 0.01	0.95 ± 0.76	0.51 ± 0.01
72		0.40 ± 0.08	0.69 ± 0.71	0.35 ± 0.05	0.91 ± 0.32
73		1.85 ± 0.19	1.42 ± 0.38	0.54 ± 0.34	2.04 ± 0.05
74		0.42 ± 0.05	0.15 ± 0.04	0.29 ± 0.14	0.81 ± 0.44
75		0.12 ± 0.02	0.13 ± 0.07	0.29 ± 0.18	0.71 ± 0.25

A new class of bifunctional molecules **80–82** (Fig. 22) was synthesized by combining acridine and redox-active NDI rings, both directly and through a flexible linker. The adducts with a rigid bond of acridine and NDI showed activity with respect to the chloroquine-sensitive strain of the human malaria pathogen *P. falciparum* in micromolar to submicromolar concentration range (compound **80**: IC$_{50}$ 0.419·10^{-6} M). Compounds with a flexible linker between aromatic rings showed high activity both against chloroquine-sensitive (compounds **81** and **82**: IC$_{50}$ 4.33·10^{-9} and 3.65·10^{-9} M) and against chloroquine-resistant *P. falciparum* strains (compounds **81** and **82**: IC$_{50}$ 28.53·10^{-9} and 52.20·10^{-9} M) .[108]

Table 11. Antiparasitic (against *L. major*, *T. brucei*, and *P. falciparum)* and antiproliferative activity (against MRC-5 human diploid cells) of NDIs **70–75**

Com-pound	IC$_{50}$, 10^{-6} M			
	L. major	*T. brucei*	*P. falciparum*	MRC-5
70	0.244 ± 0.007	0.024 ± 0.001	1.350 ± 0.636	1.15 ± 0.29
71	1.041 ± 0.027	0.089 ± 0.007	0.360 ± 0.071	0.51 ± 0.01
72	0.184 ± 0.009	0.017 ± 0.007	0.225 ± 0.120	0.91 ± 0.32
73	0.921 ± 0.051	0.099 ± 0.010	0.370 ± 0.085	2.04 ± 0.05
74	0.306 ± 0.019	0.021 ± 0.003	0.180 ± 0.099	0.81 ± 0.44
75	0.537 ± 0.030	0.017 ± 0.009	0.275 ± 0.191	0.71 ± 0.25

Figure 22. Bifunctional acridine–NDI adducts **80–82** with activity against the human malaria pathogen strain *P. falciparum*.

Ligands **70–75** showed strong antiparasitic activity when tested on *T. brucei*, *L. major*, and *P. falciparum*, whose genomes contain a large number of G4 capable of interacting with ligands (Table 11). IC$_{50}$ values are in the nanomolar range with high selectivity against *T. brucei* compared to human MRC-5 cells. The data obtained by confocal microscopy indicate the localization of the ligand within the nucleus and kinetoplast of *T. brucei*. Studies of cytotoxicity and toxicity in zebrafish have shown that carbohydrate conjugation significantly reduces the toxicity of compounds.[109]

Bifunctional compounds **83** (Fig. 23) containing NDI and coumarin rings showed a pronounced antibacterial effect (on the level of ciprofloxacin, MIC 18.75–37.5 μg/ml) against Gram-positive (*S. aureus*, *S. epidermidis*) and Gram-negative (*E. coli*, *P. aeruginosa*) bacteria.[93]

Chronic *H. pylori* infection is a major risk factor for gastric cancer. Moreover, infection of tumor cells with *H. pylori* bacteria makes them more resistant to apoptosis caused by chemotherapy. Compound **84** (Fig. 23) showed growth inhibition of *H. pylori* at a submicromolar concentration (MIC 0.5·10^{-6} M), 50% growth inhibition was observed at a concentration of 0.1·10^{-6} M. NDI **84** inhibits bacterial growth in concentrations below its IC$_{50}$ in gastric cancer cells, thus providing the opportunity to use it as an antibacterial and anticancer agent simultaneously.[110]

NDI **85** (Fig. 23) showed moderate antimicrobial activity against Gram-positive *S. aureus* in nutrient agar (MIC 118 μg/ml). High activity of NDI **85** against *S. aureus* was noted in LB medium (MIC 29.4 μg/ml). Compound **85** inhibits the growth of *E. coli* starting at a concentration of 500 μg/ml. This indicates that NDI **85** specifically inhibits the growth of Gram-positive bacteria. In addition, NDI **85** does not lyse red blood cells even at a concentration of 2.5 mg/ml and shows a higher selectivity for bacterial cells than for mammalian cells (IC$_{50}$, 1.1 mg/ml for HeLa cells).[111]

The antimicrobial activity of NDI–peptide conjugates depends primarily on the value of the positive charge of the molecule. So, the best antimicrobial activity against *E. coli* and *B. subtilus* was shown by compounds containing one unit of NDI and at least seven lysine fragments. In addition, the investigated derivatives of NDI are inactive against mammalian cell lines, which makes them promising antimicrobial agents. The results of the study showed that the combination of a positive charge with aromatic and/or hydrophobic elements can be a promising approach to the synthesis of new antimicrobial agents.[112]

Noncovalent binding with nucleic acids is characteristic of all representatives of the diazapyrene group. This is primarily due to the peculiarities of the geometry of the pyrene ring, as well as the high π-acidity of the aromatic system. Substituents in the diazapyrene ring have a significant effect on the binding selectivity and stability of the complex due to electrostatic and steric interactions with the DNA chain. In addition, some representatives of

