The Search of DNA-Intercalators as Antitumoral Drugs: What it Worked and What did not Work

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Abstract: The discovery of new compounds with antitumoral activity has become one of the most important goals in medicinal chemistry. One interesting group of chemotherapeutic agents used in cancer therapy comprises molecules that interact with DNA. Research in this area has revealed a range of DNA recognizing molecules that act as antitumoral agents, including groove binders, alkylating and intercalator compounds. DNA intercalators (molecules that intercalate between DNA base pairs) have attracted particular attention due to their antitumoral activity. For example, a number of acridine and anthracycline derivatives are excellent DNA intercalators that are now on the market as chemotherapeutic agents. Commercially available acridine and anthracycline derivatives have been widely studied from a variety of viewpoints, such as physicochemical properties, structural requirements, synthesis and biological activity. However, the clinical application of these and other compounds of the same class has encountered problems such as multidrug resistance (MRD), and secondary and/or collateral effects. These shortcomings have motivated the search for new compounds to be used either in place of, or in conjunction with, the existing compounds. Unfortunately, the results of this search have not met expectations. The vast majority of candidate intercalator compounds tested for use as anticancer agents have shown little or no biological activity. Research in this area has not been without benefits, however, for it has produced much information on the synthesis and antitumoral properties of hundreds of compounds, which have been tested on diverse tumoral cell lines. This review considers the structural and biological considerations relevant to the use of DNA intercalators and bis-intercalators as antitumoral agents, with an emphasis on the relationship between structure and activity, produced in last decade.

Keyword: DNA- intercalators, cytotoxic compounds, DNA-binding.

INTRODUCTION

Intercalators as Cytotoxic Agents

It is 50 years since Watson and Crick determined that genetic material exists structurally as a double helix with now well-established characteristics [1]. Its role in the control of cellular functions immediately suggested it as an excellent target for treating illnesses of genetic origin, such as cancer. The first compounds discovered to act on DNA were the sulfur mustards, but their high toxicity prompted to a search for less toxic and more efficient compounds [2]. In the 1960s, some compounds with cytotoxic activity were discovered to act as anticancer agents, although their mechanism of action was unknown. Interestingly, after Lerman reported the occurrence of a noncovalent interaction between acridine and DNA, suggesting an intercalative process, it was established that some of these anticancer agents worked by interacting with DNA [3,4].

Intercalators are molecules that insert perpendicularly into DNA without forming covalent bonds. The only recognized forces that maintain the stability of the DNA–intercalators complex, even more than DNA alone, are van der Waals, hydrogen bonding, hydrophobic, and/or charge transfer forces [5,6,7,8]. A frontier orbital interaction has also been suggested [8]. This means that such a process has the possibility of being reversed, and as a consequence it must have an equilibrium constant. It has been argued that the 9-amino group is important in the DNA-recognizing region because of its ability to form hydrogen bonds [5,9]**.** Experiments in which inosine was replaced by guanine indicated the relevance of this group. However, many intercalators specifically recognize AT bases**,** which indicates that there are factors other than hydrogen bonding to consider [10]

Based on the difference in stabilities between DNA alone and its intercalator complex, the stability of DNA when heated is frequently used to measure DNA intercalation. The melting temperature (T_m) is taken as the temperature at which half of the DNA has denatured. In other words, half of the bases of a single strand do not interact in any way with their complementary bases on the second strand. In an oligonucleotide, the first bases to break the interaction will be those in the middle of the strand and not those at the extreme ends. When DNA is subjected to UV spectrophotometry and the temperature is varied, the resulting absorption data (which represents denaturation) is a sigmoidal curve, from which T_m can be determined. If the same DNA sequence or oligonucleotide is complexed with

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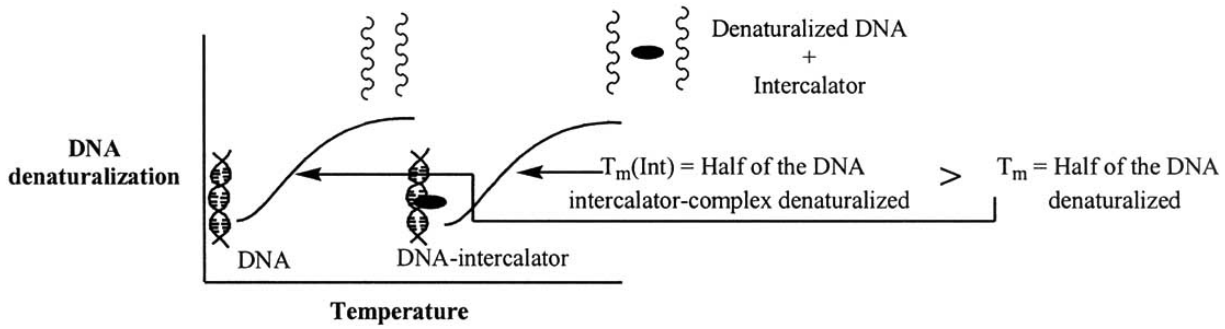


Fig. (1). Curve of temperature Vs. DNA denaturalization in DNA and DNA-Intercalator complex, indicating T_m.

an intercalator and then subjected to the same thermal conditions, another sigmoidal curve is obtained, but this curve is shifted to higher temperature compared to that obtained with the DNA alone, as shown in (Fig. **1**). Due to the higher stability of the complex, T_m will be higher. The most common forms of DNA that have been examined in this kind of experiment are calf thymus and salmon sperm DNA.

Of course Tm is not selective in the determination of DNA-intercalation, since it can be influenced by other kinds of molecules that recognize DNA such as groove binders. Methods more selective are viscosity measurements [11] and ethidium bromide displacement [12].

Other frequently used methods for the measurement DNA intercalation are circular dichroism (CD) and fluorescence spectroscopy [13,14]. More complicated, but also useful, are nuclear magnetic resonance (NMR) and mass detection using the electrospray technique. Finally, the technique that provides the most unequivocal results is X-ray diffraction [15]. NMR and X-ray diffraction are arguably the best methods by which to examine DNA intercalation. NMR can be used to extract information on the geometry dynamics and NMR and X-ray diffraction yield indispensable physicochemical data; however, they are among the most time-consuming and expensive of the available methods.

Measurement of the binding constant and biological activity of DNA-intercalator complexes in the 1970's, and QSAR studies in the 1980's, leads to the conclusion that there should exist a relationship between cytotoxic activity and binding force [16,17,18]. For some time after Lerman's discovery, the cytotoxic agents were thought to cause inhibition of RNA synthesis as a result of distortion of the shape of the genetic material. However, this and other similar proposals remained equivocal, since some potent DNA intercalators, such as daunomycin, did not cause inhibition of RNA synthesis. A satisfactory explanation was proposed when proteins that were thought to be involved in the control of the shape of DNA were found in the 1970's. This group of enzymes, called the topoisomerases (Topos), have helped to clarify the situation, and to date some interesting conclusions have emerged [19].

Although there is a relationship between the binding energy of a DNA intercalator and its biological activity, as discussed earlier, cytotoxicity is not only dependent on the ability to interact with DNA, since there are many DNA intercalators that are incapable of working as cytotoxic agents. To be effective, a drug must first overcome many barriers, including metabolic pathways, and cytoplasmic and nuclear membranes. Once the drug is situated in the nucleus, it must be capable of interacting with DNA by intercalating that is, forming a stable complex with a relatively long half-life. Achieving entry into the nucleus and forming a DNA complex are only the first stages of a series of events that underlie the cytotoxic activity of DNA intercalators; thus cytotoxicity is more than just an interaction with DNA. Cytotoxicity is a consequence of the poisoning of Topos, enzymes that are directly involved in DNA recognition, in the fundamental steps of cellular growth when DNA replication is active, in the S phase of the cell cycle, in which the topology of DNA plays a significant role. Topos also work and can be poisoned, in the M phase of the cell cycle, arranging the chromatin.

The spatial arrangement of DNA before, during, and after replication is essential to a high-quality cell-division process. In this way, DNA topology is governed by Topos [20]. Three parameters describe the topology of DNA: linking number, twist, and writhe. Topos regulate the topology of DNA, ensuring that it is arranged adequately for DNA replication by reversibly modifying the linking number, twist, and writhe, breaking one or two strands of the DNA, depending on its mechanism of action. Topos can be classified into two main classes: Topo I, which breaks only one strand of the DNA [21], although both strands are involved in the interaction with the enzyme [22], and Topo II [23], which breaks both strands of the duplex [24,25]. A third group, Topo III, has also been described but it remains unclear how the Topos in this group act and to what degree they are useful with regard to cancer chemotherapy. A fourth class, known as the gyrases, have been found in bacteria. Topo I and Topo II are good leads for DNA intercalators [26,27]. For an excellent discussion of the mechanisms of action of the Topos, we refer the reader to the reviews by Pommier, Osheroff and Stivers [28,29,30,31].

A DNA intercalator has cytotoxic activity when it poisons the Topo by stabilizing the ternary, DNA–intercalator–Topo complex in such a way that the enzymatic process cannot continue forward or backward. The structure of DNA–Topo complexes has been obtained by X-ray diffraction. Some Authors have also suggested that an interaction occurs between the cytotoxic compound camptothecin and a Topo, by interacting with Arg364, Asp533, and Asn722, (Fig. **2**). It is noteworthy that this last amino acid lies in the vicinity of Tyr723, which is responsible for breaking DNA in the phosphate region [32].

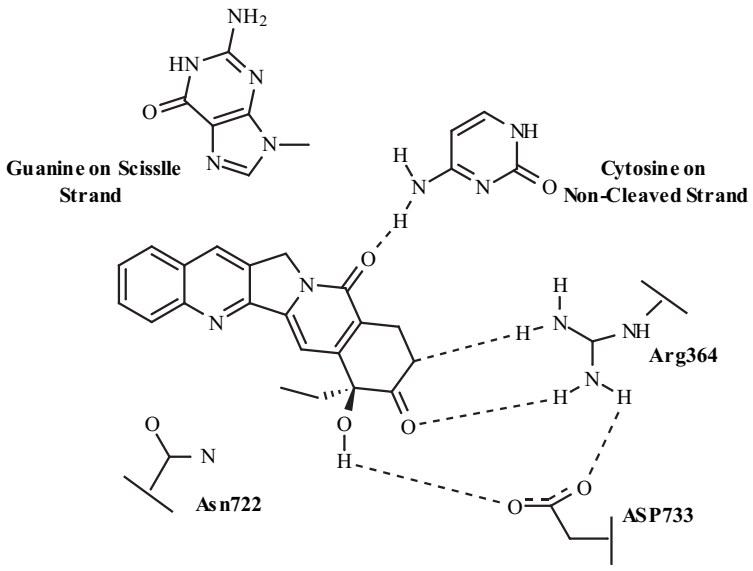


Fig. (2). Interaction suggested between camptothecin and Topoisomerase in a DNA-Intercalator.

Finally, once the enzyme–DNA complexes are poisoned by intercalators, the ternary complex is detected by the cell as a damaged portion, which triggers a series of events; one of the more important events involves p53 protein, which induces cell apoptosis (programmed cell death) (Fig. **3**) [33,34,35]. Since Topos are ubiquitous in eukaryotic cells, both healthy and damaged cells can be affected during treatment with this class of poisons [36], although cells that are constantly and frequently replicating (i.e., tumor cells) will be affected more than healthy cells.

At this point it results useful to remark that although inhibition is commonly used in literature to describe inhibition or poisoning, these terms are not exactly related. Strictly, inhibition refers to an interaction with the enzyme inhibiting its catalytic activity. Inhibition does not necessarily implicate the ternary complex mentioned above; it could be carried out with non-bonded substrates or free enzymes, changing its conformation or simply recognizing the active site complicating or impeding the union to the DNA. On the other hand, poisoning does produce damage in the complex enzyme-substrate. In this review, both terms inhibition and poisoning will be used as synonyms.

Inhibition of Topo *in vitro* requires a probe that will inhibit the enzyme, but cytotoxicity is one of the definitive characteristics for a compound to be evaluated as an antitumor agent in clinical trials. A new compound can be evaluated as a cytotoxic agent either *in vitro* or *in vivo*. The most common practice is to carry out *in vitro* studies because it is then feasible and relatively simple to evaluate whole series, from one to thousands, of compounds. The most accepted method is that which has been reported and used by the National Cancer Institute (NCI). In this methodology a panel of cells is incubated with different concentrations (less than 100 μM) of the potential antitumor compounds [37,38]. Daunomycin and 5-fluorouracyl are often used as references. If the IC_{50} is less than 100 μM, other probes and concentrations are evaluated. The criteria of whether or not a compound is "active" or "inactive" is subject to the goal of the study (design, synthesis, or isolation from a plant), so it is relative and must be considered carefully [39].

Resistance and Selectivity

Two of the principal goals of the design, discovery, and use of DNA intercalators are selectivity and drug resistance. Oligo sequences joined by intercalators give better selectivity in DNA sequences that have been exposed to

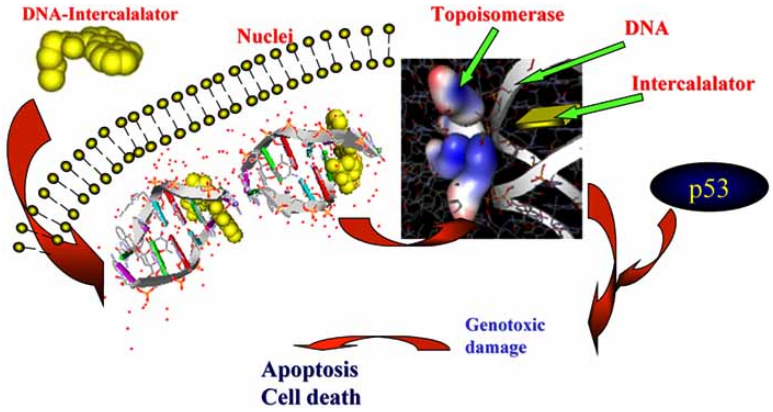


Fig. (3). Schematic representation of the mechanism of cytotoxicity of a DNA-Intercalator.

triplex formation by Hoogsteen base pairs [40,41,42,43]. In practice, one of the biggest problems is the susceptibility of phosphate linkage to breakage by nucleases, causing them to be inactivated. Many efforts have been made to improve the selectivity and stability of triplexes. An interesting proposal is found in the peptide nucleic acids (PNAs), which contain peptide chains or analogs thereof instead of sugar phosphate [44,45]. Resistance to nucleases and high affinity constants has been observed.

Quadruplexes are also a potential target, since sequences of guanosine-rich oligos form these complexes with the aid of Hoogsteen base pairs, presumably in Telomeres [46]. One class of molecules found to interact with quadruplexes is the cationic porphyrines. To improve selectivity, many strategies have been devised including, for example, linking intercalators to oligo sequences, and antibody-directed and gene-directed enzyme prodrug therapy (ADEPT and GDEPT, respectively) [47].

Drug resistance has lead researchers to synthesize and test many compounds. The mechanism underlying drug resistance to Topo poisons can be intrinsic or acquired: the former is due to poor uptake, poor drug activation, or increased drug catabolism, and the latter has been attributed to an ability to repair the drug-induced damage by increasing the expression of DNA repair enzymes or by altering Topo binding [48].

Non-Anti-Tumor Activity of Topo Poisons

Topos are also present in bacteria (gyrase), in which they act in a manner similar to the other Topos enzymes [49]. Quinolones are very useful and are probably the best example of antibacterial gyrase inhibitors in clinical use [50]. Some viruses also have a code for Topos; in this context, is noteworthy that camptothecin, a potent Topo poison, inhibits human immunodeficiency virus (HIV) transcription. *In vitro* experiments have also demonstrated that cellular Topo I enhances HIV-1 reverse transcriptase activity [51].

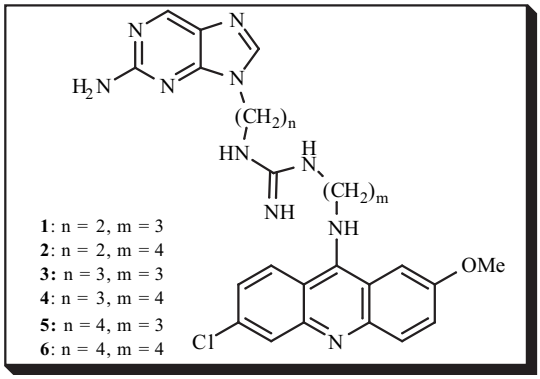
As it can be seen that intercalators have a broad range of properties. The variety of compounds in this field is very extensive and some of them are found in the clinic trials. In this review, we will focus on new compounds that act as intercalators or potential intercalators according to their physicochemical and structural properties such as flatness. The period search cover publications reported on the last decade. For a complement discussion of the theme, excellent reviews of the topic have been written by Braña [52], Haq [53], Denny [54], Graves [55], Holden [56], Bischoff [57] and Wilson [58].

MOLECULES

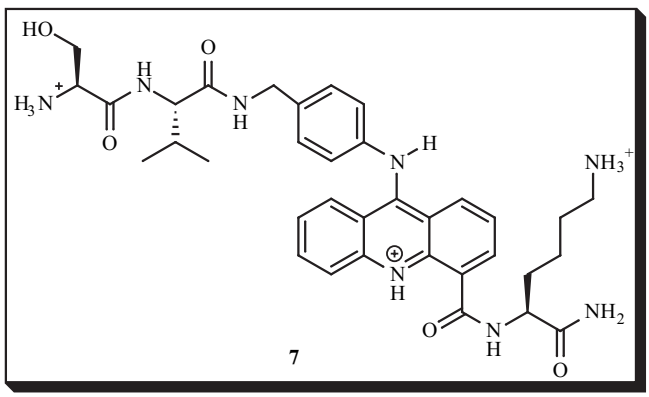
Acridines

Acridine derivatives constitute an important class of DNA-intercalating antitumor drugs. Recently, Demeunynck [59], Denny [60] and Tsann-Lang [61] have reviewed properly this topic.

Heterodimeric molecules that interact at apurinic/apyrimidinic (AP)sites constitute an attractive tool with which to potentiate the action of known anticancer agents. These molecules are composed of three units: (1) an intercalator for targeting DNA, (2) a nucleic acid base for the recognition of the abasic site, and (3) a polyamino linker that is able to stabilize the drug–DNA complex by electrostatic interaction with the phosphate backbone [62]. One example of these molecules are compounds **1-6**, in which a 2,6-diaminopurine is linked to a 9-aminoacridine chromophore [63]. The linking chain contains a central *N*,*N*-disubstituted guanidine connected to the two chromophores via polymethylene units of variable length. The molecules bind strongly to native DNA, with the exception of **3**, however, this compound causes stabilization of the abasic TX duplex at a ratio of 1:1. These data suggest a very specific interaction of **3** at the abasic site. The relative affinity of these heterodimers for abasic sites was determined on synthetic oligonucleotides containing a chemically stable analog of the abasic lesion. Cytostatic activity on L1210 cells was weak and cytotoxicity on A549 cells was moderate. No apparent relationship was found between the length of the linker and toxicity.



Solid-phase synthesis has improved the rate of discovery of small molecules that bind to DNA [64]. Threading intercalation is a binding mode wherein the intercalating group directs substituents into both grooves of a duplex simultaneously [65]. 9-Anilinoacridine-4-carboxamides belong to this group of intercalators [66]. The synthesis of an acridine–peptide conjugate **7** using solid-phase methodology has been described recently [67]. This nonnatural amino acid shows a bathochromic shift in its visible absorption spectrum in the presence of DNA consistent with intercalative binding to DNA.



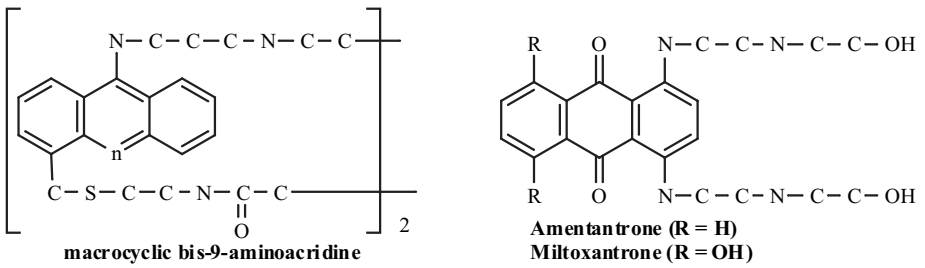
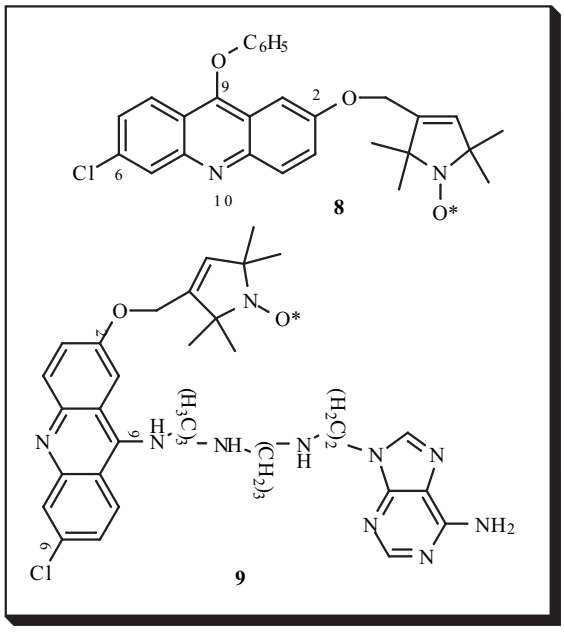


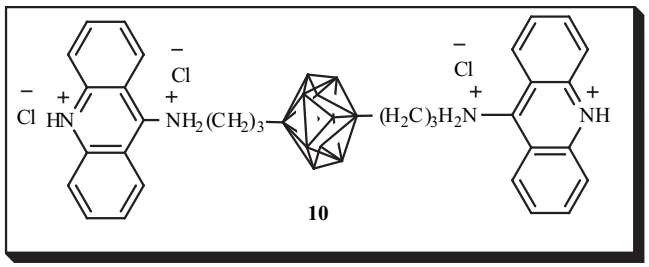
Fig. (4). Molecular formula of the macrocyclic bisacridine and mithoxanthrone.

X-ray diffraction analysis of the complexes between macrocyclic bisacridine and the antitumor intercalator ametantrone, (Fig. **4)** and CGTACG showed that only one acridine of the bisacridine drug binds at the C5pG6 step of the DNA, with the other acridine plus both linkers being completely disordered [68]. An unusual intercalation platform is formed by bringing four complexes together (involving 222 symmetry) such that the intercalator cavity is flanked by two sets of GC base pairs (i.e., C5G8 and G6C7*) on each side, joined together by G6G8* tertiary base-pairing interactions. In the bisacridine-CGTACG complex, the intercalation platform is intercalated with two acridines, whereas in the amentantrone-CGTACG complex, only one amentantrone is bound. NMR titration of the bisacridine to AACGATCGTT suggests that the bisacridine prefers to bridge more than one DNA duplex by intercalating each acridine to different duplexes.

In the search for spin-labeled intercalators that can be introduced into DNA-binding conjugates, and to study their fate in the cell, a nitroxide group was introduced at position 2 of 9-phenoxyacridines **8.** Reaction of this intermediate with a suitable amine gave a labeled 9-amino-substituted acridine conjugate **9**. Moreover, the binding properties of this conjugate do not suffer appreciable alterations [69].



With the aim of improving the water solubility and DNA-interacting properties of potential candidates for Boron neutron capture therapy, compound **10** was synthesized. These compounds have an acridine system that would serve as a DNA intercalating fragment, and a spermidine residue that would function both as a water-solubilizing and DNA-interacting element [70].

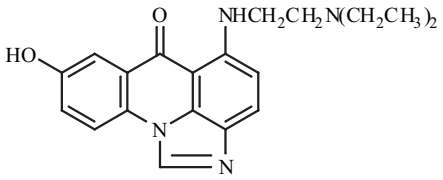


Imidazoacridinones are cytotoxic *in vitro* and *in vivo* against human and murine cell lines. The most prominent analog, C-1311, is currently being tested in clinical trials [71]. It has been found that C-1311 must bind noncovalently to DNA to induce its cytotoxic effects. On the other hand, irreversible binding to DNA in the cell-free system occurs only in the presence of the horseradish peroxidase (HPR) and hydrogen peroxide(H_2O_2) system, in a manner dependent on the drug: H_2O_2 ratio [72]. In the case of ratios of 1:3 and 1:5, the reaction gave highly reactive species that were quickly transformed into the products p2 and p3, which were unable to intercalate into DNA (Fig. **5**). In the presence of DNA, C-1311 first intercalated into DNA, and the intercalated compound was then oxidized. This oxidation produced only one product. Since peroxidase-type enzymes are present in the cell nucleus, the proposed sequence of events may also be expected to take place in the cellular environment *in vivo*.

In contrast, studies of drug-DNA interactions of compounds **11** that share the same core structure of C-1311, with two varying elements (ring substitution in positions 5, 7, or 8, and a diaminoalkyl side chain of variable length) showed that all such derivatives bind to DNA by intercalation. However, none of the analyzed drug–DNA binding parameters was significantly correlated with the biological activity of the drug [73]. Likewise, the need for a technique to analyze C1311 in mouse and human plasma leads to the development of a high-performance liquid chromatography method. This method is selective, sensitive, and reproducible [74].

Synthesis of a series of bis{[(dihydroacridine-4-carbonyl)amino]alkyl}alkylamines **12** was achieved and their antiproliferative activity was tested against HT-29 cell lines [75]. Both **12a** and **12b**, showed interesting cytotoxic profile. DNA experiments suggest that the high cytotoxic activity of these compounds may be related to their strong bis-intercalative binding to DNA. Modelling studies on **12a**

demonstrating the marked preference of its chromophores to bind in parallel orientation relative to each to other and preferentially through the minor groove of a DNA hexamer. An excellent revision on the synthesis and antitumor activity of pyrazolo and pyrimidinoacridines has been published by Antonini´s group [76].



C1311

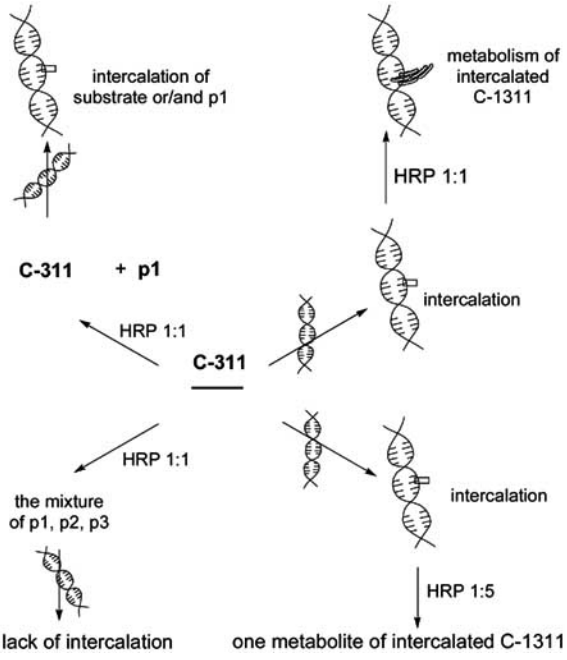
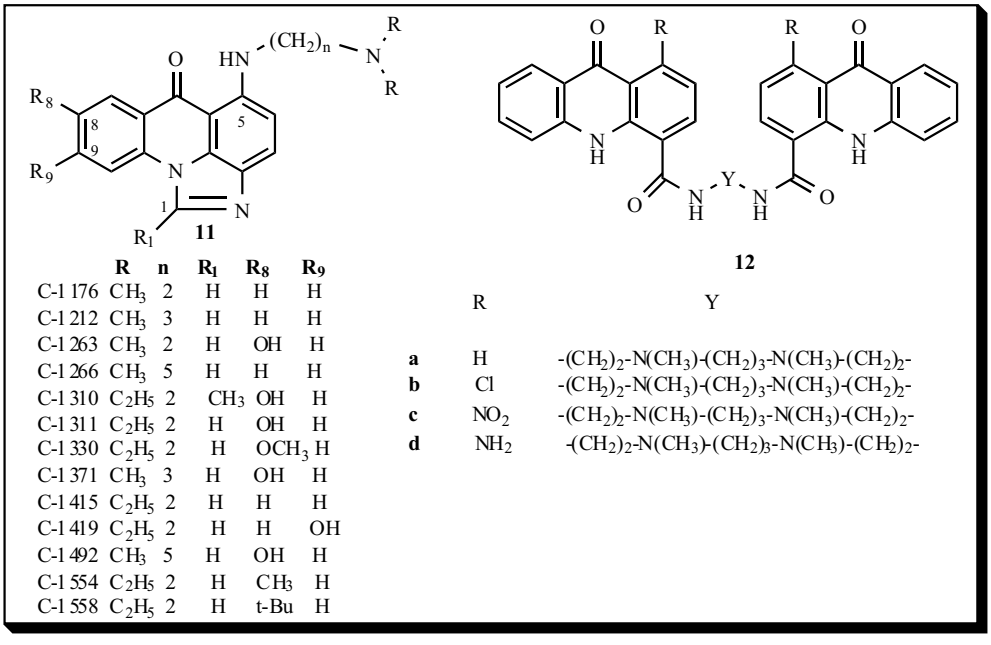


Fig. (5). HRP-mediated activation of C-1311 with different drug: H_2O_2 ratios.

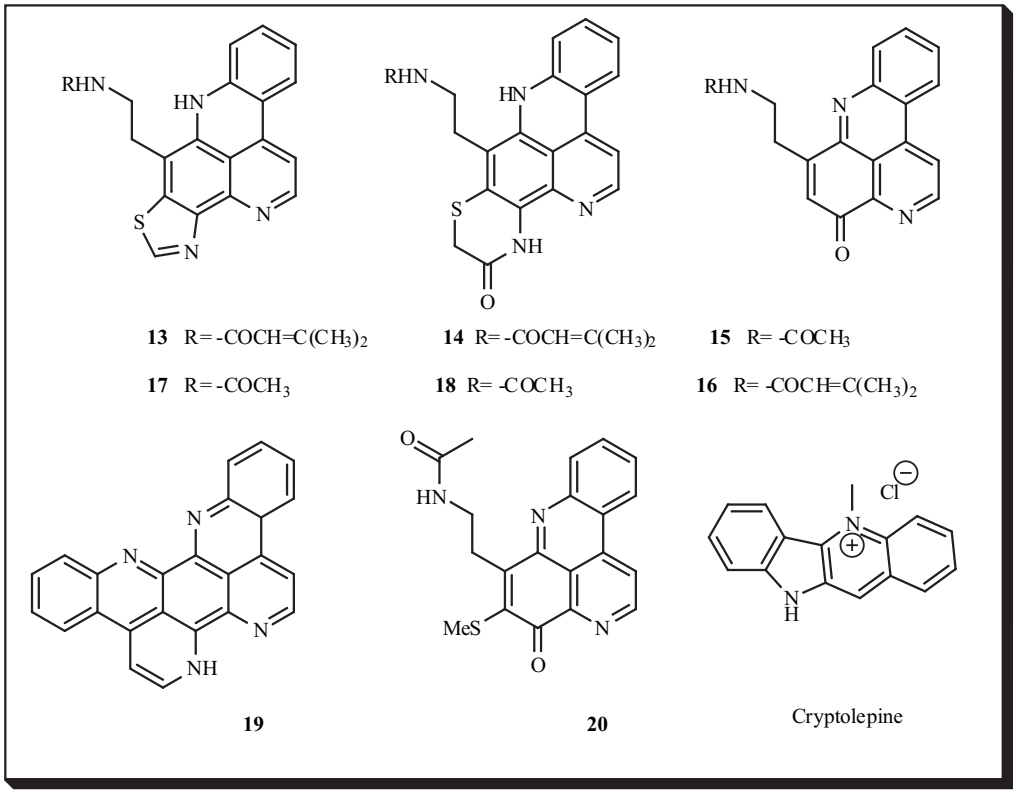
Alkaloids

The pentacyclic DNA-intercalating alkaloids are very interesting natural products because of their cytotoxic properties. In 1994, seven pyridoacridine alkaloids – dehydrokuanoniamine B (**13**), shermilamine C (**14**), cystodytin J (**15**), cystodytin A (**16**), kuanoniamine D (**17**), shermilamine B (**18**), and eilatin (**19**)- were isolated from a Fijian ascidian (Cystodytes sp) [77]. These compounds, along with diplamine (**20**), exhibit dose-dependent inhibition of proliferation in human colon tumor cells *in vitro*. In addition, all compounds inhibited the Topo-II-mediated decatenation of kinetoplast DNA in a dose-dependent manner. This ability correlated with their cytotoxic potencies and their ability to intercalate into calf thymus DNA. Cystodytin J and diplamine are the best intercalators, and Topo II inhibitors are the most potent cytotoxins of the series; two of their structural characteristics are that they have only four rings and are iminoquinones. It has also been reported that ascididemin (ASC) isolated from the Mediterranean ascidian *Cystodytes dellechiajei*, as well from *Didemnum* species [78, 79], is also highly toxic to several cancer cells lines [80]. Furthermore, ASC stimulates Topo-II-induced DNA cleavage preferentially at sites that possess a C on the 3´ side of the cleaved bond; in contrast, ASC has minimal, if any, effects on Topo I. However, it has been demonstrated that neither Topo I nor Topo II could be considered as a potential cellular target for ASC [81]. This alkaloid also induces apoptosis in HL-60 and P388 leukemia cells, an effect that is mediated by the enzyme caspase-3. Therefore, an alternative mechanism of cytotoxicity should be considered. Recently, another study has indicated that ASC-mediated DNA cleavage can occur via the production of reactive oxygen species [82]. Cryptolepine is an indoloquinoline alkaloid that was first isolated from the roots of *Cryptolepsis triangularis*, and afterward from *C. sanguinolenta* [83, 84, 85, 86]. It has been reported that cryptolepine has many pharmacological properties, such as anti-inflammatory, antibacterial, hypotensive, antipyretic, and antimalarial properties. Cryptolepine also possesses