R^1 = H, Cl, Br, OMe; R^2 = H, Cl, Br; R^3 = H; R^2, R^3= CHCH$_2$CH$_2$CH
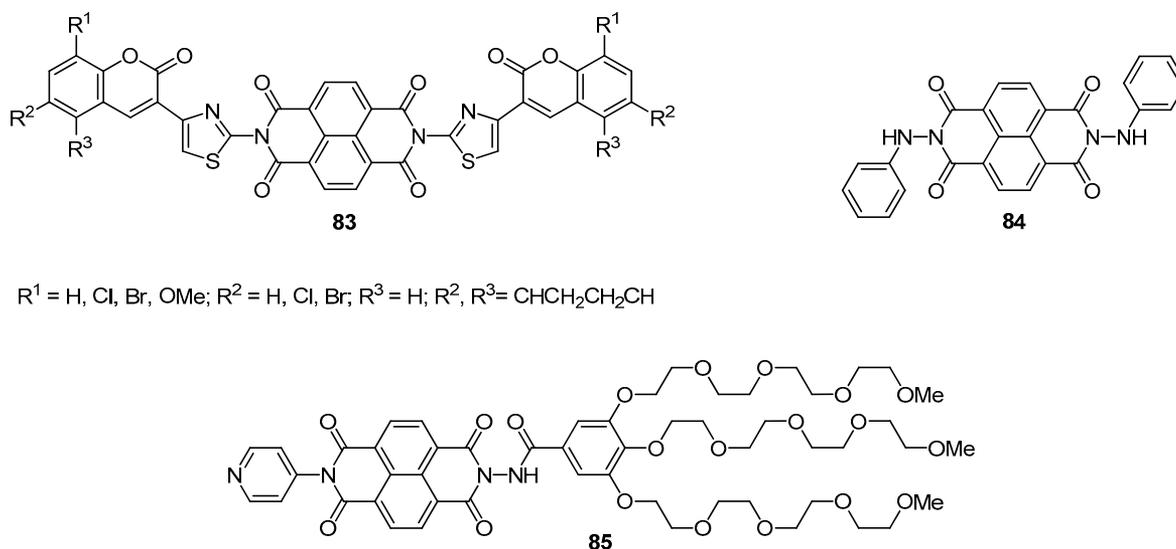
Figure 23. NDI-based compounds **83**–**85** with antibacterial properties.

diazapyrenes are able to photosensitize oxygen and introduce breaks in the DNA chain in the presence of light.

The biological activity of diazapyrene derivatives is determined mainly by their interaction with nucleic acids. Binding to DNA disrupts the functioning of enzymes such as DNA polymerase, telomerase, and topoisomerase, and stabilization of G4 in the promoter regions of genes suppresses their expression. Considering that in many oncogenes G4 are located in the promoter region, many G4 ligands exhibit antitumor activity. However, a clear correlation between antitumor activity and the strength of binding to quadruplex DNA was not found. In addition, the ability to introduce breaks in the DNA chain is an important factor determining the biological effect of diazapyrenes derivatives. The cleavage of the nucleotide chain can be both a consequence of direct photooxidation and the result of photosensitization of molecular O$_2$.

Additionally, the mechanism of the expression of the biological activity of diazapyrene derivatives may include action on lipid membranes. Thus, *N,N*-dimethyl-2,7-diazapyrenium chloride has a destabilizing effect on the lipid bilayer. The mechanism of action of *N,N'*-dimethyl-2,7-diazapyrenium, apparently, differs from the mechanism of action of classical destabilizing agents, such as nonionic surfactants. Modeling of molecular dynamics shows that the destabilization of the liposome membrane with *N,N'*-dimethyl-2,7-diazapyrenium chloride is due to its ability to surround itself with H$_2$O molecules even in highly hydrophobic media, such as the liposome bilayer, which, apparently, promotes the formation of transitional pores in the lipid bilayer.[113] Oligo(*p*-phenylene)naphthalene diimide facilitates the transport of anions through the lipid bilayer with a rare VI halogen selectivity (Cl$^-$ > F$^-$ > Br$^-$ >I$^-$).[114] Transmembrane transition of an ion occurs *via* the ion-slip mechanism without the need for pore formation in the classical sense.[115] It has been shown that compounds that disrupt transmembrane transport of chloride ions cause cell death by stimulating caspase-mediated apoptosis.[116]

The spectrum of biological activity and the mechanism of action of NDIs is determined mainly by the structure of substituents in the diazapyrene ring. The complexity of the functionalization of diazapyrenes is the main limiting factor in obtaining a large number of compounds for screening and in the rational modification of the structure. The comprehensive study of the biological activity of NDIs, unlike other diazapyrenes, is largely due to the simplicity of synthesis and the presence of a large number of methods for their functionalization.[117] Hybrid compounds containing NDI and pharmacophore groups of compounds with a studied biological effect, such as sulforaphane,[101] scriptaid,[103] and amonafide,[118] have a great potential. Currently, a number of 1,8-naphthalenimide derivatives have shown significant biological activity,[119] certain 1,8-naphthalenimides are in the process of undergoing clinical trials. Taking into account that the biological activity of naphthalene diimide analogs of 1,8-naphthalenimide was in some cases higher than that of the original compounds,[96,103,118] and their toxicity was less pronounced,[118] we can suggest the prospect of finding effective and safe biologically active compounds among them.

The combination of high biological activity, DNA binding selectivity, and multifactorial action makes diazapyrene derivatives a promising class for the development of chemotherapeutic agents and dictates the need for a comprehensive study of the mechanism of their biological activity.

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