	R	n	R_1	R_8	R_9
C-1176	CH_3	2	H	H	H
C-1212	CH_3	3	H	H	H
C-1263	CH_3	2	H	OH	H
C-1266	CH_3	5	H	H	H
C-1310	C_2H_5	2	CH_3	OH	H
C-1311	C_2H_5	2	H	OH	H
C-1330	C_2H_5	2	H	OCH_3	H
C-1371	CH_3	3	H	OH	H
C-1415	C_2H_5	2	H	H	H
C-1419	C_2H_5	2	H	H	OH
C-1492	CH_3	5	H	OH	H
C-1554	C_2H_5	2	H	CH_3	H
C-1558	C_2H_5	2	H	t-Bu	H

	R	Y
a	H	$-(CH_2)_2-N(CH_3)-(CH_2)_3-N(CH_3)-(CH_2)_2-$
b	Cl	$-(CH_2)_2-N(CH_3)-(CH_2)_3-N(CH_3)-(CH_2)_2-$
c	NO_2	$-(CH_2)_2-N(CH_3)-(CH_2)_3-N(CH_3)-(CH_2)_2-$
d	NH_2	$-(CH_2)_2-N(CH_3)-(CH_2)_3-N(CH_3)-(CH_2)_2-$

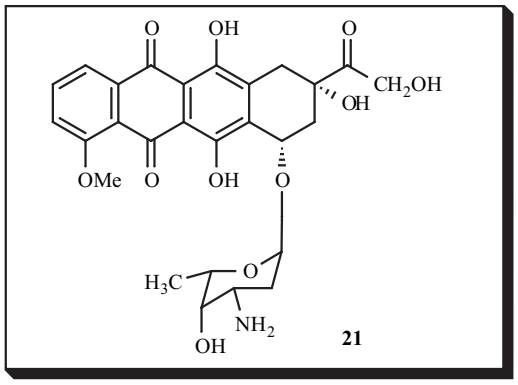


cytotoxic activity and inhibits DNA synthesis in B16 melanoma cells. In addition, it is a potent inhibitor of Topo II [87, 88]. Recently, it was reported that cryptolepine binds to DNA in a different manner to other intercalators; specifically it binds to CG-rich sequences containing no alternating CC sites [89].

Anthracyclines

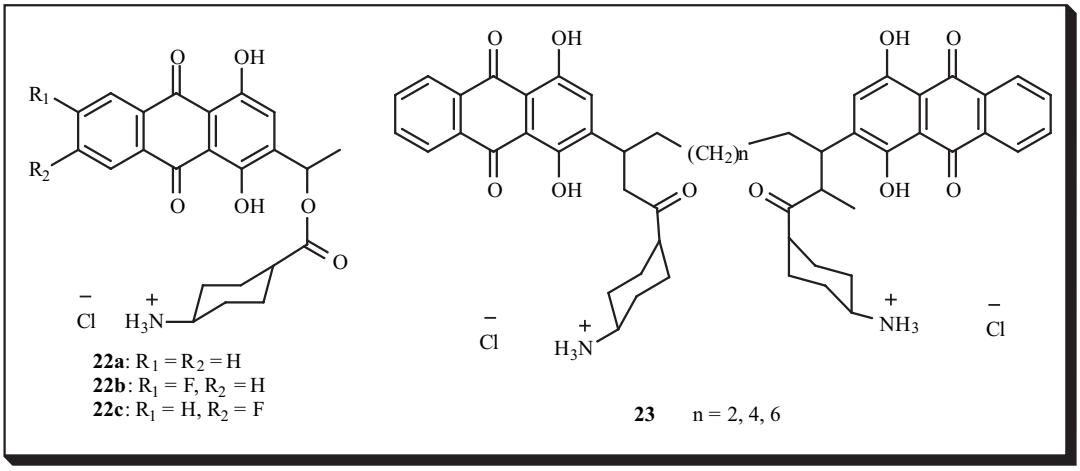
Doxorubicin **21** is the precursor of anthracycline molecules [90]. Although the majority of these compounds are potential DNA intercalators, this property correlates poorly with their anticancer activity [91]. Structural modification is an effective approach that has been used both to understand the mechanism of drug action and as a route to designing better drugs. So, the simple anthracycline analogs **22** were prepared. The main structural modifications to doxorubicin that were effected are that the basic chain linked to the anthraquinone aglycone was not restricted stereochemically, a fluorine atom was inserted at position 2 or 3 and the cyclohexane moiety is missing. Preliminary evaluation of drugs that have been designed as inhibitors of *in vitro* growth of the P388 cell line demonstrated a potency of one order of magnitude less than that of daunosaminyl derivatives [92].

In the light of this result, it was reasoned that if a linker joined two molecules of **22** it might be possible to create a new class of DNA bis-intercalators **23**, and consequently these new compounds should have enhanced affinity for DNA. However, preliminary results suggest that the bis-intercalating molecules **23** are less effective inhibitors of *in vitro* growth in the P388 cell line than the simple anthraquinones described above [93].



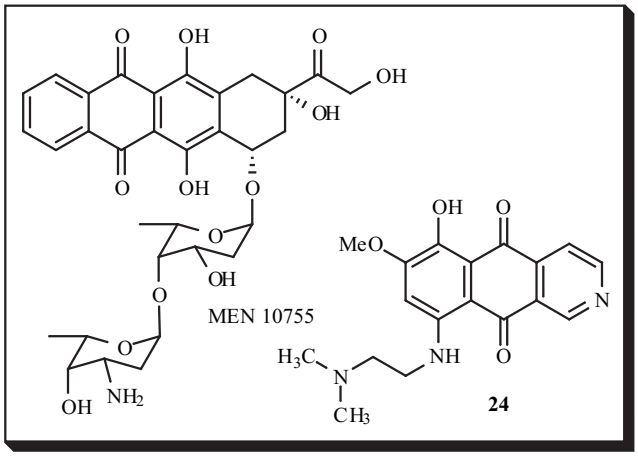
The bioisosteric modification of the C-2 carbon atom of the anthracenedione chromophore by a nitrogen atom produces the aza derivative **24** [94]. This compound intercalates into poly (dA-dT) poly (dA-dT), and poly (dA) poly (dT) sequences, inhibiting the DNA gyrase and the activity of mammalian Topos I and II.

A parallel study of the behavior under physiological conditions of doxorubicin and the novel disaccharide MEN 10755 showed that their chemical properties in solution and the pH-dependence in their properties are practically identical [95]. These results imply that the presence of a second sugar moiety and the different spatial location of the charged amino group on MEN 10755 do not appreciably modify the stability of DNA adducts. In addition, both compounds act as inhibitors of Topo II, through MEN 10755 seems to be more effective. Preliminary studies have revealed significant differences in the pharmacokinetic behavior of MEN 10755 compared to doxorubicin. A revision on the synthesis and antitumor activities of anthracyclines has been published by Bineschi [96].



22a: $R_1 = R_2 = H$
22b: $R_1 = F$, $R_2 = H$
22c: $R_1 = H$, $R_2 = F$

23 n = 2, 4, 6



MEN 10755

24

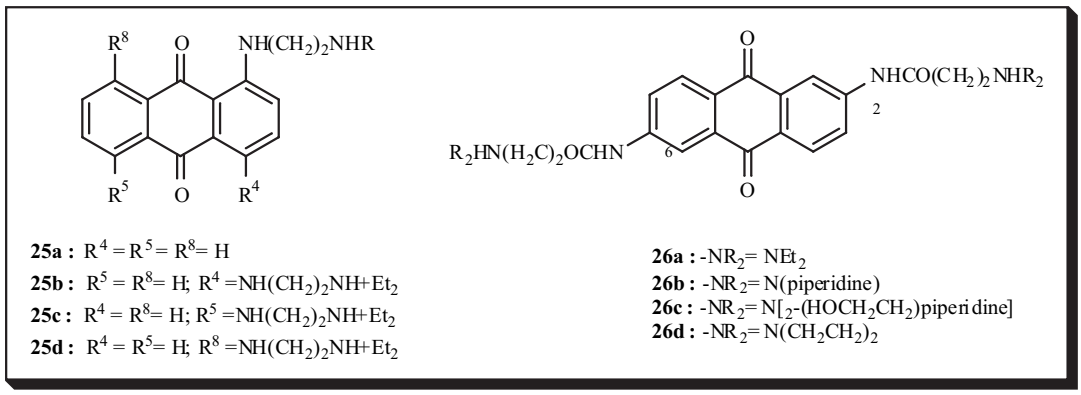
Anthracenediones

Mitoxantrone, and other anthracenedione derivatives, are DNA intercalators that are able to poison Topo II [97]. The bioisosteric modification of two carbon atoms by nitrogen atoms, at positions 2 and 3 of the anthracenedione chromophore, produces the aza derivatives BBR2853 and BBR 2894, which are characterized by less negative reduction potentials, a lower affinity for DNA, and a modified geometry of intercalation [98]. In addition, they show a marked decrease in cytotoxic potency and efficacy in tumor systems that are responsive to classical Topo II inhibitors of the anthraquinone family. These results suggest that 2,3-diaza anthracenediones are able to use another mechanism of cytotoxicity that is probably associated with oxidative damage following drug activation.

Molecular modeling studies have suggested that anthraquinone derivatives substituted at the 1,4 and 1,8 positions **25** with a –NH(CH2)2NH(CH2CH3)2^+ side chain intercalate with DNA with both substituents in the same groove (classical intercalation), while a similarly substituted 1,5 derivative intercalates in a threading mode, with a side chain in each groove [99]. Modeling studies also suggest that anthraquinone derivatives substituted at the 2,6 positions **26** should bind to DNA by the threading mode [100]. Stopped-flow kinetics association and dissociation experiments on the interaction between these anthraquinones and calf thymus DNA, and with DNA polymer with alternating AT and CG bases pairs allowed the elucidation of both the binding mode and the way in which the threading mode affects intercalation rates relative to similarly substituted classical intercalators. The experimental binding modes agree completely with the modes predicted by molecular modeling. This series of compounds demonstrated that it is possible to design intercalators with very similar binding constants but with significantly different binding kinetics [101].

Arylaminoalcohols

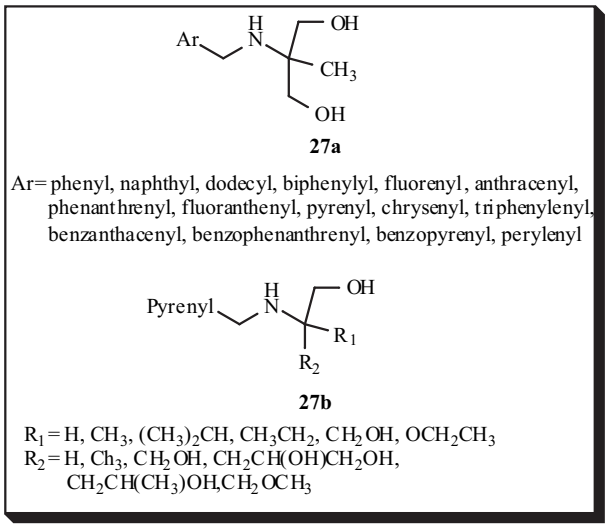
Studies of the interaction between DNA and carbocyclic 2-[(arylmethyl) amino]-2-methyl-1,3-propanodiols (AMAPs) **27a** were carried out to identify and eliminate possible



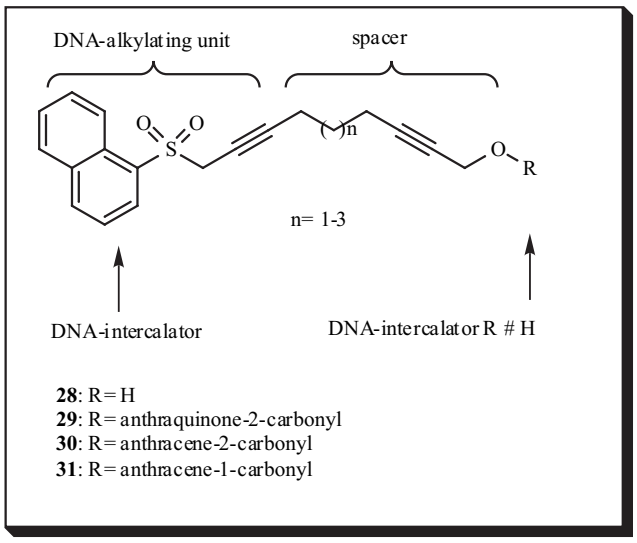
25a : $R^4 = R^5 = R^8 = H$
25b : $R^5 = R^8 = H$; $R^4 = NH(CH_2)_2NH{+}Et_2$
25c : $R^4 = R^8 = H$; $R^5 = NH(CH_2)_2NH{+}Et_2$
25d : $R^4 = R^5 = H$; $R^8 = NH(CH_2)_2NH{+}Et_2$

26a : $-NR_2 = NEt_2$
26b : $-NR_2 =$ N(piperidine)
26c : $-NR_2 =$ N[2-($HOCH_2CH_2$)piperidine]
26d : $-NR_2 = N(CH_2CH_2)_2$

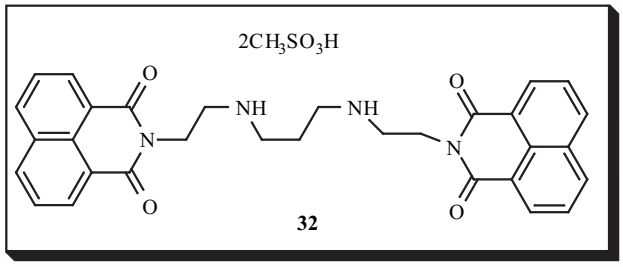
effects due to systematic heteroatoms. In general, the interaction between AMAPs and DNA increases as the intercalating ring system grows in area [102]. Antitumor activity is not a function of the ring system per se, but rather appears to be related to the shape of specific molecule. The variation of the amine side chains **27b** produced enhanced DNA binding due to electrostatic interactions, and the best chain was the 2-amino-1,3-propanediol moiety [103].



The synthesis and evaluation of DNA binding affinity and cleavage activity of novel 2-naphthyl propargylic sulfones **28** confirmed that these compounds are DNA intercalators and DNA cleavers [104]. In addition, their cytotoxic activity correlates with their DNA cleaving activity. However, a significantly reduced tethering effect is noted for the ester conjugates **29-31** as a result of interference of the aromatic ester moiety with the intercalation of the 2-naphthalene nucleus. Another finding was that the 2-naphthyl nucleus is a much better DNA intercalator than is the phenyl group, and its intercalating capacity with DNA is correlated with its DNA cleavage potency.



Naphthalimides and bis-naphthalimides are a class of compounds with high antitumor activity upon a variety of murine and human tumor cells. One of them, elinafide **32** is currently being used in clinical trials against solid tumors [105]. For an excellent and exhaustive revision on the synthesis and anticancer activity on naphthalimides we refer the reader to the review by Braña [52].



1,4,5,8-Naphthalenetetracarboxylic diimides (NDI) are successful intercalating molecules, and when tethered to one or both termini of a third strand can contribute significantly to the overall stabilization of DNA triplexes. The synthesis and triplex stabilizing properties of the oligodeoxyribonucleotides (ODNs) functionalized at the 5′- and/or 3′-termini with a naphthalene-diimide-based (NDI) intercalator (Fig. **6)** indicate that NDI intercalators are very effective as ligands to enhance binding of the third strand to the target duplex in DNA triplexes [106, 107]. The presence of a single conjugated NDI as **33a** results in two transitions, the first with a change in T_m (ΔT_m) of 17°C, represents denaturation of the third strand, while the second, with a ΔT_m of at least 26°C represents unstacking of the NDI conjugate from the target. Complexes with two terminal NDI conjugates exhibit ΔT_m enhancements as great as 41°C. Subsequently, it was found that alterations to the linker tethering the DNA sequence and the intercalator can also enhance stabilization. Less flexible linkers, in particular those with a phenyl ring present **33c**, appear to permit the stabilization afforded by the bound intercalator to be transferred more effectively to the three-stranded complex. The conjugate containing the phenyl linker exhibits a T_m 28°C higher than that of the unconjugated triplex. That the linker itself contributes to the observed stabilization is clear, since introduction of the phenyl linker increases the observed T_m by 11°C relative to a simple flexible linker.

Polyintercalators consisting of NDI units bonded in a head-to-tail arrangement via a peptide linker produced the first tetrakis intercalator, which spans at least eight base pairs [108]. Recently, the synthesis of the first octakis-intercalating derivative with eight units of NDI was reported (Fig. **7**). UV spectroscopy and viscometry measurements indicate that the molecule binds to a double-stranded DNA with all eight NDI units intercalated simultaneously, and has a preference for binding to G-C regions [109].

It is well-known that the stacked bases of the DNA double helix provide an efficient medium for charge transport, and that this charge migration on oligonucleotide duplexes can produce permanent base lesions at distances of at least ten base pairs from the damaging agent [110]. Since naphthalene diimide molecules intercalate into DNA and are potent electron acceptors, the distribution damage along a DNA restriction fragment was explored through the site-specific targeting of NDIs using oligonucleotide-directed triplex formation [111]. When covalently tethered to the center of a triplex-forming oligonucleotide **38** and delivered by triplex formation within a pyrimidine-purine-pyrimidine motif to a specific site on a restriction fragment, NDI can

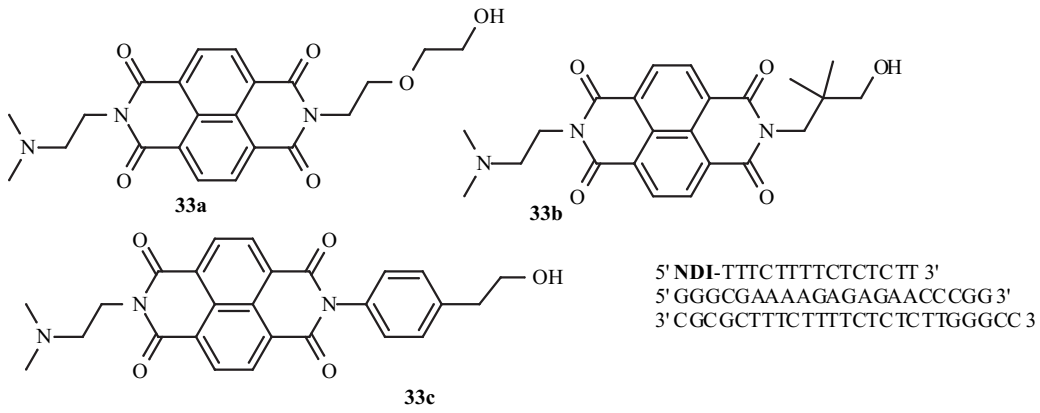


Fig. (6). NDI-conjugates triplexes **33**.

photo-oxidize guanine over at least 25–38 base pairs in each direction from the site of binding. These triplex-directed NDI intercalators demonstrate that charges can migrate through genomic DNA over 25–34 base pairs (~200 Å) in both directions down the helix and along both duplex strands, to generate permanent base lesions.

and 9(*N*-ethyl)aminomethylanthracene (N-Et-AMAC) were performed to determine the basis of DNA sequence recognition by small molecules. In these studies, a planar hydrophobic fluorescent moiety, anthryl, which is capable of intercalation, is attached to a hydrophilic cation function through an appropriate linker. While the planar anthryl

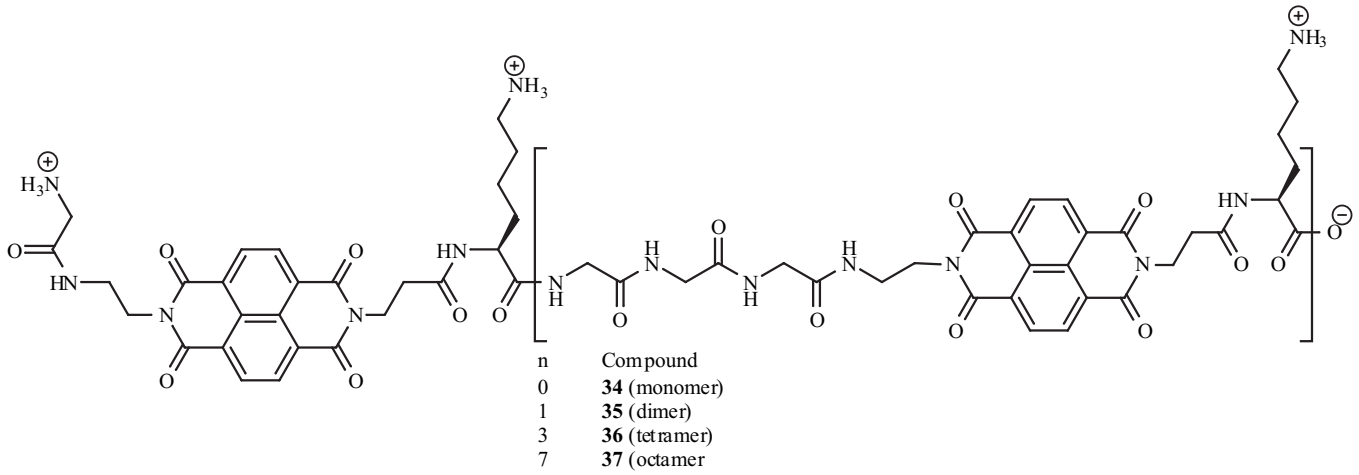
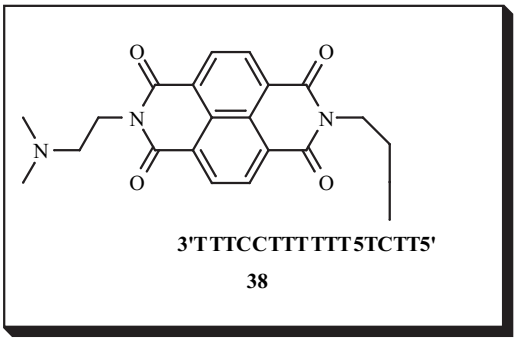


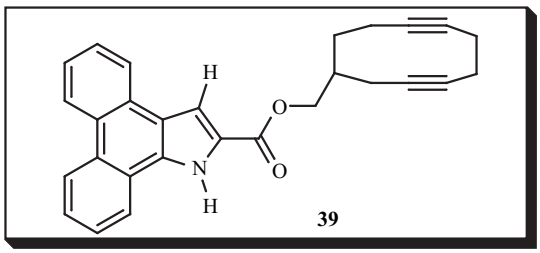
Fig. (7). General structure of polyintercalators.



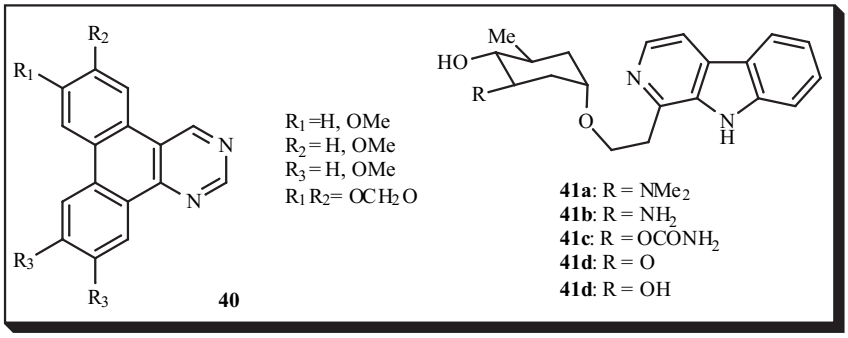
Molecular association is a common strategy used to devise more potent antitumor agents. Pyrrolo[9,10-b]phenanthrene-enediyne conjugate **39** was synthesized using this strategy. Preliminary bioassays of an enediyne conjugate suggest DNA interaction with this conjugate is favorable, and that strand scission can be induced at low concentrations [112].

Studies on the synthesis and DNA binding properties of the geometric isomers 9(3-aminopropyl) anthracene (APAC)

moiety is expected to intercalate into the helix, the cationic function can provide favorable electrostatic interactions. The linker connecting these two functionalities is varied to test how the linker controls the DNA recognition properties of the anthryl probe. Spectroscopic and thermal studies indicate intercalation of the probe into the helix. The binding properties, however, depend on the side chain as well as the DNA sequence. It is noteworthy that APAC intercalates into alternating AT and IC sequences, while discriminating against GC sequences [113].



Recent advances have shown that the pharmacological properties of phenanthro[9,10-f]heterocycles may be



correlated with their good DNA-chain intercalating ability. This researches to synthesize phenantrho[9,10-d]pyrimidines **40** via an intramolecular Stille-type biaryl coupling [114]. β-Carboline-carbohydrate hybrids **41** were synthesized as part of the search for photochemical DNA cleaving agents. These compounds were found to cleave DNA at the guanine site upon irradiation with UV light with a long wavelength without any additives. In addition, it was suggested that the DNA cleaving activity is dependant on the C3 substitution of the sugar moiety in the hybrids [115].

Coumarins

Oligonucleotide derivatives containing attached heterocyclic compounds, such as acridine, phenazine, ethidium, are attractive compounds for use in antisense and antigene strategies due to their ability to form specific complexes of high stability with complementary oligo- and polynucleotides (biostability) [116]. Another feature of these oligonucleotides bearing stabilizing agents (SAs) is the ability to cross cellular and nuclear membranes rapidly (bioavailability). To find new stabilizing agents, ODNs of different lengths have been prepared and linked to molecules related to the coumarin family. These ODNSAs, (Fig. **8**), were tested against acridine-connected oligomers of the same sequence. T_m experiments demonstrated that all ODNSAs formed complexes of increased stability with complementary sequences of deoxyribo-20-mer. The order of stability of duplexes showed that the coumarins stabilize the complexes more than acridine and the chromone derivatives[117].

Psoralens are a group of natural and synthetic linear furocoumarins with photobiological properties. The biological activity of psoralens is due mainly to their ability to photoreact with DNA. Upon irradiation with UV-A light, photoaddition of the 4′,5′ double bond of their furane ring and/or the 3,4 double bond of their pyrone ring with the 5,6 double bond of pyrimidine bases (usually thymine) occurs, leading to the formation of mono- or di-adducts[118]. Compounds that cause bifunctional damage to the macromolecules are usually more mutagenic and provoke skin phototoxicity [119]. Searching for psoralen derivatives that retain a high affinity toward the macromolecule while inducing only monoaddition lead to the synthesis of derivatives **42** and **43** [120]. In the first compound, a cyclopentane ring is fused to a 4′,5′ double bond, and derivative **42** is able to photobind with DNA, forming cross links; damage to the macromolecule is responsible for the antiproliferative activity. In compound **43**, the cyclopentane ring is fused to the 3,4 double bond, and it is devoid of biological activity. The differential behavior of compounds **42** and **43** may be explained by theoretical calculations, which demonstrate that introduction of the cyclopentane ring leads to different molecular curvatures. Furthermore, linear dichroism measurements of compounds **42** and **43** in the presence of DNA indicate that the complexed **32** and **33** molecules assume a position parallel to the plane of DNA bases, this orientation would be expected if the ligand was intercalated between two base pairs.

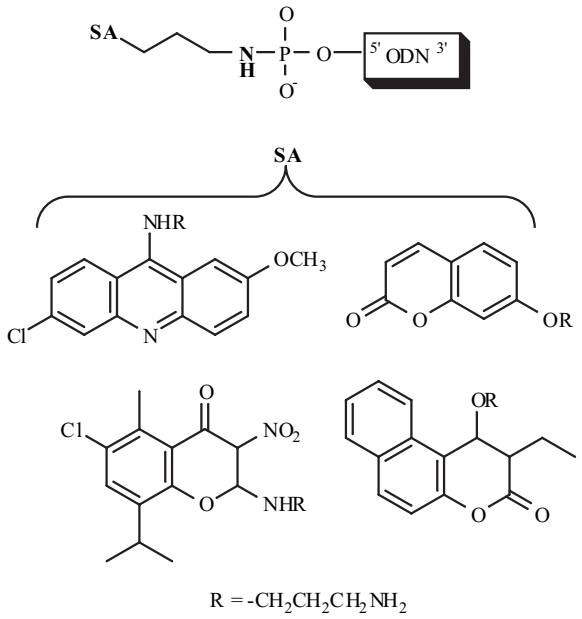
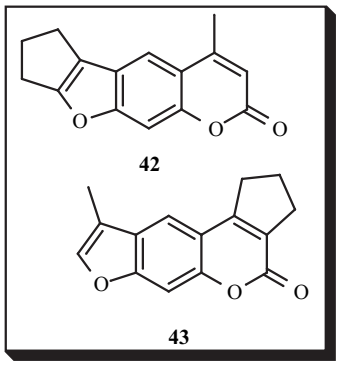


Fig. (8). General structure of ODNSAS

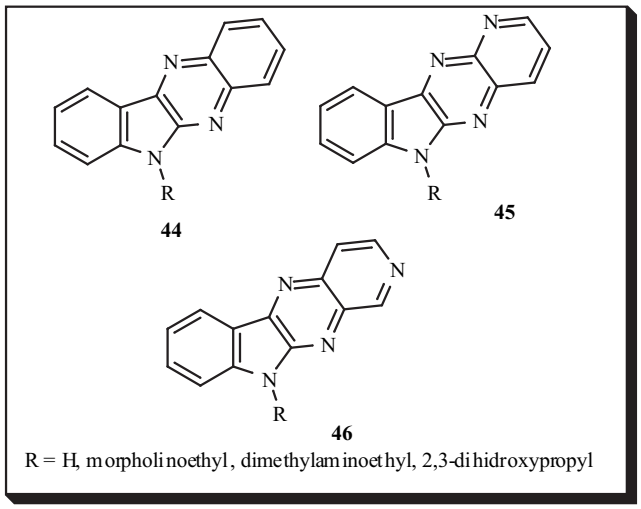


Indoles

Compound B-220 has been shown to have cytotoxic and antiviral activity [121]. In order to determine its mechanism

of action on DNA, a series of compounds was designed that incorporated an additional nitrogen atom in the indolo [2,3-b] quinoxaline chromophore **44** to afford the pyridopyrazino [2,3-b]indole derivatives of groups **45** and **46**, and a dimethylaminoethyl, morpholinoethyl or 2,3-dihydroxypropyl side chain on the indolic nitrogen [122]. It was reasoned that this chemical modification may significantly influence both the capacity of the drug to interact with DNA and to inhibit cell growth. Electric linear dichroism (ELD) measurements indicate that the compounds behave as typical DNA intercalating agents, and the negative reduced dichroism values measured for compounds **46 (**R= morpholino, dimethylaminoethyl) indicate that the pyridopyrazino[2,3-b] quinoxaline chromophore of these two ligands is oriented parallel to the DNA base pairs, as expected for intercalating binding. Compound **46(**R= dimethylaminoethyl) is equally toxic compared with its indoloquinoxaline counterpart **44**, whereas the regioisomer **45** is about five times less toxic. It is plausible that the orientation of the pyridine nitrogen with respect to the indole ring exerts a weak but noticeable influence on the capacity of the molecule to interact with DNA. Regarding the role of side-chain moieties, the compounds containing a dimethylaminoethyl side chain attached to the chromophore exhibit the highest affinity for DNA, and exhibit a preference for GC-rich DNA sequences. Apparently this aliphatic side chain reinforces the stability of the intercalated complex by anchorage into the minor groove. Weaker DNA interactions were detected for those bearing a morpholinoethyl side chain. The incorporation of a 2,3-dihidroxypropyl side chain does not reinforce the DNA interaction compared with the unsubstituted analogs.

The indolocarbazole compounds (R-6 and R-95) and one benzopyridoquinoxaline derivative (BPQ-1256) have been shown to have antitumor activity and to stimulate DNA Topo-I-mediated cleavage in a non-sequence-specific manner [123]. It has been found that these Topo I poisons are covalently attached to the 3′ end of a 16-mer triple-helix-forming oligonucleotide (TFO), (Fig. **9**). The conjugates proved to be stronger binders than the unsubstituted oligonucleotides and they were able to induce Topo-I-mediated DNA cleavage specifically at the 3′ end of the triple site [124]. The TUC-R-6 conjugate, in which the B-cycle and the sugar moiety of rebeccamycin were expected to be located in the minor groove upon triplex formation and intercalation of R-6, gave the highest cleavage efficacy. Therefore, the cleavage yield depends on the chemical nature of the Topo I poison and on the site at which it attaches.



The antitumor antibiotic AT2433-B1, originally isolated from a culture broth of *Actinomadura melliaura*, also belongs to the indolocarbazole series [125]. It contains a unique disaccharide consisting of methoxy glucose and an

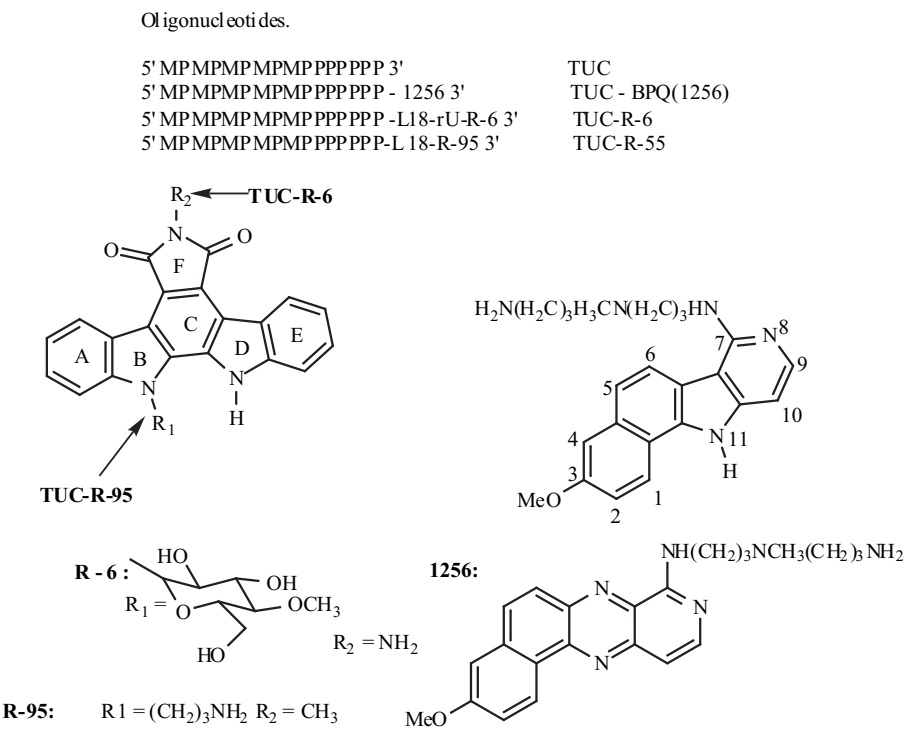


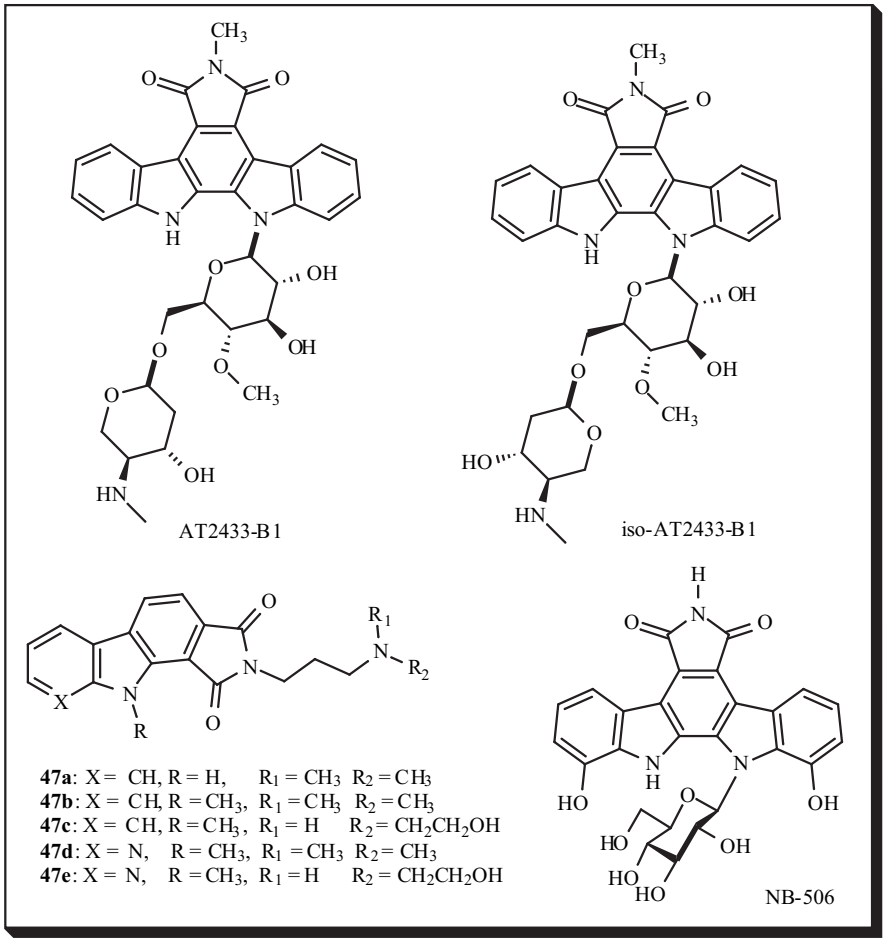
Fig. (9). Indolocarbazole and benzopyridoquinoxaline oligonucleotides(TFO)S.

aminosugar subunit, 2,4-dideoxy-4-methylamino-L-xylose. The configuration of the amino sugar distinguishes AT2433-B1 from its diastereoisomer iso-AT2433-B1. Previous studies with rebeccamycin monoglucoside derivatives have shown that the presence of an amino group in a suitable position significantly reinforces DNA interaction; based on that finding, these antibiotics were investigated to establish whether the configuration of the amino-sugar moiety influences their DNA interactions [126]. Accurate binding measurements using the BIAcore surface plasmon resonance method revealed that AT2433-B1 binds strongly to hairpin oligomers containing a $[CG]_4$ block but not to oligomers with a central $[AT]_4$ tract. In contrast, the diastereoisomer iso- exhibits a very weak sequence preference, thus showing that the configuration of the xylose subunit of AT2433- B1 is essential for DNA intercalation.

The interaction of the tetrahydropyrrolo[3,4-*a*]carbazole-1,3-diones **47a-c** and two tetrahydropyrido[3,2-*b*]pyrrolo[3,4-*g*]indole-1,3-diones **47d-e** with DNA was investigated by absorption spectroscopy and thermal melting studies. Compounds vary in the substitution pattern of the aromatic ring and the substituents on the N-pyrrolo and the propylamine side chain [127]. ELD and CD measurements showed that the compounds behave as typical DNA-intercalating agents. In addition, it was found that the strength of interaction with DNA is dependent on the nature of the side chain. Compounds with a hydroxyethyl-aminoethyl side chain demonstrated higher affinities for poly(dA-dT)$_2$ than compounds bearing a dimethylaminoethyl side chain. Furthermore, they stabilize DNA–Topo II covalent complexes but their Topo II inhibitory properties do not correlate with their cytotoxic potential. However, compounds **47c, 47d** and **47e**, exhibited a high toxicity to P388 murine leukemia cells and produced a marked accumulation in the G2/M phase of the cell cycle, whereas compounds **47a** and **47b** were essentially inactive.

NB-506 (13-*N*-glucopyranosyl-6-*N*-formylamino-1-hydroxy-indolocarbazole) is a Topo I inhibitor with noteworthy antitumor activity [128]. An investigation of the structural selectivity of the nucleic acid binding of NB-506 among 12 different nucleic acid structures and sequences revealed that NB-506 has a pronounced preference for binding to the DNA triplex poly[dA]:(poly[dT])$_2$. This selectivity was attributed to a complementary shape between its extended aromatic ring system and the planar triple stack [129]. A general revision on indolocarbazole chemistry and their cytotoxic activity has been described by Pindur [130].

Oligonucleotide–intercalator conjugates consisting of benzopyridoindole and benzopyrido quinoxaline derivatives were synthesized successfully by joining the intercalator to the 5′ end or an internucleotide position in the center of a 14-mer, (Fig. **10**). All the derivatives were observed to form a stable DNA triple helix with a DNA target duplex under physiological conditions [131]. In particular, the derivatives B[h]PQ attached to the 5′ end and B[e]PI attached to an internal position on the phosphate diester backbone stabilized the triple helix. In addition, it was found that the



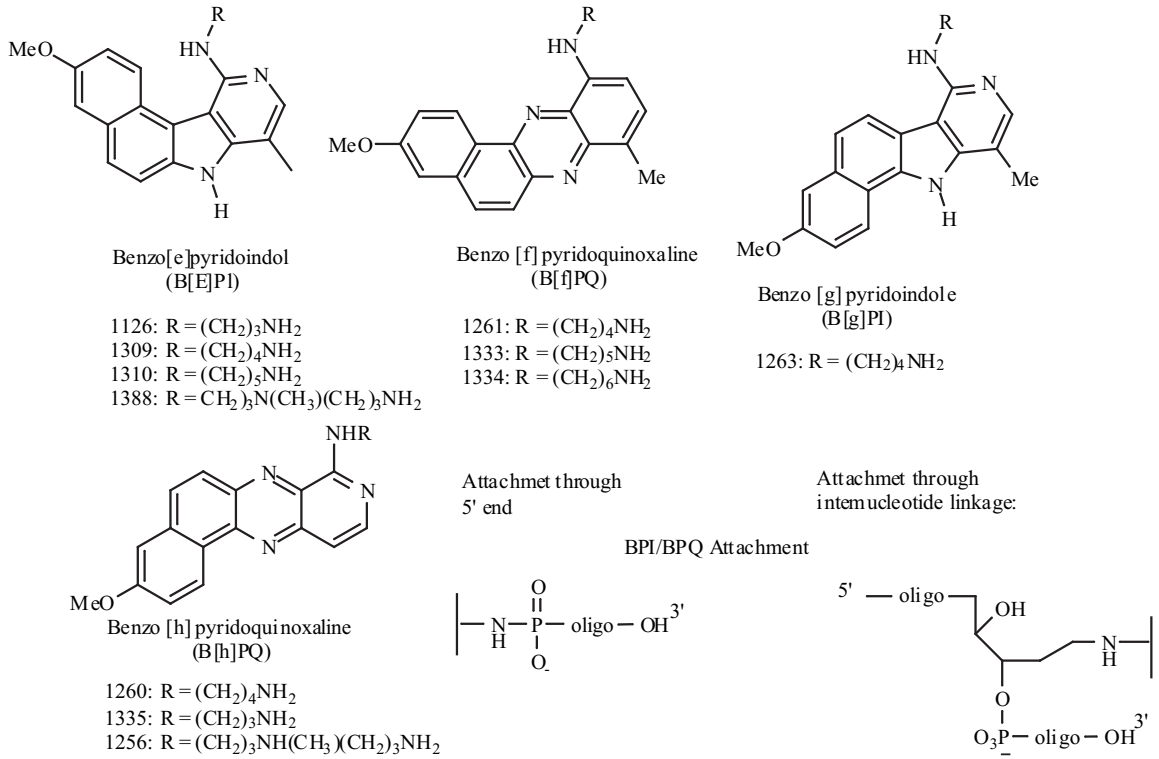


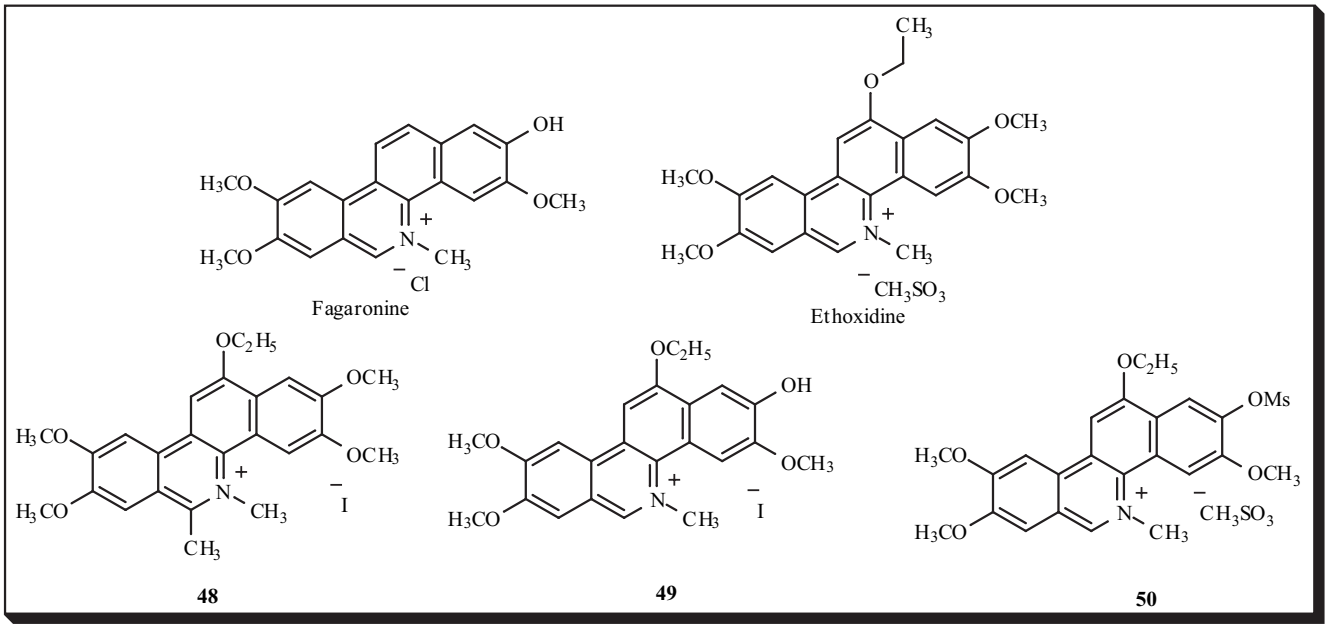
Fig. (10). Benzopyrindole and benzopyridoquinoxaline oligonucleotide conjugates.

stability of these triplexes is higher than the acridine conjugates. Molecular modeling studies support these findings.

Phenanthridines

Surface-enhanced Raman scattering (SERS) spectroscopy and flow linear dichroism are powerful techniques for selective analysis of the structure of anticancer agents and their complexes with DNA [132]. When these techniques were used to study the interactions of the potent anticancer agent fagaronine and its derivative ethoxidine with DNA, the results confirmed that both compounds are strong major groove intercalators. In addition, SERS spectroscopy reveals pronounced differences in molecular interactions of Fagaronine and Ethoxidine with DNA [133]. These differences were explained in terms of the chemical nature of the substituent groups of the drugs. It was supposed that the OH group of fagaronine is directed to the minor groove, whereas the OCH_2CH_3 group of ethoxidine protrudes into the minor groove where it is accessible for interactions with the DNA-binding intracellular enzymes.

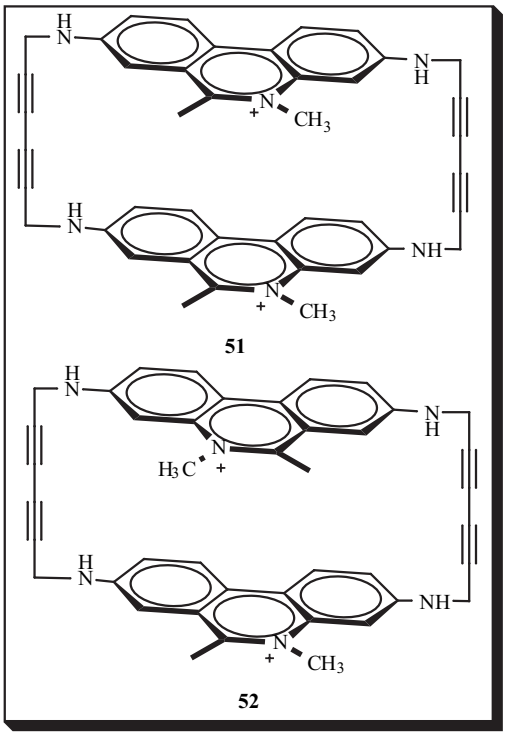
The search for better anticancer drugs has resulted in the synthesis of three new derivatives **48**, **49**, and **50**, which are



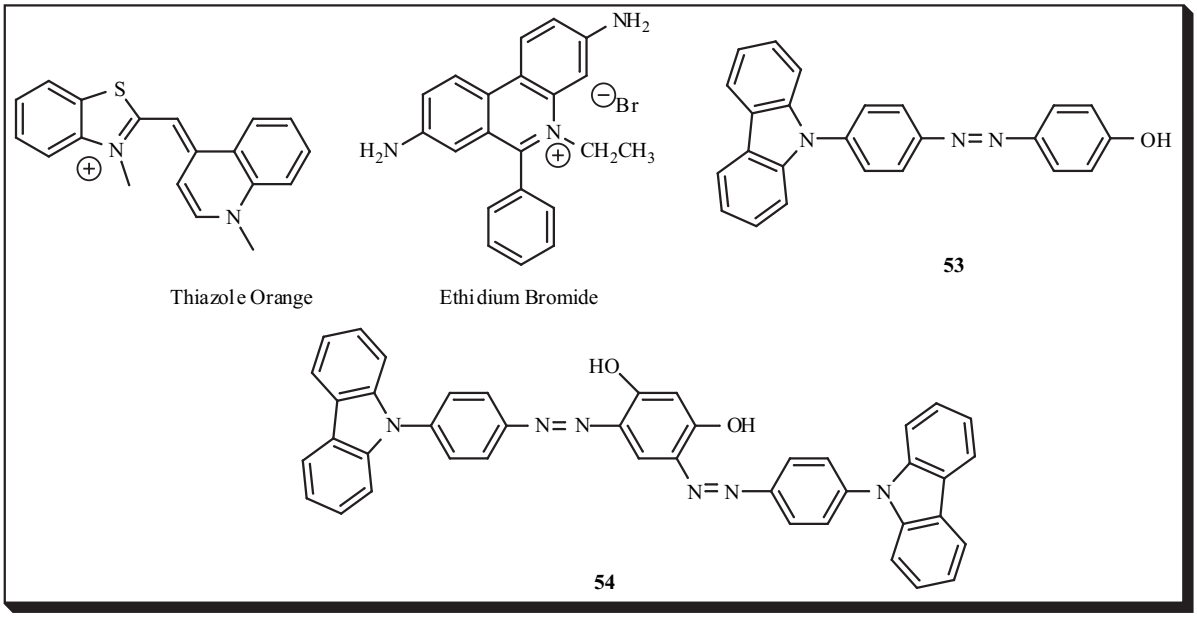
analogs of nitidine and fagaronine. The role of the 12-O-ethyl, 2-hydroxy, and 6-methyl substituents in the biological activity of these derivatives has been examined [134] Analysis of DNA binding and the inhibitory activity of Topo I in human DNA demonstrates that the new compounds combining 2-, 6-, and 12-position substitution interact strongly with DNA and exhibit important Topo I inhibition. Although fagaronine and ethoxidine were cytotoxic toward all cell lines evaluated, compounds **49** and **50** exhibited weaker activities in all *in vitro* tests. Finally, poor activity was found with compound **48**, in which 6-position substitution is known to reduce cytotoxicity. These results indicate that compounds **48**, **49**, and **50** experience major problems in reaching their cellular targets.

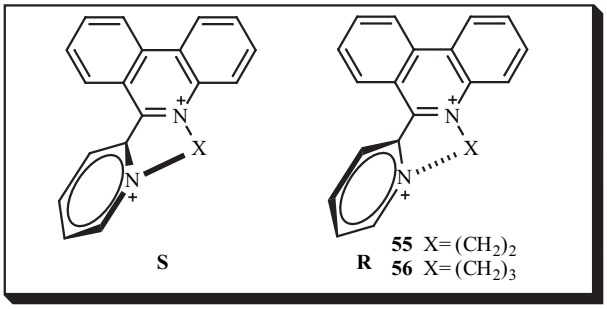
Cyclo-bis-intercaland receptors constructed by bridging two acridinium or phenatrhidinium intercalator units, bind nucleotides strongly in water by π–π stacking interactions between receptor units and the inserted nucleobase [135]. The strand binding selectivity is influenced by the length and flexibility of the connecting bridges. The synthesis of diastereoisomeric macrocycles **51** and **52**, and evaluation of their affinities for ss- and ds-polynucleotides of RNA and DNA type showed that they strongly bind AMP in water (long Ks 5.8), with significant emission increase upon complexation [136]. In contrast with GMP and UMP, only a slight change in fluorescence was observed. The ligands bis-intercalate preferentially to single-stranded rather than double-stranded polynucleotides.

Studies of small-molecule–DNA interactions demand techniques that are inexpensive, rapid, and accurate. The fluorescence intercalator displacement (FID) assay for establishing DNA binding affinity is a technique that fulfils these criteria. The FID assay relies on the fluorescence decrease that results from the displacement of DNA-bound ethidium bromide by a binding compound [137]. Recently, thiazole orange was used as a good alternative to ethidium bromide in the exploration of DNA affinity and the sequence selectivity of netropsin [138]. In addition, the azo compounds **53** and **54** were synthesized as promising fluorescence systems, and they showed an intercalative binding mode in the presence of calf thymus DNA [139].

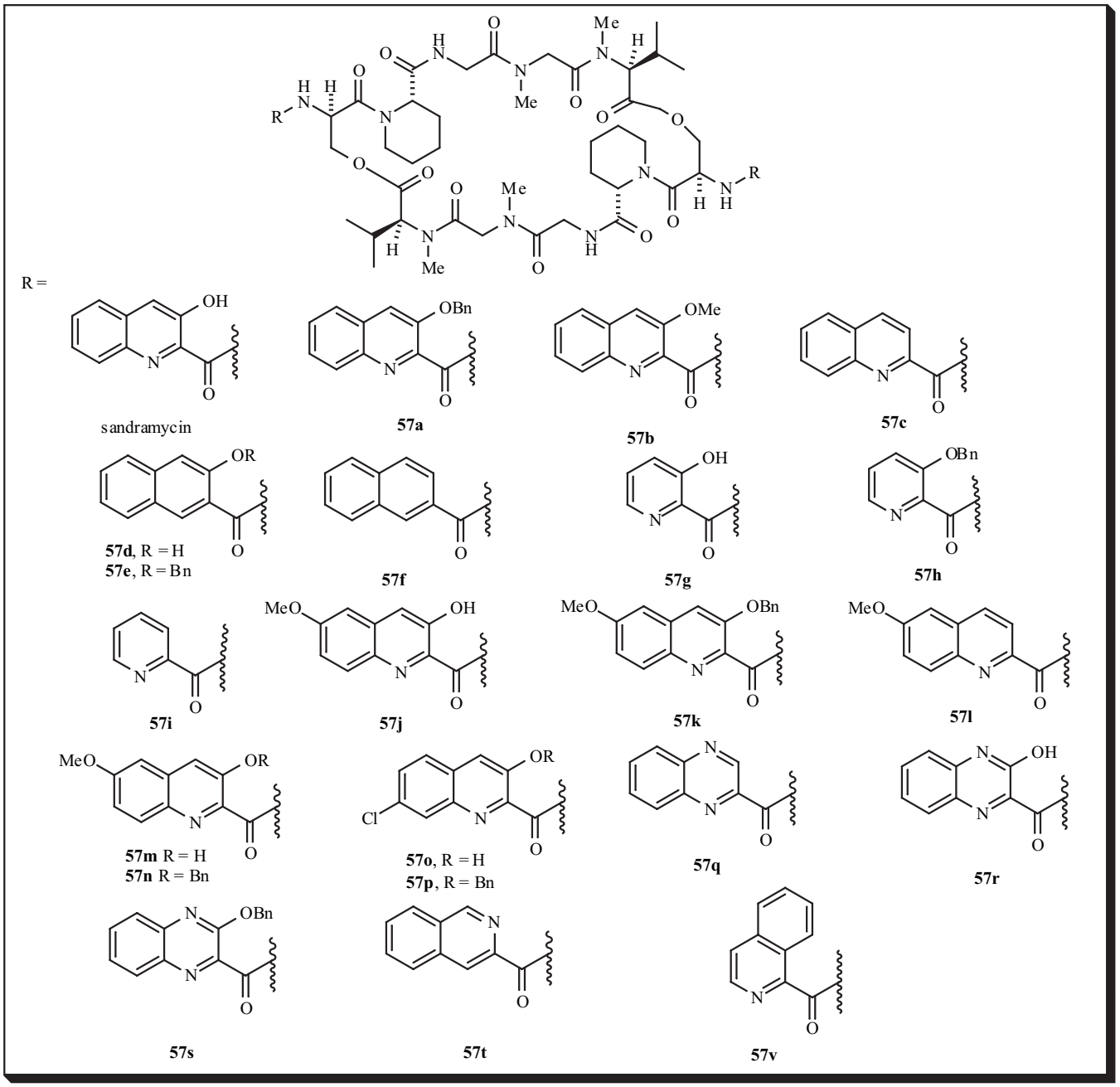


N,N′-dialkyl-6-(2-pyridyl) phenanthridine compounds are chiral photoactive drugs that target nucleic acids. The chiral nature of these compounds, which originates from the existence for each drug of two minimum-energy atropoisomeric conformations with opposite pyridine orientations (R and S), enables an enantiospecific interaction with DNA, a characteristic that has been demonstrated both theoretically and experimentally [140]. Unlike ethidium, their fluorescence is completely quenched by DNA, and it has been proposed that this quenching is the initial step in the mechanism of photosensitized DNA cleavage by these drugs. A study of the electronic transition moments and





excited states of two pyridinium-phenanthridinium compounds, **55** and **56**, revealed two different electronic transitions, a lower-energy transition that is polarized toward the pyridine ring, and a higher-energy transition that is parallel to the long axis of phenatrhidinium [141]. A second band, peaking at 250 nm, can be modeled by assuming four additional transitions, each with a different polarization. All of the transition moments lie in the phenanthridinium plane. The highest occupied molecular orbital (HOMO) of compounds **55** and **56** is essentially that of the phenanthridinium moiety, while the lowest unoccupied molecular orbital (LUMO) results from the interaction of the degenerate LUMOs of the pyridinium and phenanthridinium moieties. These results explain the observed high efficiency of the photo-oxidation, since the single occupied HOMO orbital is centered on the phenanthridinium moiety, and this ring becomes intercalated between two base pairs upon binding to DNA. Therefore, a large overlap between the HOMO of phenanthridinium moiety and the molecular orbitals of the DNA bases occurs in the complex, enabling very effective electro transfer.

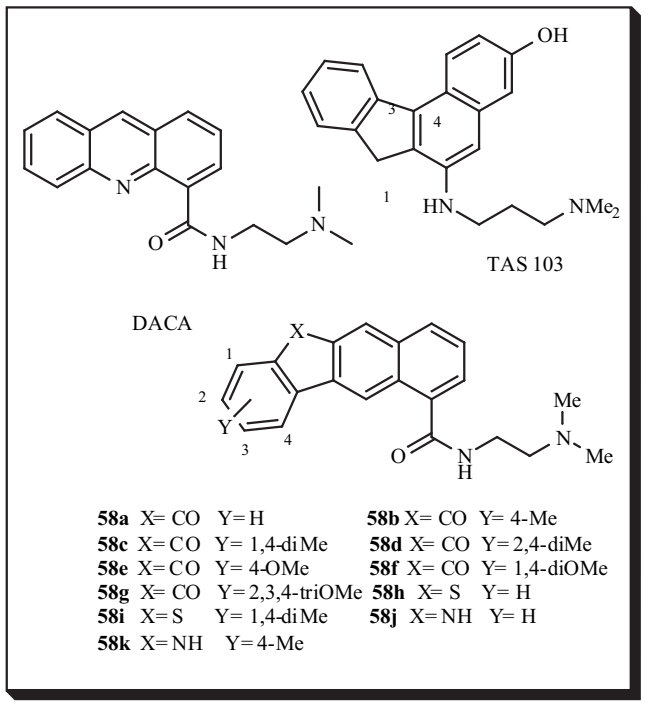


Quinolines and Quinoxalines

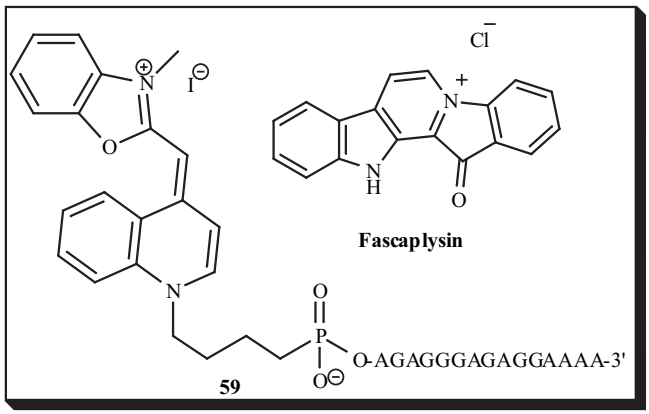
Sandramycin is a cyclic decadepsipeptide that possesses two heteroaromatic chromophores and it is a potent antitumor antibiotic [142]. To determine the influence of the intercalator moiety on DNA binding affinity, a systematic study of sandramycin analogs **57a-v** was made using calf thymus DNA, within a single high-affinity bis-intercalation binding site, 5′-d(GCATGC)$_2$. The sandramycin analogs studied differ with regard to the type and position of the substituents on the aromatic ring (OH, OMe, OBz) and the class of aromatic ring (phenanthrenyl, quinoxalyl, isoquinolyl, and pyridyl) replacing quinolinyl on the compound [143]. The role of the individual structural features of the chromophore was evaluated with the high-affinity duplex sequence 5'-d(GCATGC)$_2$. To a first approximation, the cytotoxic properties were found to parallel trends established in the DNA binding affinities, with the exception of compound **57c**, which lacks the sandramycin chromophore phenol. In addition, it was determined that sandramycin binds to 5′-(GCXXGC)$_2$, where XX=AT, TA, GC, or CG, showing a preference that follows the order: 5′-d(GCATGC)$_2$ > 5′-d(GCGCGC)$_2$ > 5′-d(GCTAGC)$_2$ > 5′-d(GCGCGC)$_2$.

The DNA intercalators 6-[[2-(dimethylamino)et-hyl]-amino]3-hydroxy-7*H*-indeno[2,1-*c*]quinoline-7-one dihydro-chloride (TAS 103) and *N*-[2-(dimethylamino)ethyl]-acridine-4-carboxamide (DACA) are dual Topo I/II inhibitors with potent cytotoxicity in a panel of leukemia lines [144,145]. One of their most prominent structural characteristics is the presence of a carboxamide group. The biological activity and binding sequence specificity of this group is considered essential to its mechanism of action. Thus, new intercalators **58a-k** were synthesized in which indeno[1,2-b]quinoline-carboxamides, [1]benzothieno[3,2-b]quinoline-4-carboxamides, and 10H-quindoline-4-carboxamides were substituted. The cytotoxic effects observed in a panel of cell lines revealed that small lipophilic substituents in the noncarboxamide ring, in a pseudo-peri position to the side chain, increased the cytotoxic potency; in particular the methyl-substituted indeno[1,2-*b*]quinoline-6-carboxamide showed substantially increased effectiveness (20-day growth delays) in a subcutaneous colon 38 *in vivo* tumor mode that is comparable to that reported for DACA, which is currently being tested in clinical trials. Crystallographic studies of drug–oligonucleotide complexes suggest that these compounds bind via van der Waals interactions between the substituent and the cytosine residue, forming the intercalation site [146]. In addition, TAS-103, was found to bind to DNA by two binding modes [147]. The major binding mode is an outside binding to the major groove and the minor binding mode is an intercalation. It has also been discovered that TAS-103 self-associates in aqueous solution through π–π stacking and hydrophobic interactions.

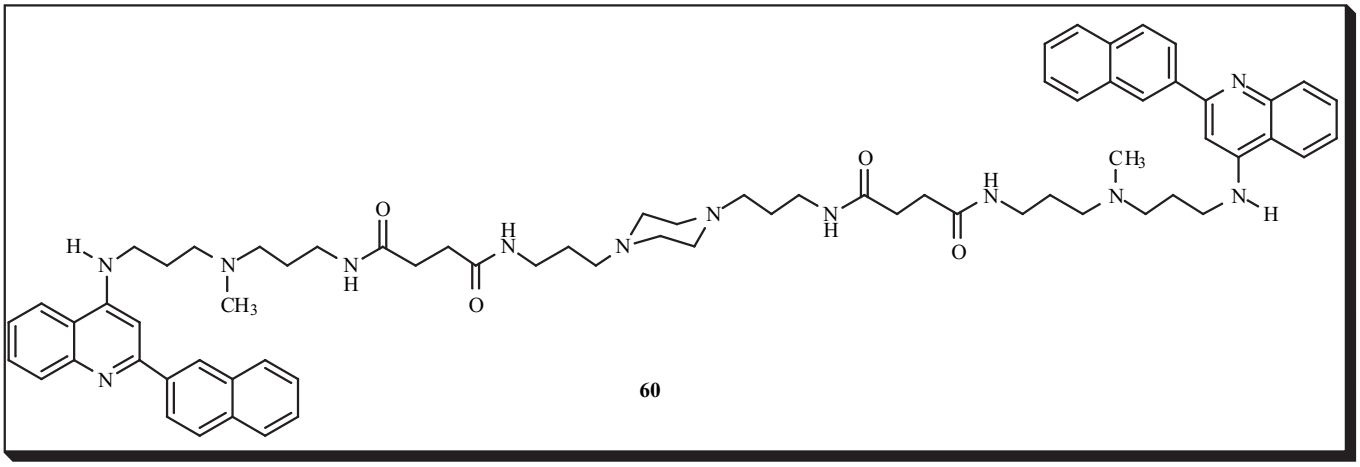
Fascaplysin is an indoloquinoline derivative that was first isolated from the marine sponge *Fascaplysinopsis* berquist sp [148]. This natural product inhibits the growth of several microbes and suppresses the proliferation of leukemia cells and a G1 arrest of tumor and normal cells. To investigate its interaction with DNA, studies involving isothermal titration calorimetry, absorption spectroscopy, and CD were performed. The results showed that fascaplysin is an intercalator of DNA with a base-pair-to-drug ratio of 2:1 [149]. These results indicate that some of its biological activity could be attributed to interference with the genetic material.



Oxazole yellow exhibits enhanced fluorescence upon binding to DNA and behaves as a typical intercalating agent [150]. The search for an intercalator-linked oligonucleotide that can form a triple helix with interleukin-2 receptor α-chain promoters led to the synthesis of an oxazole yellow-linked oligonucleotide **59** [151]. Upon light illumination, this compound exhibited a linear increase in fluorescence during triple helix formation with double-stranded DNA, and induced photocleavage of the targeted DNA in the presence of spermine. The cleavage site of one strand was seven or eight bases away from the intercalation site, whereas the other strand was cleaved at the intercalation site.



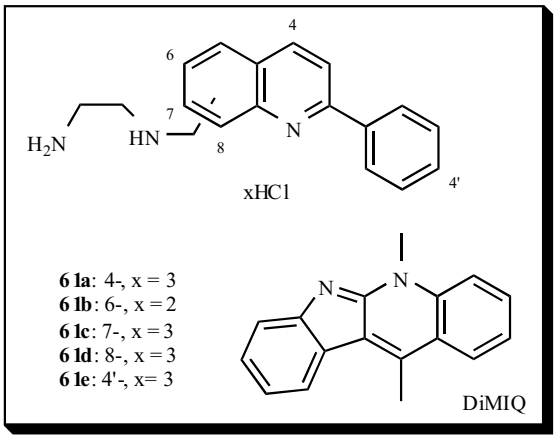
Bis-intercalators have two clear advantages over monofunctional derivatives. Firstly, bis-intercalators have a higher binding affinity and hence can be used at lower concentrations to facilitate triplex formation, thereby reducing the incidence of unwanted side effects. In theory, the affinity of an ideal bis-intercalator should be the product



60

of its monofunctional components. Such dramatic increases in binding are rarely achieved, as there are additional steric and entropic constraints. For efficient bis-intercalation, the length and nature of the linker needs to be optimized. A triplex-specific bis-intercalator, naphthylquinoline dimer **60**, was designed with this in mind. It was found that naphthylquinoline dimmer I stabilizes DNA triplexes at least 30 times more effectively than the monofunctional compound [152].

Since phenylquinolines and benzimidazoles are "minimal intercalators", and when an amino-alkyl side chain is attached to the chromophore the compounds produced exhibit the highest affinity for DNA, 2-phenylquinoline derivatives **61a-e** that have a (2-aminoethyl) aminomethyl group at positions 7, 6, or 4 of the aromatic system were synthesized [153]**.** It was found that the order of strength of binding to DNA was **61d** > **61e** > **61c** > **61a**. In addition, the supercoiled DNA unwinding assay supports the intercalation of these drugs. Likewise, the trend of cytotoxicity of the compounds, **61d** > **61c** > **61a** = **61e**, is in quite good agreement with the DNA-binding ability. These results demonstrate that the location of the ethylenediamine side chain in these derivatives has a large effect on the control of binding ability, DNA binding mode, and cytotoxicity of the compounds.

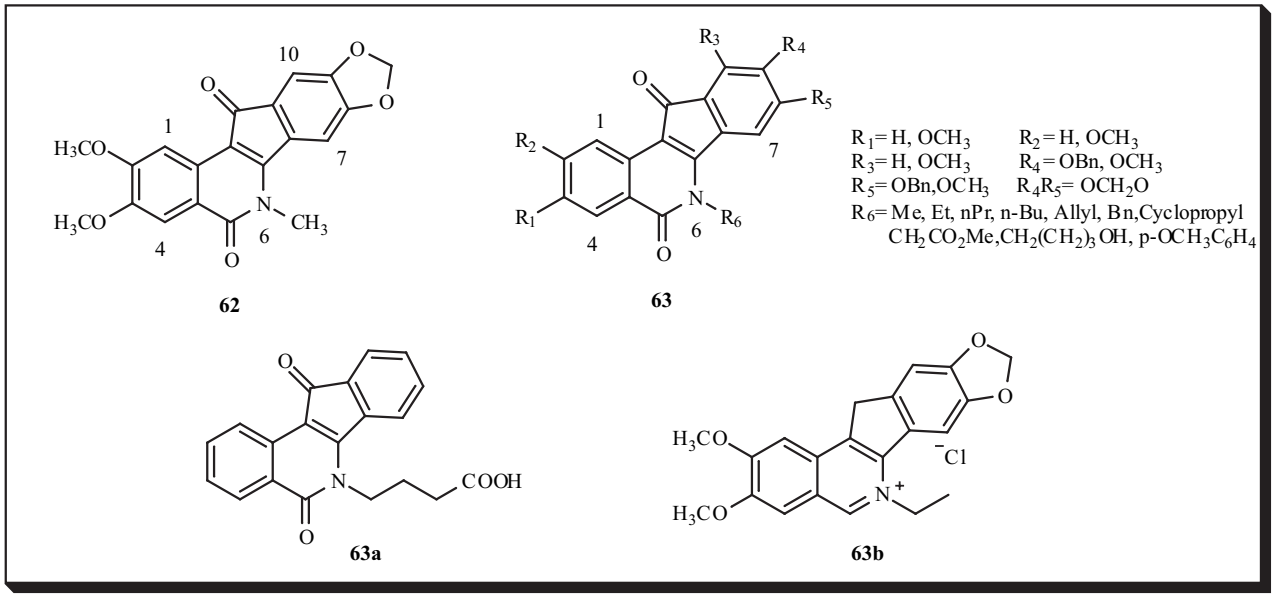


5,11-Dimethyl-5H-indolo[2,3-b]quinoline (DiMIQ) is a DNA intercalator that displays significant cytotoxic activity [154]. The mechanism of its action depends on its ability to induce and stabilize drug–Topo-II–DNA cleavable complexes. Site-specific intercalation of DiMIQ was investigated *in vitro* by DNAse I footprinting and by molecular modeling [155]. The footprinting experiment revealed that the DiMIQ molecule binds preferentially to pBR322 plasmid in the 5'-TGCTAACGC-3' region between adjacent adenine bases. The molecular modeling results corroborate the intercalation preference between adjacent adenines and indicate that the position of DiMIQ inside the DNA duplex is in an orientation parallel to the long axis of DiMIQ and the neighbor base-pair axes.

In order to maximize the cytotoxicity of the lead compound, **62** [156] in human cancer cell cultures, as well as its activity as a Topo 1 poison, several indenoisoquinolines **63** were synthesized and evaluated for cytotoxicity and for activity against Topo I in 55 human cancer cell cultures [157]. Nine compounds displayed a lower Mean graph midpoint (MGM) than the lead compound **62**, and four rivaled its Topo I activity. Two of the most potent Topo I inhibitors were compounds **63a** and **63b**, both of which also inhibited Topo II, unwound DNA, and are assumed to be DNA intercalators. However, the potencies of these compounds as Topo I inhibitors did not correlate with their potencies as cytotoxic agents.

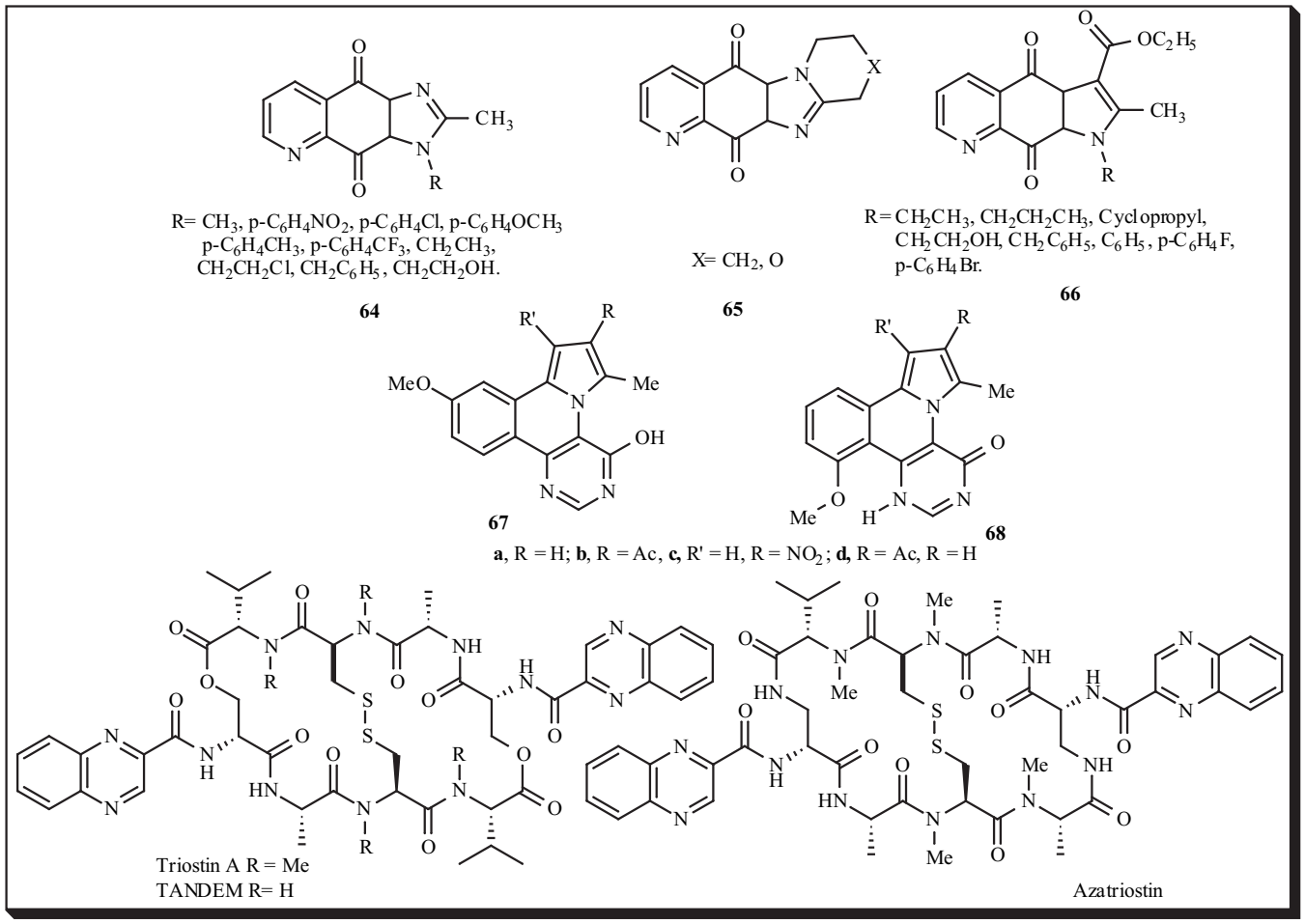
Three-dimensional quantitative structure activity relationship (QSAR) analysis, comparative molecular field analysis (CoMFA), and comparative molecular similarity indices analysis (CoMSIA) were carried out for the imidazo- and pyrrolo-quinolindione derivatives **64**, **65** and **66** to predict their biological activities [158]. Excellent agreement was obtained among the three analyses, with an error range of 0.01–0.15 between the calculated values and their measured *in vitro* cytotoxic activity against human lung A-549 cancer cell lines. The planar aromatic character of the pyrimido[5,4-c]pyrrolo[2,1-a]isoquinoline system suggests that it is acting as a DNA intercalator. The synthesis of derivatives **67** and **68**, and preliminary molecular modeling show that they are potential DNA-interactive compounds [159].

Triostin A is a member of the quinoxaline family of antitumor antibiotics that bind to DNA by bis-intercalation [160]. Triostin A binding in the minor groove exhibits a sequence preference for CG. This GC selectivity is removed

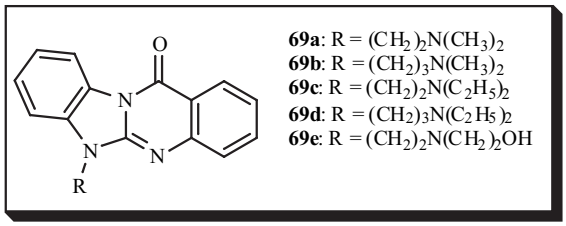


by replacement of the *N*-methyl amino acids with the natural unmethylated amino acids; the synthetic bis-intercalator TANDEM binds selectively to AT sequences [161]. Using a solution-phase strategy, a synthesis of analog Azatriostin was developed, enlisting only liquid–liquid acid/base extractions in the isolation and purification of the synthetic intermediates. Azatriostin is derived from the natural antibiotic by replacing the D-serine amino acid with D-β-aminoalanine, affording an amide versus ester linkage in the cyclic peptide backbone.

Many antitumor compounds containing a benzimidazole moiety have been described [162]. Recently, the benzimidazoquinazolines **69a-e** has been reported, which



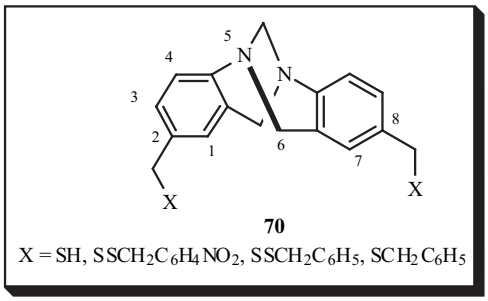
either position 6, a dialkylamino alkyl side chain or a hydroxyethylaminomethyl side chain [163]. These derivatives exhibit antiproliferative activity toward human tumor cell lines in *in vitro* assays. The cytotoxic effect depends on the type of side chain inserted into the planar nucleus, and in some cases it is comparable to the well-known drug ellipticine. Studies of the interaction between these molecules and DNA revealed that the molecular plane of the ligand chromophore is preferentially orientated parallel to the plane of DNA bases.



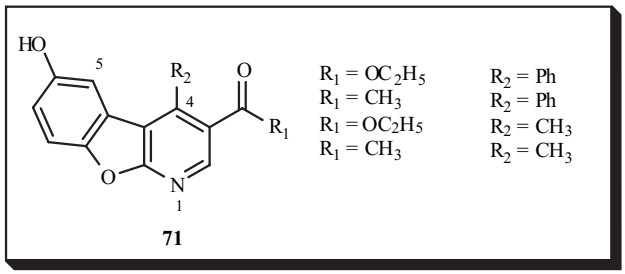
PNAs are mimics of DNA that have potential applications in molecular biology [164]. Targeting of double-stranded DNA by homopyrimidine PNA occurs by a process of invasion of the double helix, whereby two PNAs bind to the complementary DNA strand while the non-complementary polynucleotide is displaced as a single strand. A DNA-binding ligand capable of specifically promoting the strand invasion process is usually employed to reinforce the binding of PNA to double-stranded DNA. With the aim of identifying ligands that promote PNA binding, more than 50 ligands were tested, including groove binders, intercalators, and bis-intercalators [165]. Almost all of drugs have either no effect or inhibit PNA binding; however, the members of the quinoxaline family of antibiotics (echinomycin, triostin A, 2QN, Tandem) increase the binding of PNA to a double-stranded DNA target. It was suggested that quinoxalines enlarge the binding of PNA to DNA by deformation of the double helix, which facilitates the PNA invasion. A revision on the use of PNA as a tool for the development of gene expression has been published by Gambari [166].

Miscellaneous

Recently, there has been interest in the synthesis of Tröger´s base derivatives due to their DNA intercalative properties [167]. Compounds **70** were synthesized using 4-nitrobenzyl chloride as the starting material, resulting in good yields [168].



The clinical failure of most intercalator cytotoxic drugs is attributed to their poor solubility, which in turn is attributable to their polycyclic structures [169]. Their solubility can be enhanced by synthesizing small-sized tricyclic systems. Therefore, novel 1-aza-9-oxafluorenes **71** were obtained from 3-carbonyl-substituted 1,4-dihydropyridines and p-benzoquinone [170]. These are similar to α-carbolines, with the indolo-nitrogen replaced by oxygen. Cytotoxic evaluation of various cancer cell lines showed that these derivatives are more potent than the intercalative carboline systems, and that the 4-phenyl substituent is more active than the 4-methyl substituent. ^{1}H-NMR data show that the 4-phenyl substituent does not lie within the plane of the aromatic system. These experimental results suggest that the novel cytostatics do not act as intercalators.

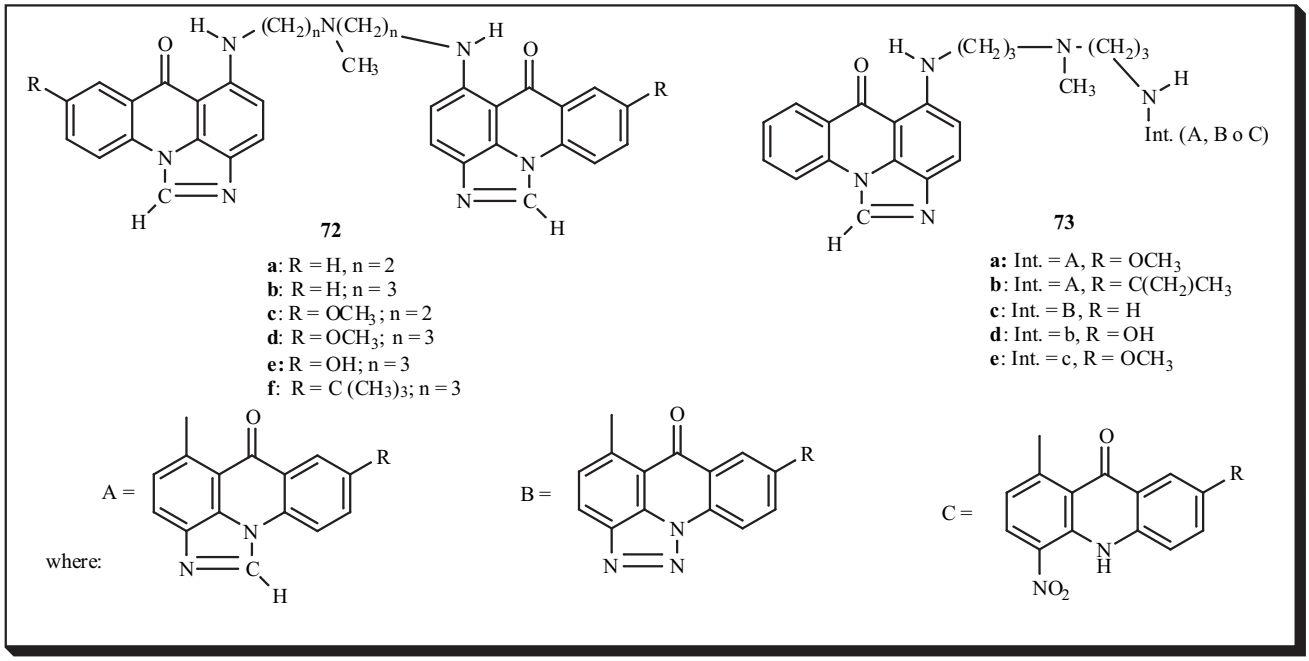


Compounds **72** were initially designed to act as bis-intercalators. Either an N^2-methyldiethylenetriamine or a 3,3´-diamino-*N*-methyldipropylamine linker holds the tetracyclic ring systems together. The asymmetrical compounds **73**, in which one of the imidazoacridinone ring systems was replaced by a triazoloacridinone ring system, were found to be cytostatic and cytotoxic *in vitro* [171]. In particular, compound **73b** showed remarkably high activity. However, preliminary experimental data and modeling results suggest that these compounds have a different mode of binding to nucleic acids that does not involve intercalation.

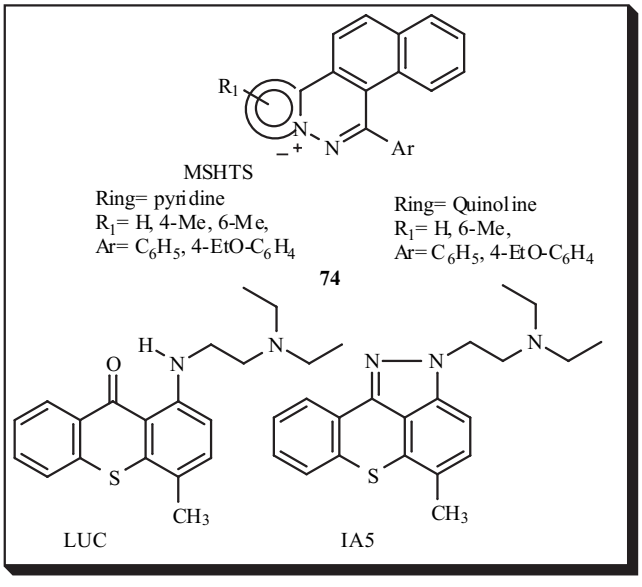
In a current research project focused on the synthesis and biochemistry of novel intercalators based on quinolizinium and aza-quinolizinium chromophores, the synthesis of the benz[f]azino[2,1-a]phthalazinium salt **74** was described using an intermolecular Westphal condensation [172].

Lucanthone is an antitumor drug that intercalates into DNA and inhibits Topo II [173]. IA-5, an indazole analog of lucanthone also inhibits Topo II and intercalates into DNA preferentially at A-T-rich sequences, but the anticancer properties of IA-5 are superior to those of lucanthone [174]. The drug stimulates DNA cleavage by Topo II with a weak preference for sites bearing a C on the 3´ side of the cleavage bond. However, binding to DNA and Topo II inhibition are two distinct processes that contribute separately to the cytotoxic activity of IA-5.

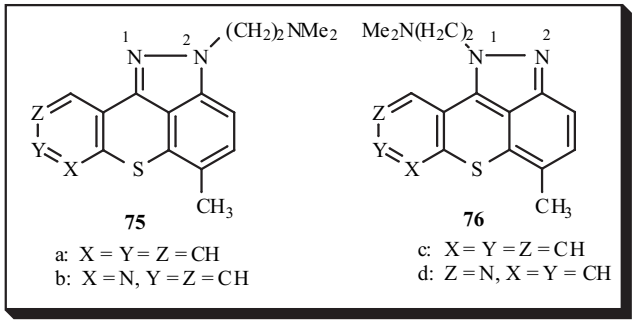
Aza-bioisosteric modification of a N,N-dimethyl derivative of IA-5 has produced several potent antitumor agents, and it has been found that the position of the nitrogen atom has an influence on the antitumor activity. The synthesis and antitumor activity of compounds **75a-d** and **76a-c** have been described in studies aimed at determining the best antitumor drugs [175]. The *in vitro* cytotoxicity of these drugs were evaluated in five different cell lines, which included murine leukemia L1210, murine sarcoma S-180, murine sarcoma resistant to Doxorubicin, S180/Dx), human colon adenocarcinoma, LoVo, and human



colon adenocarcinoma resistant to Doxorubicin, LoVo/Dx. The data clearly shows a pronounced effect of the position of the nitrogen atom on the observed cytotoxicities. Comparison of the cytotoxicity of compounds **75a** (a carbocyclic chromophore), **75b** (a 7-aza analog), and **75c**, (an 8-aza analog) reveals little difference in potency between the five cell lines. On the other hand, the analog **75d** (9-aza) exhibits a dramatic increase in potency compared to **75a-c** in all cell lines studied. In the LoVo and LoVo/Dx cell lines, the 9-aza analog **75d** is more potent than doxorubicin or mitoxantrone. The 1-substituted aza-benzothiopyranoindazoles **76a-c**, in comparison with the corresponding 2-substituted analogs, exhibits a much lower potency.



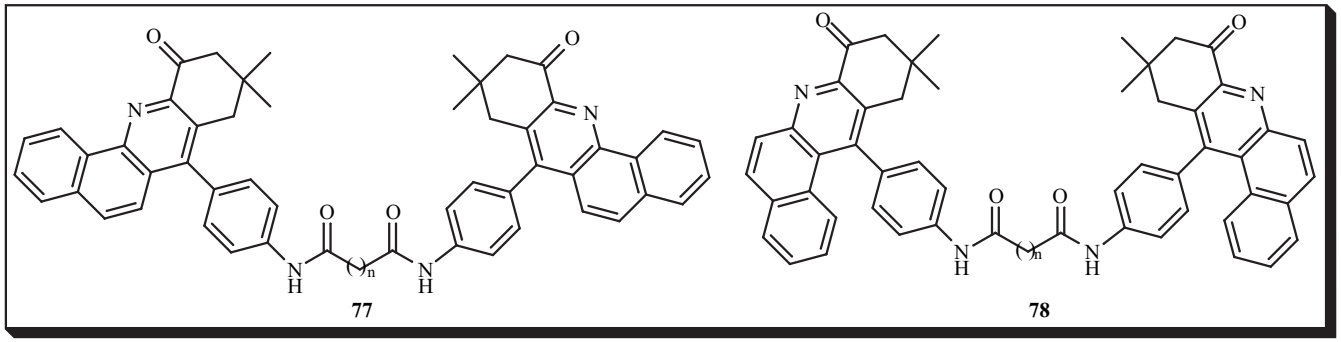
Bis[benzo[c]acridin-7-yl]phenyl]alkanediamides **77** and bis[benzo[a]acridin-12-yl]phenyl] alkanediamides **78** were synthesized in four steps using 5,5-dimethyl-1,3-cyclohexanedione as the starting material [176]. These compounds were evaluated against 60 tumor cell lines. Whereas the benzo[c]acridin-7-yl]phenyl]alkanediamides display significant cytotoxic activity, the corresponding [benzo[a]acridin-12-yl]phenyl]alkanediamide derivatives were found to be less cytotoxic. This difference was explained in terms of fusion of the benzene moiety to the acridine system in these drugs.



Pyrrolo[2,3-e]indole derivatives **79** and a dihydrobenzoindol analog **80** were synthesized to elucidate the major structural requirements for their cytotoxic activity in six different tumor cell lines [177]. The results showed that cytotoxic activity appears to be influenced by the substituents on the phenyl group, the presence of an amide group generating strong interactions such as hydrogen bonding and increased solubility.

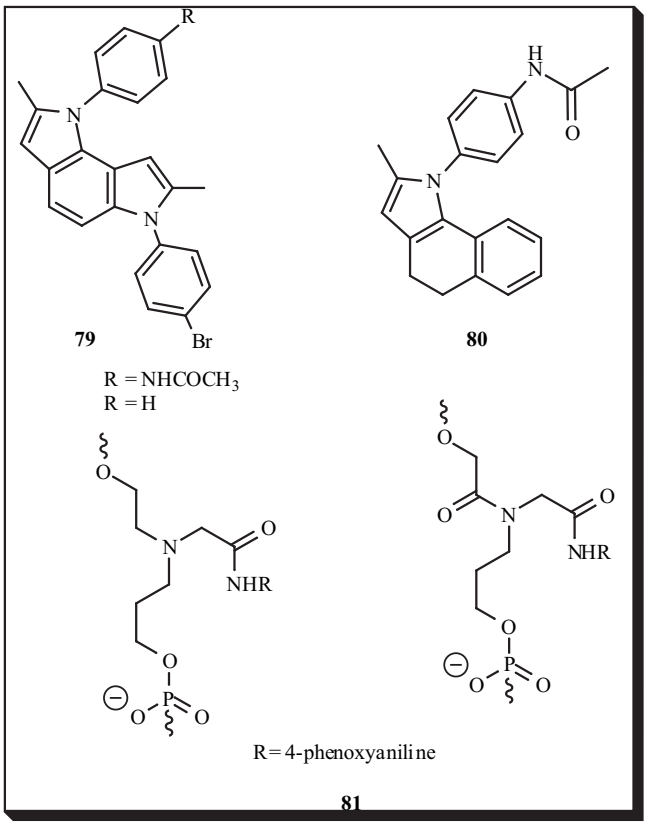
In general, in the synthesis of oligodeoxynucleotides that are stabilized by intercalators (ODNSAs), the linkers lie between the nucleobase and the intercalating agent or the intercalator is directly linked to the sugar part [178]. The DNA-conjugated phenoxyaniline intercalators **81** were synthesized, and their novelty lay in the fact that these intercalators are linked to neither a sugar part nor a nucleobase. However, only moderate stabilization of DNA three-way junctions was observed [179].

New pyrazole-annulated azathioxanthenes **82a-e** were synthesized as potential intercalators using 2-chloronicotinic acid as a starting material[180]. In addition, the synthesis of



77 78

benzo[b]carbazoles **83** from 2-arylmethylindoles has been described [181]. Noncytotoxic data has been reported in both cases.



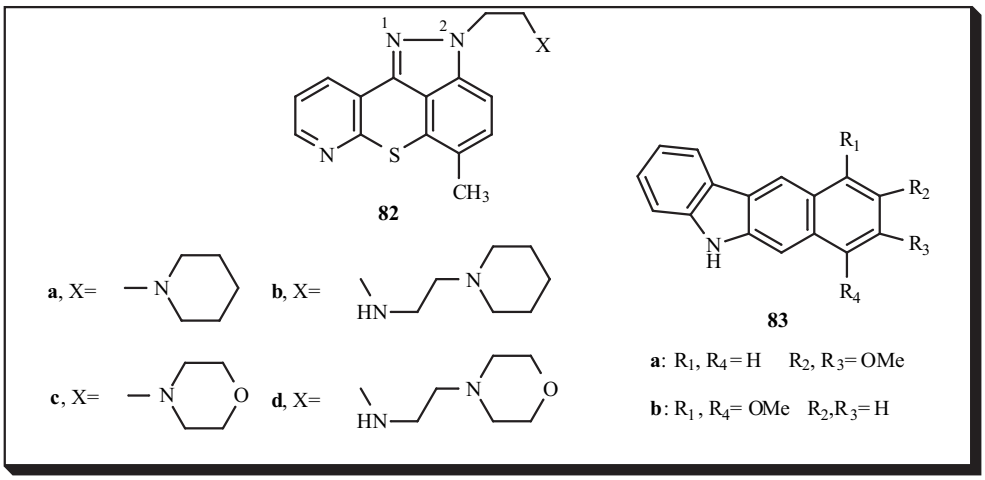
79 80 81

CONCLUSIONS AND TRENDS

This survey shows that over the past ten years, the search for new cytotoxic intercalators has mainly followed classical approaches: structural modifications to conventional molecules, new natural products and modifications of them, potential synthetic compounds, and chemical conjugates. Modifications were made to the core structure of traditional intercalators such as doxorubicin, daunomycin, and mitoxantrone, changing and/or sorting the size of rings, and varying the type and/or length of the side chains. It is well-known that natural product searching has produced outstanding antitumor drugs, some of which are either currently in use or being tested in clinical trials; cryptolepins is an excellent example, which is reviewed in this manuscript. Good results have been achieved from the qualitative structural analysis of cytotoxic intercalators and synthesis of new compounds based on general structural intercalator characteristics.

Before interacting with DNA, cytotoxic agents must overcome many barriers, including metabolic pathways and the cytoplasmic and nuclear membranes. As such, clinical failure of most intercalator cytotoxic drugs is attributed more to pharmacokinetics than to pharmacodynamics. This problem has been at least partially solved by synthesizing the salts of these compounds, such as ammonium or hydrochloride salts, or decreasing the molecular size.

Once the drug is situated in the nucleus, it must be capable of interacting with DNA to form a stable complex with a relatively long half-life. Much of the progress that has been made in trying to solve this problem is related to



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tethering intercalator moieties to nucleic acid bases, PNAs, and ODNSAs, with the aim of generating TFO and quadruplex-forming oligonucleotides. This strategy will assure the synthesis of the best intercalators with enhanced affinity and selectivity, without losing the goal of improved cytotoxic activity.

One of the main problems has been the improvement of drug selectivity. Although protein engineering has contributed much in recent years to research into ADEPT and GDEPT, there remains a considerable amount of work to be done in the area. Thus far, research has concentrated mainly on compounds that are currently in clinical use, such as daunomycin**.** In order to overcome drug resistance, it appears that the best alternative is to probe all new potential or modified compounds.

Finally, 50 years have passed since the structure of DNA was elucidated, and methods of *in silico* design are now available to any chemist and QSAR, COMFA and structure-based drug design methods are well explored. Common and useful alternatives by which to seek new antitumor compounds include the synthesis of potential cytotoxic compounds based on their structural and physicochemical characteristics, high-throughput screening, the search for natural products, and synthesis of numerous quantities of compounds to obtain data on the structure–activity relationship. It is also fascinating to see that these procedures will provide interesting information about the phenomenon of DNA intercalation and its relationship to cytotoxic activity. It should be remembered that although they are toxic, nonselective, and sometimes expensive, at the present time DNA intercalators are among the most important drugs for treating cancer.

ACKNOWLEDGEMENTS

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